ABSTRACT BOOK

2025 NF CONFERENCE

JUNE 21-24, 2025

OMNI SHOREHAM HOTEL
WASHINGTON, DC

CHILDREN'S TUMOR FOUNDATION
NF CONFERENCE

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Session Co-Chairs: Session Co-Chairs: Laura Klesse, MD, PhD, *UT Southwestern Medical Center;* Tena Rosser, MD, *Children's Hospital of Los Angeles;* Nicole Ullrich, MD, PhD, *Harvard University*

Treatment of Meningiomas in *NF2*-SWN

Friday, June 20, 1:15pm – 1:45pm

Brian Na, MD, *University of California, San Francisco*

Meningiomas are the most common primary intracranial tumors in adults, with approximately 42,000 new cases diagnosed annually in the United States and over 350,000 individuals currently living with the condition. Despite their prevalence, there remains limited consensus on treatment strategies beyond surgical resection. This challenge is particularly relevant for individuals with neurofibromatosis type 2-related schwannomatosis (*NF2*-SWN), in whom meningiomas are the second most common tumor type. This presentation will explore current approaches to the diagnosis and management of meningiomas, emphasizing the importance of a multidisciplinary care model. Case studies will be presented to illustrate key diagnostic considerations and therapeutic decision-making in this complex patient population.

Platform: Use of Case Control Data to Classify Germline Variants in *LZTR1* Occurring in Schwannomatosis

Friday, June 20, 2:30pm – 2:45pm

D Gareth Evans, MD, *University of Manchester*

Classification of variants in *LZTR1* is complicated by the high rate of Loss of Function (LoF) variants at ~1 in 323 people compared to only 1 in 527,000 people with *LZTR1*-related schwannomatosis. This ratio suggests that <1% of those with *LZTR1* LoF variants will become affected with schwannomatosis. However, once schwannomas occur in the context of *LZTR1* LoF variants, at least 50% of first-degree relatives with the variant will develop schwannomas (12/24 in our series). We therefore assessed the six most common LoF variants in gnomAD, using UK-Biobank (UKBB) frequencies as controls. All six variants were present in our series of 555 index cases who had been screened for *LZTR1.* All but one of these had an odds ratio (OR) of >29-fold and <65-fold **(Table)**. The exception was: c.1407G>A p.(Trp469Ter). This variant was found in two Finnish patients, representing 2/10 (20%) Finnish patients with schwannomatosis. However, it is also found in 0.33% of all Finnish Europeans in gnomAD, while being present at a very low frequency in UKBB controls. These data are reassuring that all LoF variants in *LZTR1* occurring in the context of schwannomas are likely causative. We therefore used case control data in variants classified in our laboratory as class 3 variants of uncertain significance (VuS) which all had inconsistent classifications on ClinVar. Two synonymous changes c.1530C>T p.(His510His) and c.2187C>T p.(Tyr729Tyr) had odds ratios close to 1.0 with one case losing the variant in a schwannoma sample. Two missense variants c.2428C>T p.(Arg810Trp) and c.353G>A p.(Arg118His) had ORs in keeping with LoF variants at 34.45 and 45.6-fold. In addition, one patient with each variant showed loss of heterozygosity (LOH) of wildtype (WT) allele. Finally a frameshift variant in the last exon of *LZTR1* c.2463dupA p.(Asp822ArgfsTer29) which cannot be assumed to be LoF as nonsense mediated decay will not occur showed an OR=225 (UKBB data was not available) with LOH in one schwannoma. These data are compelling that we can help classify all five VuS, including two likely benign synonymous variants and two likely pathogenic missense variants. In conclusion, case control data, which are usually not helpful in very rare diseases, can be extremely helpful in classifying common variants in *LZTR1.*

Table 1: Population heterozygote frequencies in gnomAD data and tumour associations of LZTR1 LoF variants and Variants of uncertain significance

LZTR1 Variant	UKBB Number With variant	Homo-zygote	Total tested	% with variant	Schwannoma-tosis index cases	Total tested	% with variant	Odds Ratio (95%CI)	P value	Somatic Evidence+
c.628C>T, p.Arg210Ter	106	0	468516	0.023%	5	555	0.901%	39.31 16.3-98.9	<0.0001	3 tumours with LOH WT
c.27del, p.Gln10AlafsTer24	101	0	389101	0.026%	5	555	0.901%	35.01 14.2-86.3	<0.0001	3 tumours with LOH WT
c.1084C>T, c.Arg362Ter*	85	0	468529	0.018%	3	555	0.541%	29.95 9.4-95.0	<0.0001	3 tumours with LOH WT
c.1407G>A, p.Trp469Ter$	2	0	468529	0.0004%	2	555	0.360%	847.25 119.1-6025.5	<0.0001	1 tumour with LOH WT
c.774del, p.Phe258LeufsTer93	70	0	416554	0.017%	6	555	1.081%	65.02 28.1-150.3	<0.0001	
c.1018C>T, p.Arg340Ter	68	0	468530	0.015%	4	555	0.721%	50.02 18.2-137.6	<0.0001	2 tumours with LOH WT
Variants in LZTR1 classified as VuS occurring in at least 2 index cases with schwannomatosis										
c.1530C>T p.(His510His)	1580	1	468529	0.337%	2	555	0.36%	1.07 0.26-4.16	NS	
c.2187C>T p.(Tyr729Tyr)	3630	8	468530	0.773%	3	555	0.58%	0.70 0.11-1.78	NS	One tumour variant lost
c.2428C>T p.(Arg810Trp)	49	0	468371	0.010%	2	555	0.36%	33.98 8.2-40.2	<0.0001	1 tumour with LOH WT
c.2463dupA p.(Asp822ArgfsTer29)	N/a			0.004%*	5	555	0.90%	225.23	<0.0001	1 tumour with LOH WT
c.353G>A p.(Arg118His)	37	0	468519	0.008%	2	555	0.36%	43.17 10.4-179.6	<0.0001	1 tumour with LOH WT

*Variant present in 0.004% of White Europeans subjects in gnomAD -not available in UKBB

+all tumours with Loss of heterozygosity (LOH) of wildtype (WT) had LOH for *NF2* plus point pathogenic variants in *NF2*

$*Variant present in 0.33% of Finnish subjects in gnomAD.

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KEYNOTE: WHAT DRIVES PLEXIFORM NEUROFIBROMA FORMATION? PROGRESS AND FUTURE PROSPECTS

Saturday, June 21, 2:00pm – 3:00pm

Nancy Ratner, PhD, *Cincinnati Children's Hospital*

Dr. Ratner is interested in the brain in Neurofibromatosis type 1 and Rasopathies, and in peripheral nerve tumors that occur in the Neurofibromatoses, NF1 and NF2. She uses genomics, animal, and cell culture models to study neurofibroma formation and neurofibroma therapeutics. Ratner received her bachelor's degree from Brown University, her doctorate from Indiana University, Bloomington (during which time she was a student in the Neurobiology Course at MBL), and was a postdoctoral fellow at Washington University in St. Louis. A member of the faculty at the University of Cincinnati from 1987 – 2004, she is currently a Professor in the Department of Pediatrics, Cincinnati Children's Hospital, University of Cincinnati, and the Program Leader for Cancer Biology and Neural Tumors Program in the Cancer and Blood Disorders Institute where she also co-Leads the Rasopathy Program and holds the Beatrice C. Lampkin Endowed Chair in Cancer Biology. She has served on numerous national and international review panels and authored over 100 peer-reviewed manuscripts and 30 reviews. She was awarded the von Recklinghausen Award in 2010, and received a Jacob K. Javits NIH Neuroscience Investigator Award in 2014.

MICROENVIRONMENTS AND THE IMMUNE RESPONSE

Session Co-Chairs: Thomas DeRaedt, PhD, *Children's Hospital of Philadelphia;* Andrea McClatchey, PhD, *Massachusetts General Hospital*

Platform: Combined SHP2 and CDK4/6 Inhibition Depletes Intratumoral Tumor-Associated Macrophages in Malignant Peripheral Nerve Sheath Tumors

Saturday, June 21, 3:25pm – 3:40pm

Lindy Zhang, MD, PhD, *Johns Hopkins University*

Purpose: Malignant peripheral nerve sheath tumor (MPNST) tumorigenesis is driven by the loss of the tumor suppressor protein, neurofibromin, which leads to hyperactivation of RAS effector pathways, suggesting that targeting of RAS-ERK signaling could be effective. We previously reported that the combination of SHP2 and CDK4/6 inhibitors had antitumor activity in immunocompromised preclinical models of MPNST. It is unknown, however, whether the presence of intratumoral immune cells may enhance the efficacy of these targeted therapies or how the therapies impact tumor immunity. Furthermore, there is emerging evidence regarding the immunomodulatory effects of molecularly targeted therapies, suggesting that immunotherapy-based combinations may provide more durable responses. Our studies of human MPNST have revealed that pathological, intratumoral myeloid cells contribute to immune surveillance evasion. Utilizing small molecule inhibitors holds the potential to directly target tumor cells as well as alter the immunosuppressive tumor microenvironment, thereby enhancing responses to immunotherapies when used in novel combinations. We therefore aim to determine the interaction between molecularly targeted therapies, with SHP2 and CDK4/6 inhibitors, and the tumor immunobiology in preclinical models of MPNST.

Methods: We used an immunocompetent, syngeneic *NF1*$^{-/-}$*;Ink4a/Arf*$^{-/-}$ mouse model of MPNST to investigate the tumor immune microenvironment (TIME) interactions with targeted inhibitors. Tumor-bearing mice received treatment with TNO155 (SHP2i), ribociclib (CDK4/6i), and their combination. We extracted treated tumors for analysis via immunohistochemistry and multiparameter flow cytometry to immunophenotype the intratumoral immune cells.

Results: The combination of SHP2 and CDK4/6 inhibition had profound anti-tumor efficacy in the syngeneic mouse model. Treatment with TNO155 resulted in depletion of tumor-associated macrophages (TAM) and enrichment of CD8+ T cells infiltrating the tumor. The addition of ribociclib further diminished TAM populations. Furthermore, TNO155 treatment depleted intratumoral myeloid-derived suppressor cells (MDSC), but the addition of ribociclib did not further modulate this compartment.

Conclusions: SHP2 and CDK4/6 inhibitors lead to alterations in the TIME. The immunomodulatory effects of SHP2 inhibitors provide rationale for combinations with immune checkpoint inhibitors. Further investigations on the adaptive signaling changes within the TIME following exposure to targeted agents can aid in designing novel combinations in the treatment of MPNST.

Full List of Authors: Lindy Zhang, MD, PhD[1,2], Stavriani Makri, MD[1], Lisa Fridman, MS[1], Rebecca Dodd, PhD[3], Jiawan Wang, PhD[1], Nicolas J. Llosa, MD[1,2], Christine A. Pratilas, MD[1]

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Disclosures: C.A.P. and J.W. had a previously fulfilled sponsored research agreement (unrelated); and have material transfer agreements providing investigational agents (related) from Novartis Institute for Biomedical Research.

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Platform: Intratumoral Plasma Cells are Required for a Durable Response to Adjuvant PD-L1 Therapy in *De Novo* MPNSTs

Saturday, June 21, 3:40pm – 3:55pm

Dawn Quelle, PhD, *University of Iowa*

Malignant peripheral nerve sheath tumors (MPNSTs) lack effective therapies. In immune competent mice bearing *de novo* MPNSTs, we reported that CDK4/6-MEK dual inhibition caused tumors to regress, delayed the outgrowth of resistant tumors, and improved survival relative to monotherapies. Drug-sensitive tumors that regressed contained plasma cells and increased CD8+ T cells. In other cancers, intratumoral plasma cells correlate with better patient survival and response to immune checkpoint blockade (ICB). Impressively, CDK4/6-MEK inhibition sensitized MPNSTs to anti-programmed death ligand 1 (PD-L1) ICB therapy with apparent cure in some mice. To directly test the importance of plasma cells in this setting, *de novo* MPNSTs were generated in *AID-/-; μS-/-* mice that selectively lack plasma cells. Tumor initiation rates were identical in wild-type and plasma cell deficient mice, as anticipated. However, treatment with CDK4/6-MEK inhibitors no longer sensitized MPNSTs in plasma cell deficient mice to anti-PD-L1 therapy. Indeed, the sustained tumor regression and curative potential of combination therapy targeting CDK4/6, MEK, and PD-L1 was completely lost in mice lacking plasma cells. Single cell RNAseq revealed that plasma cells are required for CD8 T cell infiltration and activation in MPNSTs following CDK4/6-MEK inhibition. ***These results provide the first evidence in any tumor type that plasma cells are necessary for mounting an effective, CD8 T cell-mediated antitumor immune response to ICB therapy.*** Such data highlight the potential value of intratumoral plasma cells in predicting patient response to immune-based therapies and underscore a need to better understand their role in the tumor microenvironment and anti-tumor immunity.

Full List of Authors: Joshua J Lingo[1,2], Jordan L Kohlmeyer[3], Ryan Reis[1,4], Juan A Raygoza-Garay[2], Courtney A Kaemmer[5], Elizabeth C Elias[6,7], Ellen Voigt[1,2,8], Patrick J Breheny[9], Alexander W Boyden[4], David K Meyerholz[4], Ali Jabbari[2,4], Jon Houtman[2,7], Benjamin W Darbro[2,10], Rebecca D Dodd[1,2,3,8,11], Dawn E Quelle[1,2,3,4,5,8]

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Platform: Development of a Novel Regeneration-Driven Orthotopic Patient-Derived Xenograft (PDX) Mouse Model for *NF2*-Related Schwannomatosis (*NF2*-SWN)

Saturday, June 21, 4:40pm – 4:55pm

Lars Björn Riecken, PhD, *Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany*

Purpose: Effective development and preclinical validation of therapies for human pathologies strongly rely on suitable cellular and organismal model systems. With patients in mind, usage of human cells is desirable but often limited to cell culture systems, which fall short in replicating certain in vivo conditions, particularly for systemic treatment. Instead, in vivo models often rely on transgenic animals, potentially posing later translatability challenges, especially when targeting species-specific elements. Patient-derived xenografts (PDX), introducing patient cells to mice, have been established for various cancer types to overcome this limitation, combining human target cells with in vivo, systemic testing conditions. However, for *NF2*-SWN, previous PDX attempts have had limited success due to poor engraftment and/or lack of tumor progression. Considering our established multifactorial schwannoma model, we hypothesized that previous PDX models may have suffered from overlooking microenvironmental cues that promote tumor formation, such as *Nf2*-deficient axons and injury-induced regenerative responses. With this in mind, we set out to develop a new *NF2*-SWN PDX model incorporating these cues to better reflect schwannoma formation and progression observed in human patients and thus facilitate effective therapy screening and validation.

Methods: PDX model development followed a stepwise approach, progressing from mouse-on-mouse allografts to human-on-mouse xenografts. Cells were permanently fluorescently labelled to enable in vivo tracking. Schwann cells were orthotopically injected into sciatic nerves immediately before unilateral nerve crush injury. Initially, primary mouse Schwann cells were allografted to assess engraftment feasibility and define microenvironmental requirements. Next, different immunodeficient strains were evaluated for their ability to support nerve regeneration. Finally, *NF2*-deficient human Schwann cells were xenografted into SCID mice and engraftment success assessed histologically at 1 and 3 months.

Results: Early allografts comparing wild type and *Nf2*-deficient donor Schwann cells showed a significant impact on schwannoma size. These findings may suggest potential for ATMP cell therapy, where healthy transplanted Schwann cells suppress schwannoma growth. Xenografted *NF2*-deficient human Schwann cells integrated efficiently and persisted in murine sciatic nerves for at least 3 months, maintaining healthy morphology. Long-term xenografts further exhibited morphological features suggestive of increased vascularization, similar to human schwannomas.

Conclusions: Leveraging a regenerative nerve microenvironment, we established efficient, stable xenografts of human Schwann cells in mouse sciatic nerves. Future work will further refine and validate our xenograft model and assess its responsiveness to existing therapies (e.g., Brigatinib) with the goal of developing a preclinical platform for screening and validating of novel therapeutic approaches using human *NF2*-SWN tumor cells.

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Platform: Window of Opportunity Study of Nivolumab and Ipilimumab in People with Neurofibromatosis Type 1 and Newly Diagnosed Malignant and Pre-Malignant Peripheral Nerve Sheath Tumors

Saturday, June 21, 5:20pm – 5:35pm

Jaishri O. Blakeley, MD, *Johns Hopkins University*

Purpose: There are no medical curative therapies for NF1-associated malignant peripheral nerve sheath tumors (MPNST) and most MPNST clinical trials focus on recurrent disease. We explored the safety and feasibility of administering two doses of neoadjuvant nivolumab and ipilimumab immediately after diagnosis and before conventional therapy (surgery, chemotherapy, radiation) in people with NF1 and newly diagnosed atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) or MPNST.

Methods: Participants ≥12 years old with NF1 and newly diagnosed ANNUBP or MPNST planned for surgical resection were recruited across four centers. Nivolumab 4.5mg/kg IV and Ipilimumab 1mg/kg were administered together for two doses, three weeks apart. Cycle1 Day1 was within one week of biopsy results. All participants then received institutional-defined standard of care (SOC). Blood samples were collected at various intervals to assess pharmacodynamic and immunomodulatory markers. Tissue samples were collected to evaluate tumor-infiltrating lymphocytes and identify neoantigen-specific T cell clones. The primary endpoint was the proportion of participants completing two doses of Nivolumab/Ipilimumab and starting SOC within 8 weeks of diagnosis, with success defined as at least 60% (9 of 15) achieving on-time SOC. Secondary endpoints included safety, tolerability, Numeric Rating Scale-11 (NRS-11) and Pain Interference Index, radiographic response rate, progression-free survival (PFS), clinical benefit rate at 4 months, and overall survival (OS).

Results: Eleven participants completed treatment with Nivolumab/Ipilimumab (42% female, median age 35 years, all MPNST). Ten of 11 (90%) received SOC treatment within 8 weeks, meeting the primary endpoint. There was one dose-limiting toxicity (DLT) across 11 participants (9%) of decreased lymphocyte count. Common adverse events (AEs) related to Nivolumab/Ipilimumab included fatigue, pain, and decreased lymphocyte count. Among 75 AE events attributed to Nivolumab/Ipilimumab, 40% were also attributed to SOC chemotherapy or radiation. No participant stopped treatment due to toxicity. Six of 11 participants (55%) had progressive disease; current estimated PFS is 4-12 months. Five of 11 participants opted to continue nivolumab with SOC chemotherapy or radiation therapy. Four of 11 participants (36%) have died; current estimated OS is 9-31 months from diagnosis. Enrollment will be completed by April 2025, with blood and tissue analyses and central MRI review to follow.

Conclusions: The combination of the programmed death 1 (PD-1) inhibitor nivolumab and the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor ipilimumab is safe, tolerable and feasible for people with newly diagnosed NF1 associated MPNST in advance of immune suppressing SOC therapies.

Full List of Authors: Xiaobu Ye, MD, MS[1]; Jolene Steibel[2]; Ping Chi, MD, PhD[3]; Sameer Farouk Sait, MBBS[3]; Anna Piotrowski, MD[3]; AeRang Kim, MD, PhD[4]; Stavriani C. Makri, MD[1]; Christine Pratilas, MD[1]; Jessica Wollett[1]; Amber Michalik[1]; Allan Belzberg, MD[1]; Michael Caterina, MD, PhD[1]; Shivani Ahlawat, MD[1]; Michael Lim, MD[5]; Angela Hirbe, MD, PhD[2]

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KEYNOTE: WHAT DOESN'T KILL THE TUMOR CELL MAKES IT…SENESCE

Sunday, June 22, 9:00am – 9:40am

David A Gewirtz, PhD, *Virginia Commonwealth University*

Malignant Peripheral Nerve Sheath Tumors (MPNSTs) represent the sixth most common type of soft tissue sarcoma. MPNSTs tend to grow rapidly, and generally demonstrate a high risk of local recurrence and distant metastases. A significant fraction of MPNST cases (approximately 50%) are associated with Neurofibromatosis Type 1. The most effective treatment is full surgical resection. However, this approach is often not feasible. In those cases where the tumors are not amenable to resection and for metastatic disease, chemotherapy is considered an alternative option utilizing drugs such as doxorubicin, ifosfamide, carboplatin, and cisplatin as well as targeted therapies, and in certain cases, radiation therapy. However, while radiation provides effective local control, most current chemotherapies are largely ineffective.

While the goal of these treatment strategies is tumor cell killing, primarily through the promotion of programmed cell death via a process termed apoptosis, all of these therapies have in common their capacity to promote tumor cell senescence. Senescence is a specialized (and generally prolonged) form of cell growth arrest that occurs in response to cancer therapy (as well as other stress stimuli). Senescence is characterized by tumor cell flattening and granulation, expression of SA-β-galactosidase [SA-β-gal]), secretion of a spectrum of chemokines and cytokines comprising the senescence-associated secretory phenotype (SASP), and development of epigenetic changes.

While senescence has traditionally been considered an *irreversible* form of growth arrest, it has become evident during the course of the last decade that tumor cells can recover/escape and re-emerge from therapy-induced senescence. Our laboratory has demonstrated escape/proliferative recovery of breast cancer, lung cancer, colon cancer and prostate cancer from multiple forms of therapy-induced senescence. Studies from our laboratory as well as others now strongly support the premise that senescence is unquestionably a *durable* form of growth arrest, but that *the arrest is not necessarily permanent.* Although the *majority* of senescent cancer cells may remain indefinitely arrested or eventually die by apoptosis or mitotic catastrophe and/or be eliminated by the immune system, cells that escape from a senescence-arrested state to generate surviving and replicating progeny ***have the potential to contribute to cancer progression and recurrence.***

It appears to be highly likely, if not a certainty, that Malignant Peripheral Nerve Sheath Tumors treated with chemotherapeutic agents or radiation will also enter into a temporary state of senescence from which at least some tumors will recover and thereby limit the effectiveness of these therapeutic strategies.

My presentation will discuss this phenomenon of therapy-induced senescence and current strategies that are being investigated for eliminating the surviving senescent tumor cell population.

NEXT GENERATION TREATMENTS

Session Co-Chairs: Angela Hirbe, MD, PhD, *Washington University School of Medicine, Missouri;* Christopher L. Moertel, MD, *University of Minnesota;* Brigitte C. Widemann, MD, *NIH National Cancer Institute*

Platform: Deconvoluting and Targeting Mechanism of Resistance to SHP2 Inhibition in Malignant Peripheral Nerve Sheath Tumors

Sunday, June 22, 9:40am – 9:55am

Jiawan Wang, PhD, *Johns Hopkins University*

Background: The most recurrent genomic alterations underlying the pathogenesis of malignant peripheral nerve sheath tumor (MPNST) are loss of function of neurofibromin (*NF1,* 90%), *CDKN2A* (60-80%), PRC2 (70-90%), and gain of chromosome 8 (80%), yet molecular targeting of these alterations represents a unique challenge. Novel rationally-designed strategies are therefore desperately required for the treatment of this rare disease.

Purpose: We reported the anti-tumor activity of targeting SHP2 in models of MPNST (alone or in combination), and here seek to identify mechanisms of adaptive and acquired resistance to clinical-stage SHP2 inhibitors (SHP2i), and determine the activity of SHP2i-centric combinations in NF1-MPNST utilizing clinically available agents.

Methods: *In vitro* cell-line models with acquired resistance to SHP2i were established through chronic drug exposure, and resistance was confirmed from ERK signaling, cell proliferation and growth, comparing resistant/ parental lines. Mechanisms of resistance were explored using biochemical studies focused on selected pathways suggested by **preliminary data.** Comprehensive investigations are ongoing based upon whole genome sequencing on four resistant/ parental pairs and RNA sequencing (RNAseq) on two of these pairs. Traditional and patient-derived NF1-MPNST cell lines were used for the *in vitro* evaluation of ERK signaling and cell growth in response to loss/gain of function of specific driver genes or drug treatment, using nuclear and cytoplasmic fractionation, active RAS pull down, immunoblotting, IncuCyte real-time monitoring, cell proliferation assay measuring metabolic activity, 2-dimension colony formation assay, RNAseq. Genetic manipulation was performed using lentivirus-based shRNA-mediated inducible knockdown and doxycycline-inducible gene expression system. *In vitro* potency of single agents and their combinations was assessed, and *in vivo* efficacy will be evaluated in four patient-derived xenograft models.

Results: We established and validated five cell-line models with acquired resistance to SHP2i, and demonstrated that YAP activity is markedly upregulated in three resistant models. A panel of NF1-MPNST cell lines proved differential sensitivity to pharmacological inhibition of YAP/TAZ—TEAD, but achieved combination benefit when combined with a SHP2i through more potent suppression of downstream oncogenic signaling in both traditional and patient-derived, and SHP2i-resistant models of NF1-MPNST.

Conclusions: Enhanced YAP activity represents a mechanism of resistance to SHP2i, and combined inhibition of SHP2 and pan-TEAD using clinically available agents holds promising translational potential for NF1-MPNST and can be readily advanced into the clinic for patients with NF1-deficient tumors.

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Disclosures: Christine Pratilas and Jiawan Wang had a previously fulfilled sponsored research agreement (unrelated); and have material transfer agreements providing investigational agents (related) from Novartis Institute for Biomedical Research.

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Platform: Inhibition of Focal Adhesion Kinase Impairs Tumor Formation and Preserves Hearing in a Murine Model of *NF2*-Related Schwannomatosis

Sunday, June 22, 9:55am – 10:10am

Dana Mitchell, MD, *Indiana University School of Medicine*

Purpose: NF2-related schwannomatosis (NF2-SWN) is an autosomal dominant cancer predisposition syndrome caused by germline haploinsufficiency of the *NF2* tumor suppressor gene[1]. A hallmark of NF2-SWN is the development of bilateral vestibular (VS) and spinal schwannomas secondary to loss of heterozygosity of *NF2* in Schwann cells and their precursors. While benign, VS are associated with significant morbidity[1, 2]. Compromise of cranial and spinal nerve function due to compression or inflammation can cause significant neurological deficiencies including hearing loss, vertigo, facial paralysis, chronic neuropathic pain, and even death[1, 2]. Surgical excision remains the standard of care, but due to nerve involvement and proximity to adjacent vital structures, this can be associated with significant risks[3]. At present, no long-term effective therapies have been identified and as such, the development of pharmacologic approaches that halt or reverse the growth of these tumors remains a critical unmet need.

Methods: Through advanced biochemical and transcriptomic analyses, we evaluated the tumor-intrinsic and microenvironmental impact of both genetic and pharmacologic inhibition of FAK in a murine model of NF2-SWN.

Results: Here we demonstrate that genetic ablation of focal adhesion kinase 1 *(FAK/PTK2)* impairs tumor formation and preserves hearing in a murine model of NF2 **(Figure 1A&B)**. Mechanistically, we show that *Fak* deletion decreases macrophage infiltration **(Figure 2A&B)**, suppresses the HGF-MET axis **(Figure 2C&D)** and attenuates NLRP3 inflammasome activation. Pharmacological inhibition of FAK with single agent VS-4718 did not significantly reduce macroscopic tumor volume, however, its use in combination with the MEK inhibitor selumetinib resulted in both a significant reduction tumor volume, preservation of dorsal root ganglion (DRG) architecture and diminished collagen deposition **(Figure 3A-C)**.

Conclusions: Our findings establish a critical role for FAK in schwannoma development and provide rationale for evaluation of combination FAK plus MEK inhibition in future clinical trials for NF2-associated schwannomas.

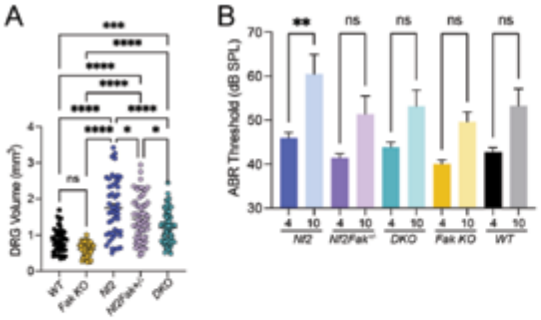

Figure 1. (A) Scatter plot depicting dorsal root ganglion (DRG) volumes (mm³) from 10-month-old mice of the indicated genotypes. Dots represent individual DRG (n=4 per mouse). Error bars reflect the standard error of the mean (SEM). P-values represent one-way ANOVA with Tukey's multiple comparisons test. **(B)** Bar plot depicting the auditory brainstem response (ABR) measured in decibels (dB) of mice with the indicated genotypes at 4 and 10 months of age P-values represent one-way ANOVA with Šídák's multiple comparisons test. Error bars reflect the SEM. ****=p≤0.0001, ***=p≤0.001, **=p≤0.01, *=p≤0.05, ns=not significant

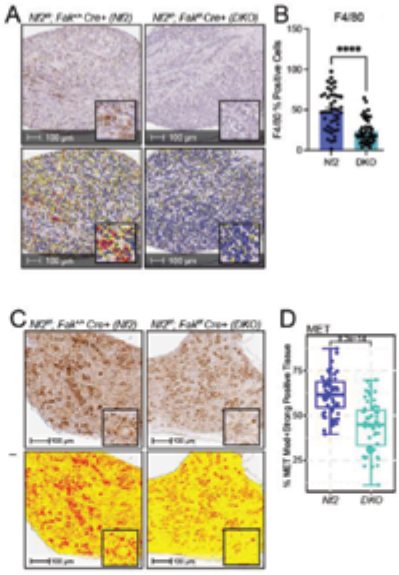

Figure 2 (A,C) Representative photomicrographs of DRG immunohistochemically stained for F4/80 **(A)** or MET **(C).** Magnification is denoted by 100 µm scale bars with inset high-power magnification as shown. The HALO area mask used to quantify the percentage of total tissue area stained positively for F4/80 or MET are shown in the bottom panel. Yellow indicates weak positive (1+), orange, moderate positive (2+) and red, strong positive (3+). **(B, D)** Plots depicting F4/80 **(B)** and MET **(D)** protein expression in DRG obtained from *Nf2* and *DKO* mice. Dots represent individual samples. P-value represents unpaired, two-tailed t-tests between groups. ****=p≤0.0001

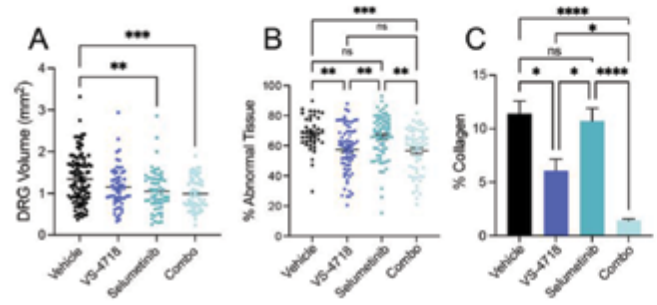

Figure 3 (A-C) Plots depicting the volume (mm³) (A) percentage of abnormal tissue (B) and collagen content (C) in DRGs obtained from mice treated with VS-4718 (n=14) and selumetinib (n=14), alone or in combination (n=15) and compared to vehicle controls. Dots represent individual DRG (n=4 per mouse). P-values represent one-way ANOVA with Tukey's multiple comparisons test. Error bars reflect the standard error of the mean (SEM). ****=p≤0.0001, ***=p≤0.001, **=p≤0.01, *=p≤0.05, ns=not significant

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Funding: National Institutes of Health grant R01-NS129769-03 (D.W. Clapp)

Platform: Proof-of-Principle of NF1 Gene Therapy in Plexiform Neurofibroma Mice Models

Sunday, June 22, 10:35am – 10:50am

Jean-Philippe Brosseau, PhD, *Université de Sherbrooke*

Purpose: The main objective is to demonstrate the conceptual feasibility of NF1 gene therapy in novel pNF mouse models with reversible NF1 expression.

Method: We build two novel NF1 xenograft mouse models with reversible *NF1* expression. In the first model, the human *NF1* $^{-/-}$ Schwann cells ipNF95.11b were genetically modified with a doxycycline-inducible full-length mouse *Nf1* gene. In the second model, the human *NF1* $^{+/-}$ Schwann cells ipNF95.11c were genetically modified with a doxycycline-inducible potent shRNA against the *NF1* mRNA transcript.

Results: First model. One month after ipNf95.11b-TetOne-Nf1 cells implantation (time to develop pNF) in the sciatic nerve, mice were split into two groups. Strikingly, all sciatic nerves from mice allowed to drink doxycycline water for one month display complete normalization of the sciatic nerve histologically (n=6 sciatic nerves) whereas 83% (5 out of 6 sciatic nerves) develop or maintain a neurofibroma when drinking regular water.

Second model. 24h after implantation of ipNF95.11c-TetON-shNF1 cells, mice were allow to drink doxycycline to allow NF1 knockdown. Strikingly, doxycycline withdrawal after neurofibroma establishment allowed complete normalization (n=4 sciatic nerves), whereas all sciatic nerves showed evidence of neurofibroma when kept on doxycycline (n=4 sciatic nerves).

Conclusion: We proof-of-principle NF1 Gene Therapy in plexiform neurofibroma mice models, providing experimental evidence supporting the development of a NF1 gene therapy solution for NF1 patients.

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Funding: This research was supported by an operating grant from the Cancer Research Society, seeding grants from Association de la Neurofibromatose du Quebec and sponsorship from NTAP for the purchase of cell lines

Platform: Combined Inhibition of eIF4A and XPO1 Synergistically Enhances Anti-Tumor Effects in MPNST Models

Sunday, June 22, 10:50am – 11:05am

Janet L Oblinger, PhD, *Nationwide Children's Hospital*

Objective: Malignant peripheral nerve sheath tumors (MPNSTs) are frequently found in patients with neurofibromatosis 1 (NF1) and are a leading cause of severe morbidities and decreased lifespan. We previously showed that MPNSTs over-express the protein translation initiation factor eIF4A (eukaryotic initiation factor 4A), facilitating uncontrolled tumor growth. We also found that the eIF4A inhibitors (eIF4Ai's), rocaglamide (Roc) and didesmethylrocaglamide (DDR), potently suppress MPNST growth. However, despite impressive tumor inhibition, not all tumor cells were eradicated, suggesting a need for combination therapy. High-throughput screening of the MIPE (Mechanism Interrogation Plates) oncology compound library identified exportin-1 inhibitors (XPO1i's) to highly synergize with Roc and DDR in killing MPNST cells. This study evaluates Roc and DDR combined with two XPO1i's, the FDA-approved selinexor and the second-generation inhibitor with improved tolerability eltanexor, in MPNST models.

Methods: Various *NF1*-related and sporadic MPNST cell lines were evaluated for synergistic growth inhibition by eIF4A/XPO1 inhibitor combinations using combinatorial proliferation matrix arrays. The mechanisms of action of eIF4A/XPO1 inhibitor combinations were analyzed by flow cytometry, Incucyte® apoptosis assay for live-cell imaging analysis, immunoblotting, and RNA-sequencing. Immunohistochemistry was used to examine XPO1 expression in MPNST tissues. The antitumor efficacies of the combinations of Roc/selinexor and DDR/selinexor were assessed in a patient-derived xenograft (PDX) model of MPNST.

Results: All MPNST cell lines and tumor tissues tested over-expressed XPO1 compared to normal Schwann cells and nerve tissues. Five XPO1i's were tested and exhibited growth inhibition of multiple *NF1*$^{-/-}$ and *NF1*-expressing MPNST cell lines at sub-micromolar doses. These XPO1i's, when combined with Roc and DDR, synergistically suppressed MPNST cell growth. Combined eIF4A/XPO1 blockade more effectively promoted G_2/M arrest and apoptosis in *NF1*$^{-/-}$ ST8814 and *NF1*-expressing STS26T cells, even at sub-IC_{50} doses. Interestingly, while eIF4Ai or XPO1i alone modestly reduced the levels of XPO1 protein, the eIF4Ai and XPO1i combinations abolished XPO1 expression to nearly undetectable levels and effectively diminished AKT and IGF-1R. Transcriptomic analysis revealed that Roc/eltanexor and DDR/eltanexor combination treatments resulted in 946 differentially expressed genes (519 upregulated and 427 downregulated) that were shared by these combinations but not induced by the monotherapies. Reactome pathway analysis revealed that most of the 427 downregulated transcripts were involved in DNA replication, DNA repair, cell cycle progression, and metabolism. Further, treatment with the Roc/selinexor and DDR/selinexor combinations led to improved tumor suppression of MPNST PDXs.

Conclusion: Combined eIF4A/XPO1 inhibition is a promising strategy to eradicate MPNSTs including those found in patients with NF1.

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Funding: CancerFree KIDS, NIH-NExT Program, Rally Foundation, US Department of Defense

Platform: Therapeutic Targeting of PRC2-Driven MPNST Metastasis

Sunday, June 22, 11:45am – 12:00pm

Alexa P. Sheehan, BS, *University of Iowa*

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas with poor clinical prognoses. Up to 50% of MPNSTs metastasize, and the 5-year survival rate of 20-35% has not improved in recent years. The overall goal of this study is to disrupt mechanisms of MPNST metastasis using clinically relevant agents. The majority of MPNSTs have loss-of-function mutations in the polycomb repressive complex 2 (PRC2), a master regulator of gene expression. Dysregulation of PRC2 activity is linked to poor prognosis, metastatic disease, and chemotherapy resistance across multiple cancer types. We previously used a combination of in vitro metastasis assays and orthotopic mouse models to identify PRC2-dependent mechanisms of metastasis in multiple paired, isogenic MPNST cells. From these, we determined that loss of PRC2 drives MPNST metastasis through remodeling of the extracellular matrix (ECM). Specifically, PRC2 deletion increases collagen-dependent invasion in vitro and drives lung metastasis in orthotopic mouse models. Additionally, clinical sample analysis demonstrates that PRC2 loss correlates with metastatic disease, increased fibrosis, and decreased survival in patients with MPNSTs. This PRC2-driven metastasis is associated with increased expression of lysyl oxidases (LOX), a family of matrix-remodeling enzymes. Several next-generation LOX inhibitors are currently being evaluated in clinical trials for the treatment of fibrotic disease and some cancers. Importantly, these agents have minimal side effects and are well-tolerated in patients. In this study, we examined two of these inhibitors: the pan-LOX inhibitor PXS-5505 and the LOXL2 inhibitor PXS-5382. Both agents selectively reduce matrix-dependent invasion with minimal impact on cell viability, suggesting these enzymes are promising therapeutic targets in MPNST patients. Current studies focus on evaluating these agents in vivo. This characterization of targeted inhibitors has high potential to improve the clinical management of MPNSTs and has broad implications for PRC2 function across multiple cancers.

Full List of Authors: Alexa P. Sheehan, Nina C. Carnevale, Qierra R. Brockman, Megan K. McGovern, Akshaya Warrier, Gavin R. McGivney, Rebecca D. Dodd

Funding: Children's Tumor Foundation Young Investigator Award (APS)

Platform: Voluntary Aerobic Exercise Attenuates Tumor Growth in a Rat Model of NF1-Driven Mammary Cancer

Sunday, June 22, 12:00pm – 12:15pm

Semira Ortiz, PhD, *Pennington Biomedical Research Center*

Purpose: Women with germline inactivation of NF1 have a significantly higher risk of developing breast cancer (BC) and 3.5-fold higher likelihood of death due to BC. Additionally, 30% of all breast cancers have somatic loss of NF1. These cases are earlier onset and exhibit drug resistance in ER positive BCs. BC is highly correlated with adiposity and physical activity. The purpose of this study was to test if diet-induced obesity or aerobic exercise modify tumor growth in Nf1 deficient rat mammary adenocarcinoma. We hypothesized that high-fat diet would increase tumor growth, whereas aerobic exercise would decrease growth and delay tumor onset.

Methods: $Nf1^{+/-}$ rats develop aggressive mammary tumors beginning at 6 weeks of age. By 13 weeks old, more than 60% of KO animals have at least one tumor. $Nf1^{+/-}$ (KO) rats and $Nf1^{+/+}$ (WT) littermates were weaned on to 10% or 45% fat diet and followed until 15 weeks of age (n=6 WT and 10 KO per group). Body weight and food intake were measured weekly. Tumor onset was noted, and size was measured biweekly using calipers. In a separate study, $Nf1^{+/-}$ (KO) rats were weaned into cages containing exercise wheels (n=10) or locked control wheels (n=9) and observed until 15 weeks old. Tumor onset was noted, and size was measured biweekly using calipers. Kaplan-Meier survival estimates were performed, where survival is defined as being tumor free. Tumor growth kinetics were analyzed using a generalized linear mixed model.

Results: Diet composition did not modify tumor onset or tumor growth kinetics in KO rats. No WT animals developed tumors. There was no significant difference in body weight between 45% and 10% fat diet groups due to reduced food intake. Contrastingly, aerobic exercise significantly improved the probability of remaining tumor-free compared to sedentary controls (median survival 11.9 and 9 weeks, respectively, $p<0.01$).

Conclusions: Our results demonstrate that exercise significantly delays tumor onset in Nf1 deficient rats. While diet did not impact tumor outcomes, this may be due to the limited time of dietary treatment before tumor onset (3 weeks) and the use of a moderate (45% rather than 60%) high fat diet. Overall, our findings highlight the potential for physical activity as an adjuvant therapy for NF1-related breast cancers.

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[1]Pennington Biomedical Research Center, [2]University of Alabama at Birmingham

Funding: Research reported in this abstract was supported by the NIDDK and NCI of the National Institutes of Health under award numbers 2T32DK064584-21 and 1R01CA265933-01A1.

Platform: Dual-Acting Inhibitors Target RAS/RAF/MERK/ERK, mTOR, and Autophagy to Treat MPNST

Sunday, June 22, 1:40pm – 1:55pm

Frank Huang, PhD, *Mayo Clinic*

Purpose: NF1associated malignant peripheral nerve sheath tumors (MPNSTs) are driven by hyperactive RAS signaling, which not only activates downstream pathways (RAF/MEK/ERK and PI3K/AKT/mTOR) but also induces ER stress. Our research has uncovered that Cyclophilin B (CyPB) directly binds activated RAS (RASGTP) and cooperates with it to amplify oncogenic signaling. Furthermore, CyPB synergizes with PIKfyve—an essential regulator of autophagy—to maintain proteostasis and support tumor cell survival. This study aims to evaluate SBI298, a novel dualacting inhibitor targeting both CyPB and PIKfyve, for its ability to disrupt these convergent survival pathways in MPNST.

Methods: In vitro experiments were conducted in NF1mutant and sporadic MPNST cell lines to assess SBI298's potency using CCK8 viability assays. We confirmed that 100 nM SBI298 significantly downregulates CyPB and PIKfyve expression by Western blot, highlighting the dual inhibition strategy. In vivo, orthotopic xenograft models were established by injecting patientderived MPNST cells into the sciatic nerve of C57BL6J mice. Tumor growth was monitored via IVIS bioluminescence imaging. A cohort of mice received SBI298 at 6 mg/kg intraperitoneally twice daily, while control animals received vehicle. Survival, tumor burden, and functional outcomes (limb impairment) were recorded.

Results: SBI298 demonstrated low nanomolar potency (IC50 range: 0.5–52 nM) in vitro. Its dual action effectively disrupts both the CyPB–RAS-GTP interaction and PIKfyve activity, resulting in marked suppression of the RAF/MEK/ERK and PI3K/AKT/mTOR pathways. In the orthotopic xenograft model using the JW23.3 murine MPNST cell line, SBI298 treatment significantly reduced tumor size and improved overall survival ($p = 0.00001$). These findings validate the synergy between inhibiting CyPB and PIKfyve as a means to impair RASdriven oncogenic signaling, autophagy, and ER stress responses.

Conclusion: Our results indicate that dual inhibition of CyPB and PIKfyve by SBI298 disrupts critical oncogenic and survival pathways in NF1associated MPNST, leading to significant tumor growth suppression and improved survival in preclinical models. This innovative strategy may pave the way for more effective therapeutic interventions for MPNST and other RASdriven malignancies.

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Funding: Mayo Clinic Foundation

Platform: Merlin Restoration Prevents Schwannoma Growth in Genetically Engineered Mouse Models of *NF2*-SWN

Sunday, June 22, 1:55pm – 2:10pm

Jeremie Vitte, PhD, *University of California, Los Angeles*

Purpose: Patients with *NF2*-related schwannomatosis (*NF2*-SWN) present with hallmark bilateral vestibular schwannomas, but also schwannomas on other cranial, spinal, and peripheral nerves, as well as meningiomas and ependymomas, caused by germline mutations in the tumor suppressor gene *NF2.* Current therapies involving surgery and radiosurgery are effective for individual tumors but are not always a viable option for patients with multiple tumors, and harbor significant risk of neurological deficits and morbidity. Gene replacement therapy is becoming a promising new treatment strategy for several neurologic diseases. This study aims to understand if re-expression of a functional merlin protein, gene product of the *Nf2* gene, in *Nf2*-deficient tumor cells, can provide preclinical therapeutic efficacy.

Methods: We have developed a new *Nf2* allele *($Nf2^{FRT}$)* that allows us to conditionally restore *Nf2* expression by activation with the Flp recombinase. We generated a new mouse model using the $Nf2^{FRT}$ allele in combination with $Nf2^{flox}$ and *Postn-Cre* alleles. As an approach to gene replacement therapy, we used adeno-associated virus (AAV) vectors expressing merlin, administered to the established schwannoma mouse model *Postn-Cre;*$Nf2^{flox/flox}$*.*

Results: Using the new conditional schwannoma mouse model *Postn-Cre;*$Nf2^{FRT/flox}$*;*$R26^{FlpoER}$*,* we validated the hypothesis that re-expression of *Nf2* reduces the growth of schwannoma. Moreover, early injection of AAV expressing merlin showed a significant reduction of schwannoma cell density in *Postn-Cre;*$Nf2^{flox/flox}$ mice.

Conclusion: Demonstrating that merlin restoration effectively controls schwannoma growth is a crucial first step toward developing this concept as a new therapeutic strategy for potentially treating schwannomas. The new mouse model will be used to better understand the cellular and molecular mechanisms involved in stopping the growth of *Nf2*-deficient tumors.

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Disclosures: MW and SRP are co-founders of NF2 Therapeutics. SRP is consultant for Akouos. No disclosures were reported by the other authors.

Funding: Funding provided by the Department of Defense CDMRP NFRP (W81XWH2110448) to AIM and MG, and NF2 Therapeutics to MG.

Platform: *In Vivo* Evaluation of MERTK Inhibitors UNC2025 and Clinically Tested MRX-2843 in Periostin-Cre;NF2$^{fl/fl}$ Schwannoma and Orthotopic Xenograft Meningioma Mouse Models

Sunday, June 22, 2:10pm – 2:25pm

Sylwia Ammoun, PhD, *University of Plymouth, UK*

Purpose: To assess the efficacy of MER Proto-Oncogene, Tyrosine Kinase MERTK inhibitors UNC2025 and clinically tested MRX-2843 *in vivo,* following successful *in vitro* validation in a patient-derived schwannoma and meningioma model (Dave et al., Oncogene. 2024;43:3049-3061).

Methods: Mice were treated daily via oral gavage with vehicle (10% DMSO/corn oil), 50 mg/kg UNC2025, or 50 mg/kg MRX-2843. Periostin-Cre;NF2$^{fl/fl}$ mice (spontaneous NF2-null schwannoma model) were treated for 21 days. KT21-*luc* NSG (orthotopic meningioma mouse model: NOD SCID gamma (NSG) mice implanted with luciferase-expressing human NF2-null grade 3 KT21 meningioma cell line) were treated for 28 days. On day 20 (Periostin-Cre;NF2$^{fl/fl}$) or day 27 (KT21-*luc* NSG), animals were pulsed with EdU (100 mg/kg) for 24 hours. Tissues were fixed for immunofluorescence or lysed for Western blot analysis. Tumour size in KT21-*luc* NSG mice was monitored weekly via bioluminescence imaging.

Results: UNC2025 and MRX-2843 significantly reduced the number of EdU+ cells in dorsal root ganglia (DRG) and vestibular ganglia (VG) in Periostin-Cre+;NF2fl/fl mice compared to vehicle controls **(Fig. 1A-D)**. MRX-2843 also significantly reduced macrophage numbers in the VG **(Fig. 2)**. In KT21-*luc* NSG mice, MRX-2843 significantly reduced bioluminescence levels compared to vehicle controls, indicating slower tumor growth. Additionally, smaller tumour size was observed in MRX-2843 -treated mice compared to vehicle-controls **(Fig. 3)**.

Conclusion: MERTK is a promising therapeutic target for schwannoma and meningioma. The clinically tested MERTK inhibitor, MRX-2843, effectively inhibits tumour cell proliferation, slows tumour growth, and reduces tumour-associated macrophages *in vivo.*

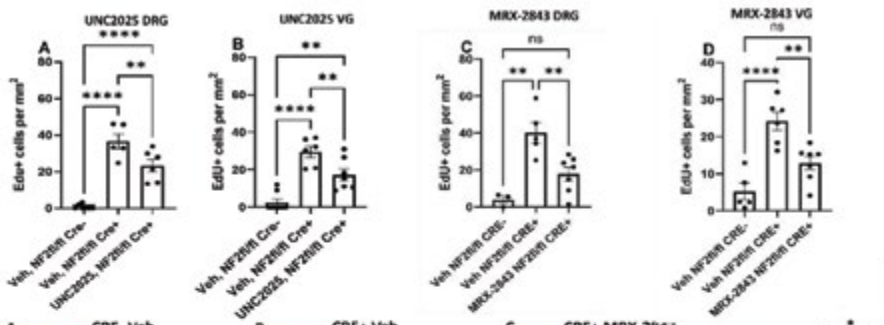

Fig. 1. UNC2025 and MRX-2843 decreased proliferation of schwannoma tumour cells in the DRG and VG of 5-month Periostin-Cre+;NF2fl/fl (NF2 null) schwannoma mice (A-D) Graphs illustrating the fluorescence immunohistochemistry results showing the number of EdU+ proliferating cells per mm². Statistical analysis was performed using one-way ANOVA with Tukey's test. Error bars ± SEM. p < 0.05 = *, p < 0.001 = ***, p < 0.0001 = ****.
NF2fl/fl Cre- Floxed mice with intact NF2
NF2fl/fl Cre+ NF2 null mice

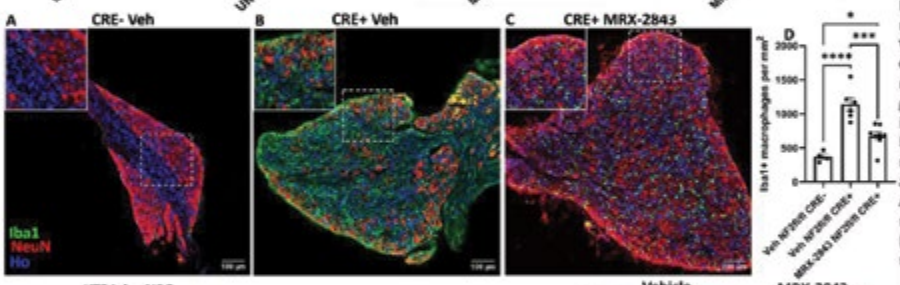

Fig. 2. MRX-2843 reduced macrophage/microglia number in the VG from 5-month Periostin-Cre+;NF2fl/fl (NF2 null) schwannoma mice. (A-C) Confocal images. **(D)** A graph depicting the fluorescence immunohistochemistry results, highlighting the number of Iba1+ macrophages per mm². Statistical analysis was performed using one-way ANOVA with Tukey's test. Iba1= macrophage & microglia marker, NeuN (neuronal nuclear protein)=neuron marker, Ho (Hoechst)=DNA stain.

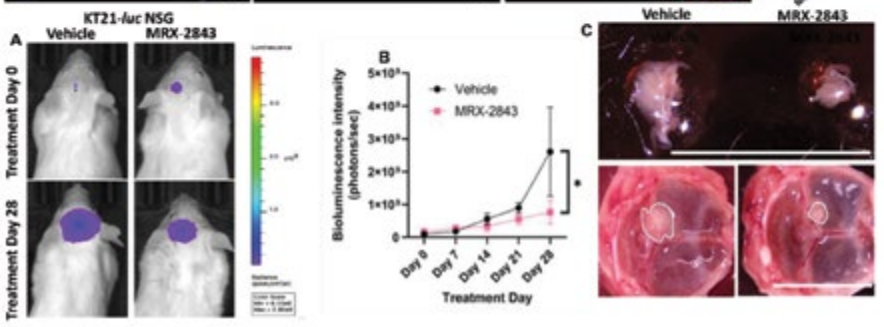

Fig. 3. MRX-2843 decreased tumour growth in KT21-*luc* NSG mice (NSG mice with engrafted luciferase-expressing KT21 cells, orthotopic patient-derived xenograft meningioma model. (A, B) The raw bioluminescence values show a significant difference between vehicle and MRX-2843-treated mice.**(C)** Images of the dissected tumours show a clear size difference, indicating inhibited tumour growth in the MRX2843-treated mice compared to the vehicle-treated group. Statistical analysis was performed using one-way ANOVA with Tukey's test.

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Funding: Drug Discovery Initiative (DDI) program.

Platform: Human Induced Pluripotent Stem Cell (iPSC) and Murine Immune-Proficient Preclinical Models of ANNUBP Reveal Sensitivities to MDM2 Inhibition and Low Dose Methotrexate

Sunday, June 22, 3:05pm – 3:20pm

Garrett Draper, BS, *University of Minnesota*

Purpose: To create novel human iPSC and immunocompetent murine models of ANNUBP amenable to high throughput drug discovery and mechanistic validation.

Methods: We generated *NF1*$^{+/+}$ human iPSC-derived Schwann cells (hiSC) and isolated *Nf1*$^{-/-}$ primary murine Schwann cells (mSC) from mice of the following genotype: *Dhh-Cre; Nf1*$^{flox/-}$*; Rosa26-LSL-Cas9-IRES-eGFP.* Schwann cells from both species were subjected to CRISPR-Cas9 knockout to generate *NF1*$^{-/-}$ or *NF1*$^{-/-}$ + *CDKN2A/B*$^{-/-}$ plexiform neurofibroma (PNF) or ANNUBP, respectively. The resulting PNF-like and ANNUBP-like cells were examined through *in vitro* tumorigenic assays. Additionally, cells were treated to 12-point drug dose response curves assessed by alamarBlue viability. ANNUBP mSCs were implanted into immune proficient and NRG mice and are currently undergoing *in vivo* drug combination treatments of MEKi and MDM2i or methotrexate to assess long-term tumor control and survival.

Results: Targeted *in vitro* Cas9 knockout of *NF1* and *CDKN2A/B* achieved 80-95% editing in hiSCs, and similar efficiency of *Cdkn2a/b* was observed in *Nf1*$^{-/-}$ mSCs. ANNUBP Schwann cells demonstrated increased proliferation, migration, and anchorage independent growth *in vitro* compared to PNF Schwann cells. We subjected PNF and ANNUBP Schwann cells to 12-point drug response studies utilizing recently described drug candidates from our laboratory. These studies revealed loss of *Cdkn2a/b* sensitized the Schwann cells to MDM2 inhibition, dependent on *Trp53* status, and uncovered a striking sensitivity to methotrexate (MTX) at nanomolar concentrations. Implantation of *Nf1*$^{-/-}$ + *Cdkn2a/b*$^{-/-}$ mSCs orthotopically into the sciatic nerve pocket of *immunocompetent Nf1*$^{+/-}$ recipient animals led to decreased tumor latency and overall median survival times compared to *Nf1*$^{-/-}$ mSCs. Additional efforts are ongoing to characterize differences in tumor infiltrating immune cells, metabolomic sensitivities which may be contributing to the observed MTX toxicity, and *in vivo* drug studies combining MEK inhibition with either MDM2i or MTX treatment.

Conclusion: We conclude that both hiSC and primary mSC can be genetically modified to replicate ANNUBP tumors, and these driving mutations carry with them unique druggable vulnerabilities. There are currently a small number of preclinical ANNUBP models despite their importance as the likely precursor to life-threatening malignant peripheral nerve sheath tumors. These current models lack the ability for medium to high throughput drug screening. Our human and murine ANNUBP models can assess numerous drug candidates and identify ANNUBP-specific drug vulnerabilities both *in vitro* and *in vivo.* Future studies using these models include identifying potential targets for immunotherapeutic intervention, all in the context of an immune proficient *Nf1*$^{+/-}$ microenvironment.

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Disclosures: D.A.L. is the co-founder and co-owner of several biotechnology companies, including NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immusoft, Inc.), B-MoGen Biotechnologies, Inc. (recently acquired by Biotechne Corporation), and Luminary Therapeutics, Inc. The business of all these companies is unrelated to the contents of this research. All other authors have no disclosures.

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KEYNOTE: THE NIH UNDIAGNOSED DISEASES PROGRAM: DISCOVERY, DIAGNOSIS, COMMUNITY, SHARING [CME]

Monday, June 23, 8:30am – 9:30am

William Gahl, MD, PhD, *NIH, National Human Genome Research Institute*

Meningiomas are the most common primary intracranial tumors in adults, with approximately 42,000 new cases diagnosed annually in the United States and over 350,000 individuals currently living with the condition. Despite their prevalence, there remains limited consensus on treatment strategies beyond surgical resection. This challenge is particularly relevant for individuals with neurofibromatosis type 2-related schwannomatosis (*NF2*-SWN), in whom meningiomas are the second most common tumor type. This presentation will explore current approaches to the diagnosis and management of meningiomas, emphasizing the importance of a multidisciplinary care model. Case studies will be presented to illustrate key diagnostic considerations and therapeutic decision-making in this complex patient population.

MANAGEMENT OF DIVERSE CLINICAL MANIFESTATIONS – CME SESSION

Session Co-Chairs: Tena Rosser, MD, *Children's Hospital of Los Angeles;* David Stevenson, MD, *Stanford University;* Kaleb Yohay, MD, *NYU Langone*

Platform: Deep Intronic *NF1* Splice Variant Consistently Causing Spinal Neurofibromatosis in Five Patients

Monday, June 23, 11:40am – 11:55am

Kimia Hashemi, MSc, *Medizinische Universität Innsbruck, Austria*

Purpose: Spinal neurofibromatosis (SNF) is a rare, distinct type of Neurofibromatosis type 1 (NF1), characterized by presence of neurofibromas affecting all spinal nerve roots, leaving no segment intact. Patients with SNF often lack or have only few cutaneous NF1 manifestations. Currently, several familial SNF cases have been reported, and there is a higher prevalence of missense and splice-site *NF1* pathogenic variants (PVs) in SNF patients compared to the general NF1 population, suggesting a potential genotype-phenotype correlation. To get better insight in the underlying molecular and cellular mechanisms driving SNF, which are still largely unexplored, we investigated germline and tumour DNA of a SNF case.

Methods: The male index patient has multiple neurofibromas, located paravertebral along the nerve roots of the cervical, brachial, and lumbosacral plexus, as well as along the intercostal nerves and throughout the extending peripheral nerves. Notably, he lacks typical NF1-associated skin manifestations such as café-au-lait spots, freckling and cutaneous neurofibromas. Aged 18 years, due to localized pain, the patient underwent tumor excision from the right gluteal and pretibial regions, histologically evaluated as neurofibroma and schwannoma, respectively. Ten years later, severe neurological symptoms arouse from high-grade cervical spinal cord compression caused by intraspinal components of multiple cervical neurofibromas, of which several were removed.

Germline genetic testing included *NF1* transcript analysis. To determine whether the multiple (spinal) tumors have independent 2nd-hit somatic *NF1* alterations or are manifestations of a single tumor linked by a common 2nd-hit, SNP array and next generation sequencing (NGS) were conducted on DNA extracted from a cervical neurofibroma, followed by ddPCR to screen for the identified somatic mutation in other tumor samples.

Results: We identified a deep intronic PV, NM_000267.3(NF1):c.5547-363T>G, causing exonisation of 176 nucleotides from *NF1* intron 38 (r.5546_5547ins5547-538_5547-363), leading to a premature stop codon, p.Ser1850Leufs*2. This PV has been found in four additional NF1 patients, all with multiple spinal neurofibromas and minimal/no other NF1 features. In the cervical neurofibroma, we identified a somatic frameshift variant which was absent in the pretibial tumor. No alternative 2nd-hit variant was identified in this tumour and the analysis of the right gluteal neurofibroma was prevented by low cellularity.

Conclusion: Identification of NM_000267.3(NF1):c.5547-363T>G in five patients, all with massive spinal, but minimal/no cutaneous involvement suggests an association of this PV with SNF. Moreover, our data suggest different somatic *NF1* 2nd-hit alterations in the patient's tumours arguing against them having a shared origin.

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Platform: Somatic *KRAS*$_{G12V}$-Variant as a Driver for Localized Hypertrophic Neuropathy Mimicking Plexiform Neurofibroma

Monday, June 23, 11:55am – 12:10pm

Pia Vaassen MD, *Sana Kliniken Duisburg, Germany*

Purpose: Localized hypertrophic neuropathy (LHN) is a neoplastic disease characterized by hypertrophy of single peripheral nerves. Affecting spinal nerve roots it can mimic plexiform neurofibroma (PN) growth. Since pathogenic somatic *KRAS* variants have been identified in biopsies from LHN patients it is supposed that LHN is caused by dysregulation of the RAS-MAPK-signaling pathway and subsequent hyperproliferation of neural cells. Therefore, RAS-induced LHN might be a novel therapeutic target for the MEK inhibitor selumetinib in addition to the approved use for NF1-associated PN.

Methods: A 15-year-old boy presented with a deep ulcer under the right foot due to persistent numbness over 1 year. Clinical examination revealed gait disturbance and hypesthesia of both legs but was not suggestive of a neurocutaneous disease. MRI showed massive localized tumor formation of the lumbar spinal roots reminiscent of PN growth in neurofibromatosis type 1 (NF1). Sequence analysis was unable to uncover a pathogenic *NF1* variant in germline DNA of the patient. Biopsy excluded a PN and demonstrated normal neural tissue dominated by mature ganglion cells. Furthermore, sequencing and SNP array of this nerve biopsy revealed no indication for *NF1* inactivation. However, it revealed the somatic *KRAS*$_{G12V}$-variant.

Results: This *KRAS*$_{G12V}$-variant likely being the somatic driver of nerve hypertrophy opened a new opportunity for a targeted therapy. However, specific KRAS-inhibitors are only available for *KRAS*-$_{G12C}$-variants. We decided to use the MEK inhibitor selumetinib to downregulate KRAS-induced hyperactivity of the MAPK pathway and reduce nerve hypertrophy. To date the patient still is on selumetinib treatment, which is tolerated with manageable adverse events.

Conclusion: This patient adds to a growing number of cases that show oncogenic somatic *KRAS*-driver mutations might cause LHN, which can mimic PN growth in NF1. Selumetinib could provide a novel therapeutic option for LHN as previously shown in other reports (PMID 32388560, PMID 39385823, PMID 27450488).

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BASIC / PRECLINICAL PLATFORMS – CONCURRENT SESSION

Session Co-Chairs: Elliot Robinson, MD, PhD, *Cincinnati Children's Hospital Medical Center;* Miriam Smith, PhD, *University of Manchester (UK)*

Platform: Differential Expression Analysis of Schwannomas Compared to Normal Tibial Nerve Samples Identifies Potential Druggable Targets in Non-*NF2* Schwannomatosis

Monday, June 23, 3:45pm – 4:00pm

Sasha Scott, PhD, *Sage Bionetworks*

Purpose: Non-*NF2* Schwannomatosis (SWN) is a rare, understudied syndrome that causes the development of nerve sheath tumors. Recent efforts to study the genomics of non-*NF2* schwannomatosis patients have increased our understanding of the disorder, but the genetic modifiers of this non-*NF2* schwannomatosis remain poorly understood. A major challenge when evaluating transcriptomic data in many NF datasets is a lack of patient-matched non-tumor tissue that can be used as a comparison point for tumors. We piloted a methodology that allowed us to compare non-patient-matched expression data. We collected data from 413 normal tibial nerve samples from the GTEx dataset and compared them to data from Synodos SWN schwannomas.

Methods: To account for varying experimental designs, we developed a novel k-means clustering-based approach to separate the GTEx samples into 18 clusters and performed differential expression analysis between each cluster and the schwannoma RNA-seq samples. We identified consensus genes that were significantly differentially expressed across all clusters and evaluated the direction of effect. After identifying significantly differentially expressed genes, we performed functional enrichment analyses in multiple gene set databases using enrichR.

Results: We identified 3,504 genes that are significantly differentially expressed in schwannoma samples compared to normal tibial nerves and used these genes to help us identify important pathways and potential drug targets. We also identified 34 significantly upregulated genes in the schwannomas from patients with pain but not in schwannomas from patients without pain when compared to the tibial nerve samples. Two of these genes, *CD109* and *SLITRK6,* are also differentially expressed in the painful schwannomas compared to non-painful schwannomas. Finally, we identified three drugs whose targets are significantly enriched in genes that are significantly differentially expressed in schwannomas from patients with pain but are not significantly altered in non-painful schwannomas.

Conclusions: By comparing SWN schwannoma expression data to RNA-sequencing data from normal tibial nerves, we were able to identify and prioritize a list of potential druggable candidates. Furthermore, by performing gene set enrichment analyses against various drug target databases, we were able to identify potential novel drug candidates for *in vitro* screening.

Full List of Authors: Sasha Scott, PhD and Robert J Allaway, PhD

Funding: CTF-2021-04-007 to SS and RJA

Platform: A New Hypomorphic *NF2* Isoform Induced by Antisense Gene Therapy is Able to Partially Recover *NF2* Deficiency on *NF2*-Related Schwannomatosis iPSC-Based Cell Model

Monday, June 23, 4:00pm – 4:15pm

Gemma Casals-Sendra, MSc, *Germans Trias & Pujol Research Institute*

NF2-related Schwannomatosis (*NF2*-related SWN) is an inherited autosomal dominant disorder resulting from loss-of-function (LOF) mutations in the *NF2* gene, currently, there is no effective treatment available.

Since truncating variants in *NF2* gene are associated to the severest phenotype comparing to in frame or missense variants, we have expressed an hypomorphic *NF2* isoform harbouring nonsense variants on exon 11 by inducing skipping of this exon through Phosphorodiamidate Morpholino Oligomers (PMOs) antisense treatment in patient's primary fibroblast[1].

Here, we aim to test the potential of this PMOs treatment in a developed iPSC-based model at the state of Schwann Cell-like (SC-like) spheroids carrying heterozygous and homozygous truncating variants on exon 11, to study the capacity of this therapy approach of recovering the merlin function and rescuing the *NF2* phenotype in those cells that give rise the schwannomas[2].

To achieve this, we differentiated the obtained cell lines into SC-spheroids and treated them with PMOs after 7 days of differentiation. After 3 days of treatment, we observed a restoration of merlin levels in both *NF2* +/- and *NF2* -/- lines. RNA sequencing analysis revealed a recovery of key pathways associated with *NF2*-deficiency in schwannomas and Schwann cells, such as the mTORC pathway and cell cycle progression. Upon further analysis, we noted a trend toward the normalization of expression levels for several key genes, including *ANKRD1, CCND1,* and *COL1A2,* and a slight recovery in *ITGA6* and *PDGFRB* as well. We confirmed the recovery of Cyclin D1 protein level, which suggests a reduction in cell cycle progression, as well as a restoration of the pS6/S6 and pAkt/Akt ratios, indicating mTORC pathway inhibition, both relevant pathways upregulated in *NF2*-deficient Schwann cells. We are currently investigating additional aspects as toxicity, cell proliferation and cell adhesion, among others.

Full List of Authors: Gemma Casals-Sendra[1], Ignasi Jarne-Sanz[1], Irene Boluda[1], Núria Catasús[1], Emilio Amilibia[3], Angela Callejo[3], Eduard Serra[4], Ignacio Blanco[1,2], and Elisabeth Castellanos[1,2] on behalf NF-SWN Reference Center (CSUR on Phakomatosis).

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References:
1. Catasús N, Rosas I, Bonache S, Negro A, Torres-Martin M, Plana-Pla A, et al. Antisense oligonucleotides targeting exon 11 are able to partially rescue the NF2-related schwannomatosis phenotype in vitro. Mol Ther Nucleic Acids. 2022 Dec 13;30:493–505.
2. Núria Catasús, Miguel Torres-Martin, Alex Negro, Bernd Kuebler. Merlin-Deficient iPSCs Show Altered Pluripotency and Constitute a Potential in Vitro Model for NF2-Related Schwannomas. Genes & Diseases 2025. Accepted.

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Platform: NF1 Loss Confers Susceptibility to Cell Death in Schwann Cell Tumors

Monday, June 23, 4:15pm – 4:30pm

Liang Hu, MD, *Cincinnati Children's Hospital Medical Center*

Background: Loss of the NF1 tumor suppressor causes Neurofibromatosis type 1, which is characterized by peripheral nervous system tumors. These tumors include benign plexiform/cutaneous neurofibromas and aggressive malignant peripheral nerve sheath tumors **(MPNSTs)**. Current mainstay therapies for NF1-associated neurofibromas rely on MEK inhibitors, which are not cytotoxic to NF1-deficient tumorigenic Schwann cells **(SCs)**, and require long-term administration that leads to compliance challenges and pose toxicity risk. For MPNSTs, conventional approaches, including chemoradiotherapies and surgical resection, and MEK inhibitiors have so far proven ineffective. These limitations underscore the critical need for more durable and effective therapies targeting NF1-deficient tumors, particularly MPNSTs.

Methodology and Results: Using biochemical and genetic approaches, we find that KRAS is the RAS protein essential for growth and tumorigenesis in NF1-deficient primary SCs and SC lines *in vitro.* We also demonstrate that PKC agonism, known to suppress RAS-mutant tumorigenesis, selectively induces CASP8/CASP3 activation and cell death in NF1-deficient SC lines. *In vivo* treatment with PKC agonists induced cell death in mouse neurofibroma (from *Dhh-Cre; Nf1$^{fl/fl}$* mice) and human MPNST xenograft tissues. Importantly, PKC agonism effectively treated prototypical NF1-loss-driven tumors *in vivo,* including plexiform neurofibromas and NF1-deficient MPNSTs, while showing no therapeutic effects in NF1-sufficient MPNST xenografts. Mechanistically, this selective cell death depends on PKCδ activation and KRAS inhibition, followed by CASP8/CASP3 activation.

Conclusions: Our findings suggest that NF1 loss creates a KRAS-dependent vulnerability to cell death in SCs, indicating PKC agonism as a promising therapeutic strategy for both prevention and treatment of NF1-deficient SC tumors.

Full List of Authors: Liang Hu[1], Yuting Tang[1,2], Yuan Lin[1], Jay Pundavela[1], Katherine Chaney Scheffer[1], Tilat Rizvi[1], Jianqiang Wu[1,3], Yi Zheng[1,3], and Nancy Ratner[1,3]

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Platform: Using Patient-Derived Stem Cells to Model the Neurodevelopmental Phenotype of NF1

Monday, June 23, 4:30pm – 4:45pm

Kiymet Bozaoglu, PhD, *Murdoch Children's Research Institute*

Purpose: NF1 is associated with significant neurodevelopmental challenges, but the underlying pathogenesis is poorly understood. Patient-derived induced pluripotent stem cell (iPSC) models provide a powerful platform to investigate how genetic variation affects brain development and function. We hypothesize that analysis of iPSC derived from affected individuals will identify the molecular mechanisms underlying clinical phenotypes associated with NF1.

Methods: We generated iPSCs from 12 individuals and created isogenic controls using CRISPR Cas9 gene editing. Participant phenotyping included neuropsychological evaluation and pathogenic *NF1* variants were identified by exome sequencing. iPSCs were differentiated into cortical neurons using a lentiviral-mediated NGN2 overexpression protocol. Neurofibromin levels were assessed using immunofluorescence and western blot analysis. Structural analysis of neuron morphology and synaptogenesis was performed using confocal microscopy. RNA sequencing was performed to identify genes and pathways dysregulated in patient compared to isogenic neurons. A well-established cerebral organoid model was used to assess size differences between patient and isogenic control iPSC lines.

Results: Quantitative analysis demonstrated a significant reduction in neurofibromin levels, in the NF1 patient neurons compared to the isogenic controls ($p<0.001$). Structural analyses revealed significantly shorter neurites ($p<0.01$) and, significantly fewer pre-synaptic puncta in NF1 derived neurons compared to isogenic controls neurons, ($p<0.001$) suggesting that *NF1* haploinsufficiency compromises neurite extension and synapse formation. Multielectrode array analysis demonstrated significantly reduced neuronal activity in NF1-derived neurons compared to isogenic controls ($p<0.0001$), consistent with the reduction in pre-synaptic termini. Transcriptomic analysis demonstrated significant downregulation of cellular pathways important for neuron development including synaptic signalling ($p < 0.0001$), synaptic plasticity regulation ($p < 0.0001$), long-term synaptic potentiation ($p < 0.0001$), and neurogenesis regulation $p < 0.001$). To further investigate these observations, we have established 3D cerebral organoid models. Preliminary results ($n=6$ patient lines) suggest a significant overgrowth phenotype in patient-derived organoids compared to isogenic controls ($P<0.0001$).

Conclusion: Our iPSC modelling pipeline has provided valuable insights into the pathomechanisms and molecular pathways dysregulated in NF1. These models may provide a valuable advanced platform for *in vitro* preclinical trials using patient-derived models, enabling the evaluation of targeted therapeutic strategies before clinical trials.

Full List of Authors: Kiymet Bozaoglu (PhD)[1,2], Sarah Massie[1], Friederike Irion[1], Wei Shern Lee[1,2], Mai Raabus[1], Kristina M Haebich[1,2], Kathryn N North[1,2], Paul J Lockhart[1,2], Jonathan M Payne[1,2,3].

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Platform: Behavioral and Metabolic Phenotypes Exhibit Differential Requirement on Signaling Cascades Downstream of Ras

Monday, June 23, 4:45pm – 5:00pm

Seth Tomchik, PhD, *University of Iowa*

Purpose: To test whether signaling cascades downstream of Ras differentially affect two NF1 phenotypes - behavioral and metabolic regulation.

The suite of symptoms in neurofibromatosis type 1 includes cognitive/behavioral symptoms and metabolic alterations, which are recapitulated in animal models ranging from flies to rodents to pigs. Mutations in the NF1 gene impact multiple signaling cascades, including Ras and its ramifying downstream signaling pathways (Raf/MEK/ERK, PI3K/AKT/mTOR, RalGEF/Ral, etc.). Each of these pathways have individually been implicated in neurofibromatosis type 1 phenotypes. However, the extent to which each phenotype is dependent on one pathway or another (or multiple) is unknown, particularly for cognitive/behavioral symptoms. To approach this question, we utilized the *Drosophila* model, which exhibits key NF1 phenotypes and allows deep genetic dissection of signaling pathways.

Methods: We knocked Nf1 down and quantified two phenotypes: behavioral hyperactivity and metabolic alterations. Both of these phenotypes depend on Ras GAP-related domain function. To determine which downstream signaling pathways are necessary for the phenotypes, we combined Nf1 knocked with simultaneous knockdown of a second downstream signaling molecule (MEK, ERK, AKT, mTOR/Raptor, S6K, or 4E-BP). Validity of the genetic manipulations were confirmed molecularly. Restoration of a normal phenotype in the double knockdowns indicated a requirement for that signaling molecule for the phenotype. In complementary experiments, we tested sufficiency of the downstream signaling molecule by expressing constitutively-active versions of the protein in wild-type animals.

Results: These studies revealed an unexpected divergence of the requirements for different signaling pathways downstream of Ras in Nf1-dependent regulation of behavioral phenotypes and metabolic regulation.

Conclusions: Different symptoms in neurofibromatosis type 1 may result from divergent actions of signaling pathways downstream of Ras. Interventions targeting different symptoms may be targeted to signaling pathways that exert stronger influence on each underlying process.

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Platform: Mitochondrial Respiration as a Readout of NF1 Function in Mouse Models with Patient Mutations

Monday, June 23, 5:00pm – 5:15pm

Semira Ortiz, PhD, *Pennington Biomedical Research Center*

Purpose: Neurofibromatosis type 1 (NF1) is associated with altered metabolism, including reduced muscle function and low body mass/adiposity. Reduced mitochondrial function has been reported in several models of NF1 loss and may contribute to these metabolic features. NF1-null cells exhibit impaired NADH-linked respiration and succinate dehydrogenase activity. The purpose of this study is to characterize whole body and tissue metabolism in a mouse model of adult biallelic inactivation of *Nf1.* We hypothesized that loss of *Nf1* would result in reduced energy expenditure, decreased electron transport and oxidative phosphorylation.

Methods: To measure the metabolic effect of the nonsense allele c.2041C>T; p.Arg681*, *Nf1Arg681*$^{*/+}$ and *Nf1*$^{+/+}$ littermates were placed in Promethion indirect calorimetry cages for 4 days (n=15 per group). To measure mitochondrial function upon loss of heterozygosity, we used a tamoxifen-inducible systemic knockout model. *CAGGCre-ER*^{TM}*;Nf1*$^{4F/Arg681*}$ (n=6) and *Nf1*$^{4F/+}$ (n=4) littermates were treated with tamoxifen for 5 days. 48 hours post tamoxifen, brain, heart, gastrocnemius, liver, and brown adipose samples were collected. Crude mitochondrial fractions were isolated for high-resolution respirometry. Using an Oroboros O2k machine, we measured NADH- and succinate-linked oxidative phosphorylation (OXPHOS) and electron transfer (ET) capacity and complex IV (CIV) activity. Citrate synthase activity was measured to confirm equal mitochondrial loading.

Results: *Nf1*$^{Arg681*/+}$ mice exhibited metabolic and behavioral abnormalities compared to wildtype littermates. Heterozygous animals had significantly reduced energy expenditure ($p<0.05$), water consumption ($p<0.05$), and a profound reduction in pedestrian locomotion ($p<0.01$). After loss of heterozygosity, *CAGGCre-ER*^{TM}*;Nf1*$^{4F/Arg681*}$ mice exhibited wasting, with 10% reduction in body weight ($p<0.001$) compared to controls. In the brain and gastrocnemius, NADH-linked OXPHOS and ET, succinate-linked ET, and CIV activity were significantly lower in *CAGGCre-ER*^{TM}*;Nf1*$^{4F/Arg681*}$ mice ($p<0.05$). A similar trend was observed in the heart, but there were no significant differences. We also found no significant differences in liver or brown adipose tissue. There was no difference in citrate synthase activity between groups.

Conclusions: In a heterozygous mouse model of the pathogenic NF1(p.Arg681*) nonsense allele, we confirmed that reduced NF1 leads to metabolic abnormalities. Our findings also demonstrate that NF1 stabilizes complex I (NADH-linked) OXPHOS and ET in multiple tissues. Without NF1, the overall capacity for tissues to generate energy (ATP) is reduced. This is particularly true in the brain and skeletal muscle. Importantly, our findings are consistent with previous studies using respirometry in cell culture. These results suggest that mitochondrial respiration parallels NF1 function and that improving mitochondrial performance could be therapeutically beneficial.

Full List of Authors: Semira Ortiz[1], Elizabeth Zunica[1], Christopher Axelrod[1], Erik Westin[1], Analisa Taylor[1], Deeann Wallis[2], and Robert Kesterson[1,2]

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Platform: Aberrant Cortico-Striatal Neural Activity Underlies Impulsivity and ADHD in a Preclinical Model of Neurofibromatosis Type 1

Monday, June 23, 5:15pm – 5:30pm

Jodi Lukkes, PhD, *Indiana University School of Medicine*

The purpose of this study is to investigate attention deficit hyperactivity disorder (ADHD) in Neurofibromatosis type 1 (NF1) using treatment and neural recordings in a preclinical model.

Introduction: Learning differences are common in NF1, with ADHD being most prevalent, affecting around 60% of patients with NF1. Our recent data demonstrate that male mice haploinsufficient for the neurofibromin gene *(Nf1$^{+/-}$)* exhibit hyperactivity in an open field, increased risky behavior in a cliff avoidance test (CAR), and increased impulsivity in a delay discounting task (DDT) compared to wild-type males. These deficits were all attenuated with systemic treatment with a commonly prescribed, non-stimulant ADHD drug, guanfacine. Preclinical studies using murine experimental systems of ADHD have shown that lesions of the prefrontal cortex (PFC) or nucleus accumbens (NAc) increase impulsivity in a delay discounting task (DDT).

Methods: We measured neural firing using an electrode simultaneously implanted in NAc and PFC of male and female wildtype and *Nf1* haploinsufficient *(Nf1$^{+/-}$)* mice while they performed behavioral tasks measuring hyperactivity and impulsivity. Electrodes recorded individual neuron activity called spikes and group neuron activity called local field potentials. We also determined sufficiency of *Nf1* knock-down in cortical-striatal circuitry causing executive dysfunction using clinically-relevant behavioral tasks through the use of *Nf1$^{flox/flox}$* male and female mice. *Nf1$^{flox/flox}$* male and female adult mice were injected bilaterally with either control virus (AAV5-CMV-GFP) or the Cre virus (AAV5-CMV-Cre-GFP) into the PFC or NAc.

Results: Recording studies demonstrated underlying neural differences in male mice. “Power” indicates strength of neural signal while “synchrony” measures how well two brain areas are communicating. *Nf1$^{+/-}$* mice exhibited increased power in the prefrontal cortex and nucleus accumbens but decreased synchrony between the brain areas. We also found that selective deletion of the neurofibromin gene *(Nf1)* in the NAc increased hyperactivity to a novel open field and increased risky behavior in a CAR in males but not females. However, both sexes of *Nf1$^{flox/flox}$* mice injected with AAV5-CMV-Cre-GFP into the NAc exhibited deficits in behavioral inhibition measured by increased frequency of small reward choice in DDT. In contrast, selective deletion of *Nf1* in the PFC of males only increased impulsive behavior during the DDT in males but not in females.

Conclusions: This project is the first to use *in vivo* awake-behaving recordings in corticostriatal circuitry in a preclinical NF1 model. Corticoaccumbal circuitry is involved in decision making and motivated action. Our findings of increased power but decreased synchrony underlies impulsivity. In other words, although each of the brain regions is making increased effort to communicate a signal (increased power), the signal does not go through (decreased synchrony), resulting in impulsive decision-making. Our data also suggest that selective deletion of *Nf1* has region- and sex-specific effects on hyperactivity and impulsivity. Furthermore, our data show that the NAc plays an integral role in modulating the observed deficits in behavioral inhibition of Nf1 animals. Overall, these studies will help elucidate underlying molecular and neural mechanisms driving impulsivity.

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CLINICAL PLATFORMS – CONCURRENT SESSION

Session Co-Chairs: Radhika Dhamija, MBBS, *Mayo Clinic;* Chelsea Kotch, MD, *Children's Hospital of Philadelphia*

Platform: CAVS-NF1: AI-Powered Webtool for MR-T1 Volumetric Analysis of NF1 Optic Pathway Gliomas

Monday, June 23, 3:45pm – 4:00pm

Abhijeet Parida, MSc, *Children's National Hospital*

Purpose: Optic pathway gliomas associated with neurofibromatosis type 1 (NF1-OPG) affect the anterior visual pathway (AVP), which includes the optic nerves, chiasm, and tracts. Our recent studies have established a link between MRI-derived AVP shape and volume with visual acuity loss [1]. To enhance accessibility and clinical utility, we introduce *-CAVS-NF1 (Central AI-enabled Volumetric Service for NF1)-* a publicly available, AI-powered web tool designed for automated segmentation and volumetric analysis of the optic nerves, chiasm, and AVP from T1-weighted MRI scans.

Methods: We developed a fully automatic end-to-end framework for segmentation and volumetric analysis using the T1 MRI sequences. The framework comprised of a vision transformer-based segmentation network called SwinUNETR trained on MRIs of 135 children with NF1-OPG from Children's National Hospital (CNH, GE platform, n=60) and Children's Hospital of Philadelphia (CHOP, Siemens platform, n=75). Volumetric ground truth was established by expert annotation of the AVP. The SwinUNETR model was implemented using the MONAI framework, ensuring efficient model deployment in a clinical setting. After the segmentation of the AVP, a template-based registration method was used to isolate the optic nerve and chiasm region. A secure web-based platform was developed to allow privacy-preserving interactions with the trained algorithm without requiring software installation.

Results: The automated AVP segmentation and optic nerve chiasm isolation achieved an accuracy of 79% (Dice Score: 0.79 $\pm$ 0.08) in an average runtime of 98 $\pm$ 3 seconds, surpassing inter-observer variability observed in manual segmentation (Dice Score: 0.75 $\pm$ 0.06). *CAVS-NF1* is publicly accessible, privacy-preserving, free of cost, and available at https://nf1.hope4kids.io/.

Conclusion: By integrating deep learning-based MRI analysis into a web-based tool, *CAVS-NF1* enable clinicians and researchers to perform fast and automated for volumetric analysis of NF1-OPGs. This initiative aims to democratize AI-driven healthcare, enabling early intervention and personalized treatment strategies without requiring specialized software or extensive computational resources.

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References:
1. Z. Jiang *et al.,* "Automatic Visual Acuity Loss Prediction in Children with Optic Pathway Gliomas using Magnetic Resonance Imaging," *2023 45th Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC),* Sydney, Australia, 2023, pp. 1-5, doi: 10.1109/EMBC40787.2023.10339961.

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Platform: Treatment Heterogeneity and Survival Outcomes in an International, Multi-Institutional Cohort of Individuals with NF1-Associated High-Grade Glioma and High-Grade Astrocytoma with Piloid Features

Monday, June 23, 4:00pm – 4:15pm

Chelsea Kotch, MD, MSCE, *Children's Hospital of Philadelphia*

Purpose: Neurofibromatosis type 1 (NF1)-associated high-grade glioma (HGG) and high-grade astrocytoma with piloid features (HGAP) are rare tumors with poor prognoses, yet their clinical and molecular characteristics remain poorly defined. Prior studies have lacked standardized, comprehensive data collection, limiting insights into optimal management and survival outcomes. Consequently, there is no established standard of care and treatment approaches vary widely. This international, multi-institutional study aimed to assemble the largest cohort of NF1-associated HGG and HGAP to date to provide critical insights into current treatment approaches, prognostic factors, and survival outcomes, and guide future interventional clinical trial design.

Methods: Children and adults with a diagnosis of NF1 and pathologic and/or molecularly confirmed HGG and methylation array-confirmed HGAP across 17 institutions diagnosed between 2005 and 2021 were included. Retrospective clinical, molecular, treatment, and imaging data were analyzed.

Results: Eighty-nine subjects met inclusion criteria. The most prevalent diagnoses included glioblastoma (26), high-grade astrocytoma (18), anaplastic astrocytoma (14), and HGAP (9). The median age at diagnosis was 24.1 years (range 0.2-67.4), with 25 subjects (28%) diagnosed before 18 years of age. Among 53 tumor specimens with molecular testing, the most common somatic variants involved the *NF1, CDKN2A/B, ATRX,* and *TP53* genes. Treatment approaches were highly variable, with 23 unique frontline treatment regimens; the most common was focal radiation with concurrent temozolomide (40%). Overall survival (OS) at 2 years from diagnosis was 45% (95% CI 34,55). Two-year OS was prolonged among children/adolescents compared to adults (<18 years 56% vs ≥18 years 40%; P=0.013), and in HGAP compared to HGG (78% versus 40%; P=0.121). *TP53*-mutated tumors had significantly poorer OS compared to wild-type tumors regardless of histologic subtype (P=0.002). Although two subjects with HGAP demonstrated prolonged response to targeted therapies, treatment of NF1-HGG and HGAP with mitogen activated protein kinase inhibitors generally lacked durable responses (18 subjects, median treatment duration 3.1 months). Inclusion of radiation therapy at any time did not significantly impact OS. Bevacizumab-based regimens resulted in modest increases in treatment duration (28 subjects, median 6.1 months).

Conclusion: Frontline treatment approaches for NF1-HGG and HGAP are highly variable and largely unsuccessful, highlighting the urgent need for effective therapies. This study characterizes the demographic, genetic and treatment features of these rare tumors, identifies novel prognostic risk factors, and establishes a benchmark for future clinical research. The findings will also inform the design of future interventional clinical trials, including eligibility criteria and power calculations.

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Platform: Baseline Characteristics and Cross-Sectional Analysis of a Cutaneous Neurofibroma Natural History Study in 494 People with NF1

Monday, June 23, 4:15pm – 4:30pm

Mandi Johnson, MBA, *Johns Hopkins University*

Purpose: Cutaneous neurofibromas (cNFs) are benign tumors that occur in over 95% of people with NF1. Although they are often cited as the most bothersome manifestation of NF1, the natural history remains poorly characterized. This study addresses this gap by creating prospective and systematic data about cNF longitudinally to aid in design and execution of future clinical trials, identify populations at highest risk, and validate endpoints.

Methods: Individuals of all ages with NF1 were recruited. Demographic data, treatment history, and patient reported outcomes (PROs) were collected. 3D whole body (WB) photography with Vectra WB360 was performed. Genetic testing was performed via Invitae NF1 assay. cNF burden was classified on digital images as: low (≤10 cNFs), moderate (11-50 cNFs), and high (>50 cNFs). Baseline characteristics were analyzed descriptively.

Results: 494 participants enrolled, median age: 29 years [0.9-91]; 289 (58%) female, 205 (42%) male. Self-reported race: 63% White, 20% Black, 5% Asian, 4% Hispanic, and 8% ≥2 races. Fitzpatrick skin phototypes: 2% Type I, 48% Type II, 21% Type III, 10% Type IV, 16% Type V, and 3% Type VI. 179 (36%) had familial NF1, 255 (52%) sporadic, 60 (12%) unknown inheritance pattern. Constitutional NF1: 476 (96%), mosaic/segmental: 10 (2%), unknown: 8 (2%). Genetic testing revealed: frameshift/nonsense variants: 364 (74%), microdeletions 25 (5%), other 45 (9%), negative 35 (7%). 15 (3%) received treatment with a MEK inhibitor and are stratified separately. 102 (21%) had high cNF burden, 87 (18%) moderate, 301 (61%) low, and 4 (<1%) were unevaluable. All high-burden cases were >20 years old. The most common cNF-related concerns based on Skindex: appearance (n=261), worrying about cNF (n=250), annoyance (n=246), embarrassment (n=237), frustration (n=235). Itching (n=210), hurting (n=161) and irritation (n=184) were also frequently reported. CNF interference with daily activities was reported by 140 people (28%).

Conclusion: This study presents the baseline evaluation of whole body cNF burden in a large, diverse NF1 cohort. Findings confirm that cNFs affect all races and are visible in people as young as 2yo, but high burden (≥50) only occurs in adults >20. Independent of cNF burden, 68% of people report some degree of symptoms or impairment (including children) reinforcing the need for therapies and awareness regarding cNF impact. Ongoing work explores the ability to detect cNF change over time, correlate size and symptoms with biomarkers, and optimize this large and diverse cohort to serve as a potential control group for future therapeutic studies for cNF.

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Platform: MRI Features and the Role of Image Guided Biopsy for Assessment of Pre-Malignant Versus Malignant Peripheral Nerve Sheath Tumors in People with Neurofibromatosis Type 1

Monday, June 23, 4:30pm – 4:45pm

Shivani Ahlawat, MD, *Johns Hopkins University*

Purpose: Magnetic resonance imaging (MRI) plays a central role in diagnosing both benign and malignant peripheral nerve sheath tumors (PNST) in people with Neurofibromatosis Type I (NF1). This study compared the anatomic and functional MRI features of histologically proven pre-malignant/intermediary PNST with high-grade malignant PNST and explored the utility of core needle biopsy (CNB) for confirming these diagnoses in people with NF1.

Methods: This study analyzed MRIs obtained in people with NF1 and histologically confirmed pre-malignant PNST (atypical neurofibromatous neoplasm of uncertain biologic potential (ANNUBP) or low-grade MPNST) or malignant PNST (high-grade MPNST) who had pre-diagnostic MRI 02/2014 - 02/2024. Demographic data and method of diagnosis (CNB, excision or both) and final pathological diagnosis were collected. Qualitative (heterogeneity, architecture ("target sign"), perilesional edema/enhancement, necrosis, and surrounding plexiform tumor at T2-weighted, T1-weighted pre-contrast, and T1-weighted post-contrast images) and quantitative (size and minimum apparent diffusion coefficient (ADCmin) on diffusion-weighted imaging (DWI)) features on MRI were recorded. CNB diagnosis was compared with final pathology on excision. Pearson's Chi-Squared and Mann-Whitney U tests were used accordingly for statistical analysis. Benjamini-Hochberg method was applied to adjust false discovery rate.

Results: A total of 41 tumors in 40 patients (20 females [median age: 35, range: 14-66] with high-grade MPNST (n=30) or pre-malignant PNSTs (n=11) were included in the MRI analysis. The presence of intralesional necrosis, perilesional edema, and perilesional enhancement ($p<0.05$); a higher heterogeneity in T2-weighted images ($p = 0.001$); a largest diameter and average diameter ($p = 0.014$ and $p = 0.018$, respectively); and a lower ADCmin ($p < 0.001$) were associated with high-grade MPNST. The remaining features, including the absence of a "target sign" ($p = 1.0$) or a plexiform background ($p = 0.350$), were not significantly different between pre-malignant and high-grade MPNST. When applying a binary classification scheme of pre-malignant PNST versus high-grade MPNST, image guided CNB offered the correct diagnosis in 93% (26/28) of the PNSTs.

Conclusion: NF1-associated high-grade MPNST can be distinguished from pre-malignant grade PNST (neurofibroma/ANNUBP/low-grade MPNST) with MRI based on the presence of intralesional necrosis, perilesional edema, and perilesional enhancement; higher T2-weighted heterogeneity, larger size (>10.5cm); and a lower minimum ADC value ($<0.7\times 10^{-3}$ mm^2/s). When applying a binary classification scheme of pre-malignant PNST versus high-grade MPNST, diffusion-weighted image-guided core needle biopsy yields the correct diagnosis in 93% of the PNSTs.

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Platform: Age Trends of ADHD Symptoms in Children with Neurofibromatosis Type 1: An Integrative Analysis of Data from Eight Institutions

Monday, June 23, 4:45pm – 5:00pm

Yang Hou, PhD, *Florida State University*

Purpose: Children with Neurofibromatosis type 1 (NF1) are at an increased risk of experiencing Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms than their typically developing peers. However, the age progression of ADHD symptoms in children with NF1 and the correlates remain poorly understood. This study aims to explore the age trends of ADHD symptoms in children with NF1 and how these age trends vary based on demographic and NF1-related disease factors.

Methods: Integrative data analysis was adopted to combine individual-level data from 904 children with NF1 (ages 3–18 years) from eight institutions. Inattentive and hyperactive/impulsive ADHD symptoms were collected through parent-reported Conners Rating Scales and ADHD Rating Scales. Time-varying effect modeling (TVEM) was used to examine the age patterns of ADHD symptoms and their age-varying associations with sex, parental education, and NF1 heritability. TVEM is a nonparametric statistical method that can estimate associations between variables continuously across ages, providing curvilinear estimates of the intercepts and slopes with 95% confidence intervals (CIs). Significant deviations from the normative group (i.e., 50) are indicated by CIs that do not include the normative mean of 50, and significant age or group differences (e.g., boys versus girls) by non-overlapping CIs between age points or groups.

Results: Scores of inattention and hyperactivity/impulsivity symptoms were consistently elevated relative to normative means across ages 3-18 years. Age trends of both symptoms followed an inverted U-shaped pattern, increasing in early childhood, peaking at around age 10, and gradually declining through adolescence (see **Figure 1** for an example). Significant variability was observed across subgroups. Girls (versus boys) exhibited higher levels of inattention from ages 8–15 **(Figure 2)** and higher levels of hyperactivity/impulsivity from ages 5–9. Children with high (versus low) parental education demonstrated lower levels of inattention from ages 5–8 and lower levels of hyperactivity/impulsivity from ages 5–14. The age trends of ADHD symptoms did not significantly vary between children with familial versus sporadic NF1.

Conclusion: Children with NF1 consistently showed more severe ADHD symptoms than typically developing peers from early childhood to late adolescence, with symptoms peaking during early adolescence. Some subgroups, including girls (vs. boys) and those with low (vs. high) parental education, exhibited greater symptom severity at certain ages. These findings highlight the need for age-sensitive, individualized interventions. Targeted support strategies, such as early intervention for high-risk groups and tailored behavioral management approaches, may improve outcomes for children with NF1.

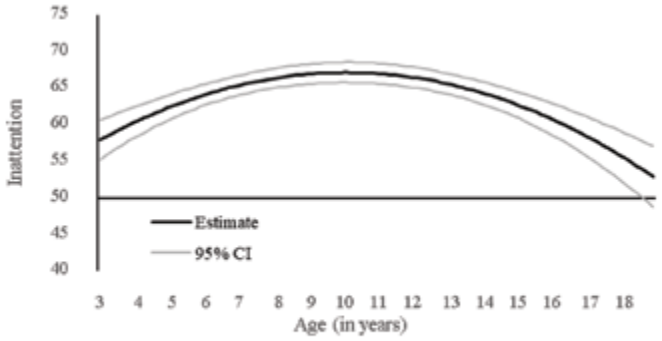

Figure 1. Age trend of inattention.

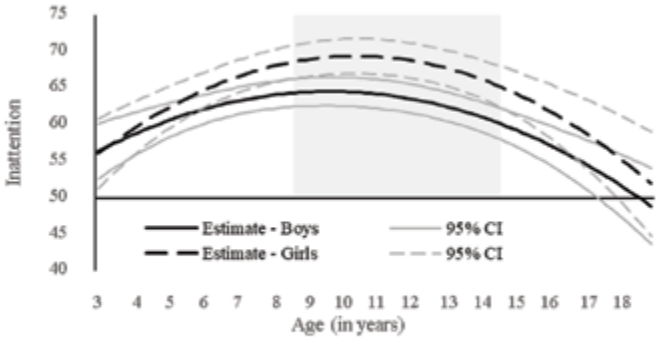

Figure 2. Age trends of inattention for boys versus girls. The shaded grey area represents the age range of significant group differences.

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Platform: Update From the Long-Term Follow-Up (LTFU) Phase of ReNeu: A Pivotal Phase 2b Trial of Mirdametinib in Children and Adults With Neurofibromatosis Type 1 (NF1)-Associated Symptomatic Plexiform Neurofibroma (PN)

Monday, June 23, 5:00pm – 5:15pm

Angela C. Hirbe, MD, PhD, *Washington University School of Medicine, St. Louis, MO*

Purpose: Mirdametinib is the first FDA-approved MEK1/2 inhibitor for both adults and children (≥2 years) with NF1 who have symptomatic PN not amenable to complete resection. We report updated results from the LTFU phase of the pivotal, phase 2b ReNeu trial (NCT03962543) evaluating the efficacy and safety of mirdametinib.

Methods: In ReNeu, mirdametinib capsules or tablets for oral suspension (2 mg/m^2 BID, max 4 mg BID) are administered in 3-weeks-on/1-week-off 28-day cycles. Following the 24-cycle (~2 years) treatment phase, patients could continue mirdametinib in an optional LTFU phase. These analyses are inclusive of the treatment and LTFU phases (data cutoff: June 12, 2024; an additional 9 months of cumulative data). Exploratory analyses reported here included confirmed objective response rate (ORR; proportion of patients with ≥20% reduction of target PN volume from baseline on consecutive MRI scans within 2 to 6 months, assessed by blinded independent central review), duration of response (DoR), change from baseline in target PN volume, and safety.

Results: Fifty-eight adults and 56 children received mirdametinib in the treatment phase. Median (range) duration of mirdametinib treatment was 21.8 (0.4, 54.4) months in adults and 25.4 (1.6, 48.5) months in children. Among LTFU-eligible patients, 26/31 (84%) adults and 32/37 (86%) children entered the LTFU. Confirmed ORR was 47% (27/58; 95%CI, 33%, 60%) in adults and 55% (31/56; 95%CI, 42%, 69%) in children. Of the patients with a confirmed objective response, 18 adults and 19 children achieved a deep response (best target PN volume reduction from baseline of >50%). Median (range) best percentage change from baseline in target PN volume was –41% (–90%, 13%) in adults and –43% (–98%, 48%) in children. Median DoR was not reached. Treatment-related adverse events (TRAEs) in ≥25% of patients were dermatitis acneiform, diarrhea, nausea, and vomiting in adults; and diarrhea, dermatitis acneiform, and paronychia in children. Grade ≥3 TRAEs occurred in 17% of adults and 25% of children. Dose interruptions, dose reductions, and discontinuations due to TRAEs occurred in 9%, 17%, and 22%, respectively, of adults; and 14%, 14%, and 9%, respectively, of children.

Conclusions: Longer duration of mirdametinib treatment improved confirmed ORR, with deep responses achieved in both adults and children with NF1-PN. Mirdametinib continued to be well tolerated with a manageable safety profile and no new safety signals.

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Disclosures:
CLM: Employment with OX2 Therapeutics; leadership role with OX2 Therapeutics; equity interest in OX2 Therapeutics; consultancy/advisory role with Alexion Pharmaceuticals and SpringWorks Therapeutics; patents, royalties, or other intellectual property with OX2 Therapeutics; travel expenses from Alexion Pharmaceuticals;
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DV: Honoraria from Alexion Pharmaceuticals and SpringWorks Therapeutics, Inc.; consultancy/advisory role with AstraZeneca and Sanofi-Genzyme; research funding from Levo Therapeutics, Nflexion Therapeutics, Soleno Therapeutics, SpringWorks Therapeutics, Inc., and Takeda Pharmaceuticals; speakers' bureau for Alexion Pharmaceuticals and SpringWorks Therapeutics, Inc.; travel expenses from Alexion Pharmaceuticals;
AS: Research funding from SpringWorks Therapeutics, Inc.;
JM: Consultancy/advisory role with Alexion Pharmaceuticals; travel expenses from Alexion Pharmaceuticals;
ACH: Consultancy/advisory role with Alexion Pharmaceuticals, AstraZeneca, Intellisphere, LLC, and SpringWorks Therapeutics, Inc; patents, royalties, or other intellectual property with Boehringer Ingelheim RCV GmbH & Co KG- Licensing- T-019044 Development of a Preclinical NF1-MPNST Platform Suitable for Precision Oncology Drug Discovery and Evaluation, paid through my institution; Deutsches Krebsforschungszentrum-licensing agreement for PDX cell lines, paid through my institution; travel expenses from Alexion Pharmaceuticals and SpringWorks Therapeutics, Inc;
LW: No relevant disclosures;

AJL, MDW: Employment with SpringWorks Therapeutics, Inc; equity interest in SpringWorks Therapeutics, Inc;
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RA: Consultancy/advisory role with Alexion Pharmaceuticals; honoraria from Alexion Pharmaceuticals;
DS: Consultancy/advisory role with Orbus Therapeutics, PRA, Anheart Therapeutics, Servier (I), SymBio Pharmaceuticals; royalties for submissions to UpToDate;
AMR: No relevant disclosures;
KN: Consultancy/advisory role with Servier Pharmaceuticals, royalties for editorial role with Oxford University Press;
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Platform: Long-Term Efficacy and Safety of Bevacizumab for Progressive Tumors in Patients with *NF2*-Related Schwannomatosis (*NF2*-SWN)

Monday, June 23, 5:15pm – 5:30pm

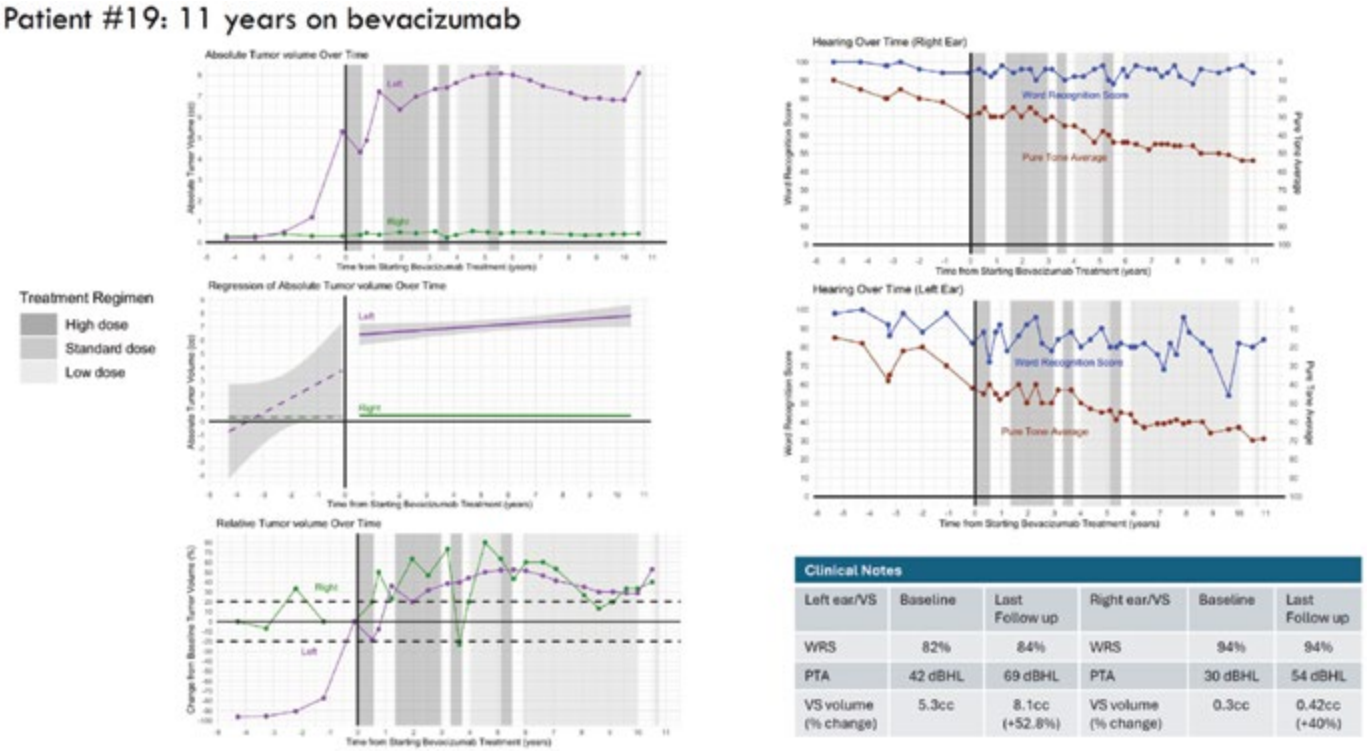
Natalie E. Stec, MD, *Massachusetts General Hospital*

Purpose: To determine outcomes for patients with *NF2*-SWN treated with bevacizumab for more than 2 years

Methods: We retrospectively reviewed the clinical records of patients with *NF2*-SWN seen at Massachusetts General Hospital (MGH) who received bevacizumab for at least 2 years for treatment of progressive tumors. Treatment doses were defined as low (<2.5 mg/kg/week), standard (2.5 mg/kg/week), or high (>2.5 mg/kg/week). Treatment regimens were defined as continuous or as intermittent (defined as intentional treatment breaks to minimize adverse effects). Radiographic responses were calculated from baseline MRI and hearing responses were calculated from baseline audiogram as described previously (PMID: 31626572). Adverse events resulting in treatment holds or discontinuation were recorded.

Results: Fifty patients were included in this preliminary review. Median age at treatment initiation was 27.2 years (13-73 years). Median duration of treatment was 9.9 years (2.3 – 16.7 years). Forty-nine percent of evaluable VS were stable or smaller (n=40/82) at last MRI (median follow-up 8.2 years). Median rate of relative growth across all tumors was 2.8% per year. Thirty-two percent of patients required surgical resection of VS after a median of 4.7 years of treatment and 18% of patients received radiation to one or both VS. Forty-four percent of evaluable ears (n=24/63) demonstrated stable or improved hearing at last audiogram (median follow-up, 6.5 years). The most common adverse events included proteinuria (68%), treatment-emergent hypertension (48%), impaired wound-healing (22%), and venous thromboembolism (VTE) (6%). Two patients developed glomerulonephritis necessitating renal biopsy and oral corticosteroids, respectively. Treatment was temporarily held for adverse events in 35% of patients with proteinuria, 17% with hypertension, 45% with impaired wound-healing, and 33% with VTE. Median duration of treatment holds for adverse events was 3.1 months. Treatment was discontinued for adverse events in 8% of patients for proteinuria (n=2), hypertension (n=1), and impaired wound-healing (n=1). All patients received bevacizumab for VS. Additional indications included ependymoma (n=6), non-vestibular schwannoma (n=4), and VS-related post-radiation facial weakness (n=1). Most patients were treated with multiple dosing schedules. Forty-nine patients received standard-dose bevacizumab, 37 received low-dose after a period of standard-dose (primarily through intermittent treatment), and 30 required high-dose bevacizumab at some point to optimize treatment response. Eighty-four percent of patients received both continuous and intermittent treatment regimens (see figure for example).

Conclusions: Long-term treatment with bevacizumab using flexible dosing that included low, standard, and high doses resulted in durable hearing and radiographic responses with a tolerable safety profile in this patient population.



Clinical Notes

Left ear/VS	Baseline	Last Follow up	Right ear/VS	Baseline	Last Follow up
WRS	82%	84%	WRS	94%	94%
PTA	42 dBHL	69 dBHL	PTA	30 dBHL	54 dBHL
VS volume (% change)	5.3cc	8.1cc (+52.8%)	VS volume (% change)	0.3cc	0.42cc (+40%)

Full List of Authors: Natalie E. Stec [1],Veronica Foureaux-Lee [1],Fred G Barker II[1], D. Bradley Welling[2], Alona Muzikansky[1], Vanessa Merker[1], Christina Orr[1], Justin T. Jordan[1], Scott R. Plotkin[1]

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Disclosures: Scott Plotkin is co-founder of NFlection Therapeutics and NF2 Therapeutics; consults for Akouos; and is on the scientific advisory board of SonALAsense. Dr. Welling is a consultant for Salubritas. Justin Jordan is a paid consultant for Recursion Pharmaceuticals, Alexion Pharmaceuticals, Springworks Therapeutics, Navio Theragnostics, Shepherd Therapeutics, Michigan State University, and the Children's Tumor Foundation; he has equity in Akeila Bio, Shepherd Therapeutics, Khora TX, and Navio Theragnostics.

Platform: Updated Results on Brigatinib Treatment for Progressive Tumors in Patients with *NF2*-Related Schwannomatosis: A Sub-Study of the INTUITT-NF2 Trial

Monday, June 23, 5:30pm – 5:45pm

Scott R. Plotkin, MD, PhD, *Massachusetts General Hospital and Harvard Medical School, Boston, MA*

Introduction: Brigatinib is an oral ALK inhibitor that inhibits multiple tyrosine kinases. We previously published outcomes of patients with *NF2*-related schwannomatosis treated with brigatinib for a median of 10.4 months (range, 1.1-31.1). We now report updated results of these patients with extended follow-up.

Methods: This multicenter, phase II, open-label study included participants ≥12 years old with *NF2*-SWN and progressive target tumors (baskets: VS, NVS, meningioma, or ependymoma). Patients were treated with brigatinib 180 mg daily. Patients discontinued treatment upon progression of target tumors but were allowed to continue treatment with isolated progression of non-target tumors if deemed clinically acceptable by the treating team and patient. One target and up to 5 non-target tumors were followed in each participant. Tumor response was evaluated by MRI every 3 months in year 1 and every 6 months thereafter with radiographic response (RR) defined as ≥20% decrease in volume compared to baseline.

Results: Forty participants were treated for a median time of 23 months (range, 3 to 40 months). Updated imaging data is available on 162 tumors (45 VS, 59 non-VS, 53 meningiomas, and 5 ependymomas) in this preliminary analysis. The RR rate for all tumors was 28%. By tumor basket, RR was 31% for non-VS, 28% for meningioma, 22% for VS, and 60% for ependymomas. The 12-month freedom from tumor progression was 77.1% (68.4%-86.8%) for all tumors, 81.5% (68.7%-96.6%) for non-VS, 78.1% (61.9%-98.4%) for meningioma, 69.2% (54.2%-88.5%) for VS, and 100.0% (inestimable) for ependymoma. The 24-month freedom from tumor progression was 73% (65.2%-81.7%) for all tumors, 81.3% (69.4%-95.2%) for non-VS, 72.4% (59.3%-88.3%) for meningioma, 62.4% (48.4%-80.3%) for VS, and 100.0% (inestimable) for ependymoma. There were no grade 4 or 5 treatment-related adverse events (AEs). The most common AEs were diarrhea (65%), nausea (40%), muscle cramps (40%), hypertension (35%, increased LDH (35%), increased AST (33%), and increased ALT (25%).

Conclusion: Brigatinib treatment was associated with high rates of tumor stability at 24 months across multiple tumor types in patients with *NF2*-related schwannomatosis.

Full List of Authors: Scott R. Plotkin, MD, PhD[1]; Kaleb H. Yohay, MD[2], Phioanh L. Nghiemphu, MD[3], Christine T. Dinh, MD[4], Dusica Babovic-Vuksanovic, MD[5], Vanessa L. Merker, PhD[1], Annette Bakker, PhD[6], Geoffrey Fell, MS[7], Lorenzo Trippa, PhD[7], Jaishri O. Blakeley, MD[7] for the INTUITT-NF2 Consortium*

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Disclosures: Dusica Babovic-Vuksanovic: Advisory Board for Alexion and SpringWorks; Annette Bakker: Employee, Children's Tumor Foundation (President); Christine Dinh: None; Geoffrey Fell: None; Vanessa Merker: None; Leia Nghiemphu: Consultant for Alexion; scientific advisory board for SpringWorks; Scott Plotkin: co-founder of NFlection Therapeutics and NF2 Therapeutics; scientific advisory board for SonALAsense; consultant for Akouos; Lorenzo Trippa: None; Kaleb Yohay: Scientific advisory board for Infixion, scientific advisory board for NF2 Biosolutions, consultant for Alexion; Jaishri Blakeley: Medical Advisory Board for SpringWorks.

Funding: Takeda Pharmaceuticals and Children's Tumor Foundation

Platform: Development of the QUEST Patient-Reported Measure: QUality of Life Evaluation for *NF2*-Related Schwannomatosis Trials

Monday, June 23, 5:45pm – 6:00pm

Sophia C. Carias, BA, *Massachusetts General Hospital, Boston, MA*

Purpose: Disease-specific quality of life (QOL) is an important patient-reported outcome measure for clinical trials for *NF2*-related schwannomatosis (*NF2*-SWN). Prior research indicates that existing *NF2*-SWN QOL measures may not be optimal for use in clinical trials in which an intervention is designed to improve outcomes. This research details the development and content validity of a new measure for this context.

Methods: *Concept elicitation interviews,* focused on exploring *NF2*-SWN symptoms and QOL impacts relevant to clinical trials, were conducted with individuals with *NF2*-SWN from the INTUITT-NF2 trial and international *NF2*-SWN clinicians. Interview transcripts were coded and analyzed using the Framework Method to document QOL concepts. A modified scale-level content validity index (CVI-S) method was applied independently for the patient and clinician groups to identify the most common QOL concepts. A *survey* completed by individuals with NF2-SWN from the NF Registry evaluated the importance of these concepts, which were prioritized for the draft measure. Four rounds of *cognitive debriefing interviews,* focused on evaluating question interpretation, were completed with individuals with *NF2*-SWN from the NF Registry or the Massachusetts General Hospital NF clinic to refine the measure wording.

Results: We conducted *concept elicitation interviews* with 16 individuals with *NF2*-SWN and 10 *NF2*-SWN clinicians **(Table 1)**. Interview analysis yielded 83 unique NF2-SWN symptoms or QOL impacts [Figure 1]. CVI-S analysis indicated that 35 of the 83 were highly rated among patients, clinicians, or both groups. These concepts were evaluated in a survey, completed by 57 individuals with NF2-SWN [Table 1]. Ultimately, 21 of the 35 concepts were selected for the draft measure based on statistical cut-offs from either the interviews or the survey. Finally, we conducted *cognitive debriefing interviews* with 16 individuals with *NF2*-SWN **(Table 1)**. Feedback led to changes in formatting, response scale options, the sequence of questions, and item wording to improve readability and comprehension. The final 21-item QUality of life Evaluation for *NF2*-related Schwannomatosis Trials (QUEST) measure encompasses a range of physical, emotional, and social well-being concepts **(Figure 2)**. It assesses how individual symptoms interfere with day-to-day activities and how multiple symptoms collectively impact key life areas that trial treatments may affect.

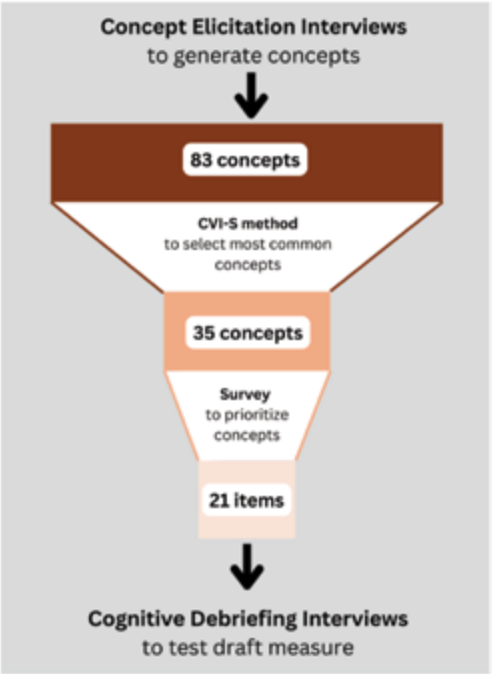
Conclusion: Through a multistage research process, we demonstrate the content validity of the QUEST measure as a comprehensive, relevant, feasible, and understandable QOL assessment for trial outcomes. It is currently being validated for internal reliability, test-retest reliability, and construct validity. Future analysis will determine its sensitivity to change in clinical trials.

Table 1. Demographic Information on Study Participants Across Each Phase of Measure Development

	Clinician Concept Elicitation Interviews (N=10)	Patient Concept Elicitation Interviews (N=16)	Patient Survey (N=57)	Patient Cognitive Debriefing Interviews (N=16)
Median age (range)	NC	**25 years (15-54)**	**43 years (15-85)**	**31 years (12-58)**
Sex (N, %)				
Female	5, 50%	11, 69%	38, 67%	8, 50%
Male	5, 50%	5, 31%	19, 33%	8, 50%
Race (N, %)				
White	NC	14, 88%	55, 96%	13, 81%
Asian	NC	1, 6%	2, 4%	3, 19%
Black	NC	0, 0%	1, 2%	1, 6%
Native Hawaiian/Pacific Islander	NC	0, 0%	0, 0%	0, 0%
American Indian/Other Indigenous	NC	0, 0%	1, 2%	0, 0%
Other	NC	1, 6%	1, 2%	1, 6%
Ethnicity (N, %)				
Non-Hispanic/Latino	NC	12, 75%	52, 91%	16, 100%
Hispanic/Latino	NC	2, 13%	5, 9%	0, 0%
Unknown	NC	2, 13%	0, 0%	0, 0%
Self-Reported Disease Severity (N, %)				
No symptoms	N/A	NC	0, 0%	1, 6%
Mild	N/A	NC	10, 18%	3, 19%
Moderate	N/A	NC	35, 61%	10, 63%
Severe	N/A	NC	12, 21%	2, 13%
Has participated in clinical trial (N, %)				
Yes	N/A	16, 100%	16, 28%	10, 63%
No	N/A	0, 0%	35, 61%	5, 31%
Unsure	N/A	0, 0%	6, 11%	1, 6%
Medical Practice Specialty (N, %)				
Neuro-oncology	3, 30%	N/A	N/A	N/A
Neurology	2, 20%	N/A	N/A	N/A
Surgery	2, 20%	N/A	N/A	N/A
Advanced Practice Nursing	1, 10%	N/A	N/A	N/A
Psychology	1, 10%	N/A	N/A	N/A

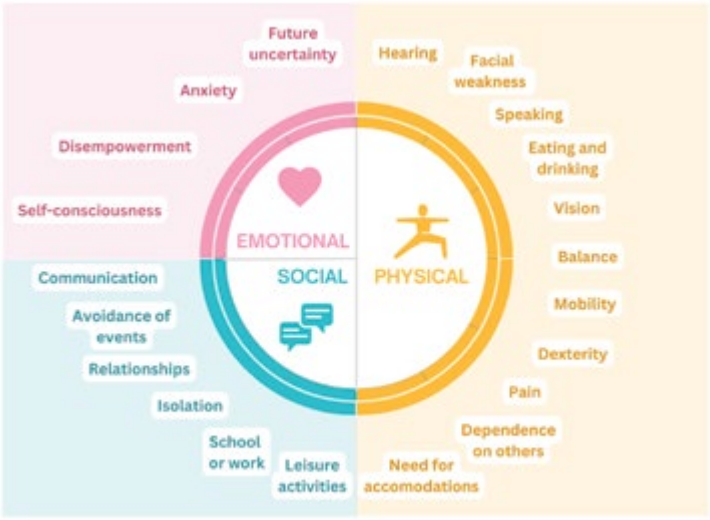
Legend: Each vertical column indicates one phase of measure development. N indicates the number of participants. N/A indicates fields that are not applicable to the participants. NC indicates fields that were not collected. Participants could be considered for multiple race options, so percentages may not add up to 100%. All percentages are rounded to the nearest integer value.

Figure 1. Flowchart of Measure Development



Legend: Detailing the major steps in the development of the measure and the number of *NF2*-SWN QOL concepts or items generated after each step.

Figure 2. Concepts Assessed in the Draft QUEST Measure



Legend: Listing of the 21 QOL concepts assessed in the draft QUEST measure across physical, emotional, and social areas of well-being.

Full List of Authors: Sophia C. Carias, BA[1], Frank D. Buono, PhD[2], Rosalie E. Ferner, MD[3], John F. Golding, PhD[4], Scott R. Plotkin, MD, PhD[1], Vanessa L. Merker, PhD[1]

[1]Massachusetts General Hospital, Boston, MA; [2]Yale School of Medicine, New Haven, CT; [3]Guy's and St. Thomas' NHS Foundation Trust, London; [4]University of Westminster, London

Funding: The described study was funded by the Children's Tumor Foundation (CTF) Clinical Research Awards 2020-10-001 and 2023-10-003 to VM; Takeda Pharmaceuticals and CTF provided funding for the brigatinib arm of the INTUITT-NF2 trial.

Platform: Clinical Evaluation of PRG-N-01 in *NF2*-Related Schwannomatosis: Interim Findings from a Phase 1/2 Study

Monday, June 23, 6:00pm – 6:15pm

Beom Hee Lee, MD, PhD, *Asan Medical Center, Seoul, Korea*

Purpose: PRG-N-01, a novel therapeutic candidate for neurofibromatosis type 2-related Schwannomatosis (*NF2*-SWN), has demonstrated preclinical efficacy in suppressing tumor growth and modulating tumorigenic pathways. Given the lack of approved pharmacological treatments for *NF2*-SWN and the high recurrence rate of associated tumors after surgery, we initiated a Phase 1/2 clinical trial to assess the safety, tolerability, and preliminary efficacy of PRG-N-01 in *NF2*-SWN patients.

Methods: An ongoing investigator-initiated Phase 1/2 clinical trial is being conducted at Asan Medical Center, Seoul, South Korea. The study evaluates the safety and efficacy of PRG-N-01 in patients diagnosed with symptomatic or progressive *NF2*-related SWN, including vestibular schwannoma, non-vestibular schwannoma, meningioma, or ependymoma. Patients are assessed every three months via MRI to monitor tumor progression, along with safety assessment including dose-limiting toxicity (DLT), organ toxicities, and vital parameters. Audiological evaluations, including hearing and word recognition tests, are conducted alongside quality-of-life (QoL) assessments. The trial, initiated in June 2024, aims for completion of Phase 1 by the third quarter of the year.

Results: Preliminary findings indicate that PRG-N-01 exhibits a favorable safety profile, with no reported adverse events to date. MRI-based evaluations have demonstrated a potential inhibitory effect on tumor growth. Functional assessments, including hearing and word recognition tests, are being analyzed to determine potential symptomatic improvements. Additionally, patient-reported QoL surveys are being conducted to assess treatment impact on daily life.

Conclusions: The interim clinical data suggest that PRG-N-01 is well-tolerated and demonstrates potential therapeutic benefits in *NF2*-SWN patients, which continue to be evaluated in this on-going study. The study's safety and efficacy outcomes will provide crucial data for the planned Phase 2 trial in the United States, aimed at further evaluating PRG-N-01 as a viable treatment for *NF2*-SWN.

Full List of Authors: Beom Hee Lee[1]*, Yeon-Ho Chung[2], Bae-Hoon Kim[2,3], Minju Kim[2], Soyoung Park[3], Tae Gyun Woo[2], Bum-Joon Park[2,3]

[1]Medical Genetic Center, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea, [2]PRG S&T Co., Ltd, Busan, Republic of Korea, [3]Department of Molecular Biology, College of Natural Science, Pusan National University, Republic of Korea

Funding: This research was supported by Korea Drug Development Fund funded by Ministry of Science and ICT, Ministry of Trade, Industry, and Energy, and Ministry of Health and Welfare (RS-2024-00442484, Republic of Korea).

KEYNOTE: UNLOCKING THE FUTURE OF ONCOLOGY: HUMAN-CENTRIC ADVANCED CELL MODELS IN PRECLINICAL DRUG DEVELOPMENT

Tuesday, June 24, 9:00am – 10:00am

Pelin Candarlioglu Deacon, PhD, *3D and 3Rs*

In the ever-evolving world of cancer research, the development of innovative, human-relevant advanced cell models is paving the way for breakthroughs in preclinical drug development. These cutting-edge models are not just a leap forward in technology; they represent a crucial shift towards more effective and personalized therapies for patients battling cancer. By mimicking real human biology more accurately, we can enhance the predictive power of our studies, ultimately leading to safer and more effective treatment options. Join us as we explore how these advanced cell models are transforming the landscape of oncology and setting the stage for a brighter future in cancer care!

I will begin with an introduction to Organ-on-Chip technology, discussing the current capabilities of various players in the field, practical achievements that support the submission of new drugs, and the regulatory perspectives on this technology. Next, I will focus on neurofibromatosis, highlighting the potential applications of Organ-on-Chip technology in supporting drug discovery for this condition.

TRANSLATIONAL MODELS AND NOVEL RESEARCH APPROACHES

Session Co-Chairs: Sara Gosline, PhD, *Pacific Northwest National Laboratory;* Lu Q. Le, MD, PhD, *University of Virginia School of Medicine;* Eva Trevisson, MD, PhD, *University of Padova, Italy*

Platform: Leveraging a Patient-Derived Xenograft Microtissue Platform to Identify Patient Specific Drug Combinations in NF1 Malignant Peripheral Nerve Sheath Tumors

Tuesday, June 24, 10:25am – 10:40am

Sara Gosline, PhD, *Pacific Northwest National Laboratory, Seattle, WA*

Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are associated with overall survival rates of 20-50%. The tumors are very aggressive and often fail to respond to conventional therapies, both targeted and chemotherapies[1]. This, together with the genetic heterogeneity of the tumor[2], has motivated the study of drug combinations to treat MPNST patients. To date, numerous potential drug combinations have been identified using high throughput in vitro screening platforms[3,4]. However, there are inherent limitations to in vitro approaches that fail to recapitulate three-dimensional cell-cell interactions and inter-patient diversity. Toward this end, we leveraged our patient-derived xenograft microtissue (PDX-MT) framework[5] to identify putative drug combinations in MPNST through the integration.

Methods: We have optimized our PDX-MT platform[5] to evaluate the effect of sixteen drugs on four distinct PDX-MTs. We measured the reduced growth of the samples upon drug treatment via dose response experiments with CellTiter-Glo as a readout of drug effect. We also measured the transcriptional changes that occur upon early drug treatment using RNA-sequencing of each sample compared to a time-matched DMSO control. We used transcriptomic measurements for each drug to identify transcription factors and pathways that were activated upon drug treatment. We then identified specific drugs that both targeted these up-regulated pathways and were proven to be effective in in vitro cell line data with similar transcription responses.

Results: We have established a computational workflow that can process the gene expression measurements across distinct samples, drugs, and time points to identify biologically relevant pathways that are activated upon drug treatment and secondary drugs that can serve as potential combination treatments. In one sample alone, the number of genes differentially expressed ranges from 208 genes differentially expressed upon mirdametinib treatment to 4745 drugs differentially expressed upon trabectedin treatment. We were able to identify specific transcriptional effects such as the down-regulation of the activity of FOXC1, previously found to be essential in PRC2-null MPNST[6], by both trabectedin and doxorubicin. Furthermore we could identify broader patterns of drug expression such as the similar responses elicited by MEK inhibition (selumetinib, trametinib, mirdametinib) as well as similarity across combinations drugs such as trabectedin and Olaparib which have been found to be synergistic in other cancer systems[7]. By querying published drug screen data in cancer cell lines, we also predicted potential combinations including MEK and HDAC inhibitors, which have previously been found to be effective for mitigating treatment resistance in other cancers[8-9].

Conclusion: The paucity of efficacious drug combinations in MPNST is partially driven by the absence of patient-relevant ex vivo systems in which we can identify and test drug pairs that effectively shrink MPNST *in vivo.* Through the development of our comprehensive *in vivo/ex vivo* experimental platform together with an integrative computational pipeline, we can now rapidly identify and test putative drug combinations across heterogeneous patient samples.

Full List of Authors: Alex Larsson[1], Belinda Garana[2], Yang Lyu[3], Ritu Shah[1], Zach Seeman[1], Elizabeth Rono[1], Lisa Fridman[4], Kyle Williams[1], Kuangying Yang[3], Kangwen Xiao[3], Trisha Gannu[3], Dana Borcherding[3], Xiaochun Zhang[3], David Largaespada[1], Christine Pratilas[4], Angela Hirbe[3], David Wood[1], Sara Gosline[2,5]

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Disclosures: D.A.L. is the co-founder and co-owner of NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immusoft, Inc.), B-MoGen Biotechnologies, Inc. (recently acquired by Bio-Techne Corporation), and Luminary Therapeutics, Inc. The business of all these companies is unrelated to the contents of this abstract.

Funding: Department of Defense NFRP, Gilbert Family Foundation

Platform: Dissecting the Role of CDKN2a Loss in Regulating Antioxidant Pathways to Promote MPNST Tumorigenesis Using *In Vivo* CRISPR/Cas9 Models

Tuesday, June 24, 11:25am – 11:40am

Akshaya Warrier, *University of Iowa*

Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are highly aggressive and metastatic soft tissue sarcomas that arise from benign precursor tumors, plexiform neurofibromas (pNFs). Homozygous loss of *NF1* in Schwann cells gives rise to pNFs. Additional mutations in the *CDKN2A* locus are associated with progression to MPNSTs. The *CDKN2A* locus encodes two potent tumor suppressors, *p16INK4a,* and *ARF.* Our lab has pioneered the use of the CRIPSR/Cas9 technology to generate MPNSTs *in vivo.* We use adenovirus to deliver Cas9 and gRNAs targeting known genetic drivers of MPNST formation into the sciatic nerves of mice. We used this approach to directly compare the impact of *Nf1*/*Arf* loss, *Nf1*/*Ink4a* loss, or combined *Nf1*/*Arf*/*Ink4a* for complete *Cdkn2a* loss in MPNSTs. Our data show that MPNST tumor onset is driven by *Nf1*/*Arf* loss, while loss of *Nf1*/*Cdkn2a* accelerates tumor onset. Loss of *Nf1*/*Ink4a* is insufficient to generate tumors. Our overall goal is to examine the cooperativity of *Arf* and *Ink4a* in MPNST progression.

Cancer metabolism is altered over the course of MPNST progression. Previous data from our lab show that MPNSTs with *Nf1* and *Cdkn2a* loss have dysregulated Nrf2- modulated G6pd activity, resulting in high levels of NADPH. Based on this finding, we are interested in examining the cooperativity of *Arf* and *Ink4a* loss in MPNST metabolism. We determined that primary cell lines derived from MPNSTs with *Nf1*/*Cdkn2a* deletion are more sensitive to pharmacologic inhibition of G6pd when compared to those with only *Nf1*/*Arf* deletion. Based on these data, we hypothesize that *Ink4a* loss cooperates with *Arf* loss to accelerate tumor onset through cellular antioxidant response pathways. Current studies are using functionals assays to dissect the metabolic pathways driving MPNST progression. This study is significant as it will identify key metabolic pathways in MPNSTs that are susceptible to pharmacologic inhibition, thereby improving patient outcomes.

Full List of Authors: Akshaya Warrier[1,2], Gavin McGivney[1], Mandy Scherer[1], Vickie Knepper-Adrian[1], Alexa Sheehan[1], Benjamin Darbro[1], Dawn Quelle[1], Rebecca Dodd[1]

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[2]University of Iowa MSTP

Platform: Immune Competent Models for NF1-Associated Glioblastoma: Allografts Accurately Recapitulate Primary Tumors

Tuesday, June 24, 11:40am – 11:55am

Stephanie N. Brosius, MD, PhD, *Children's Hospital of Philadelphia*

Purpose: Compared to the general population, individuals with Neurofibromatosis Type 1 have a 50-fold higher risk of developing a high-grade glioma (NF-HGG) in their lifetime. Despite improved understanding of molecular and cellular drivers of these neoplasms, we have yet to translate this knowledge into therapies that improve overall survival. One limitation has been the paucity of *in vivo* models for drug testing within this population. We generated 3 distinct glioma stem cell lines from spontaneous high-grade gliomas arising in mice with *Nf1* and *Trp53* mutations in cis (NP-cis mice).

Methods: NF1-HGG stem cell lines were orthotopically allografted in immune competent wild-type C57Bl/6 or $Nf1^{+/-}$ mice which were monitored for tumor formation. Tumors were harvested for single cell RNA sequencing and compared to the NF-HGG stem cell lines as well as the primary murine tumor. Overall survival was assessed via Kaplan-Meier curves with log-rank testing.

Results: Glioblastoma tumor cells have distinct differentiation states including glioma stem cells, neuronal precursors, oligodendrocyte precursor, astrocyte precursors, and mesenchymal-like states. All of these differentiation states are typically present within a given tumor. Importantly, we observe the same differentiation states in primary glioblastoma that arise in our NPcis mouse model. Because our cell lines are grown in stem cell media, there is an inherent reduction in differentiation states observed *in vitro.* However, orthotopic allografts of the glioma stem cell lines again recapitulate the differentiation states observed in high-grade glioma based on single cell RNA sequencing. Furthermore, the allografted cells also faithfully model the primary tumor as well as its microenvironment both histopathologically and on sequencing. Next, we investigated if the Nf1 heterozygous microenvironment altered any behavior of these tumors by injecting the NF1 HGG stem cell lines in syngeneic NF1 wildtype or heterozygous mice. Murine survival was not altered when comparing allografted wild-type C57Bl/6 mice to $Nf1^{+/-}$ mice.

Conclusion: Allografts of NF1-HGG stem cell lines faithfully recapitulate the tumor differentiation states and microenvironment observed in the primary tumors. While germline mutations within the tumor microenvironment do not appear to cause marked changes in baseline survival within this model system, the genetic background remains an important consideration for therapies targeting the glioma microenvironment. These data indicate that allografted glioma-lines from NP-cis mice are an effective model of NF-HGG that can be used for larger scale *in vivo* drug screening and validation.

Full List of Authors: Stephanie N. Brosius, MD, PhD[1,2]; Jonathan Sussman, PhD[3]; Katherine Labella, BS[1,2], Isabella Di Stefano, BS[1,2]; Una Barkardottir, BA[1,2]; Kai Tan, PhD[1,2]; Thomas De Raedt, PhD[1,2]

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[3]Medical Scientist Training Program, University of Pennsylvania School of Medicine, Philadelphia, PA

Funding: Gilbert Family Foundation (TDR), DHART SPORE (TDR), NCI K12 (SNB)

Platform: A Haploinsufficiency Restoration Strategy Corrects Neurobehavioral Deficits in *Nf1*$^{+/-}$ Mice

Tuesday, June 24, 11:55am – 12:10pm

Steven Angus, PhD, *Indiana University School of Medicine*

Purpose: Neurofibromatosis type 1 (NF1) is a genetic disorder caused by mutations of the *NF1* tumor suppressor gene resulting in the loss of function of neurofibromin, a GTPase activating protein (GAP) for Ras. While the malignant manifestations of NF1 are associated with loss of heterozygosity of the residual wild type allele, the nonmalignant neurodevelopmental sequelae, including autism spectrum disorder (ASD) and/or attention deficit hyperactivity disorder (ADHD) are prevalent morbidities that occur in the setting of neurofibromin haploinsufficiency. We reasoned that augmenting endogenous levels of wild type neurofibromin could serve as a potential therapeutic strategy to correct the neurodevelopmental manifestations of NF1.

Methods: Here, we utilized a combination of in vitro genetic screening, biochemical and cell-based assays, genetically engineered murine models (both germline and floxed *intercrossed* F-box specificity factors with *Nf1*$^{+/-}$ mice), neurobehavioral assays, stereotactic Cre injection, and immunohistochemistry (IHC) and biochemical assay of murine brain tissue.

Results: We identify two key mediators of neurofibromin degradation by the ubiquitin proteasome pathway (UPP). Genetic intercross to generate compound heterozygous *Nf1*$^{+/-}$*;F-box* mice restored neurobehavioral deficiences observed in *Nf1*$^{+/-}$ mice. Targeted ablation of the target in the nucleus accumbens of mice likewise led to resolution of neurobehavioral deficiency. IHC and biochemical evaluation of murine brain tissue revealed a corresponding increase in neurofibromin protein level, reduction in active Ras, and suppression of hyperactivated ERK1/2 in *Nf1*$^{+/-}$ mice with *F-box* loss as compared to control *Nf1*$^{+/-}$ mice.

Conclusions: Our data highlight a role for F-box specificity factors in the regulation of neurofibromin protein levels and regulation of Ras/MAPK pathway activity. Furthermore, the neurobehavioral assays in *Nf1*$^{+/-}$ mouse models establish a potential paradigm for overcoming haploinsufficiency by blocking the UPP and increasing neurofibromin protein levels from the functional wild type allele.

Full List of Authors: Su Jung Park#, Jodi L. Lukkes#, Ka-Kui Chan#, Hayley P. Drozd, Callie B. Burgin, Shaomin Qian1, Morgan McKenzie Sullivan, Cesar Gabriel Guevara, Nolen Cunningham, Stephanie Arenas, Makenna A. Collins, Jacob Zucker, JinHee Won, Abbi Smith, Li Jiang, Dana K. Mitchell, Steven D. Rhodes, Steven P. Angus*, D. Wade Clapp*

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Platform: Exploring the Interplay Between Lipid Metabolism and LZTR1 in Peripheral Nerve Pathologies

Tuesday, June 24, 1:25pm – 1:40pm

Georgia Daraki, MSc, *Fritz Lipmann Institute-Leibniz Institute on Aging, Jena, Germany*

Schwannomatosis is a condition primarily characterized by severe neuropathic pain, with patients harboring LZTR1 deletions (*LZTR1*-related schwannomatosis) experiencing more intense pain compared to those with other genetic mutations associated with the disease. Despite this well-established correlation, the underlying mechanisms of neuropathic pain in *LZTR1*-related schwannomatosis and the specific role of LZTR1 deletion remain poorly understood. To elucidate these mechanisms, we employed a mouse model with *Lztr1* specifically knocked out in Schwann cells (*Lztr1*-KO P0). A variety of molecular analyses and sensory assessments were performed, including the Von Frey filament assay to assess mechanical sensitivity and the Hot Filament assay to measure heat sensitivity. At an early age, *Lztr1*-KO P0 mice exhibit increased mechanical sensitivity, which shifts to heightened heat sensitivity with age, partially mirroring the clinical phenotype observed in patients.

Electron microscopy of sciatic nerves from Lztr1-KO P0 mice revealed notable hypermyelination of axons, coupled with a significant reduction in the total number of myelinated axons compared to wild-type controls. This is accompanied by an inflammatory response typically seen in demyelinating diseases. Interestingly, these mice also display progressive nerve enlargement, which worsens with aging, a symptom consistent with other LZTR1-related disorders. Proteomic analysis revealed a previously unrecognized role for LZTR1 in lipid metabolism regulation, highlighting that LZTR1 deficiency disrupts both cholesterol and sphingolipid metabolism, as well as the stoichiometry of structural myelin proteins.

LZTR1 functions as a substrate adaptor for the E3 ubiquitin ligase CUL3, with RAS being a key substrate. By regulating the degradation of RAS, LZTR1 modulates MAPK signaling activity. In LZTR1 knockout HEK293T cells, serum deprivation leads to hyperactivation of the ERK component of the MAPK pathway, independent of MEK activation, and disrupts de novo lipogenesis, as indicated by SREBP dysfunction. Given the crucial role of lipid metabolism in maintaining peripheral nerve function and myelination, we propose that LZTR1 deficiency disrupts lipid homeostasis, potentially contributing to myelination defects and the development of neuropathic pain in *LZTR1*-related schwannomatosis.

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Funding: Children's Tumor Foundation - Young Investigator Award

Platform: Neurofibromatosis Type 1 is Associated with Extensive, Independent Somatic Mutation of the Wild-Type *NF1* Allele in Normal Tissues

Tuesday, June 24, 1:40pm – 1:55pm

Thomas R. W. Oliver, MD, PhD, *Wellcome Sanger Institute, Hinxton, UK; Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK*

Purpose: Neurofibromatosis type 1 is a cancer predisposition syndrome caused by germline *NF1* gene mutation. Many of its associated lesions (café au lait spots, neurofibromas and low-grade gliomas) form after developing a second somatic mutation, or "hit", in the unaffected allele. Whether patient-derived normal tissues can sustain this biallelic *NF1* loss without morphological transformation is unclear. In this study, we examined normal tissues from affected individuals for the presence of second *NF1* hits.

Methods: We performed whole genome (WGS) or whole exome sequencing (WES) on 479 bulk and microdissected tissues collected at autopsy from a child with neurofibromatosis type 1, alongside 610 control samples from two non-predisposed children. Sampling encompassed all germ layers, including intra- and extracranial viscera. Variant allele fraction was used to estimate clone size, and haplotype phasing was implemented to detect low-level *NF1* loss of heterozygosity. We applied targeted duplex sequencing to the same tissues, and those from an additional nine adults with neurofibromatosis type 1, to validate our findings. This novel technology enabled accurate driver mutation detection at single DNA molecule resolution. We measured the selective pressure for *NF1* mutation across cell types by comparing the non-synonymous and synonymous mutation rate (dN/dS ratio).

Results: The normal tissues of the child with neurofibromatosis type 1 commonly harbored somatic loss-of-function variants in *NF1* but those of the non-predisposed children did not. Affected cells clonally expanded to account as many as ~56% of cells within a biopsy. In the WGS/WES data, these events were confined to the central nervous system, of neuroectodermal origin. Mutations called from the much more sensitive targeted duplex sequencing not only corroborated this burden, but dN/dS analysis revealed strong positive selection for truncating *NF1* mutations across all germ layers. Of note, selective pressure was strongest within the neuroectoderm and spleen, common sources of tumors in neurofibromatosis type 1. Bulk nerve (neuroectoderm) from predisposed adults, but not muscle and blood (both mesoderm), showed a similar mutation pattern.

Conclusion: Second *NF1* hits frequently affect the normal tissues of individuals with neurofibromatosis type 1, conferring a selective advantage. Although they may be insufficient for immediate tumor formation, their tissue-specific selection patterns could represent a quantifiable link between genotype and phenotype. This challenges the traditional model of tumorigenesis in this syndrome, as it might in similar genetic conditions. Further work is needed to identify other factors governing neoplastic transformation and understand the relationship between mutation burden and cancer risk.

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Disclosures: I.M. is a co-founder and consultant of Quotient Therapeutics. D.H. provides consultancy to AstraZeneca/MedImmune, Alexion Pharmaceuticals, Bayer, Biodexa, Roche/Genentech, and Novartis, as well as expert testimony to AstraZeneca and Novartis, and his expenses are covered by Alexion Pharmaceuticals, Boehringer Ingelheim, Roche/Genentech, and Novartis. The remaining authors have no relevant financial relationships to declare.

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Basic / Preclinical - NF1 (The Poster Sessions are a Non-CME Activity)

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Basic / Preclinical - NF1 (The Poster Sessions are a Non-CME Activity)

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Volz	Avery	5555	Development of an Adeno-Associated Virus (AAV) Toolkit to Modulate Signaling Pathways Altered in Neurofibromatosis Type 1 (NF1)
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Fertitta	Laura	5596	Promising Efficacy of LIMK Inhibitors in Reducing Cutaneous Neurofibromas in an *Nf1*-KO Mouse Model
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Basic / Preclinical - NF1 (The Poster Sessions are a Non-CME Activity)

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Zheng	Tingting	5487	Gene Therapy Strategies and Prospects for Neurofibromatosis Type 1

TUMOR BIOLOGY AND DISEASE MECHANISM

CDKN2A Deletion Drives Malignant Transformation in NF1 Deficient Glioma

Wai Chin Chong, *Brain Tumor Institute, Center for Cancer and Immunology Research, Children's National Hospital, Washington, DC; Center for Genetic Medicine, Children's National Research and Innovation Campus, Children's National Hospital, Washington, DC*

Background: Neurofibromatosis type 1 (Nf1) is a common autosomal dominant disorder that is caused by mutations in *neurofibromin 1 (NF1)* gene and affecting 1 in 3000 children worldwide. Nf1 patients have higher risk of developing different tumors, including glioma. Importantly, comprehensive genomic analysis revealed high frequency of *cyclin-dependent kinase inhibitor 2A (CDKN2A)* inactivation in malignant NF1 mutant glioma as compared to its benign counterpart, suggesting its role in driving malignant transformation in NF1 mutant glioma. However, little is known about this *CDKN2A* dependent malignant transformation, due to lack of appropriate preclinical glioma models for comprehensive profiling. To address this knowledge gap, we utilized CRISPR editing platform to generate isogenic lines with different combinations of *NF1* and *CDKN2A* mutation, aiming to investigate the role of CDKN2a in malignant transformation of NF1 mutant glioma.

Method: We utilized CRISPR technology to knock out *NF1* and *CDKN2A* genes in NF1 and CDKN2A wildtype RES186 glioma cell. The cell growth and migration potential of the generated isogenic lines were assessed and compared using cell proliferation and Boyden chamber invasiveness assays. The mRNA-seq was performed on these isogenic lines to compare the global transcriptomic changes and enriched molecular events. Further, these isogenic lines were mcherry-luciferase labelled and engrafted in the NSG immunodeficient murine models to evaluate their proliferation and invasiveness potential under in-vivo condition.

Results: Our findings revealed that RES186 $NF1^{KO}$ has similar growth and invasive rate as its parental line. Conversely, RES186 $CDKN2A^{KO}$ possessed both increased growth and invasive rate. Strikingly, the RES186 $NF1^{KO}$ $CDKN2A^{KO}$ has the highest growth and invasive rate as compared to its parental and other isogenic lines. The global transcriptomic analysis further revealed additional enrichment in collagen degradation process solely in RES186 $NF1^{KO}$ $CDKN2A^{KO}$ cells. These results highlight the interplay of NF1 and CDKN2a in regulating the growth and invasiveness dynamics of glioma cells.

Conclusion: In conclusion, our preliminary study suggests that the concurrent loss of NF1 and CDKN2a enhanced growth and invasiveness in gliomas. The established CRISPR isogenic model will provide a platform to explore the collaborative role of these genetic alterations in tumor transformation.

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Curtailing the Exit Strategy of Lymphocyte Migration to Regulate Neurofibroma Growth

Jay Pundavela, PhD, *Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

Plexiform neurofibroma (PNF) is a benign peripheral nerve sheath tumor associated with Neurofibromatosis Type 1 (NF1). Beyond the presence of neoplastic Schwann cells, an inflammatory milieu enriched with immune cells, including myeloid subsets (macrophages and dendritic cells) and T lymphocytes, contributes to PNF pathogenesis. Our recent findings highlighted the critical involvement of CD8 T cells in neurofibroma tumorigenesis, after loss of Nf1 in Schwann cells (Pundavela et al., Sci. Adv., 2024). However, the impact of manipulating T cell trafficking on neurofibroma growth remained unexplored.

Aim: In this study, we investigated the modulation of T cell migration through the administration of Fingolimod—a sphingosine-1-phosphate receptor modulator approved for use in Multiple Sclerosis—on established neurofibromas in a clinically relevant preclinical mouse model (Nf1 f/f; DhhCre), which closely mimics the pathological features of NF1 in humans.

Methods: We administered Fingolimod at a daily dose of 10 mg/kg orally for 30 days to Nf1 f/f; DhhCre mice, with a parallel vehicle control group. Toxicity assessments included monitoring body weight and histological evaluations of liver, lung, and kidney tissues. Migration of lymphocytes across lymphoid organs and tumors was assessed using multiparametric flow cytometry and immunofluorescence.

Results: No significant toxicity was observed, as evidenced by stable body weights and unremarkable tissue morphology in Fingolimod-treated mice compared to controls. Importantly, Fingolimod treatment resulted in a notable reduction in tumor size. Flow cytometry analysis revealed a significant decrease in circulating T cells, with a marked reduction in CD8 T cells while CD4 T cell levels remained unchanged. Conversely, an increased frequency of CD8 T cells was noted in the lymph nodes of Fingolimod-treated mice, indicating an inhibition of CD8 T cell egress from lymphoid compartments. No alterations were detected in the CD8/CD4 T cell ratio within the spleen across both treatment groups.

Conclusion: Our findings support a pivotal role of T cell trafficking in the growth of neurofibromas and suggest that Fingolimod may serve as an immunomodulatory agent for neurofibroma treatment. This research supports testing of therapeutic strategies that target T cell dynamics to effectively manage neurofibromas.

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Proteomic Analysis to Study Selumetinib Effect in Adolescents with Neurofibromatosis Type-1 and Inoperable Plexiform Neurofibroma (PN)

Haydee Lara, PhD, *Alexion-AstraZeneca Rare Disease Unit, Translational Sciences*

Background & Purpose: The samples analyzed in this proteomics study correspond to clinical study NCT05101148 "Phase I Study to Assess the Effect of Food on the PK and Gastrointestinal Tolerability of Selumetinib in Adolescent children With Neurofibromatosis Type 1 Related Plexiform Neurofibromas". The clinical study NCT05101148 was designed to evaluate the effect of a low-fat meal on steady state selumetinib exposure. Selumetinib was approved in the US in 2020 and in Europe in 2021; it was approved for the treatment of NF1 pediatric patients with symptomatic and inoperable PN and until recently, the drug had to be taken in a fasted state. The results of this clinical study supported the update of the label in 2024, and selumetinib can now be taken with or without food. In the proteomic analysis discussed here, we investigated the effect of selumetinib in disease-relevant pathways in this NF1 patient population, especially in the patients with skeletal manifestations.

Methods: We evaluated plasma samples corresponding to twenty-three patients from study NCT05101148 using aptamer-based proteomics technology. We compared protein fold changes in pre-dose plasma samples corresponding to one cycle vs. two cycles of selumetinib treatment. Similarly, we studied changes in the proteome of patients with NF1-related skeletal manifestations in the same population. Pathway enrichment analysis was also performed to assess pathways involved with the significantly dysregulated proteins.

Results: We found that CCL5 (RANTES) was upregulated in NF1 patient samples corresponding to cycle two of selumetinib treatment. Proteins associated with tumor growth, such as hepcidin and HMG Co-A synthase, were downregulated. When we analyzed the proteome of NF1 patients with skeletal manifestations, we found that proteins in the collagen family are upregulated. Although the role of these dysregulated collagen proteins in NF1 is unknown, other researchers have reported their relationship with other bone diseases.

Conclusion: This proteomics study in NF1 patient samples treated with selumetinib demonstrates changes in proteins previously reported for this population, such as CCL5 (RANTES). The analysis also uncovered a potential link of collagen proteins dysregulation in NF1 skeletal manifestations with other bone diseases. Further validation of these findings is warranted.

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Contribution of Fibroblasts to Drug Resistance in 3D NF1 Plexiform Neurofibroma Cultures

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Purpose: Neurofibromatosis Type 1 (NF1) plexiform neurofibroma (hereafter called pNF1) is present in ~50% of NF1 patients, and is a complex tumor composed of abnormal Schwann cells and cells of the surrounding tumor microenvironment (TME). Fibroblasts, the most abundant cell types within the pNF1 TME, are implicated in tumor formation and regulate extracellular matrix (ECM) stiffness by producing excessive amounts of collagen. Using 3D pNF1 culture models, we previously demonstrated that fibroblasts have a critical role in tumor growth and invasiveness through their secretome. However, it is poorly understood how fibroblasts affect a long-term drug response of pNF1. Delineating molecular mechanisms of drug resistance is crucial to designing new therapies to improve therapeutic outcome in pNF1. In the present study, we will investigate how fibroblasts affect drug resistance of pNF1 via their secretome and/or their modulation of ECM stiffness.

Methods: As a pre-clinical model to evaluate drug resistance of pNF1 tumor cells, we used our unique 3D/4D (3D + real-time) cultures of human pNF1 cells in the context of their TME, i.e., fibroblasts in the present study, at a ratio of 1 tumor: ½ or ¼ fibroblasts grown in our patented culture chips (TAME chips; Patent #: US 10,227,556 B2). Our 3D/4D culture model supports growth of the 3D cultures, live-cell confocal imaging in real-time (4D) and non-invasive, high-content analysis and drug testing over extended long-term culture periods. Two immortalized human pNF1 cell lines (ipNF95.11b C and ipNF05.5; $Nf1^{-/-}$) and patient-derived primary fibroblasts ($Nf1^{+/-}$) are used for 3D cultures. FDA-approved selumetinib and mirdametinib are used for testing drug resistance of pNF1. We employed: 1) 3D parallel cocultures of pNF1 tumor cells and fibroblasts in which the two cell types share media without direct contact to test effects of fibroblast-derived secretome, and 2) two kinds of 3D ECM hydrogels, naturally derived and synthetic ones, to test effects of different ECM stiffness produced mainly by fibroblasts. We used live-cell imaging and 3D quantitative analysis to evaluate drug responses in pNF1 tumor cells.

Results: We observed a greater viability of pNF1 tumor cells in parallel cocultures with fibroblasts than in pNF1 monocultures treated with selumetinib or mirdametinib. Moreover, pNF1 tumor cells grown in 3D ECM hydrogel with higher stiffness (7 kPa) are more resistant to the drug treatment compared to ones in 3D ECM hydrogel with lower stiffness (1.5 kPa). The pNF1 tumor cells cultured with fibroblast-derived conditioned media or grown in 3D ECM hydrogel with higher stiffness displayed more increased level of p-glycoprotein, a major mechanism of drug resistance induced by actively pumping drugs out of cells.

Conclusions: Our results demonstrate that fibroblasts significantly increase drug resistance of tumor cells via their secretome, and the modulation of ECM stiffness and p-glycoprotein is a potential mechanism underlying fibroblast-mediated drug resistance in pNF1. The results of this study may lead to improvement of the therapeutic outcome of pNF1 patients by identifying means to ameliorate drug resistance of pNF1.

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Molecular and Circuit Mechanisms of Visual Hypersensitivity in Mice Modeling NF1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by loss-of-function mutations in the NF1 gene that encodes the neurofibromin protein. Neurofibromin loss leads to increased signaling in the Ras-mitogen activated protein kinase (MAPK) pathway (Ras-Raf-MEK-ERK) that is critical for nervous system development and function. The neurocognitive symptoms of neurofibromatosis type 1 (NF1) include impaired executive functioning, autistic features, speech and language delays, and sensory processing abnormalities. NF1 patients also have a high incidence of attention deficit/hyperactivity disorder (ADHD), which is associated with diminished ability to suppress distractive stimuli and increased sensitivity to aversive sensory cues. Here, we show that mice modeling NF1 (*Nf1*$^{+/-}$ mice) are hypersensitive to aversive visual stimuli, such as threatening looming discs that simulate predator approach from above. This phenotype was also observed in a novel mouse model of *MAPK1*-related Rasopathy that contains a patient-derived ERK2 gain-of-function mutation (*Mapk1*$^{A174V/+}$ mice) but not in mice with increased mTOR signaling (*Tsc2*$^{+/-}$ mice). Conditional expression of an activated MEK1 mutant in cortical excitatory neurons and glia was sufficient to enhance threat reactivity, whereas MAPK activation in the midbrain, which plays a critical role in innate behavioral responses to visual threats, or in forebrain interneurons was not. Our findings were not caused by changes in retinal sensitivity to light given that *Nf1*$^{+/-}$ mice exhibited no differences in baseline or light-evoked retinal ganglion cell (RGC) firing in intact retinal preparations *ex vivo* or in physiological assays that test RGC function *in vivo.* Taken together, our results suggest that increased MAPK activation in the *Nf1*-deficient cerebral cortex alters top-down control of subcortical sensory processing centers, which, in turn, enhances visual threat reactivity. Currently, we are employing circuit- and cell-type specific *Nf1* knockout models and calcium imaging techniques to refine our understanding of the effects of neurofibromin loss and MAPK pathway activation on attentional and sensory function.

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CD4+ and CD8+ T Cell Subsets Cooperate to Constrain Malignant Outgrowth of NF1-Peripheral Nerve Sheath Tumors (PNSTs)

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Purpose: Atypical neurofibromas exhibit increased signatures of immune cell surveillance and T cell infiltration, but the role of T cells in governing malignant transformation of MPNST precursor lesions remains unclear. We hypothesized that effector CD4+ and/or cytotoxic CD8+ T cells are critical in preventing malignant transformation of plexiform and atypical neurofibroma precursors. The goal of this work was to address a critical gap in our understanding of how T cell subsets influence the behavior of MPNST and their precursor lesions.

Methods: Single cell RNA sequencing (scRNAseq) with sub-type anchor correction for alignment in Seurat (STACAS) was utilized to integrate multiple scRNAseq datasets and characterize the functional states of infiltrating T cells in human NF1-PNSTs. Effector cytotoxic, helper, regulatory, and exhausted CD4+ and CD8+ T cell subsets were quantified in native tumor tissue by multiplexed immunohistochemistry (IHC). Finally, preclinical mouse models that recapitulate MPNST development from neurofibroma precursors were used to evaluate the impact of CD4+ and CD8+ T cell depletion on tumor progression.

Results: We observed changes in single cell transcriptional programs of infiltrating T cells in human PNSTs with a shift from naïve, effector, and cytotoxic CD4+ and CD8+ T cells in PNF to regulatory (Treg) and exhausted states in MPNST. Furthermore, multi-chromogenic IHC of human PNST showed robust T cell infiltration in atypical neurofibroma with a predominance of CD4+ T helper 1 (Th1) cells versus T cell exclusion with Treg predominance in MPNST. In parallel studies, the ratio of $CD4^{+}FOXP3^{+}$ Tregs to total $CD4^{+}$ T cells increased as MPNSTs progressed in immunocompetent C57BL/6 mice engrafted with syngeneic *Nf1*$^{+/-}$*;Trp53*$^{+/-}$ MNF463A cells. To assess the functional contribution of T cells in restraining transformation and malignant outgrowth, immunocompetent syngeneic recipient mice were orthotopically engrafted with primary *Nf1-Cdkn2a*$^{-/-}$ Schwann cell precursors or MNF463A cells and treated with monoclonal antibodies against CD4, CD8, and the combination versus an IgG2b isotype control (n=8/group). Kaplan-Meier analysis showed that while depletion of CD4+ T cells alone had minimal impact on tumor progression, CD8+ depletion and combined CD4+/CD8+ T cell depletion profoundly accelerated the latency and penetrance of MPNST ($p<0.0001$, log-rank test).

Conclusions: Interactions between CD4+ and CD8+ T cell subsets are critical in governing malignant transformation of NF1-PNSTs. Changes in T cell phenotypes across the neurofibroma to MPNST continuum may inform future diagnostic and therapeutic strategies for the early detection and prevention/treatment of malignancy.

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Characterizing the Role of ZNF423 in Neurofibromatosis Type 1 (NF1)-Related Malignant Peripheral Nerve Sheath Tumors (MPNST)

Sarah Morrow, *IU School of Medicine, Indianapolis, IN*

Purpose: Recent work in our lab utilized transcriptomics to analyze two *SUZ12*-deficient human MPNST cell lines with restored PRC2 activity, which revealed downregulation of *ZNF423,* a lineage-specific transcription factor. These results were validated in murine tumor cells-of-origin isolated from GEMMs of plexiform neurofibroma (PNF) and MPNST, which revealed *ZNF423* was significantly upregulated in MPNST compared to both wild-type controls and PNF, suggesting a potential role for this protein in MPNST tumorigenesis. Our goal is to identify potential therapeutic targets for NF1-related MPNST by elucidating ZNF423-dependent signaling mechanisms that may influence MPNST lineage, identity and proliferation.

Methods: Human MPNST cell line models were employed for RNA interference, RNA sequencing, immunoblotting, proteomic analysis of the kinome, cell-based assays, and *in vivo* implantation.

Results: Short-interfering RNA (siRNA) depletion of *ZNF423* from four human MPNST cell lines altered MPNST cell morphology, and significantly decreased cell proliferation and viability compared to controls. EdU/DAPI staining further confirmed these effects, showing a marked decrease in DNA synthesis and hence impaired cell cycle progression and proliferation in ZNF423 knockdown cells. To establish the biological mechanism underlying these phenotypic effects, we performed RNA sequencing, which revealed significant transcriptional changes, with overlapping gene expression patterns amongst the four cell lines. Shared significantly downregulated genes were enriched for pathways involved in proliferation, migration and development. In contrast, shared upregulated genes were enriched for pathways involved in UV response, DNA damage checkpoint activation and cell cycle arrest, suggesting a shift toward stress-induced inhibition of tumor growth. To assess the effects of ZNF423 knockdown *in vivo,* we generated a human MPNST cell line with stable *ZNF423* suppression using shRNA, which was implanted into the sciatic nerve of NRG mice. Tumor growth was measured over time revealing that ZNF423 knockdown led to marked reduction in tumor size compared to controls, and individual tumor growth curves show a consistent slower progression in the ZNF423 knockdown mice.

Conclusion: These studies suggest that ZNF423 regulates key pathways that maintain MPNST survival, as depletion reduces MPNST cell viability and interferes with tumor growth *in vivo.* Ongoing studies will further delineate ZNF423-dependent signaling pathways using ChIP-seq, affinity purification-mass spectrometry (AP-MS), and multiplex inhibitor bead mass spectrometry (MIB/MS) kinome profiling to reveal druggable targets within these pathways.

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Inflammation Driven Schwann Cell Reprogramming in Plexiform Neurofibroma: Role of the NF-κB Pathway in Neurofibroma Formation

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Schwann cells are the cells of origin in plexiform neurofibroma (PNF), and inflammation plays an essential role in PNF formation and growth. Tumor formation occurs long after Nf1 loss in mouse models of the disease, suggesting that Schwann cells undergo secondary changes during tumor formation. Prior single cell RNA-Sequencing implicated NF-κB expression as upregulated late in tumor formation, and thus such a potential second signal. In human and mouse neurofibromas, p-65 was nuclear (active) in many neurofibroma cells, at least some of which were Schwann cells (Kershner et al., 2022).
Purpose: Identify the tumor Schwann cells in PNF and investigate the role of the NF-κB pathway in Schwann cell reprogramming after Nf1 loss.

Methods: A multiplex antibody panel was developed for the analysis of the PNF microenvironment and to identify the tumor Schwann cells. In vitro assays and RNA-Seq were utilized to determine the effect of NF-κB pathway modulation on Nf1 mutant Schwann cells. In vivo pathway modulation was also studied in a mouse model.

Results: Multiplex imaging revealed that tumor Schwann cells, but not pre-tumor Schwann cells in the same mice, express the cell surface markers CD44 and CD49f. We used these markers to isolate the tumor cell population by flow cytometry and characterized isolated tumor cells (versus non-tumor Schwann cells) by RNA-Seq. We also cultured Nf1 mutant Schwann cells and showed that they upregulate CD44+ CD49f+ expression when exposed to of a variety of stressors, including prolonged serum depletion, Poly I:C (inflammatory agent), and certain cytokines (ie., Il1β, Tnfa), or when infected with activated IKK2, which activates the NF-κB pathway. As these cells increased expression of these surface proteins, they also showed nuclear (active) p65. Preliminary results from ongoing in-vivo experiments show that low dose treatment of DhhCre; Nf1 fl/fl mice with the NF-κB pathway inhibitor BAY 11-7082 slightly reduced tumor volume, and higher-dose treatment reduced CD44+ CD49f+ tumor cells.

Conclusion: An inflammatory microenvironment is formed with activation of the inflammation associated NF-κB pathway in Schwann cells, resulting in an upregulation of the CD44+ and CD49f+ marker expression in the Schwann cells, and an overall reprogramming of the Schwann cells within the neurofibroma. A two-step process to PNF formation is proposed, with Nf1 loss in Schwann cells being the first step and the formation of an inflammatory microenvironment via activation of the NF-κB pathway and Schwann cell reprogramming as the second step.

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Alternative RNA Splicing Analysis Elucidates Therapeutic Targets During the Transformation of Plexiform Neurofibromas to Malignant Peripheral Nerve Sheath Tumors

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Purpose: Neurofibromatosis type 1 (NF-1) is a cancer predisposition syndrome associated with the development of benign plexiform neurofibromas (pNFs) which can transform into malignant peripheral nervous sheath tumors (MPNSTs). While the genomic and epigenetic underpinnings of this transformation have been described, changes in alternative RNA-splicing (AS) during malignant transformation are not well understood. AS is frequently dysregulated in human tumors, promoting the expression of pro-tumorigenic mRNA isoforms, and represent an untapped repertoire for novel therapeutics. Here, we define the AS landscape during pNF to MPNST transformation in patient samples and cell lines, and identify targetable RNA splicing alterations.

Methods: Bulk short-read RNA-sequencing (RNA-seq) from human pNFs and MPNSTs across three cohorts (UCSF, UoT, GeM) was analyzed using rMATS to quantify AS and DESeq for gene expression. Reverse-transcription polymerase chain reaction (RT-PCR) using RNA from pNF and MPNST-derived cell lines was used to validate AS splicing differences between tumor types. Finally, splice-switching antisense oligonucleotides (ASOs) were designed to modulate AS events.

Results: Using samples from UCSF as a discovery cohort, we identified 660 AS events significantly differently spliced during transformation from pNFs to MPNSTs. Of these 233 AS events (35%) were also detected in the University of Toronto cohort and 299 (45%) in the GeM cohort. These AS events were enriched for genes associated with mitotic spindle and epithelial-mesenchymal transition signatures, including for example, increased inclusion of an alternative cassette exon in *Fibronectin (FN1)* in MPNSTs compared to pNFs, which regulates FN1 protein localization and function. We further identified 13 RNA splicing regulators differentially expressed between MPNSTs and pNFs, including *MBNL1, PTBP1, IGF2BP1* which have known roles in cancer. Finally, we demonstrate that splice-switching ASOs that promote *FN1* exon skipping decrease proliferation, migration, and invasion of MPNST cell lines. We also show that ASOs targeting AS events in known RAS/MAPK regulators *HRAS, KRAS, RRAS,* or *RASA3* decrease proliferation of MPNST cells and synergize with the MEK inhibitor selumetinib.

Conclusion: We identify multiple AS events in MPNSTs and provide proof-of-concept for targeting of AS with ASOs as single agents and to synergize with MEK inhibitors, the only FDA approved therapy for pNFs. While our work reveals that AS represents an actionable target in NF-1, further research is needed to comprehensively map the AS landscape in NF-1 patients using long-read and single cell approaches, and to test AS-targeting therapeutic approaches in preclinical models.

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The Landscape of Alternative Splicing Change in Human Neurofibroma Fibroblasts

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Purpose: The main objective is to determine the landscape of alternative splicing change in human neurofibroma fibroblasts.

Method: We performed RNAseq in 3 normal skin fibroblasts and 3 cutaneous neurofibroma fibroblasts. We performed differential percent of splicing index (PSI) analysis to identify alternative splicing events associated with neurofibroma fibroblasts. We also performed Gene ontology analysis (DAVID bioinformatics) to get insights into their functional role. We performed DESeq2 analysis to measure the change in splicing factor expression.

Results: We identified 68 alternative splicing events associated with neurofibroma fibroblasts. Gene ontology analysis indicated that this splicing isoform signature is associated with cytoplasm intracellular location. We also identified 19 splicing factors with a significant change in gene expression between normal and neurofibroma fibroblasts. Overall, our preliminary results suggest a regulated alternative splicing program where a set of neurofibroma fibroblast splicing factors control the expression of specific neurofibroma fibroblast isoforms to contribute to the function of neurofibroma fibroblast phenotype ultimately.

Conclusion: We determined the landscape of alternative splicing change in human neurofibroma fibroblasts, setting the first step toward understanding the functional contribution of alternative splicing to neurofibroma fibroblast biology.

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Exploring Chromosome 8 Gain in *NF1*-/- Schwann Cell Precursors Using a hiPSC-Based Model

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Purpose: Neurofibromatosis type 1 (NF1) is an autosomal-dominant genetic disorder and the most common cancer predisposition syndrome, caused by germline mutations in the *NF1* tumor suppressor gene. Somatic loss of the second *NF1* allele in schwann cell precursors (SCPs) leads to benign plexiform neurofibromas (PNs), which can progress to malignant peripheral nerve sheath tumors (MPNSTs). Our lab previously identified chromosome 8 (Chr8) gain as a recurrent event in NF1-MPNST patient-derived xenografts (PDX) and paired patient tumors, suggesting a role in tumor progression. To investigate the impact of Chr8 gain on MPNST pathogenesis, we generated isogenic *NF1*-/- iPSC-derived SCP models with and without Chr8 gain.

Methods: A fibroblast population with mosaic trisomy 8 was acquired from Coriell and then single-cell cloned to generate both wild-type (WT) and trisomy 8 (Chr8 gain) lines. The Genome Engineering & Stem Cell Center (GESC@MGI) at Washington University assisted in reprogramming these cells into human induced pluripotent stem cells (hiPSCs). *NF1* knockout lines were also generated by GESC@MGI using synthetic gRNA, and CRISPR/Cas9 to introduce *NF1* mutations into these cells. WT, Chr8 gain, *NF1*-/-, and Chr8 gain;*NF1*-/- iPSC cell lines were differentiated into SCPs using a differentiation protocol that included TGF-beta inhibitor (SB431542), GSK-3 inhibitor (CT099021), N2, and B27 supplements. NRG-1 was added at day 6 of the protocol. The iPSC and SCP states of the cell lines were verified with qPCR after the completion of the differentiation protocol. Cell survival and cell proliferation of SCPs were assessed by Incucyte cell survival and CellTiter-Glo® 2.0 Cell Viability Assay.

Results: First, we validated the iPSCs with FISH and karyotyping. High expression of iPSC markers OCT3/4 and SOX2 was observed in all four iPSC lines. Second, after completing the differentiation protocol, we observed high expression of SCP markers GAP43 and ITGA4 in WT, *NF1*-/-, Chr8 gain and Chr8 gain;*NF1*-/- SCP lines compared to their iPSC state, consistent with their morphology. Third, we observed that Chr8 gain;NF1-/- SCP cell line proliferates faster and have enhanced survival compared to WT, *NF1*-/-, Chr8 gain SCPs.

Conclusion: These findings collectively indicate that the presence of Chr8 gain improves the proliferative capacity and survival of cells in the context of *NF1* loss, suggesting a potential role in MPNST progression. The successful generation and characterization of isogenic *NF1*-/- iPSC-derived SCP models with and without Chr8 gain provide a valuable platform for further mechanistic studies.

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DLK1 Distinguishes Subsets of NF1-Associated Malignant Peripheral Nerve Sheath Tumors with Divergent Molecular Signatures

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Purpose: In persons with neurofibromatosis type 1 (NF1) the lifetime risk of pre-existing neurofibroma (PNST) undergoing malignant transformation to MPNST is 8-16%. MPNST is the leading cause of premature death among individuals with NF1 and beyond the few known characteristic genetic changes, the transcriptional aberrations that precede malignant transformation and contribute to MPNST tumorigenesis remain poorly defined. Alterations involving *CDKN2A* and components of PRC2 have been implicated as early drivers of PNST evolution, but these events do not occur in all MPNST. Accordingly, emerging data has begun to highlight the importance of molecular-based stratification to improve outcomes in patients with NF1-PNST.

Methods: Here we perform an integrated analysis of multiple, independent datasets obtained from human NF1 patients to gain critical insight into PNST evolution and MPNST heterogeneity.

Results: We show that Delta-like non-canonical Notch ligand 1 (DLK1) is significantly increased in MPNST **(Figure 1A&B)** and provide evidence that *DLK1* overexpression may precede histological changes consistent with malignancy **(Figure 2)**. In complementary analyses, we find that serum levels of DLK1 are significantly higher in both mice and humans harboring MPNST compared to those without malignancy **(Figure 3A&B)**. Importantly, while DLK1 expression is increased in MPNST overall, through the integration of multiple, independent datasets we demonstrate that divergent levels of *DLK1* expression distinguish MPNST subsets characterized by unique molecular programs and potential therapeutic vulnerabilities. Specifically, we show that overexpression of *DLK1* is associated with the reactivation of embryonic signatures, an immunosuppressive microenvironment and a worse overall survival in patients with NF1-MPNST.

Conclusions: Collectively, our findings provide critical insight into MPNST tumorigenesis and support prospective studies evaluating the utility of DLK1 tissue and serum levels in augmenting diagnosis, risk assessment and therapeutic stratification in the setting of NF1-PNST.

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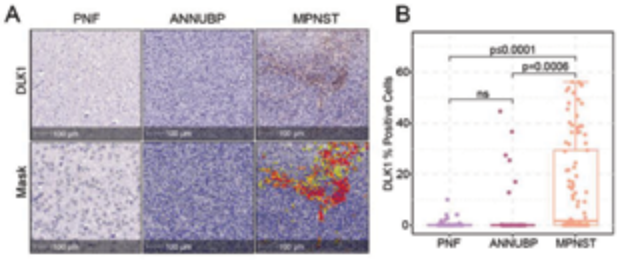

Figure 1. (A) Representative photomicrographs of human tumor sections across the PNST continuum immunohistochemically stained for DLK1. Magnification is denoted by 100 µm scale bars with inset high-power magnification as shown. The HALO cytonuclear mask used to quantify DLK1 staining is shown in the bottom panel. **(B)** Box and whisker plot depicting DLK1 positive cells as a percentage of total cells per field generated in R studio. Dots represent individual regions of interest (ROI). Error bars represent the 95% confidence interval. The center line represents the median. The box spans the 25th to 75th percentiles. Data beyond the whiskers are outliers and are plotted as individual points.

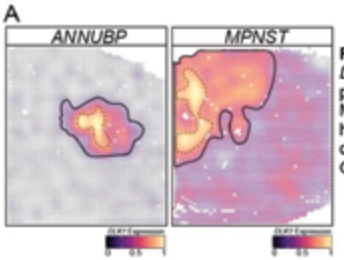

Figure 2. (A) Surface plots depicting *DLK1* expression within the spatially profiled ANNUBP and the contiguous MPNST. Yellow corresponds to highest expression and dark purple to decreased expression as indicated. Grey represents no expression.

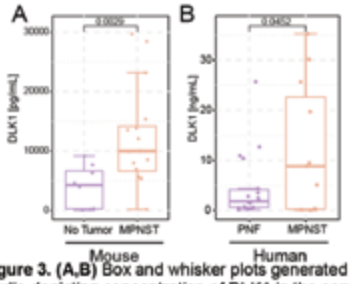

Figure 3. (A,B) Box and whisker plots generated in R studio depicting concentration of DLK1 in the serum of *Nf1/Cdkn2a Cre+* mice **(A)** and humans **(B)** with MPNST compared to those without malignancy. Dots represent individual samples. Error bars reflect the 95% confidence interval. The center line represents the median. The box spans the 25th to 75th percentiles. Data beyond the whiskers are outliers and are plotted as individual points. P-value represents unpaired, two-tailed t-tests between groups. Graph depicts single experiment.

Intratumoral Heterogeneity of MPNST has Implications for Diagnostics and Understanding of Malignant Progression

Tomas Phan, *Research Institute Children's Cancer Center, Hamburg, Germany*

Purpose: Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive tumors and should be detected and treated as early as possible. However, MPNST show pronounced intratumoral heterogeneity, comprising areas histologically corresponding to premalignant stages like plexiform neurofibromas (PNF) or atypical neurofibromatous lesions of unknown biological potential (ANNUBP) next to areas of obvious malignant morphology. Thus, CT-guided stereotactic biopsies harbor the risk of misdiagnosis. We aim to delineate the intratumoral heterogeneity of MPNST on an histologically, epigenetic, genetic, and transcriptomic level to improve diagnostic certainty and to better understand the mechanisms of progression from premalignant to malignant stages.

Methods: We carefully examined the histology of 12 MPNST and chose one area with high-grade MPNST morphology and one or more areas with histological premalignant morphology in each tumor for global DNA methylation profiling, targeted DNA panel sequencing, and single cell RNA sequencing.

Results: Clustering analysis of global DNA methylation data revealed that in 6/12 cases, malignant and premalignant areas from one tumor showed highly similar characteristics, leading them to group within the same epigenetic cluster. The remaining 6 sample pairs, however, showed clear epigenetic differences, with the premalignant sample consistently clustering alongside ANNUBP and the malignant sample clustering with MPNST. Of note, no sample clustered with PNF, despite clear PNF histomorphology in some cases. Copy number profiles inferred from the methylation data showed marked global alterations not only in the high-grade areas but also in the histologically benign areas in 7/12 cases. In the histologically premalignant areas of the remaining 5/12 cases, only subtle copy number changes were detected. Targeted gene sequencing revealed pathogenic variants in TP53, SUZ12, or EED in the malignant areas of 5/12 MPNST. In two of these cases, the respective variant was also detected in the premalignant area. First preliminary results from single cell RNA sequencing indicate that premalignant areas show significantly fewer malignantly transformed cells, defined by their gene expression profile and copy number variations.

Conclusion: Overall, our study underscores the intratumoral heterogeneity present in MPNST, which is evident histologically, epigenetically, and genetically. Our findings are highly relevant when diagnosing stereotactic biopsies of suspected MPNST. Areas that appear histologically benign can show an epigenetic signature or typical genetic variants indicative of malignancy. They also indicate that epigenetic changes might occur early during malignant progression of peripheral nerve sheath tumors and can be detected even before histological features of malignancy become apparent.

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Exploring the Role of Cutaneous Innervation in the Development of cNFs in NF1

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Cutaneous neurofibromas (cNFs) are nerve sheath tumors that develop in nearly all individuals with NF1. They typically emerge at puberty and can number in the thousands. Despite their benign nature, cNFs often cause pain and pruritus, making them a significant burden for patients. This suggests that skin innervation may play a crucial role in their pathogenesis. Supporting this hypothesis, our recent transcriptomic profiling of tumor Schwann cells (SCs) isolated from cNFs revealed an overactivation of genes involved in axonal growth and pruning, further implicating innervation in cNF development.

In this study, we investigated the role of skin innervation in cNF development in both NF1 patients and an *Nf1*-KO mouse model. First, we performed quantitative sensory testing (QST) in 21 NF1 patients by comparing a cNF area to the contralateral healthy looking skin area (HLS). 66,7% of NF1 patients showed a reduced response to mechanical stimulation in cNF vs. HLS areas, while the response to thermal stimulation varied between patients. The cNF and HLS tested were then collected and characterized using immunofluorescence (IF) with a panel of neuronal markers. Our key observations include: (i) in all NF1 patients, nerve fibers ($PGP9.5^{+}$) displayed abnormally dense and defasciculated network in cNFs compared to HLS, primarily in the lower dermis, (ii) in all cNFs, tumor SCs remained closely aligned with axons, with no naked axons observed, indicating an association between these cells and (iii) approximately 37.5% and 56.3% of cNFs showed an enrichment of CGRP-positive fibers (A-delta and C fibers) and TH-positive fibers (C-LTMR fibers), respectively. Similar findings were observed in *Nf1*-KO mice developing mature cNFs, reinforcing the value of this model for investigating the role of innervation in cNF pathogenesis and its potential as a therapeutic target.

To further characterize the peripheral neurons innervating cNFs, we performed retrograde labeling in *Nf1*-KO mice by injecting either Cholera Toxin B (CTB) or AAVrg-GFP into cNF regions. Preliminary data revealed numerous labeled sensory neurons using both approaches. No traced sympathetic neurons were detected, confirming the sensory origin of aberrant cNF innervation. Additionally, the presence of labeled small- and medium-diameter neurons suggests that multiple classes of sensory neurons are involved, and their characterization is ongoing.

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Understanding and Targeting Epigenetic Vulnerabilities in Malignant Peripheral Nerve Sheath Tumors

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Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas with very poor prognosis. MPNSTs arise from benign plexiform neurofibromas (PNs) that occur at an increased frequency in patients with Neurofibromatosis Type 1 (NF1). Some of the most recurrent mutations in MPNSTs are loss of function alterations to members of the polycomb repressive complex 2 (PRC2) including mutations in either the SUZ12 (56%) or EED (33%) genes that are associated with worse prognosis. Here, we apply quantitative mass spectrometry to characterize the effect of PRC2 loss on the proteome, histone post-translational modifications (PTMs) and DNA modifications in human PNs, MPNSTs and MPNST cell lines. We complement this data with ChIP-seq and RNA-seq to select drugs for screening.

Methods: Flash-frozen human MPNST tumors and paired plexiform neurofibromas (PNs) from neurofibromatosis 1 (NF1) patients were processed with DNA Microprep Kit (Zymo) to extract proteins and DNA. For total proteome, proteins were trypsin digested with S-Trap (Protifi) and analyzed on ZenoTOF 7600 (SCIEX). Histone PTMs were analyzed on Exploris 240 (Thermo Scientific), after in-gel trypsin digestion and derivatization. For DNA modifications, we applied our recently published SWATH Analysis of Modified Nucleic Acids (SWAMNA) workflow. Analysis for MPNST cell lines was performed as for the tumors.

Results: Histone PTM analysis from MPNSTs (11 tumors) and PNs (10 fibromas) revealed that 5 MPNSTs were PRC2 loss with decrease in H3K27me3, and simultaneous increase in H3K27ac, H3K36me2 and H4K16ac. Proteome analysis revealed that 31 proteins were differentially enriched (adj. p-value $<$ 0.05) in PRC2 loss MPNST tumors compared to retained, and 69 proteins were differentially enriched in MPNSTs compared to PNs. Proteins most strongly up-regulated in PRC2 loss tumors included three closely related RNA-binding proteins. Doxycycline-induced re-expression of WT SUZ12 in PRC2 loss cell line shows downregulation of these proteins, suggesting that their expression is repressed by intact PRC2. These findings are supported by ChIP-seq data, that shows increased H3K27me3 upstream of the transcription start site at some of these genes after dox induction. Interestingly, DNA modification analysis by mass spectrometry showed that 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC) and 8-oxoguanine (8oxoG) are significantly upregulated in PRC2 loss cells, suggesting that these DNA modifications are also influenced by PRC2. Based on the proteomic and epigenetic data, we are currently performing drug screening in MPNST cell lines with live-cell imaging.

Conclusions: Our multi-omics approach potentially identifies new biomarkers and drug targets in SUZ12 and EED mutant MPNST.

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Examining the Role of Conserved Neuronal Activity Pathways in the Progression of Neurofibromatosis Type 1-Associated Plexiform Neurofibroma

Vladimir Amani, BSc, *University of Colorado Anschutz Medical Campus*

Neurofibromatosis type 1 (NF1) is an inherited genetic disorder that is typically diagnosed in childhood or early adulthood that often results in the formation of plexiform neurofibromas (PN). During formation of PN, a complex tumor microenvironment (TME) develops, with cellular recruitment of other cell types including non-neoplastic cells being critical for growth and progression. Previously, treatment options for PN were limited to surgical debulking, but recently, targeted drug therapy options based on MEK inhibition have emerged. These treatments have caveats, however, as the risk of long-term toxicities and drug resistances are unknown.

In our recently published study, the neurexin-1 (NRXN1) and neuroligin-1 (NLGN1) pathway was proposed to be potentially involved in the progression of PN. Both receptor and ligand were found to be aberrantly expressed in PN Schwann cells and fibroblast subpopulations according to single-nuclei RNA sequencing data. The use of predictive cell-to cell communication algorithms (CellChatDB) showed strong probability of NRXN1/NLGN1 interaction between PN Schwann cells and fibroblast subpopulations. Our recent studies employed the use of *in-vitro* functional validation to determine if this directional cross-talk mechanism is in fact a driver of disease progression in PN as well as allow for testing of small molecule inhibitors to disrupt NRXN1/NLGN1 signaling, specifically the NLGN1 sheddase, ADAM10, inhibition using GI254023X and aderbasib.

In order to mimic the TME of PN, primary fibroblasts isolated from an NF1 patient's PN were cultured with validated PN cells resulting in a significant increase of growth of PN cells in co-culture conditions versus alone. Additionally, the use of aderbasib on PN cells alone resulted in no dose response; however, when co-cultured with patient-derived fibroblasts, PN cells showed dose response to ADAM10 inhibition. And finally, when PN cells are cultured with patient-derived fibroblast conditioned media, we see a significant increase in PN cell growth suggested that cellular cross-talk mechanisms contribute to the progression of PN.

Our recent studies investigate the progression of PN and how it may be linked to a neuronal activity pathway involving NRXN1 and NLGN1, a pathway we reported as a potential driver for PN progression. Our novel *in-vitro* co-culture model is a strong validation tool to confirm the importance of the NLGN1/NRXN1 signaling pathway and provide alternative targeted therapy options for PN. Future use of multispectral fluorescence immunohistochemistry on primary patient samples will be used to show direct ligand-receptor interactions between PN putative cells of origin, Schwann cells, and surrounding stromal fibroblasts.

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Neurofibroma Development in Neurofibromatosis Type 1: Insights from Cellular Origin and Schwann Cell Lineage Development

Ling-Ling Ge, MD, *Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

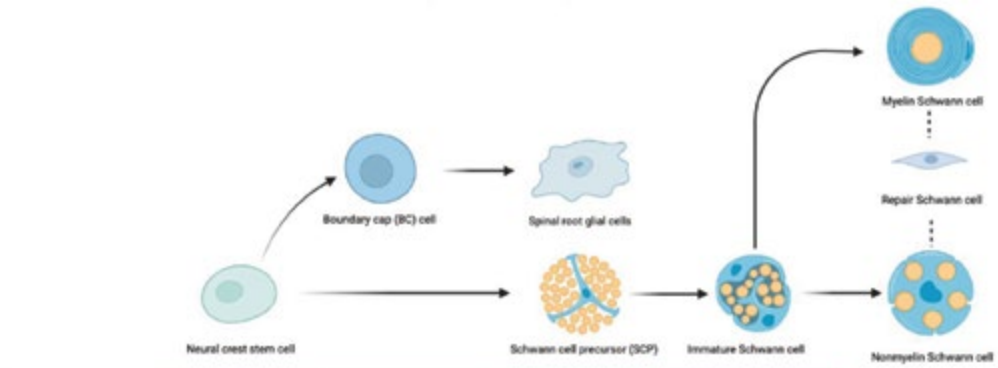
Purpose: Neurofibromatosis type 1 (NF1), a genetic tumor predisposition syndrome that affects about 1 in 3000 newborns, is caused by mutations in the NF1 gene and subsequent inactivation of its encoded neurofibromin. Neurofibromin is a tumor suppressor protein involved in the downregulation of Ras signaling. Despite a diverse clinical spectrum, one of several hallmarks of NF1 is a peripheral nerve sheath tumor (PNST), which comprises mixed nervous and fibrous components. The distinct spatiotemporal characteristics of plexiform and cutaneous neurofibromas have prompted hypotheses about the origin and developmental features of these tumors, involving various cellular transition processes.

Methods: We retrieved published literature from PubMed, EMBASE, and Web of Science up to 21 June 2022 and searched references cited in the selected studies to identify other relevant papers. Original articles reporting the pathogenesis of PNSTs during development were included in this review. We highlighted the Schwann cell (SC) lineage shift to better present the evolution of its corresponding cellular origin hypothesis and its important effects on the progression and malignant transformation of neurofibromas.

Results: In this review, we summarized the vast array of evidence obtained on the full range of neurofibroma development based on cellular and molecular pathogenesis. By integrating findings relating to tumor formation, growth, and malignancy, we revealed the role of SC lineage shift as well as the combined impact of additional determinants in the natural history of PNSTs.

Conclusion: To conclude, the wealth of work exploring the pathogenesis of neurofibromas in NF1 individuals presented in this review has brought in-depth insights into the development spectrum of these tumors. Nonetheless, significant knowledge gaps persist regarding the nuanced mechanisms of neurofibroma formation. Advancing our understanding of the cellular origins, the influence of the tumor microenvironment, and the processes leading to malignant progression will be crucial for a more exhaustive elucidation of neurofibroma pathogenesis.

Figure 1. The developmental stage of SC lineage and corresponding characteristics of different cell types.



Embryo Day	E8.5–9	E10.5–11	E12–13	E13–15
Association features	Association with ECM	Association with axons	Association with axons	1:1 association with axons
Survival Signals	Transcription factor FoxD3	GABA signaling	Axon secreted NRG-1	Autocrine survival circuits
Molecular Markers	p75, SOX10, HNK1, Slug, Snail, Twist, AP-2α	p75, P0, KROX20/EGR2, PRS556, Nestin, PSA-NCAM	p75, SOX10, BFABP, PLP, DHH, HOXB7, P0, Cadherin 19, GAP43	S100, GFAP
Differentiation ability	Neurons, glia cells, bone, cartilage, melanocytes, fibroblasts, and smooth muscle cells	Schwann cells, myofibroblasts, astrocytes, and neurons	Endoneurial fibroblasts, melanocytes, and parasympathetic neurons	Myelin Schwann cells, and nonmyelin Schwann cells

Figure 1 legend. Neural crest stem cells (NCSCs) can differentiate into multipotent boundary cap (BC) cells and SC precursors (SCPs). The SCPs further develop into immature SCs, which then differentiate into myelinating/non-myelinating SCs according to the associated axons. These mature types can de-differentiate upon specific mutation or injury into repair SCs. The corresponding embryo-genesis time of each cell type in mice and other features, including their association characteristics, survival signals, molecular markers, and differentiation capacity, are listed relative to the cells.

Figure 2. SC lineage shift and contributing factors in neurofibroma progression.

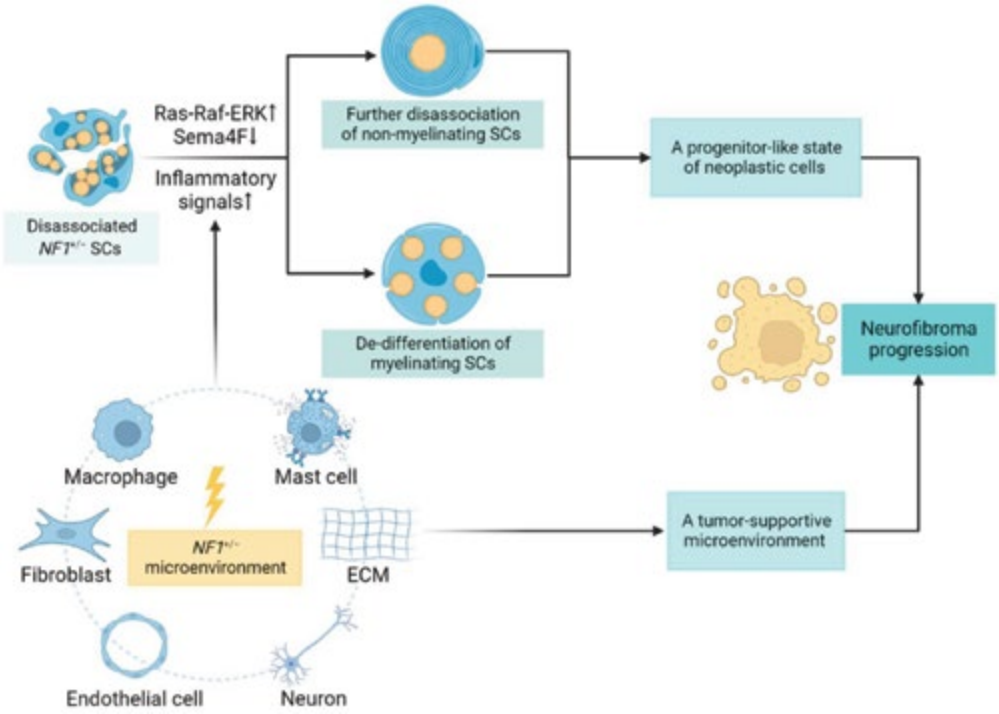


Figure 2 legend. The neoplastic SCs can rapidly de-differentiate to a progenitor-like state, disrupting SC–axonal interactions with tumor development. The underlying mechanism involves Ras-dependent downregulation of an SC surface protein, semaphorin 4F (Sema4F), together with elevated inflammatory signals, especially upon injury. Other environmental factors, including cellular and non-cellular components, further create a tumor-promoting microenvironment. The proliferative state of neoplastic cells and supportive tumor microenvironment combined to promote neurofibroma progression. ↑: upregulation of signaling pathways; ↓: downregulation in ex-pression.

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Reduced PTPRS Expression Promotes Epithelial-Mesenchymal Transition of Schwann Cells in NF1-Related Plexiform Neurofibromas

Zhichao Wang, *Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

Plexiform neurofibromas (PNFs) are a prevalent and severe phenotype associated with NF1, characterized by a high teratogenic rate and potential for malignant transformation. The growth and recurrence of PNFs are attributed to aberrant proliferation and migration of Nf1-deffcient Schwann cells. Protein tyrosine phosphatase receptor S (PTPRS) is believed to modulate cell migration and invasion by inhibiting the EMT process in NF1-derived malignant peripheral nerve sheath tumors. Nevertheless, the specific role of PTPRS in NF1-derived PNFs remains to be elucidated. The study utilized the GEO database and tissue microarray to illustrate a decrease in PTPRS expression in PNF tissues, linked to tumor recurrence. Furthermore, the down- and overexpression of PTPRS in Nf1-deffcient Schwann cell lines resulted in the changes of cell migration and EMT processes. Additionally, RTK assay and WB showed that PTPRS knockdown can promote EGFR expression and phosphorylation. The restoration of EMT processes disrupted by alterations in PTPRS levels in Schwann cells can be achieved through EGFR knockdown and EGFR inhibitor. Moreover, high EGFR expression has been significantly correlated with poor prognosis. These findings underscore the potential role of PTPRS as a tumor suppressor in the recurrence of PNF via the regulation of EGFR-mediated EMT processes, suggesting potential targets for future clinical interventions.

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Investigating the Progression of Disctinct Types of NF1-Associated MPNSTs Using an iPSC-Derived Neural Crest-Based Model System

Judit Farrés-Casas, MS, *Germans Trias i Pujol Research Institute (IGTP)*

Malignant peripheral nerve sheath tumors (MPNSTs) are a heterogeneous group of aggressive soft tissue sarcomas arising in both, the general population and, with a special high prevalence, neurofibromatosis type 1 (NF1) individuals. MPNST initiation is highly marked by the combined inactivation of tumor suppressor genes (TSGs) like *NF1, CDKN2A,* PRC2 (*SUZ12* or *EED*) and, at a lesser frequency, *TP53.* In our group, we performed a comprehensive genomic characterization of NF1-associated MPNSTs and identified different groups bearing a distinct TSG inactivation signature. To better understand the molecular mechanisms driving the initiation of the different MPNSTs, we used an iPSC-derived neural crest (NC)-based *NF1*(-/-) model to reproduce the different TSG inactivation combinations idenitified.

We used CRISPR/Cas9 technolgy to knock out key TSG in *NF1*(-/-) iPSC and derived NC cells. TSG inactivation was assessed by genetic analysis and confirmed by western blot, and selected clones underwent physiological and functional characterization. Cell identity markers were analyzed and proliferation capacity and differentiation potential was tested. These cell lines were amenable to 3D culturing as spheroids. To further investigate their tumorigenic capacity, we will engraft these spheroids into nude mice and potential tumors formed will be histologically characterized by an expert pathologist.

We will use these new NC-based cell lines to characterize the impact of different TSG losses on tumorigeneicity and cell identity in light of the existing MPNST heterogeneity. At the same time, these new cell lines may serve as a valuable preclinical resource for testing novel therapeutic strategies and comparing treatment responses in the genotypically different backgrounds.

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Metabolic Phenotyping of the Plexiform Neurofibroma Model *Periostin-Cre+;NF1$^{flox/flox}$* Demonstrates Lower Weights and Increased Energy Expenditure Compared to Non-NF1 Controls

Kathryn M. Lemberg, MD, PhD, *Johns Hopkins University School of Medicine*

Purpose: Children and adults with neurofibromatosis type I (NF1) who develop nervous system tumors such as gliomas and plexiform neurofibromas (pNF), tend towards lower body weights than general population norms[1-4], even when consuming calorie dense diets[5]. The reason for this observation is not well understood, and there are limited published studies of the interactions of energy metabolism and tumor growth in animal models of NF1[6-9]. The aim of this work was to compare the metabolic phenotype of mice with loss of *NF1* that develop pNF to litter-matched control mice.

Methods: We compared male *Periostin-Cre+;NF$^{flox/flox}$* mice[10, 11] with litter-matched *Cre-* mice (n=9-11/arm) beginning at 1 month of life. Mice were fed standard chow. We collected weekly weights, monthly QNMR body compositions, and performed indirect calorimetry at 3 months, which is reported onset of peripheral nerve hyperplasia in this model. VO_2 and VCO_2 were used to calculate respiratory exchange ratio (RER) and energy expenditure (EE). Glucose and insulin tolerance testing was also performed.

Results: *Periostin-Cre+;NF1$^{flox/flox}$* (Cre+) mice have lower body weights than *Periostin-Cre-;NF1$^{flox/flox}$* (Cre-) controls (21.36 ± 1.36 versus 22.91 ± 1.53 g; $p = 0.03$) at 3 months of life. Fat mass was significantly lower in Cre+ mice at 4 months (2.89 ± 0.88 versus 4.22 ± 1.19 g; $p=0.01$). RER was lower in Cre+ mice (0.92 ± 0.02 versus 0.96 ± 0.03; $p<0.01$) while energy expenditure per kg of body weight was ~20% higher in Cre+ mice (19.29 ± 1.32 versus 15.87 ± 1.53 kcal/kg; $p<0.0001$). Food intake was slightly higher in Cre+ mice (11.39 kcal vs 10.32 kcal, $p = 0.024$), while no differences in activity were observed between the groups. Cre+ mice had lower peak glucose than controls (174.44 ± 17.68 versus 207.55 ± 36.62 mg/dl, $p = 0.02$).

Conclusions: Similar to what has been observed in non-tumor forming models and in people with NF1, lower weights, fat mass, and higher EE were observed in the Cre+ mice compared to Cre- controls. Lower RER and lower peak blood glucose suggest differences in carbohydrate and fat metabolism in NF1 versus control animals. The study remains ongoing to collect data through at least 6 months of life. Future directions include evaluating hormone differences between the groups, assessing the metabolic response of Cre+ mice to high fat diet, and analyzing metabolomic profiles of tissues, including serum, peripheral nerve and liver, collected at study termination.

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Discovery of Resistance to Valosin-Containing Protein Inhibitor in Plexiform Neurofibromas

Lalitha Gopalan, PhD, *Division of Experimental Hematology and Cancer Biology, Cancer & Blood Diseases Institute Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

Neurofibromatosis type 1 (NF1) patients are prone to developing plexiform neurofibromas (PNFs). Previous work showed that the NF1 protein neurofibromin can bind p97/valosin-containing protein (VCP) in rat brain (Wang et al, 2011). We found VCP protein increased expression in both mouse and human PNFs versus wild type mouse dorsal root ganglia or normal human nerves. Co-immunoprecipitation confirmed that VCP binds to NF1 in purified mouse Schwann cells and tumor lysates. In vivo treatment with CB-5083, a p97/VCP inhibitor that causes poly-ubiquitinated protein accumulation and proteotoxic stress, inhibited cell proliferation, increased apoptosis, and reduced PNF volume in the *DhhCre;Nf1fl/fl* mouse model. However, some mice were resistant to CB-5083 mediated tumor shrinkage. To investigate mechanisms that underly this resistance, we developed a CB-5083-resistant cell line by exposing an NF1 knockout immortalized human Schwann cell line to stepwise increasing concentrations of CB-5083 (0.1 μM to 6.5 μM). RNA sequencing on the resistant cells identified 805 differentially expressed genes (vs non-resistant, 3-fold change, $p<0.05$), with TGF-beta signaling predicted as the top pathway. We confirmed the overexpression of TGFβ1, TGFβ2, TGFβR1, TGFβR2, and SMAD7 genes by qRT-PCR, and Western blotting confirmed the overexpression of TGFβR1 and TGFβR2 proteins. Pre-clinical testing combining CB-5083 with a TGFβ inhibitor will be carried out.

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Obesogenic Diet Exposure Modulates Risk of Nf1-OPG Formation Induced by Specific Germline Mutation

Nicole M. Brossier, MD, PhD, *Washington University in St. Louis*

Children with the cancer predisposition syndrome Neurofibromatosis Type 1 (NF1) have ~15-20% risk of developing low-grade gliomas of the optic pathway (NF1-OPG). Recent studies suggest that nonrandom factors, such as the specific germline *NF1* mutation and environmental exposures, may increase this risk. One potential factor is maternal obesity, as our laboratory has recently demonstrated that maternal obesogenic diet increased Nf1-OPG penetrance and accelerated tumor formation in murine models, consistent with data from the general population showing that children of obese mothers develop several types of tumors at increased rates. Previous research has also shown that an obesogenic diet triggers low-grade inflammation, which can promote tumor formation in other model systems. Based on these observations, we hypothesized that maternal obesogenic exposure would modulate the risk of Nf1-OPG formation through increased inflammatory signaling.

Methods: We exposed both dams and offspring to an obesogenic high-fat, high-sucrose diet (Ob) to mimic typical U.S. dietary conditions, with control diet (CD)-exposed animals serving as a comparison. At 6 weeks, we analyzed serum and optic nerves from both groups.

Results: Immunohistochemistry revealed increased microglial and T-cell infiltration in the optic nerves of Ob-exposed NF1-OPG mice, suggesting a potential increase in inflammatory stimuli. However, there were no significant differences in circulating inflammatory cytokine levels between the two groups, as measured by cytokine array and confirmatory ELISA. Similarly, quantitative PCR analysis of optic nerves did not show upregulation of inflammatory cytokines in the tumor microenvironment. RNA sequencing of optic nerves from Ob- and CD-exposed mice also revealed no evidence of increased inflammatory signaling pathways. Instead, Ob-exposed nerves exhibited upregulation of pathways related to neuronal signaling and brain development.

Conclusion: Our findings suggest that maternal obesogenic exposure does not increase the risk of NF1-OPG development through heightened inflammatory cytokine pathways. However, the observed alterations in neuronal signaling pathways raise the possibility that crosstalk between these pathways and tumor cells may contribute to tumor formation.

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Modeling NF1 Tumor Microenvironment Using 3D Invitro Co-Culture Model

Ahmad Zunnu Rain, *Biomedical Engineering, Wayne State University, Detroit, MI*

Purpose: PNs are complex tumors that include a driving Schwann cell (SC) component and support cells in the perineural environment[1]. This study focuses on understanding signal transduction pathways between neurons, SC, support cells (fibroblasts and endothelial) and immune cells that drive this aberrant proliferation and invasiveness in SCs and lead to neurofibromas. The goal of this project is to develop an invitro model of NF1 neurofibromas that can be used to understand these interactions and future drug testing. We have developed a nanofiber-based system that provides a tunable "neurite mimic" that allows us to investigate cell behavior in the presence of a neural component.

Methods and Results: *Biomimetic neurons:* Neurite mimics have been developed to provide physical (topographical and mechanical), chemical, electrical and adhesive cues to cells within the neurofibroma that are typically provided by neurons. HA nanofibrous scaffold incorporated with multi-walled carbon nanotubes (CNT)[2] have been developed to serve as the "neurite mimic". Microsphere fabrication was accomplished by making PLGA (75:25) microspheres using a water/oil/water emulsion. A gelatin layer was added to the microspheres using chemical conjugation with EDC chemistry. Dual-layered PLGA-gelatin microspheres were electrospun onto a hyaluronic acid-carbon nanotube (HA-CNT) nanofiber mat. Cytokine release is controlled by electrical stimulation of the scaffold allowing biomimetic electrical stimulation and cytokine delivery. Scaffolds were stimulated using a custom-built cell stimulation chamber. Growth factor release was evaluated using ELISA following stimulation at 50, 75, 100, and 125 mV/mm for 5 minutes every 24 hours and release was found to be linear and fully controllable based on stimulation parameters **(Figure 1)**.

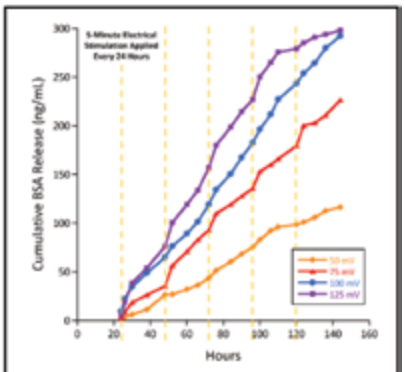

Figure 1: Drug release curves following 5 mins stimulation every 24 hours.

Cell behavior: Several cell types that are present in NF1 neurofibromas have been studied within this system to assess the effect of neuron-like stimulation and cytokine release on cell behavior. NF-SC (NF-/-) showed increased elongation and proliferation when cultured on HA-CNT scaffolds compared with WT-SC (NF+/+). Fibroblasts stimulated with FGF showed increased proliferation compared to unstimulated controls. Current work involves testing the effect of stimulation and cytokine release on immune cells, including NF1 macrophages. Following individual cell experiments, we will co-culture cells to develop an organotypic model. Initial co-culture studies with NF-SC and fibroblasts show that co-culture further increased proliferation of NF cells.

Conclusions: Neurons are a key part of NF1 neurofibromas but it is difficult to evaluate the role of neurons on tumor formation. We have developed a system to study the interaction of cells within the NF1 tumor microenvironment including neurons. Cytokine release and electrical stimulation effect NF-SC and fibroblasts behavior. Immune cells are currently being evaluated. We are also developing NF-iPSC cells to further improve this model and will evaluate genetic targets using RNAseq.

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Motor Pattern Changes as Indicators for Neuronal Dysfunction in a Model of Neurofibromatosis Type 1

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Purpose: To determine how Nf1 deficiency impacts neuronal function and behavior.

Methods: For spontaneous grooming assays flies are place into a grooming chamber and recorded at allocated time periods, followed by frame-by-frame analysis of grooming for determination of excessive grooming. For dusting flies are placed into a chamber, approximately 1mg of dust is added to the chamber and then the flies are "shaken" to be fully coated and then allotted to groom for set periods of time before being dissected and imaged. Images are then used for calculation of how much dust is left covered on each corresponding body part following the allocated grooming time.

Results: Loss of Neurofibromin (Nf1) function may alter neuronal activity levels, resulting in changes in behavioral phenotypes. We found that loss of Nf1 in *Drosophila melanogaster* alters spontaneous motor behaviors, one being an increase in grooming frequency. This phenotype can be seen when Nf1 is knocked down non-specifically in neurons, indicating a need for Nf1 in maintaining neuronal function. This has been further investigated via an induced grooming model. When flies are covered in dust, they will engage in vigorous grooming to remove the dust. Dust removal allows for the flies to better receive information from their environment. We tested whether the deficiency of Nf1 affects the pattern of sensory-evoked behaviors. It was found that the prioritization/sequence of grooming was scrambled in mutant *nf1* flies. This was repeated with knockdown of Nf1 only in neurons, also appears to have a scrambled grooming pattern. This suggests that loss of Nf1 in neurons has indications for neuronal dysfunction and/or hyperactivity. However, isolation within specific subsets of neurons has not yielded results of a behavioral phenotype or appearance of neuronal dysfunction; leading us to believe there may be compensatory mechanisms which are used to overcome loss if Nf1 in small populations of neurons. Since we found a behavioral phenotype: we chose to pick known subsets of neurons that when activated cause specific grooming behaviors. Using these we can rescue Nf1 in specified neurons to determine if Nf1 is sufficient to restore grooming to specified body parts, or if rather there are compensatory mechanisms that will still result in neuronal dysfunction.

Conclusion: We found that loss of Nf1 in neurons results in an increased grooming behavior in flies, this suggests that Nf1 is needed to maintain neuronal function.

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The Immunopeptidome of Malignant Peripheral Nerve Sheath Tumors Reveal Novel Non-Reference Peptides and Cancer Testes Antigens with Immunogenic Potential

Kyle Richards, *Masonic Cancer Center, Department of Pediatrics, University of Minnesota*

Purpose: This is a novel study of immunopeptidomics and customized bioinformatics to characterize antigen presentation of malignant peripheral nerve sheath tumors (MPNSTs), which may be useful for developing treatment or prophylactic vaccines.

Methods: Two Human MPNST cell lines and one patient derived xenograft (PDXs) were used to isolate antigens via immunoprecipitation of major histocompatibility (MHC) class I. Peptide epitopes were purified from MHC proteins and analysis of epitopes was performed using Orbitrap Eclipse LC-FAIMS-MS system. MS/MS spectra were matched to the human Uniprot reference proteome plus contaminants and novel neoantigen peptides. Novel peptides were predicted using deep RNA-Seq and Ribo-seq analyses and peptide spectra matches (PSMs) were generated, quantified and validated using FragPipe and Pepquery in the Galaxy for proteomics (Galaxy-P) platform. NetMHCpan was used to predict and rank the antigens bound to MHC I alleles present in each sample.

Results: Mass spectrometry and subsequent Fragpipe analysis was able to identify between 5,000 and 8,000 peptides per sample. More than 80% of peptides were between 8 and 14 amino acids in length, showing consensus with published amino acid lengths of MHC class I epitopes. Peptides also show consensus with published peptide binding motifs of the MHC alleles present in each sample. Technical replicates show approximately 50% overlap, suggesting a single shot can capture a large diversity of antigenic peptides.

From this initial list of peptides; 20-50 peptides per sample could be validated as deriving from non-reference ORFs. Peptides could be characterized by their aberration, such as deriving from 3' UTRs (15%), 5' UTRs (11%), frame shifts (47%), splicing junctions (6%), introns (13%), intergenic regions (4%), and lncRNA (4%). Two of these peptides, both deriving from introns, were present in all cell lines tested. Along with peptides from non-reference transcripts, peptides were also identified coming from cancer testes antigens, including peptides from cancer-enriched proteins MAGEA3, MAGEB2, MAGEC1, MAGEC2, and POTE. MAGEA3 antigen specifically has gone through a phase III vaccine clinical trial.

Conclusion: Continuing work focuses on optimization of mass spectrometry and analysis to maximize the antigenic peptides identified from each sample. This includes quantification of peptides to prioritize peptides most likely to interact with immune cells. Both the non-reference derived peptides and the tumor associated antigens identified in this study will be ranked based on their potential for immunogenicity and validated *in vitro.* These validated peptides can be used in further analysis of immunotherapy and prophylactic vaccination for patients with NF1 syndrome and MPNST.

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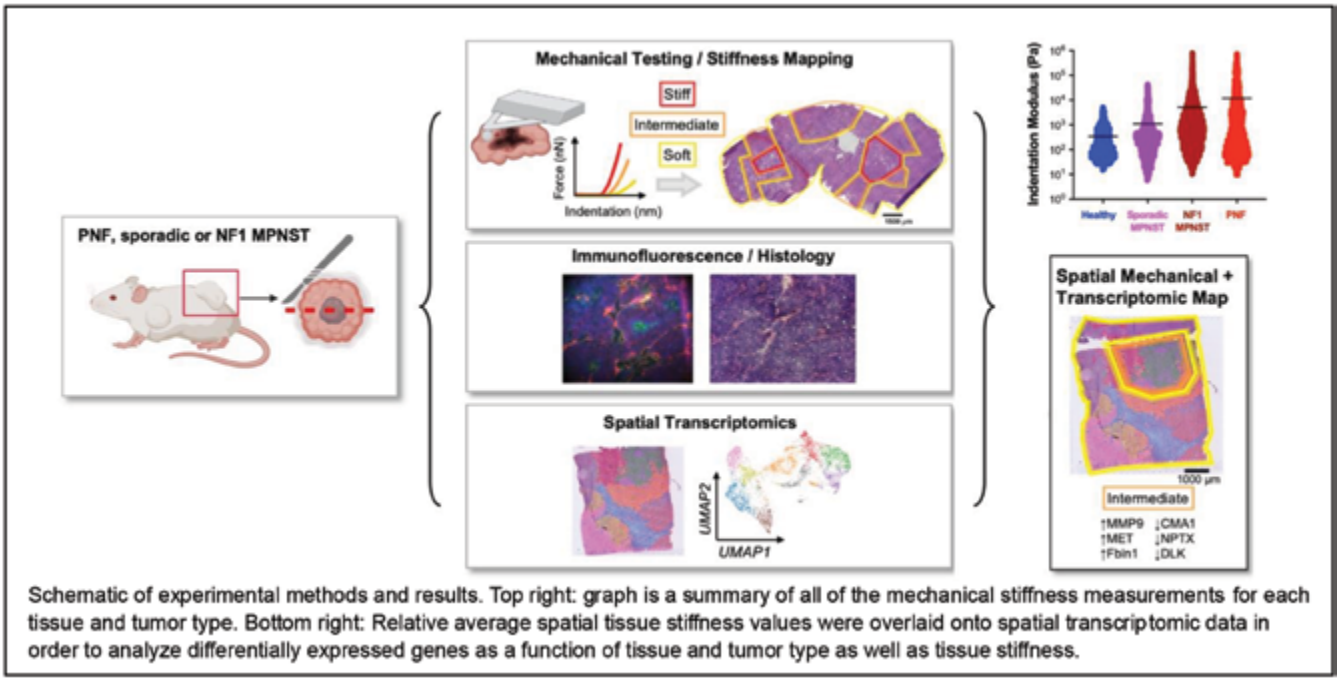
Spatial Mechanical and Transcriptomic Profiling Reveal NF1-Dependent Stiffening of Plexiform Neurofibromas and Malignant Peripheral Nerve Sheath Tumors

Micah Rambo, PhD, *Rensselaer Polytechnic Institute, Troy, NY*

Purpose: The purpose of this study is to identify and investigate differentially expressed genes associated with mechanically altered regions during malignant progression of NF1-associated tumors.

Methods: Plexiform neurofibromas (PNFs) were generated spontaneously in the dorsal root ganglion and trigeminal nerves of *Nf1*$^{Fl/-}$; *Krox20*$^{Cre/+}$ mice.[1] MPNSTs were generated via CRISPR-Cas9 injection in the sciatic nerves of NF1 and wildtype mice.[2] Tumor progression was monitored, and mice were euthanized at humane or tumor endpoints. All mouse work was approved by the RPI IACUC and performed under the supervision of a resident veterinarian. Tumors and control tissues were excised and cross-sectioned for mechanical testing, staining, and spatial transcriptomics. Nano-indentation—force versus indentation depth—curves were collected over the cross-section with an atomic force microscope and the relative resulting stiffness values—soft, intermediate, and stiff—were mapped to histological sections. Spatial transcriptomics was optimized and performed following 10x Genomics and Visium protocols for fresh frozen sections.

Results:



Schematic of experimental methods and results. Top right: graph is a summary of all of the mechanical stiffness measurements for each tissue and tumor type. Bottom right: Relative average spatial tissue stiffness values were overlaid onto spatial transcriptomic data in order to analyze differentially expressed genes as a function of tissue and tumor type as well as tissue stiffness.

The clear effect of the NF1 mutation (NF1 MPNSTs and PNFs) was to drive the stiffness of the tumor tissue to higher average values (right, top). The PNF stiffness distribution, in particular, is very interesting as it reflects an intermediate distribution with features similar to both the healthy peripheral nerve distribution and the NF1 MPNST distribution. Combining our stiffness maps with histological and spatial transcriptomic mapping (middle), we have identified tissue structure, cell types, extracellular matrix components, and differentially expressed genes that contribute to the altered mechanical micro-environment. For example, intermediate and stiff regions are associated with increased expression of proteins that interact with the extracellular matrix like matrix remodeling proteins.

Conclusion: NF1 tumors present a pathologically heterogeneous and stiffened mechanical microenvironment that we believe likely plays a role in malignant progression.

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Investigating the Role of Mek-Inhibition on Fatty Acid Metabolism and Weight Gain in NF1

Miriam Bornhorst, MD, *Ann & Robert Lurie Children's Hospital, Chicago, IL*

Purpose: MEK-inhibitors (MEKi), which decrease activity of the MAPK pathway, are being used for the treatment of Neurofibromatosis Type 1 (NF1)-associated tumors with increased frequency. Although variable amongst patients, weight gain has been reported as a side effect of MEKi treatment in all pediatric clinical trials incorporating these medications so far, with some patients requiring a temporary medication hold due to this. Yet the underlying metabolic mechanism for this is unknown.

Methods: In this study, we reviewed weight gain patterns of mice receiving treatment with MEKi. We then performed a global metabolomics analysis using liquid chromatography-mass spectometry (LC-MS; Metabolon) on plasma from *Nf1*$^{flox/flox}$*; Postn*-cre (Periostin-cre) mice and a small cohort (n=10 for each group) of patients with NF1 either receiving or not receiving therapy with a MEKi. Initial analysis was focused on the lipid pathway.

Results: *Nf1*$^{flox/flox}$*; Postn*-cre+ (NF1-deficient) and *Nf1*$^{flox/flox}$*; Postn*-cre- mice (NF-wt) both had weight gain when treated with MEKi. Global metabolomic analysis of plasma samples from *Nf1*$^{flox/flox}$*; Postn*-cre+ mice and from NF1 patients receiving treatment with a MEKi showed enrichment of pathways controlling long chain fatty acid metabolism and phospholipid signaling/synthesis.

Conclusion: This preliminary metabolomic data provides evidence that MEKi leads to a decrease in lipid oxidation, which could account for increased weight gain during treatment. Additional studies will be done to explore biomarkers that may identify patients who are at increased risk for weight gain on treatment, and adjuvant therapies that could potentially be used to mitigate while on MEKi therapy. Additional studies will also explore differences in energy expenditure (mice and patients) and non-plasma metabolism (i.e. liver, tumor) in the mouse models, to further explore how NF1-deficiency and inhibition of the Ras/MAPK pathway alters metabolism.

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High Mobility Group A2 (HMGA2) Drives Plexiform Neurofibroma Growth and Malignant Transformation

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Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are the leading cause of mortality in neurofibromatosis type 1 (NF1) and often arise from pre-existing plexiform (PN) and atypical neurofibroma (ANF). While genetic disruption *CDKN2A* and PRC2 complex members are frequently observed in ANF and MPNST, higher order transcriptional mechanisms governing the trajectories of these precursor lesions remain largely uncharted. High Mobility Group A2 (HMGA2) is a non-histone chromatin binding protein that modulates stem-like gene expression programs by altering chromatin structure. While silenced postnatally, we found that HMGA2 is aberrantly re-expressed in MPNST, leading us to hypothesize that HMGA2 drives MPNST progression by reactivating embryonic growth programs in Schwann cell precursors.

Methods: We utilized genetically engineered mouse models with conditional *Nf1, Ink4a/Arf,* and *Hmga2* alterations driven by *PostnCre,* as well as CRISPR-Cas9 editing of *Hmga2* in primary Schwann cell precursors (SCPs) and human MPNST cell lines. RNA sequencing identified *Let7*-HMGA2 axis disruption and key HMGA2-dependent target genes which we validated by qRT-PCR, western blot, and immunohistochemical staining (IHC). Orthotopic xenograft studies assessed *in vivo* tumorigenesis.

Results: RNAseq revealed a 7.6 $\log_2$ fold increase in *Hmga2* expression in MPNST versus precursor lesions (padj <0.001), confirmed by western blot and IHC across mouse and human neurofibromas and MPNST. This was accompanied by downregulation of *Let7* miRNAs, which physiologically suppress HMGA2. While conditional knockout (KO) of *Hmga2* in $Nf1^{f/f}$;$Hmga2^{f/f}$;$PostnCre^{+}$ mice did not impact PN genesis, ectopic HMGA2 overexpression in $Nf1^{flox/+}$;$Hmga2^{\Delta/+}$;$PostnCre^{+}$ mice resulted in the development of massive facial PN and impaired survival ($p < 0.0001$, log-rank test). CRISPR-Cas9 knockout of *Hmga2* in primary *Nf1-Ink4a/Arf* $^{-/-}$ SCPs delayed MPNST outgrowth (n=2 clones, 8 mice/group) and completely abolished tumor growth in human JH-2-002 MPNST cells (n=3 clones, 8 mice/group) in orthotopic xenografts. Contrastingly, *Hmga2* KO in *Nf1/Ink4a/Arf* $^{+/-}$ SCPs did not alter MPNST-free survival, suggesting a role for *Ink4a/Arf* gene dose in modulating the impact of HMGA2 on malignant growth. Compensatory upregulation of Hox-family genes in RNAseq analysis of KO-tumors suggests an adaptive resistance mechanism that merits further exploration.

Conclusions: While dispensable for PN initiation, ectopic overexpression of HMGA2 in SCPs drives PN progression and promotes malignant outgrowth *in vivo* in a subset of MPNST cell lines and primary SCPs – dependent on *Ink4a/Arf* gene dose. Future studies will characterize compensatory mechanisms that enable some tumor cells to bypass HMGA2 disruption and identify therapeutic vulnerabilities in HMGA2-addicted MPNSTs to inform clinical translation.

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A Suite of NF1 Reconstitution Systems Reveal GAP Activity Achieves Transient but Not Long-Term Efficacy in MPNST Cells

Sarah Mohabeer, *Helen Diller Family Comprehensive Cancer Center, University of California San Francisco*

Purpose: Neurofibromatosis type-1 (NF-1) is a monogenic syndrome caused by germline mutation of the *NF1* gene, making the possibility of gene therapy conceptually attractive. However, *NF1* reconstitution efforts have been hampered by effective expression systems and both the optimal cargo and cellular context for *NF1* gene therapy remain unclear. Here, we report the development of multiple *NF1* expression systems and their effects in malignant peripheral nerve sheath tumor (MPNST) models.

Methods: We optimized transient and stable expression systems for three *NF1* constructs: full length NF1 ($NF1^{full}$), the catalytically inactive arginine finger mutant ($NF1^{R1276P}$), and the minimum GAP-related domain ($NF1^{GRD\text{-}CaaX}$) membrane anchored via the HRAS prenylation signal (CaaX). Expression systems were validated in HEK293T cells followed by functional and biochemical analysis in two *NF1* null MPNST cell lines, ST88-14 and JH-2-002, to investigate the effects of NF1 reconstitution.

Results: ALFA tagged transient $NF1^{FL}$ and $NF1^{GRD\text{-}CaaX}$ fully blocked downstream phospho-MEK (pMEK) and phospho-ERK (pERK) activation in HEK293T but not MPSNT cells. Stable lentiviral doxycycline inducible expression revealed the $NF1^{GRD\text{-}CaaX}$ was superior to $NF1^{FL}$ in blocking both pERK activation and decreased cell growth in MPSNT cells. In contrast, the $NF1^{R1276P}$ showed minimal effect on both signaling and cell growth. However, continued $NF1^{FL}$ or $NF1^{GRD\text{-}CaaX}$ expression led to MPNST cells silencing expression through genetic mutation or deletion of these constructs while $NF1^{R1276P}$ expression was maintained, underscoring the selective pressure of long term catalytically competent *NF1* expression. This occurs at least in part due to the stability of the NF1 protein following doxycycline induction in MPNST cells. To overcome this limitation, we developed and validated an *E. coli* dihydrofolate reductase degron system to translationally regulate NF1 protein expression, providing a more dynamic expression window. Finally, we also designed a dual NF1 and tamoxifen inducible CRAF system to circumvent NF1 silencing through downstream RAF activation.

Conclusion: Transient and short-term stable reconstitution of catalytically competent NF1 blocks RAS signaling and cell growth in MPNST cells, but long-term reconstitution is overcome through *NF1* mutation. Moreover, our data suggests the dominant function of NF1 is through its Ras GAP catalytic activity, as the $NF1^{R1276P}$ did not affect biochemical or functional outputs and was not subject to the same selective pressure as $NF1^{FL}$ or $NF1^{GRD}$, motivating the development of alternate systems for NF1 reconstitution.

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Morphometric Analysis of Microglia in the Retina and Optic Nerve

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Purpose: Microglia promote many diseases including some clinical manifestations of Neurofibromatosis type 1 (NF1). Particularly visual impairments seen in NF1 patients can be result from damage of retinal ganglion cells (RGC) by activated microglia. This study performs morphometric analysis of microglia in the retina and optic nerve of mice with a mutation in the Neurofibromin 1 (Nf1) gene to investigate reactive microglia in NF1.

Methods: Retinal whole-mounts and optic nerves from Nf1 fl/fl mice were stained with ionized calcium binding adaptor molecule 1 (Iba1), a pan-myeloid marker, and 4′,6-diamidino-2-phenylindole (DAPI) for cell nuclei. To increase the image resolution we incubated tissues in the clearing solution and put them on the concave microscope slides mounting with clearing media. Then confocal 3D images were acquired using the z-stack function of Olympus Fluoview FV3000 microscope (160 μm depth, 2 μm steps, $\times$20 magnification). IMARIS software (Version 10.0.1) was used to reconstruct the microglial surface and Filament Tracer function was applied to reconstruct the processes and spines of microglia cells. Vantage function was utilized for quantitative analysis and classification of the microglia based on cell area and processes length.

Results: This study successfully characterized microglia morphology and cell states (homeostatic, reactive, hyper-ramified, rod-shaped) in the retina and optic nerve of Nf1 fl/fl mice. The pipeline included tissue clearing, high-resolution image acquisition, and artificial intelligence-powered image analysis. This allowed for detailed description of microglia parameters, precise cell classification, and extraction of numerous cell features (cell area, soma size, process number, process length, Sholl analysis).

Conclusions: This pipeline provides a framework for high-throughput image analysis in the retina and optic nerve, particularly relevant for NF1 research. This protocol may be adapted for other image analysis applications.

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PRC2 Loss Drives Genome Instability in Malignant Peripheral Nerve Sheath Tumors

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Purpose: Malignant Peripheral Nerve Sheath Tumors (MPNSTs) have been characterized with recurrent chromosomal imbalances, including gains, losses, and rearrangements. In most cases a component of the Polycomb Repressive Complex 2 (PRC2) is lost, which results in a global loss of the repressive mark H3K27me3. MPNSTs that have loss of the PRC2 also present with a higher number of chromosomal abnormalities, suggesting a link between genome instability and the PRC2. Topoisomerase IIα (Topo II) is located on recurrent gain site chromosome 17q, and the resulting upregulation is linked to poor survival in MPNST patients. Topo II is an essential enzyme that maintains genome stability by deconcatenating sister chromatids during mitosis to release tension from supercoils and untangle knots in DNA. The ability of Topo II to efficiently deconcatenate DNA relies on its chromatin tethering domain and that domain's interaction with methylated histones, including H3K27me3. The twin purposes of this study were to determine if loss of the PRC2 and H3K27me3 can cause mitotic defects in human Schwann linage cells and if drugs that target the mitotic apparatus are selectively toxic to PRC2-deficient cells. In this way, this study may inform therapeutic opportunities for treating PRC2-deficient MPNSTs and suggest MPNST-specific mechanisms for the acquisition of genome instability.

Methods: We utilized a human induced pluripotent stem cell (iPSC)-derived model system, iPSC-Schwann cell precursors (iPSC-SCPs) to understand how MPNSTs acquire genome instability over time with loss of PRC2. This model includes loss of *NF1, CDKN2A/CDKN2B,* and *SUZ12.* To visualize errors in mitosis, including ultra-fine bridges (UFBs) and aberrant chromosome segregation, we used fluorescence microscopy. To determine if PRC2-deficient cells are more sensitive to drugs targeting mitosis, we conducted a drug screen in immortalized NF1-deficient Schwann cells. To assess how PRC2 loss contributes to tumorigenesis, we are analyzing anchorage independent growth *in vitro.* After tumor formation, we will determine if PRC2 loss leads to more genome instability via low-pass whole genome sequencing to determine copy number alterations and structural variants.

Results: Our studies revealed that key indicators of genomic instability, UFB formation and aberrant segregation, are increased in *NF1*- and *CDKN2A/CDKN2B*-deficient iPSC-SCPs treated with an inhibitor of PRC2. We have also shown that immortalized NF1-deficient Schwann cells with PRC2 loss have a heightened sensitivity to mitosis-targeted drugs, including Haspin Kinase and Topo II inhibitors, revealing the potential for therapeutic exploitation.

Conclusion: This study will deepen our understanding of the biological consequences of PRC2 loss in MPNSTs and establish a rationale for the use of drugs targeting the mitotic apparatus in PRC2 deficient tumors. We are integrating mechanistic studies with preclinical therapeutic validation to understand the acquisition of genome instability in these tumors and inform new treatment opportunities.

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Identification of Key Genes and Pathways of Decreased Bone Mineral Density in NF1 Patients with Dystrophic Scoliosis by Microarray and Integrated Gene Network Analysis

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Purpose: To explore the differential genetic expression profile, Gene Ontology terms, and Kyoto Encyclopedia of Genes and Genomes pathways in human trabecular bone (HTB)-derived cells of dystrophic scoliosis secondary to neurobromatosis type 1 (DS-NF1) and compare these to normal controls.

Methods: Bone mineral density (BMD) was measured and compared between DS-NF1 patients and normal controls. Then, microarray analysis was used to identify differentially expressed genes (DEG) of HTBs between DS-NF1 patients and normal controls. Functional and pathway enrichment analysis was performed based on GO and KEGG pathway databases. Then, STRING database, Cytoscape and MCODE were used to construct the protein-protein interaction (PPI) network and screen hub genes. Further, the hub genes and gene clusters determined by module analysis were analyzed by pathway enrichment. Six potential key genes were selected for reverse transcription polymerase chain reaction (RT-PCR).

Results: The BMD of lumbar spine, femoral neck and total femur in DS-NF1 patients significantly decreased. Bioinformatics analysis showed that there were 401 previously unrecognized DEGs (238 up-regulated genes and 163 down regulated genes) in HTBs of DS-NF1 patients, which were mainly enriched in immune response, type I interferon signaling, TNF signaling pathway and RIG-I-like receptor signal pathway. Five hub genes such as STAT1, OASL, IFIH1, IRF7 and MX1 were identified by PPI network. These genes were mainly enriched in Jak-STAT and RIG-I-like receptor signaling pathway. PPI network analysis also found an independent dysregulated protein cluster containing CCL2, CXCL1, CXCL3, CX3CL1, TLR1 and CXCL12. The former five genes were significantly down-regulated, while CXCL12 was significantly up-regulated. All these genes are closely related to immune response, suggesting that immune response may play an important role in the pathogenesis of DS-NF1 with decreased bone mass.

Conclusion: In this study, STAT1, OASL, IFIH1, IRF7 and MX1 were identified as the key genes of DS-NF1-related bone mass reduction. Immune response may play a key role in this process, and CXCL12 mediated osteogenesis may play a protective role in the reduction of BMD. This requires further research to clarify the pathogenesis of osteopenia in DS-NF1.

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Increased Myxoid Stroma in NF1-Associated Breast Cancer

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Purpose: Women with neurofibromatosis 1 (NF1) are at an increased risk for breast cancer, and the prognosis of NF1-associated breast cancer is often poor. Data from neurofibromas and model systems have shown that NF1 can have major effects on tumor microenvironment. We now aimed to characterize histological features of NF1-associated breast cancer.

Methods: A set 26 breast cancers from women with NF1 was analyzed. A frequency-matched control cohort of 75 breast cancers from non-NF1 women was retrieved from Auria Biobank (Turku, Finland). The matching of the control breast cancers accounted for age at diagnosis ($<$40, 40-50, $\geq$50 years), estrogen receptor status, HER2 status, tumor grade and histological subtype. A pathology specialist, blinded to the NF1 status of the samples, scored hematoxylin & eosin sections for myxoid stroma and tumor budding.

Results: Myxoid stroma was observed significantly more frequently in NF1-related breast cancers (69%) than in control breast cancers (40%), yielding an odds ratio (OR) of 3.33 (95% CI 1.20-10.1, $P=0.013$). Among the breast cancers with myxoid stroma, the proportion of myxoid stroma was higher in NF1-related breast cancers than in the control breast cancers ($P=0.025$). Especially breast cancers diagnosed after 50 years of age showed increased rate of myxoid stroma in NF1 versus the controls (OR 4.03, 95% CI 1.06-17.5, $P=0.023$). Tumor budding was detected in 50% of the NF1-related breast cancers and 37% of the control breast cancers, yet the difference was not statistically significant (OR 1.67, 95% CI 0.62-4.54, $P=0.354$). Upon further stratification, many NF1-related breast cancers showed single tumor budding complexes (NF1 42%, control 15%), and abundant budding was more common in the control breast cancers (NF1 7.7%, control 23%).

Conclusion: NF1 affects the stromal composition of breast cancer, which may contribute to the development and growth of NF1-associated breast cancer.

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Neural Crest-Like Cell State in MPNSTs is Dependent on SHH Pathway Activation

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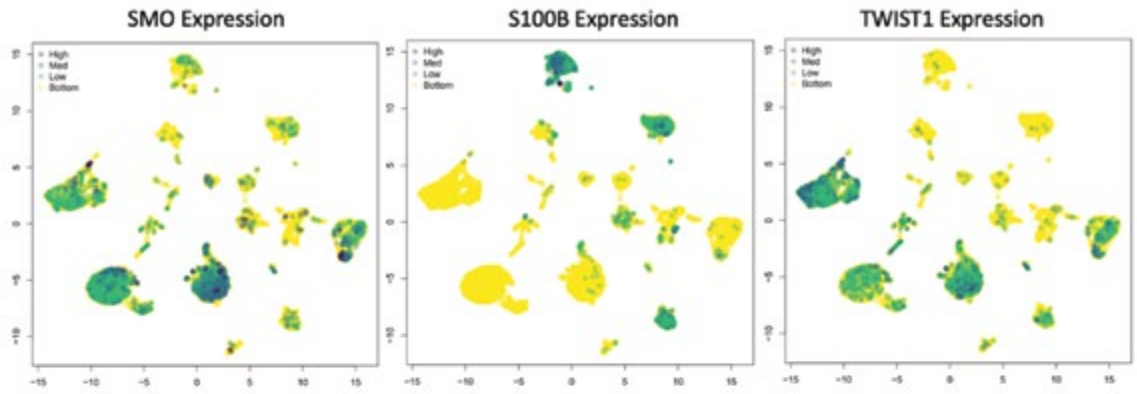
Introduction: The molecular mechanism that drives malignant transformation from a neurofibroma to a MPNST in NF1 individuals has yet to be fully understood. Our previous work has demonstrated that SHH pathway activation drives malignant transformation in a subset of malignant peripheral nerve sheath tumors (MPNSTs). Here, we demonstrate that SHH pathway activation induces a neural crest cell-like state in a subset of MPNSTs.

Methods: We performed single nuclear RNA sequencing on tumors (5 MPNSTS and 1 atypical neurofibroma). Data was generated using the 10X Chromium platform and analysed using the Suerat 3.0 pipeline. Cellular trajectories were inferred by generating of relative pseudotime through a linear transformation relative to cells with lowest and highest pseudotimes with Moncole2. We performed *in vitro* experiments to assess the correlation between SHH pathway activation and neural crest signatures. We inhibited SHH pathway with sonidegib, a SMO inhibitor, in MPNST cell lines and performed RT-PCR to assess markers of neural crest cell signatures. We also overexpressed SHH pathway in neurofibroma cell lines and performed RT-PCR to assess markers of neural crest cell signatures.

Results: A total of 43, 365 cells were analyzed, with 30, 518 tumoral cells and 12, 847 non-tumoral cells characterized. Notably, we observed that cells that express SMO at high levels, with SHH pathway activation, express neural crest cell makers. These cell populations are negative for S100B, but overexpress TWIST1 (**Figure 1**). Single cell trajectory analysis further demonstrates a pseudotemporal continuum from atypical neurofibromas (Schwann cells), MPNSTs (Schwann cell precursor cells) and other MPNSTs (neural crest cells), which supports that these tumors fall along the developmental trajectory of neural crest cell lineage. SHH pathway activity was high in S462TY MPNST cell line and low in STS-26T MPNST cell line (**Figure 2**). S462TY also demonstrated prominent elevation in expression of neural crest cell markers TWIST1, SOX9, SNAI2, OTX2, PAX4 and PAX6 (**Figure 3**). We found that treatment with sonidegib treatment attenuates the expression of neural crest cell signatures. Finally, inhibiting SHH pathway activation reduced cellular viability in MPNST cell lines. Similarly, we found that overexpressing SHH pathway in neurofibroma cell lines resulted in overexpression of neural crest cell signature in the cell lines.

Conclusion: SHH pathway activation drives dedifferentiation into a neural crest cell-like state in a subset of MPNSTs. We confirm that inhibiting SHH pathway activity in a subset of MPNSTS prevent growth and malignant progression, providing a rational for future clinical trials.

Figure 1:



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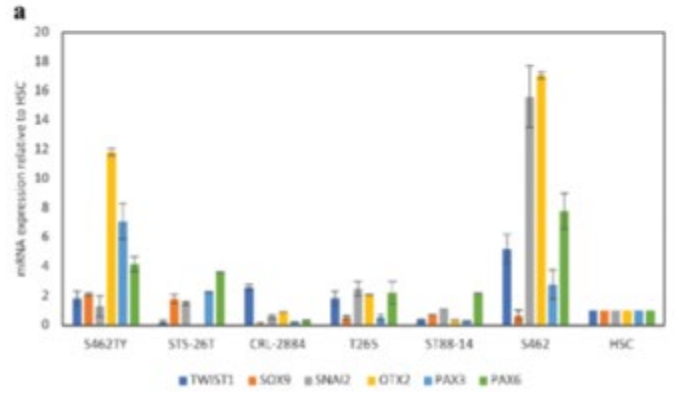
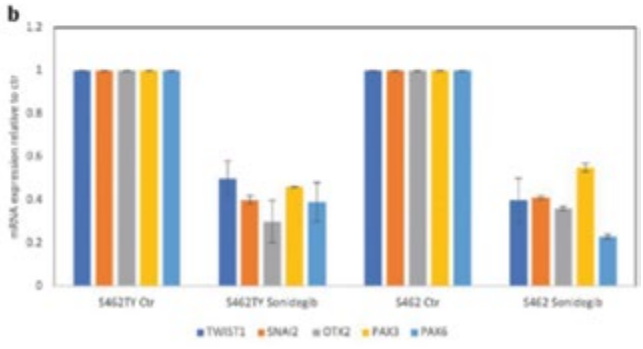


Figure 3:



Single-Center Retrospective Case Series on the Coexistence of *NF1* Mutation with Other Cancer Predisposition Syndromes

Gabriel Guevara, BS, MS, *University of Illinois at Chicago*

Purpose: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by mutations in the *NF1* gene, which encodes the neurofibromin protein, a tumor suppressor regulating RAS signaling via GTPase activity. Currently, there is limited data on the coexistence of *NF1* mutations with other cancer predisposition syndromes. This study presents three cases of patients with *NF1* mutation coexisting with mutations in other cancer susceptibility genes.

Methods: We conducted a retrospective review of three patients with both *NF1* mutations and other germline mutations associated with cancer predisposition syndromes. Clinical data, including reasons for genetic screening, oncologic history, family history, and demographics were recorded. A literature review was also conducted to identify similar reports of patients with NF1 and cancer predisposition syndromes.

Results: The first patient with no diagnosis of cancer or NF1, underwent genetic screening due to multiple café-au-lait spots and an extensive family history of various cancers (ovarian, stomach, pancreatic, lung, and liver). This screening revealed a likely pathogenic *NF1* mutation (c.6449dup (p.S2151Vfs*22)) and a *PMS2* mutation suggestive of Lynch Syndrome. The second patient, diagnosed with NF1, was found to have concurrent *CHEK2* mutation. Lastly, a patient with NF1 was found with a likely pathogenic *HOXB13* pathogenic mutation, which is linked to high risk of prostate cancer. Literature review identified one case of NF1, carrying *CHEK2* mutations, which is linked to breast, prostate, and colorectal cancers. Another study reports a patient with NF1 and colon adenocarcinoma who underwent genetic screening and was discovered with *PMS2* mutation and a novel *NF1* frameshift mutation (NM_000267: exon2: c.71_75del: p.K24fs).

Conclusion: While literature has not established a link between NF1 and other cancer predisposition syndromes, this case series reports three patients with coexistence of *NF1* mutation and other cancer-related genetic mutations. These data emphasize the importance of taking a full family history for patients with NF1 and need of testing a broad panel of cancer susceptibility genes.

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Single-Nucleus Transcriptomic Analysis Reveals Progressive Dedifferentiation and Cellular Heterogeneity in MPNSTs

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Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive cancers that arise from neurofibromas and atypical neurofibromas, afflicting 8-13% of patients with Neurofibromatosis Type 1. The molecular mechanisms driving the malignant transformation of MPNSTs remain poorly understood. Here, we use single-nucleus RNA sequencing (snRNA-seq) to define the cellular and transcriptomic landscape across these tumor types and identify key molecular events associated with malignant progression.

Methods: Single-nucleus RNA sequencing was performed on 7 neurofibromas, 8 atypical neurofibromas, and 15 MPNSTs from human patients. After quality control, Harmony was applied to correct for batch effects, and unsupervised clustering was performed using Seurat in R. Cell type annotation was conducted using canonical markers, the Human Primary Cell Atlas, and chromosomal copy number alterations. CytoTRACE2 was applied to infer cellular potency and differentiation states across tumor types.

Results: Unsupervised clustering revealed distinct malignant and non-malignant cell subpopulations, including endothelial cells, macrophages, T cells, mature non-myelinating and myelinating Schwann cells, immature Schwann cell-like cells, Schwann cell precursor-like cells, and neural crest-like cells. Neurofibromas and atypical neurofibromas exhibit greater clustering of mature Schwann cell populations, consistent with their lower malignancy potential.

Our previously described MPNST molecular subgroups exhibited distinct cellular compositions and differentiation states. MPNST Group 1 tumors, associated with shorter progression-free survival (PFS), were highly enriched for dedifferentiated cell clusters with greater dispersion in UMAP space. MPNST Group 2 tumors, associated with longer PFS, contained a higher proportion of immune cells and exhibited a more differentiated state than Group 1, but were less differentiated than atypical neurofibromas. A stepwise loss of Schwann cell identity and enrichment in neural crest-like markers was observed from neurofibromas to atypical neurofibromas to MPNST G2 to MPNST G1, supporting a model of progressive dedifferentiation and malignancy.

Conclusion: Our single-nucleus transcriptomic analysis provides a high-resolution cellular and molecular characterization of neurofibroma-to-MPNST progression. We demonstrate a continuous dedifferentiation trajectory, spanning from neurofibromas to atypical neurofibromas to MPNST Group 2 tumours to MPNST Group 1 tumours.

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Funding: Neurofibromatosis Therapeutic Acceleration Program (NTAP)

Comparative Profiling of Activated Kinome and Transcriptome in NF1 Schwann Cell Lines: Pathway Insights for Therapeutic Targeting

Stephanie J. Bouley, PhD, *Center for Genomic Medicine, Department of Neurology, Massachusetts General Hospital, Boston, MA*

Neurofibromatosis type 1 (NF1) is a tumor-predisposing condition caused by mutations in the *NF1* gene which encodes the Ras-GTPase activating protein neurofibromin. Loss of both copies of *NF1* in Schwann cells (SCs) results in hyperactive Ras signaling and the formation of benign tumors, the most common being plexiform neurofibromas (PNs). Currently, MEK inhibitors are the only FDA-approved treatments for a subset of NF1-associated tumors; however, the long-term efficacy of MEK inhibition in NF1 patients is unknown, and there are concerns about side effects and resistance. Due to the limited treatment options available for NF1 patients, there is a critical need for discovering novel targets for the rational design of strategies to treat NF1-deficient tumors.

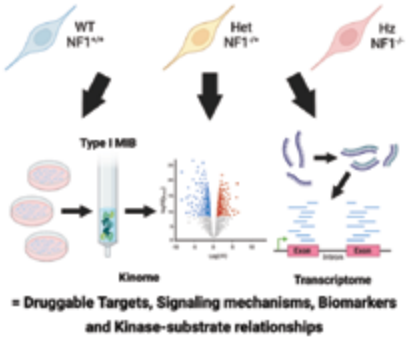

Figure 1 Overview of Scientific Approach to Understanding NF1-related Signaling Networks We established a panel of NF1 wild type, heterozygous, and homozygous Schwann cell lines to preform kinome and transcriptome analyses. We then compared changes between the different analyses to identify novel therapeutic pathways. Made using BioRender.

To discover new therapeutic targets, we have taken a multipronged approach to identify molecular signatures associated with NF1 loss in SCs. Using a panel of genetically matched pairs of *NF1*-deficient homozygous and heterozygous SCs derived either from plexiform neurofibromas or engineered using CRISPR gene-editing, we conducted profiling of the activated kinome and transcriptome signatures (**Figure 1**). While our initial analyses focused on identifying changes between *NF1* homozygous and heterozygous cell line pairs. Numerous kinases with altered activity and genes with altered transcription levels were identified.

Comparative analyses between genetically matched sets of SCs highlighted several signaling pathways with altered activity upon NF1 loss. With our kinome analyses, we found 27 kinases with increased activation in $NF1^{-/-}$ cells compared to $NF1^{-/+}$ cells in the three matched pairs. Pathway enrichment analysis of these kinases using Enrichr identified several pathway complexes, including Survivin, NF-kappaB, protein kinase CK2, and Bcl-2 family protein complexes. KEGG pathway comparative analyses additionally identified several pathways known to be altered in NF1-associated tumors, including ErbB signaling, MAPK signaling, and autophagy in addition to pathways yet to be implicated as playing a role in NF1. With our RNAseq analyses, we identified 83 genes with increased expression in $NF1^{-/-}$ cells compared to $NF1^{-/+}$ cells in the three matched pairs. Using the L1000 connectivity map, we have made prediction of compounds that $NF1^{-/-}$ cells could potentially respond to; the compound with the greatest prediction was the MEK inhibitor Selumetinib, suggesting that this platform can accurately predict potential therapeutic strategies based on our comparative analyses. We will present our follow up validation studies of top hits from our profiling assays in CRISPR-engineered, isogenic SCs, as well as new PN-derived cell lines. Additionally, we are using immunohistochemistry to investigate targets of interest in *NF1*-deficient tumors samples from patients.

Our findings support the use of comparative profiling as a method of identifying potential therapeutic targets and effective strategies to treat *NF1*-deficient tumors including PNs. This approach also reveals novel biomarkers to measure successful treatment options in NF1 patients.

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Unraveling the Development of Cutaneous Neurofibromas in Neurofibromatosis Type 1

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Purpose: Neurofibromatosis type 1 (NF1) is a genetic disorder that leads to the formation of cutaneous neurofibromas (cNFs), benign nerve sheath tumors that develop in the skin and significantly impact patients' quality of life. cNF development begins with bi-allelic NF1 loss in Schwann cell (SC) lineage, followed by the recruitment of a complex tumor microenvironment consisting of fibroblasts, immune cells, blood vessels, axons and a dense extracellular matrix. Despite their high prevalence and clinical impact, the molecular mechanisms underlying cNF formation remain poorly understood.

Methods: Here, we used an *Nf1* knockout *(Nf1-KO)* mouse model, combined with immunohistochemistry and single-cell transcriptomics, to explore the mechanisms driving cNF development.

Results: Using a trauma-induced *Nf1*-KO model with scRNAseq, we conceived a transcriptomic atlas of growing and mature cNFs, as well as adjacent, seemingly healthy skin. This analysis identified a population of non-myelinating Aquaporin1^{high}Nestin^{low} SCs as the likely cells of origin for cNFs. These cells overexpress genes involved in axon growth and guidance, potentially driving the abnormal innervation observed in cNFs from both mice and patients. Additionally, we found that tumor SCs, along with dermal and/or epineurial fibroblasts and pericytes, overexpress collagen-encoding genes, contributing to the extensive fibrosis characteristic of cNFs. Notably, all of these cells exhibit high expression of Periostin and Tenascin C, key extracellular matrix components, highlighting them as novel therapeutic targets for cNF treatment.

Conclusion: Our study provides insights into the cellular mechanisms driving cNFs formation in NF1. By identifying Aquaporin1^{high}Nestin^{low} SCs as the likely cells of origin and uncovering molecular pathways involved in fibrosis and abnormal innervation, we highlight potential therapeutic targets, such as Periostin and Tenascin C. These findings enhance our understanding of cNF pathogenesis and pave the way for future strategies aimed at preventing or treating these tumors.

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Classification of Cutaneous Neurofibromas Cell Populations

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Neurofibromas are a cardinal feature of neurofibromatosis type 1 (NF1), which results from inherited or spontaneous mutations in the neurofibromin gene *(Nf1)*. Driven by Schwann cells (SCs) that have lost expression of *Nf1*[1, 2], these benign tumors depend on a microenvironment of multiple cell types that are also haploinsufficient for *Nf1*[3, 4]. Dermal or cutaneous neurofibromas (cNFs) occur in most NF1 patients. Unlike plexiform neurofibromas, cNFs do not bear a significant risk of malignant transformation and are much less likely to cause loss of function[5]. These tumors are, however, a principal disease concern for many NF1 patients since they typically increase in number and size over a patient's lifetime, are often associated with chronic pain and itch, and may produce cosmetic disfigurement imposing a psychosocial burden[6]. We do not know how to prevent their development and treatment options are limited.

Our goal is to identify cell populations and sub-populations within cNF tissue samples to develop a better understanding of these complex tumors and make progress toward identifying effective treatments. Using fresh-frozen cNF samples from the NF1 biospecimens repository at Johns Hopkins University, we have successfully completed in-situ transcriptomics analysis of 16 cNF samples from different patients using the Visium 10x Genomics platform. Integrating the spatial cNF dataset with publicly available scRNA-seq data from skin derived Schwann from donor donation and a cNF patient will allow us to establish spatial relationships among cell populations. Bioinformatics analyses of these results are expected to reveal significant new information on the cellular microenvironment of cNF.

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THERAPEUTICS AND DRUG DISCOVERY

Targeting PRMT5 in MTAP-Deleted NF1 High Grade Gliomas

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Neurofibromatosis type 1 (NF1) is a tumor predisposition syndrome that is characterized by hyperactivation of the RAS/MAPK signaling pathway. Our analysis of NF1-associated high grade gliomas revealed that 57% of these tumors harbor homozygous deletions in chromosome 9p21.3, encompassing the tumor suppressor genes *CDKN2A, CDKN2B,* and *MTAP.* Methylthioadenosine phosphorylase (MTAP) is critical for the methionine salvage pathway and its loss leads to increased vulnerability to PRMT5 inhibition. PRMT5 methylation activity is also associated with multiple kinases in the RAS/MAPK signaling pathway, warranting further investigation on a combined PRMT5 and RAS/MAPK inhibition therapy.

Cell lines (n= 4) with loss-of-function mutations in the *NF1, CDKN2A/B,* and *MTAP* genes and CRIPSR-Cas9 MTAP-knockout (MTAP-KO) cells were first validated for MTA accumulation using liquid chromatography-mass spectrometry (LC-MS). Cell viability assays were then performed using a brain-penetrant, MTA-cooperative PRMT5 inhibitor, TNG908. Specific inhibition of PRMT5 was confirmed by immunoblotting for symmetric dimethylarginine (SDMA) expression and genes involved in the RAS/MAPK signaling pathway. Additionally, the impact of TNG908 on cell cycle progression and apoptosis was assessed using flow cytometry. Combination of TNG908 with MEK inhibitors was also assessed.

MTAP-deleted and MTAP-KO cells showed increased MTA accumulation compared to wild-type MTAP (MTAP-WT) cells, resulting in significant sensitivity towards TNG908. PRMT5 inhibition was confirmed by reduced SDMA expression in MTAP-KO cells but not in MTAP-WT cells, indicating TNG908's MTA-dependent inhibition activity. PRMT5 inhibition also significantly increased ($p<0.05$) the proportion of cells in the G2/M phase of the cell cycle and enhanced apoptotic activity ($p<0.05$). Combinatorial inhibition using TNG908 and Binimetinib demonstrated significant synergy (ZIP score= 14.36) in MTAP-KO cells and additive activity in TM31 cells (ZIP score= 0.272).

These findings highlight PRMT5 inhibition as a promising therapeutic strategy for MTAP-deleted NF1 HGGs. Moreover, the combination of TNG908 and Binimetinib shows strong potential and warrants further investigation in vivo.

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The RXR Agonist MSU-42011 and the MEK Inhibitor Selumetinib Reduce Tumor Burden by Decreasing pERK Levels and Modulating Immune Cell Populations Within the NF1 Tumor Microenvironment

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Neurofibromatosis type 1 (NF1) is a common genetic disease that predisposes approximately 50% of affected individuals to develop plexiform neurofibromas (PNFs), which can progress to highly aggressive malignant peripheral nerve sheath tumors (MPNSTs) in approximately 10% of patients. Selumetinib, a specific inhibitor of MEK1/2, is the only FDA-approved drug for NF1-associated PNFs. However, the anti-tumor effects of selumetinib are limited in MPNSTs, and the drug has dose-limiting side effects. During the formation of neurofibromatosis, a complex tumor microenvironment (TME) develops, with the infiltration of macrophages critical for growth and progression. Targeting tumor-promoting immune cells could be an alternative approach for treating or preventing the progression of NF1. The novel retinoid X receptor (RXR) agonist MSU-42011 reduces tumor growth in experimental Kras-driven lung cancers by decreasing pERK levels, reducing tumor-promoting immune cells like CD206+ macrophages and regulatory T cells, and increasing activated cytotoxic T cells.

Purpose: Given the similarities in RAS activation and immune cell infiltration in NF1 and Kras-driven lung cancer, we hypothesized that MSU-42011 can be used as an alternative approach to selumetinib and modulate immune cell populations within the NF1 TME.

Methods: We treated NF1-deficient cells and a syngeneic mouse model of MPNST with MSU-42011 and selumetinib, either alone or in combination, to evaluate their immunomodulatory and anti-tumor effects.

Results: In NF1-deficient cells, treatment with MSU-40211 or selumetinib reduced pERK protein levels, and the combination treatment enhanced this reduction. Additionally, there was a trend toward reduction in cell viability with increasing drug concentrations after the combination treatment. Moreover, conditioned media (CM) from human and mouse PNF cells increased the mRNA expression of monocyte chemoattractant *CCL2* (C-C motif chemokine ligand 2) and the secretion of IL-6 and TNFα in human THP1 monocytes/macrophages and bone marrow derived macrophages (BMDM). Notably, MSU-42011 and selumetinib alone inhibited *CCL2* mRNA expression in THP1 macrophages and BMDM stimulated with CM from human and mouse PNF cells, respectively, and the inhibition of *CCL2* mRNA expression was greatest with the combination treatment. The combination of MSU42011 and selumetinib also significantly reduced tumor growth and reduced pERK levels, tumor-promoting CD206+ macrophages, and regulatory T cells in both a LL2 model of lung cancer driven by an activating *Kras* mutation and a syngeneic mouse model of MPNST.

Conclusions: As NF1 is a complex multisystem disorder, a combination of drugs with different mechanisms will most likely be more effective than single agents. Our initial results form the justification for further preclinical evaluation *in vivo.* Currently, we are assessing the immunomodulatory and anti-tumor effects of MSU-42011 and selumetinib in a genetically engineered model of PNF and MPNST, with the eventual goal of translating these findings into clinical applications.

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Investigating the Role of NF1 Mutation in Cerebral Organoid Development and Its Impact on Glioma Phenotypic Plasticity and Drug Response

Soniya Chatterjee, *Brain Tumor Institute, Center for Cancer and Immunology Research, Children's National Hospital, Washington, DC*

Background: Neurofibromatosis type 1 (NF1) is a genetic disorder caused by NF1 gene mutations, affecting 1 in 3,000 individuals worldwide. It is characterized by cutaneous neurofibromas and an elevated tumor risk, with 15–20% of patients developing gliomas. While NF1 inactivation is linked to low-grade gliomas, the drivers of malignant transformation remain poorly understood. We hypothesized that a permissive NF1-mutant cerebral microenvironment may contribute to malignant transformation and treatment responses of gliomas. Current preclinical models, such as glioma cell lines and xenografts, fail to replicate the complex NF1 tumor microenvironment. Human induced pluripotent stem cell (hiPSC)-derived cerebral organoids provide a promising platform for modeling NF1-driven neurodevelopment and tumor biology.

Purpose: To investigate the role of NF1 mutations in cerebral organoid development, glioma plasticity, and treatment response.

Methods: Cerebral organoids were generated from healthy control and NF1-mutated (NF1+/-, NF1-/-) iPSCs reprogrammed from plexiform neurofibromas. Organoids were analyzed for cytoarchitectural development at key stages using immunofluorescence assays. mCherry-labeled glioma cells TM31 and patient-derived high-grade astrocytoma with piloid features (HGAP) cells were co-cultured with cerebral organoids to assess glioma migration (qualitative imaging), proliferation (qPCR), secretome profiles (MSD), and treatment responses (cell titer glow assay).

Results and Conclusions: Initial immunofluorescence analysis revealed no significant differences in neuronal development between wild-type and NF1-mutant organoids. A co-culture of our novel organoids with TM31 and patient-derived HGAP glioma cells was successfully established. Preliminary experiments indicate increased glioma migration and proliferation in NF1-mutant organoids compared to NF1 wild type controls. Image analysis further suggests localized glioma growth within specific zones of the organoid, warranting further exploration. Additionally, elevated levels of GM-CSF and IL-18—cytokines implicated in glioma growth and invasion—were detected in the secretome of NF1-mutant organoid-glioma co-cultures. These findings suggest that NF1 mutations foster a tumor-supportive microenvironment, enhancing glioma aggressiveness and potentially influencing treatment response.

To explore therapeutic strategies, we piloted a CDK4/6 inhibitor using TM31 cultures and showed drug's efficacy in targeting tumor cell viability. Drug combination assays with MAPK/mTOR inhibitors are underway. The most potent combinations will then be used for organoid co-cultures.

This study highlights the utility of NF1-mutant organoids as a platform to study glioma behavior and NF1-associated tumor predisposition. Future analyses, including single-cell RNA sequencing and spatial transcriptomics, will further validate NF1's role in glioma progression. These findings pave the way for ex vivo drug testing to improve personalized therapies for NF1-driven gliomas.

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FOXM1 as a Drug Target in NF1-Associated Malignant Peripheral Nerve Sheath Tumors

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Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are sarcomas that arise either spontaneously or through transformation of benign tumors, called plexiform neurofibromas (PNFs), in patients with Neurofibromatosis Type 1 (NF1). Understanding the driver mutations that cooperate to promote malignancy remains a priority in the field. One potential driver, FOXM1, is an oncogenic transcription factor that promotes malignant progression, drug resistance, and immune evasion in other cancers. There are just two published studies of FOXM1 in MPNSTs, both of which suggest it promotes the disease. We seek to understand the role of FOXM1 in these deadly tumors and *hypothesize that FOXM1 is essential for MPNST pathogenesis.*

Methods: FOXM1 mRNA and protein expression were evaluated in patient matched PNFs, ANNUBPs, and MPNSTs via RNA-Seq and IHC. For *in vitro* studies, we generated normal human Schwann cells (NHSCs) and MPNST cell lines (S462, sNF96.2) with overexpression (OE) or knockdown (KD) of FOXM1 isoforms b and c. Cell proliferation, survival, and transformation status are being measured as is their response to historical and newly developed FOXM1 inhibitors. To directly test the *in vivo* role of FOXM1 in MPNST initiation and progression, *de novo* MPNSTs were initiated by *Nf1*/*Ink4a*/*Arf* editing in the sciatic nerve of *Nf1*$^{+/-}$*, Foxm1* floxed mice relative to *Nf1*$^{+/-}$ controls.

Results: In patient matched tumor sets, *FOXM1* mRNA was significantly elevated in MPNSTs relative to patient-matched PNF/ANNUBP precursor lesions. At the protein level, FOXM1 expression rose dramatically in a stepwise manner from normal nerve to PNFs, ANNUBPs, and MPNSTs. Increased FOXM1 expression corresponded with heightened transcriptional activation, as measured by upregulation of FOXM1 target genes in MPNSTs versus PNFs. Among its targets, upregulation of programmed death ligand 1 (PD-L1) protein correlated tightly with increased FOXM1 in patient tumors. Broad acting FOXM1 inhibitors, thiostrepton and FDI-6, effectively inhibited MPNST proliferation and induced apoptosis. Excitingly, synergistic killing of MPNST cells was obtained by combining FOXM1 inhibitors with a MEK inhibitor (mirdametinib), CDK4/6 inhibitor (palbociclib), or EGFR inhibitor (gefitinib), all of which are relevant drugs for MPNST therapy.

Conclusion: Our data suggest FOXM1 is an important driver of MPNST pathogenesis. FOXM1 expression and transcriptional activity are greatly increased in human MPNSTs compared to benign precursors from the same patients. Therapeutic inhibition of FOXM1 *in vitro* kills MPNST cells and effectively synergizes with several clinical drugs that antagonize MEK, CDK4/6, and EGFR. Ongoing *in vitro* studies will define the role of FOXM1 in Schwann cell transformation while analyses of novel *Nf1*$^{+/-}$*, Foxm1* deficient mice will reveal the requirement of FOXM1 in NF1-associated MPNST development *in vivo.* To date, our evidence indicates that FOXM1 inhibition in specific combination therapies could be a new, effective strategy for treating MPNST patients.

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Developmental Analyses of Skeletal Manifestations in Knock-In Mouse Model of Neurofibromatosis Type 1 p.M992del "Mild" Patient Mutation

Alexis Murawski Stillwell, *Pennington Biomedical Research Center; University of Alabama at Birmingham*

Background: Animal models are critical for understanding disease and testing new therapeutics. Patients with the p.M992del mutation display a relatively mild clinical phenotype associated with café-au-lait spots but no neurofibromas, with a subgroup of patients displaying Noonan-like features. Therefore, we hypothesize that mutant p.M992del neurofibromin is hypomorphic and retains sufficient functional activity to suppress tumor formation, but not sufficient activity in all NF1 related functions.

Methods: Heterozygous mice ($Nf1^{M992del/+}$ on FVB background) were created using CRISPR/Cas9 and a repair template to introduce a 3 bp deletion in the homologous region of mouse Nf1 gene. We intercrossed heterozygous mice ($Nf1^{M992del/+}$) to assess viability of homozygous ($Nf1^{M992del/M992del}$) mutants, as most Nf1 mutant alleles induce early embryonic lethality when homozygous. $Nf1^{M992del/M992del}$ animals and control littermates were assessed for growth parameters, body composition, skeletal structure (histology and X-Ray), and bone density (uCT). Histological analysis of growth plates and cranial sutures was also performed. Embryos from intercrossed heterozygous mice ($Nf1^{M992del/+}$) were analyzed between E17.5 and P0.

Results: Analysis of genotype ratios revealed perinatal lethality of $Nf1^{M992del/M992del}$ pups (observed 101 pups born compared to 262.75 expected pups). Surviving $Nf1^{M992del/M992del}$ mice fail to thrive and are ~50% of the size by weight of littermate controls; only 61 of the 101 observed pups survived to weaning. Mice display impaired bone organization of the sternum and craniosynostosis. Mice surviving to 6 months also display bilateral suppurative otitis media (n=2/4) and pneumonitis (n=3/4). E16.5 $Nf1^{M992del/M992del}$ pups display double outlet right ventricle phenotype, and 6-month-old mice display mitral valve defects. Preliminary Western blots confirm the presence of mutant neurofibromin protein at levels consistent with control animals, and a significant change in pERK levels.

Conclusions: $Nf1^{M992del/+}$ mice appear to be healthy with no overt phenotype, whereas $Nf1^{M992del/M992del}$ animals display perinatal lethality, with surviving animals severely runted. Further characterization of the skeletal and heart phenotype is ongoing. Preliminary data from backcrossing the p.M992del mutation from FVB/NJ mice to C57BL/6J strain indicates genetic background plays a role in embryonic lethality as no viable homozygous mice have been identified on the C57BL/6J background.

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Connecting Sleep and Sensory Deficits in a *Drosophila* Model of NF1

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Background: Neurofibromatosis type 1 (NF1) is mainly characterized by benign tumors throughout the nervous system. However, individuals with NF1 also experience cognitive and social deficits, disrupted circadian rhythms, poor sleep quality, and altered sensory perception. Although these non-tumor manifestations affect up to 80% of the NF1 population, it is largely unknown how they arise, and whether they influence one another. Interestingly, these traits are highly conserved across species, including in *Drosophila* models of NF1. Here, we leverage this powerful animal model to examine the relationship between sensory integration and sleep disruption in NF1.

Methods and Results: Sensory input can either disrupt sleep or can be harnessed to enhance sleep quality. Gentle vibration, for example, improves sleep in rodents and humans, with recent studies indicating similar effects in Drosophila. Here, we used a vibration-induced sleep (VIS) paradigm to investigate how sleep is influenced by sensory input a fly model of NF1. Adult fly sleep was recorded at baseline for 24 hours, followed immediately by 24 hours of exposure to gentle, continuous vibration. Nf1 flies exhibited reduced and fragmented sleep at baseline, consistent with published work. Additionally, while control flies showed VIS, Nf1 flies failed to increase sleep in response to vibration, suggesting deficits in the sleep-sensory connection.

To determine if VIS or baseline sleep deficits are due to excessive sensory input, we reduced mechanosensory signals by surgically removing antenna, where most mechanosensory neurons reside. This manipulation reduced VIS in controls, as expected, but failed to normalize baseline sleep or VIS in Nf1 flies (**Figure 1**). This suggested that absence of VIS in NF1 does not result from hypersensitivity to vibration. We then used genetic approaches to directly activate mechanosensory neurons *in vivo.* In controls, we found increased sleep similar to VIS, but in Nf1 flies there was no effect.

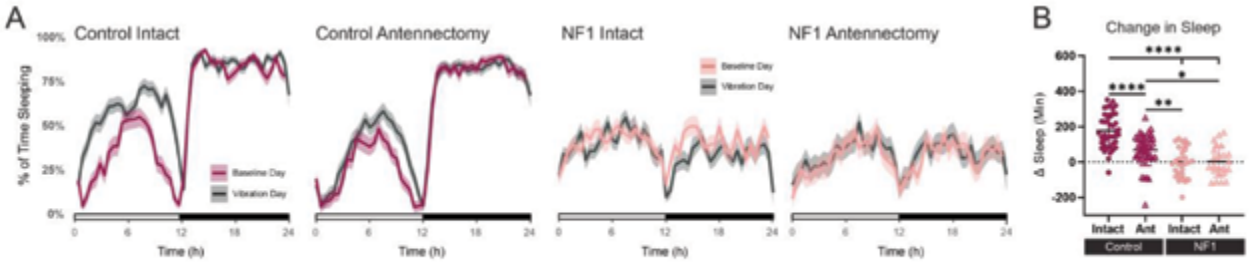

Figure 1 A) Averaged sleep traces for control and NF1 flies that underwent antennectomy or remained intact during the baseline day and vibration day. **B)** Quantification of total change in sleep (One-way-ANOVA, Tukey's multiple comparisons *p<0.05. **p<0.01. ***p<0.001. ****p<0.0001.)

To decouple potential sensitivity differences from sleep, we exposed flies to an hour of vibration while they were awake. Control flies responded with an initial spike in activity during the first 5 minutes of vibration. Surprisingly, NF1 flies showed a similar response, regardless of vibration intensity. Furthermore, mechanosensory neurons in Nf1 flies appear morphologically normal.

Conclusion: Together, these findings suggest that mechanosensory neurons in Nf1 flies function normally during wake, but their sensory input is not effectively integrated to influence sleep. This work sheds light on the relationship between sensory integration and sleep in NF1, pointing towards novel treatment strategies for disrupted sleep.

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Comparative Toxicity of Selumetinib and Binimetinib on Growth, Development, Behavior, and Cardiac Health in Zebrafish Embryos

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Purpose: Currently, there is no research clarifying whether drugs used to treat neurofibromatosis type 1 (NF1) affect the growth, development, and behavior of children. Therefore, the aim of this study was to explore the growth and developmental toxicity, as well as the effects on behavior and the heart, of selumetinib and binimetinib using zebrafish embryo models.

Methods: Embryos at 3 hours post fertilization (hpf) were exposed to selumetinib and binimetinib (0.5, 1, 2, 4, 8 and 16 μM) until 120 hpf (before the feeding stage). During this period, the survival and morphology of embryos were observed and recorded every 24 hours, and specific observation parameters would be assessed at specific developmental time points. As for the development of different systems and the heart, the corresponding transgenic zebrafish model were chosen, such as Tg (fli1a:EGFP), Tg (hb9:GFP), Tg (huc:GFP), Tg (mpeg:EGFP), Tg (mpx:EGFP) and Tg(myl7:GFP). Moreover, a deeper analysis of cardiac toxicity was conducted by performing apoptosis staining to observe the growth and development of myocardial cells. At the gene level, the expression of genes related to cardiac function and apoptosis was assessed. At the protein level, the expression of proteins related to myocardial enzyme profiles was measured.

Results: The results indicated that selumetinib showed slightly higher toxicity than binimetinib in terms of survival, deformity, locomotor behavior, and cardiac toxicity. The lethal concentration for both drugs was around 32 μM. At 16 μM, selumetinib significantly increased the malformation rate, while binimetinib showed minimal changes. Selumetinib also induced venous sinus congestion at 16 μM, which was not observed with binimetinib. In locomotor behavior, under light stimulation, selumetinib at 16 μM significantly decreased swimming distance and speed under light stimulation, while binimetinib had little effect; Under sound stimulation, both drugs increased swimming speed, with a more pronounced effect at higher concentrations. Cardiac toxicity results showed that at 32 μM, selumetinib significantly reduced heart rate and blood flow velocity, increased pericardial edema and AV-SV distance, and altered the expression of cardiac enzyme markers and related genes.

Conclusion: Excessively high concentrations of selumetinib significantly affected the growth and development of zebrafish embryos and caused severe cardiac toxicity, whereas binimetinib exhibited lower toxicity than Selumetinib. Therefore, in clinical practice, the dosage and blood drug concentration of selumetinib must be strictly controlled when used in children.

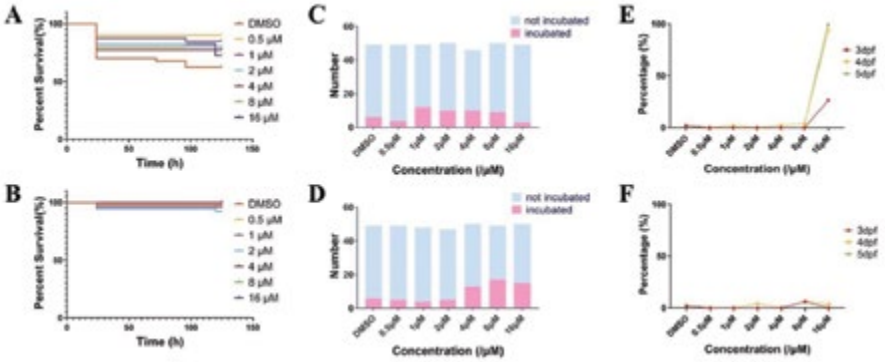

Figure 1. Survival-related parameters after selumetinib and binimetinib intervention. (A) Survival curve of selumetinib. (B) Survival curve of binimetinib. (C) Hatching rate of selumetinib. (D) Hatching rate of binimetinib. (E) Deformity rate of selumetinib. (F) Deformity rate of binimetinib.

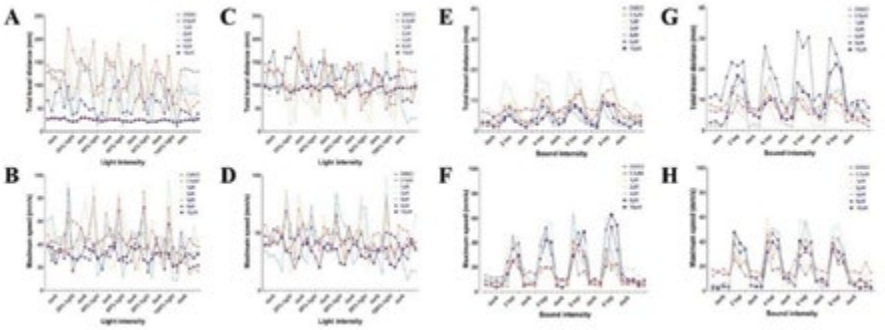

Figure 2. Swimming behavior analysis after selumetinib and binimetinib intervention. (A) Total swimming distance of selumetinib under light stimulation. (B) Maximum speed of selumetinib under light stimulation. (C) Total swimming distance of binimetinib under light stimulation. (D) Maximum speed of binimetinib under light stimulation. (E) Total swimming distance of selumetinib under sound stimulation. (F) Maximum speed of selumetinib under sound stimulation. (G) Total swimming distance of binimetinib under sound stimulation. (H) Maximum speed of binimetinib under sound stimulation.

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Repurposing of Synergistic Drug Combinations for the Treatment of NF1 Tumours

Megan Stevens

Purpose: The aim of this project is to develop effective therapies to treat tumours associated with Neurofibromatosis type 1 (NF1). NF1 affects approximately 1/3,000 live births and is associated with a variety of symptoms including the formation neurofibromas along peripheral nerves. These tumours, cause disfigurement and can disrupt nerve function leading to pain and loss of motor control. A proportion of tumours develop into malignant peripheral nerve sheath tumours (MPNSTs) that are often fatal.

While MEK inhibitors are currently used to treat a subset of neurofibromas, these are not effective in all patients and can cause side effects.

We previously generated a synthetic lethal interaction network for *NF1,* containing 54 potential drug-targets for NF1 tumours (PMID: 39129390). Initially, we focused on autophagy as a target and showed that chloroquine caused selective killing of NF1 deficient cells across a range of *in vitro* and *in vivo* models of NF1 tumours. This demonstrates the value of the network in developing new treatment options for NF1. Here, we described new results building on our synthetic lethal network to develop a synergistic drug combination with high potential as a future treatment for NF1 tumours.

Methods: To identify candidate drug-targets not conserved in *Drosophila,* we performed semantic similarity analysis based on the results of the synthetic lethal screens. Existing drugs were tested in *Drosophila* and human patient-derived cell models as well as an *in vivo* MPNST xenograft model.

Results: One challenge of screening for drug targets using *Drosophila* cells is that human targets that are not conserved in *Drosophila* cannot be directly identified. To address this limitation, we performed statistical enrichment analysis to identify human-specific genes with close functional relationships with the hits from the *Drosophila* screens. This identified TERT as a candidate target. We then showed that a repurposed inhibitor known to affect TERT function, caused selective death of NF1 deficient patient-derived cells and MPNST-derived cells both *in vitro* and *in vivo.* Further testing revealed that this inhibitor synergises with MEK inhibition to enhance efficacy.

Conclusions: Synthetic lethal screens in *Drosophila* cells combined with statistical enrichment analysis is a powerful approach to identify novel treatment strategies for NF1 tumours.

From our studies, we conclude that inhibition of the telomerase complex synergises with MEK inhibition to kill NF1-deficient tumour cells.

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Funding: Medical Research Council

Development of an Adeno-Associated Virus (AAV) Toolkit to Modulate Signaling Pathways Altered in Neurofibromatosis Type 1 (NF1)

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder caused by loss of function mutations in the *NF1* gene encoding neurofibromin. Neurofibromin is a GTPase activating protein that acts as a negative regulator of the Ras-mitogen activated protein kinase (MAPK; Ras-Raf-MEK-ERK) signaling cascade. The NF1 phenotype is characterized by a predisposition to plexiform neurofibromas (PN), peripheral nerve sheath tumors that can be locally destructive and undergo malignant transformation. Targeted gene therapies that rescue neurofibromin function could provide a method for tumor prevention and treatment. Vectors based on adeno-associated virus (AAV) represents an appealing delivery tool for gene therapy. However, its small packaging capacity prevents delivery of the entire *NF1* gene, and AAV capsids lack substantial tropism for Schwann cells (SC), the tumor cell of origin. Here, we describe *in vitro* validation of AAV-compatible transgenes that modulate signaling pathways perturbed in NF1 and *in vivo* efforts to improve transgene delivery via characterization of SC tropism by novel AAV capsids.

Selected transgenes are known to downregulate MAPK signaling or affect other pathways dysregulated in NF1. These include functional variants of neurofibromin GAP-related domain (GRD); dominant negative mutant G-proteins (HRas, NRas, KRas, Rac1); Sprouty-related, EVH1 domain-containing proteins (SPRED1, 2, 3); etc. We found all versions of the GRD AAV vectors reduced growth factor-stimulated ERK phosphorylation, proliferation of *NF1*-null immortalized human SCs and *Nf1*-null primary mouse SCs, and SC precursor (SCP) sphere formation relative to YFP control virus. These effects were dose-dependent and acted primarily by reducing proliferation rather than inducing apoptosis. Dominant negative KRas and HRas were also efficacious in ERK phosphorylation, proliferation, and SCP sphere formation assays.

In vivo assessment of several naturally occurring and evolved AAV serotypes was performed in a mouse model of PN (*Nf1*^{floxflox}*;Dhh*-Cre mice) using a GFP reporter vector containing a strong, ubiquitous promoter (AAV-CAG-GFP-WPRE-pA, 3 x 10^{12} vg/mouse, r.o.). Most candidate capsids failed to demonstrate meaningful SC tropism after intravenous administration. AAV-Pep2hSC1, a capsid evolved *in vitro* to target human SCs, proved most effective, but transfection efficiency remained low. Cre inducible overexpression of the AAV receptor (AAVR) gene *(AU040320)* in *Dhh* lineage cells significantly improved SC transduction by AAV-Pep2hSC1 variant compared to other AAVs. At the 2025 NF Conference, we will report preliminary results of *in vivo* efficacy studies using the *Nf1*^{floxflox}*;Dhh*-Cre;AAVR mouse model and AAV-Pep2hSC1 vectors. Overall, these studies represent continued progress toward the development of a diverse set of AAV gene therapies in NF1.

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Identifying New Therapeutic Targets for Neurofibromatosis Type 1 Using *Drosophila* Models

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Purpose: Neurofibromatosis type 1 is a common genetic disorder, resulting in tumours of the skin and nervous system as well as various neurological manifestations. Despite approval of selumetinib for the treatment of inoperable plexiform neurofibroma, few therapeutic strategies exist for other symptoms. We therefore aimed to identify novel potential therapeutic targets for the treatment of neurofibromatosis type 1.

Methods: The NF1 protein is well conserved in *Drosophila* (gene name *Nf1,* amino acid conservation >50%). We used a previously described *Drosophila* strain with knockout of the *Nf1* gene caused by a nonsense mutation *($Nf1^{E2}$).* Neuronally-mediated phenotypes were tested using body weight (measured by a tube weighing robot, Biomicrolab XL9), wing area (light microscopy) and feeding (measured using capillary feeding, CAFÉ, assay).

Results: We have confirmed that knockout of *Nf1* in Drosophila results in two easily measured phenotypes: reduced body size (measured as a reduction in body weight and wing area) and overfeeding. Both phenotypes are rescued by re-expression of wild-type Nf1 specifically in neurons. Re-expression of Nf1 with a patient-associated mutation in the GAP related domain rescues less potently, suggesting that these phenotypes are at least partly Ras-dependent.

We have conducted a large-scale genetic screen using body weight as a read-out. We generated flies with homozygous knockout of *Nf1* and heterozygous knockout of candidate protein kinases. So far, we have screened >120 kinases (~50% of protein kinases in the *Drosophila* genome). We aim to screen ~180 kinases (~75% of protein kinases in the *Drosophila* genome). We identified several kinases which increase body size in *Nf1* knockout but not wild-type flies. Heterozygous mutation of *par-1* (human orthologue *MARK1*), a microtubule regulating kinase, rescued defects in body size and feeding observed in *Nf1* knockout flies. We are currently assessing whether neuronal knockdown/overexpression of *par-1* affects *Nf1* loss-of-function related phenotypes. *par-1* and other candidate proteins will be pharmacologically inhibited in human cellular models.

Conclusions: We are performing a large-scale genetic screen looking for novel modifiers of neuronal dysfunction in *Drosophila* with knockout of *Nf1.* We have identified candidate genes, including *par-1* and are in the process of further validating these hits.

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Promising Efficacy of LIMK Inhibitors in Reducing Cutaneous Neurofibromas in an *Nf1*-KO Mouse Model

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Cutaneous neurofibromas (cNFs) affect 95% of individuals with neurofibromatosis type 1 (NF1), significantly impacting their quality of life. Our laboratory has developed an *Nf1*-KO mouse model that faithfully recapitulates these tumors, providing a robust platform for testing candidate compounds. We previously demonstrated that tumor Schwann cells (SCs), the cells of origin for cNFs, exhibit elevated levels of phosphorylated cofilin (p-Cofilin), a key marker of an active LIMK pathway, in both mouse and patient-derived cNF (**Figure 1**).

The aim: To evaluate the efficacy of two LIMK inhibitors (LIMKi), M1 and M2 (patent: WO2021/239727A1), in reducing cNF size in the *Nf1*-KO mouse model.

Groups of 12 six-month-old mice were treated with either M1, M2, or a placebo (P) for one month. Treatment efficacy was assessed through: (1) macroscopic analysis of 2D epifluorescence images of the back skin, taken before and after treatment, leveraging the Tomato fluorescent reporter expressed in NF1 mutant SCs; and (2) immunofluorescent (IF) profiling of cNF sections post-sacrifice using a panel of SC and microenvironmental markers. Automated image analysis was used. Animal well-being and treatment tolerance were also monitored.

While cNFs in the M1 and P groups increased in size over the treatment period, the M2 group showed a reduction compared to P (**Figure 2**): decrease in total fluorescent surface area ($p=0.059$) and fluorescence intensity ($p=0.003$). P-Cofilin staining was reduced in cNFs from both M1- and M2-treated mice, confirming LIMKi efficacy in inhibiting LIMK1/2-mediated cofilin phosphorylation. M1 treatment did not significantly alter tumor SC morphology or density, whereas M2 led to a significant decrease in SC density ($p=0.012$), along with morphological changes such as a flatter, bipolar shape and retraction of cytoplasmic protrusions (Fig 3). Additionally, periostin expression in tumor SCs was significantly reduced in the M2 group ($p=0.0449$) but remained unchanged in M1. Ki67 staining was comparable across treatment groups. Analysis of the tumor microenvironment revealed a reduction in CD45+ immune cells in M2-treated mice compared to P ($p=0.033$).

This preclinical study demonstrates that M2 significantly reduces cNF in the *Nf1*-KO mouse model. Given that the LIMK pathway regulates cell communication, motility, and morphology via actin cytoskeleton dynamics—rather than directly influencing cell survival or proliferation—its inhibition presents a promising therapeutic strategy for cNFs, where tumor cell proliferation is minimal in mature lesions. These findings support further studies to validate the efficacy of M2 and its potential translation to clinical trials in humans.

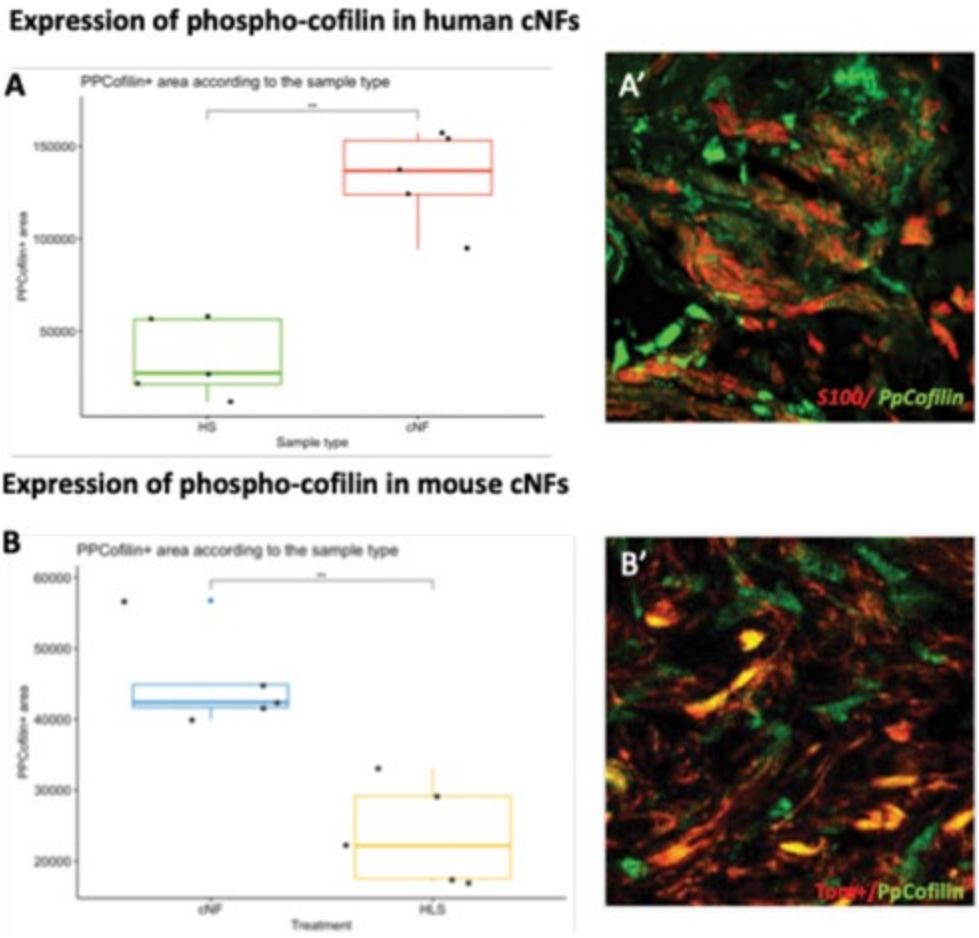


Figure 1: Expression of P-Cofilin in tumor SCs from patients and NF1-KO mice: Quantification of P-Cofilin+ area from cNFs and adjacent healthy-looking skin from patients (A) and Nf1-KO mice (B), (Statistic test: Wilcoxon test, Bonferroni FDR-adjusted p-value: > 0,05, **> 0,005 comparing to placebo group). **(A')** IHC on cNFs from patients with markers of P-cofilin and Schwann cells (S100), (B') IHC on cNFs from mutant mice with markers of p-cofilin and Schwann cells (Tom)

Figure 2: Quantification of Tom+ areas using automatic imaging analysis script. 2 parameters: (A) total fluorescent surface area (TFSA) and (B) fluorescence intensity were used for each group of mice, then represented in a Box and Whisker plot. (Statistic test: Wilcoxon test, Bonferroni FDR-adjusted p-value: * > 0,05, **> 0,005).

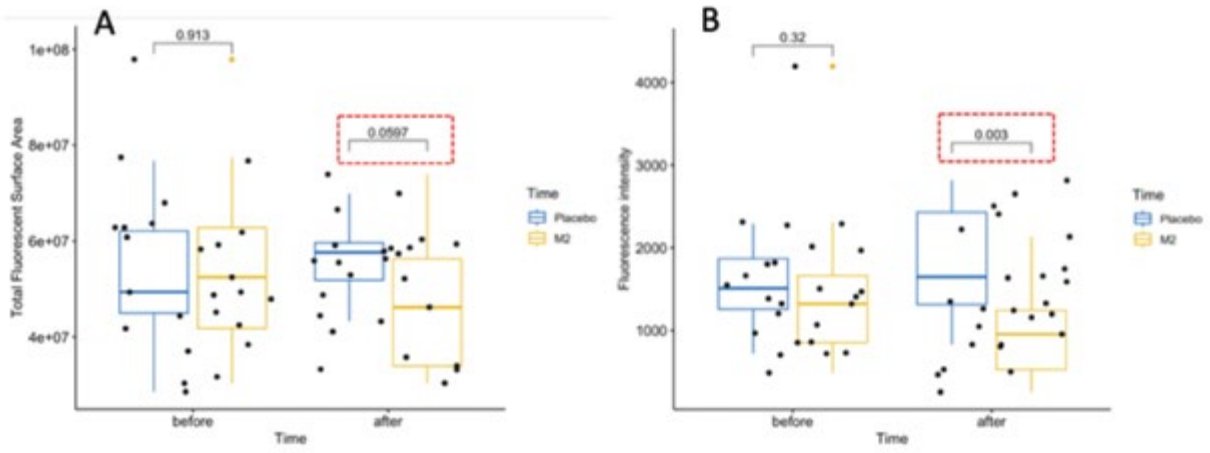
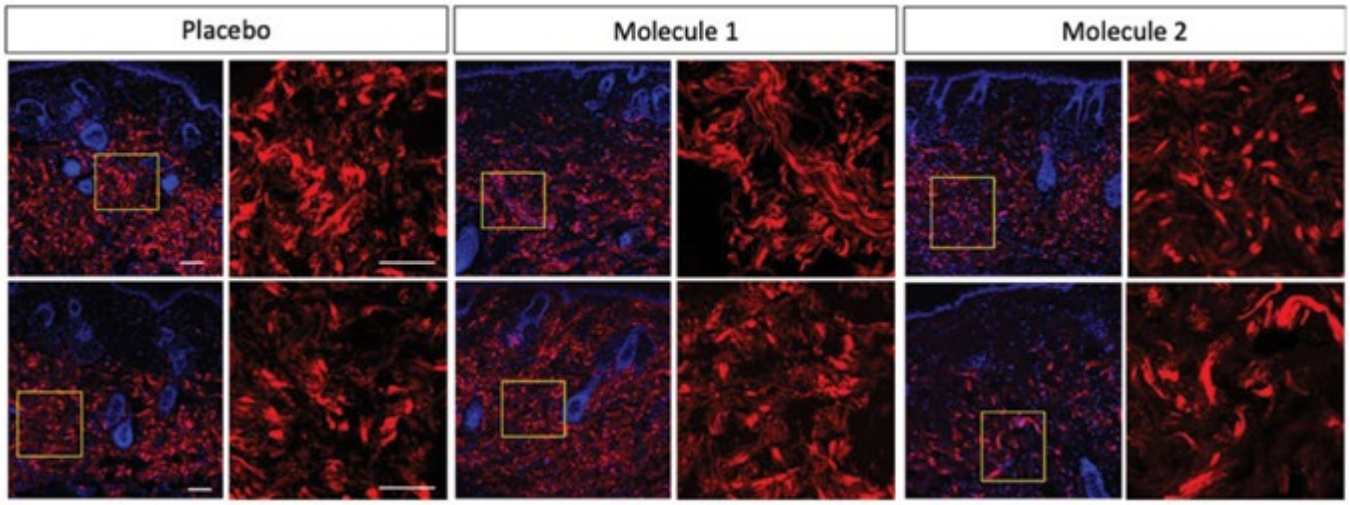


Figure 3: Immunofluorescence on cNFs sections with markers against tumor SC (Tom+) and nuclei (DAPI).



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Emerging Mechanism and Therapeutic Potential of Neurofibromatosis Type 1-Related Nerve System Tumor: Advancing Insights into Tumor Development

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Neurofibromatosis Type 1 (NF1) is a genetic disorder resulting from mutations in the *NF1* gene, which increases susceptibility to various nervous system tumors, including plexiform neurofibromas, malignant peripheral nerve sheath tumors, and optic pathway gliomas. Recent research has shown that these tumors are intricately connected to the complex, dynamic interactions within neurons, culminating in neuronal signaling that fosters tumor growth. These interactions offer crucial insights into the molecular mechanisms underpinning tumor development, as well as broader implications for therapeutic strategies. This review summarizes the mechanisms through which mutations in the *NF1* gene within neural tissues trigger tumorigenesis, while examining the role of the neuron—via factors such as visual experience, neurotransmitter, tumor microenvironment, and psychological influences—in both promoting tumor progression and being affected by the tumors themselves. By investigating the dynamic relationship between NF1-associated nervous system tumor cells and neurons, we aim to shed light on novel biological pathways and disease processes, emphasizing the potential of interdisciplinary approaches that combine neurobiology, oncology, and pharmacology to enhance treatment strategies and even inhibit the tumorigenesis.

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Finding the Best Combination of cAMP Activation and Ras/MAPK Inhibition for Cutaneous Neurofibroma Therapy

Meritxell Carrió, PhD, *Hereditary Cancer Group, Germans Trias & Pujol Research Institute*

The development of cutaneous neurofibromas (cNFs) constitutes one of the major concerns of persons affected by Neurofibromatosis type 1 (NF1). Complete loss of *NF1* in a boundary cap-derived Schwann cells (SC) is required for cNF development. MEK inhibitors (MEKis) are being analyzed as a potential therapy for cNFs, but as for plexiform neurofibromas, despite their beneficial effect, it seems that a complete remission of these tumors will require additional therapeutic players.

We showed that the activation of the proton-sensing G protein-coupled receptor GPR68 combined with the inhibition of the Ras/MAPK pathway by the MEKi Selumetinib, induces arrest and activate myelination program of cNF-derived SCs, as well as increased cell death in cNF-derived SC cultures and human iPSC-derived *NF1*(-/-) neurofibromaspheres (Mazuelas et al. 2022, 2024). At least, part of this response is mimicked by replacing GPR68 activators (Ogerin, PAM71) by cAMP elevators (forskolin, 8CPT-cAMP) in combination with MEKi (Mazuelas et al. 2024).

The aim of this study is to exploit this therapeutic strategy for targeting cNFs by identifying the best combination of cAMP elevators and MEKis *in vitro* and *in vivo.* For that, we analyzed the expression of cAMP pathway components present in cNF-derived SC cultures and identified compounds already in the clinic that elevate intracellular cAMP levels by modulating activity of these cAMP pathway proteins. We tested eight candidates in combination with Selumetinib in cNF-derived SC cultures and in the human iPSC-based neurofibromasphere model, assessing SC differentiation, viability, and apoptosis. Top compounds are currently under further analyses.

In addition, to advance towards *in vivo* mouse experiments and to evaluate the specificity of these compounds in heterozygous vs null neurofibromin contexts, we are currently testing top candidate combinations *in vitro* in 3D neurospheres model derived from a *Prss56 Nf1*-KO cNF mouse model (Gresset, et al 2015). These neurospheres consists in sphere cultures of *Nf1*(-/-) or *Nf1*(+/-) Tomato-expressing stem like glial cells present in the skin and derived from *Prss56*-expressing boundary caps. After *in vitro* selection of best combinations, we aim to assess the best combo candidate *in vivo* in different mouse models, obtaining robust pre-clinical information for potential clinical assays.

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Predictive Modeling of Differential Targeting and Additive Effects of CDK4/6 Inhibitors in MPNST

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Purpose: The leading cause of premature death for patients with Neurofibromatosis type 1 (NF1) is the development of malignant peripheral nerve sheath tumors (MPNST). These tumors render current medical treatment strategies largely ineffective, prompting an investigation of rational targeted therapies. CDK4/6 inhibitors are favorable candidates to oppose the chronic cell cycle deregulation in NF1-related MPNST, but continued characterization of tumor adaptive responses and the polypharmacology of these drugs is needed.

Methods: Multiplexed kinase inhibitor bead (MIB) affinity chromatography coupled with mass spectrometry (MIB/MS) was used to identify target spectra of CDK4/6 inhibitors abemaciclib and palbociclib. *In vitro* changes in kinase activity and expression were also analyzed by MIB/MS following treatment of MPNST cell lines. CRISPR/Cas9 knockout and shRNA knockdown of *RB1* in MPNST cell lines were used to evaluate the dependence of CDK4/6 inhibitors on RB.

Results: MPNST cell lines demonstrated sensitivity to single agent treatment by CDK4/6 inhibition, inclusive of reduced cell viability and cell cycle entry. In MIB/MS competition assays, previously reported off-target kinases exclusive to abemaciclib were identified, while palbociclib remained selective to CDK4/6. Both inhibitors elicited diverse kinome response profiles despite a shared cell cycle arrest phenotype. Inhibition of unique secondary (non-CDK4/6) targets of abemaciclib demonstrated greater additive effects in combination with abemaciclib than with palbociclib. *RB1* knockout and knockdown MPNST cell lines exhibited resistance to palbociclib treatment, but remained sensitive to abemaciclib, in long-term exposure studies.

Conclusions: CDK4/6 inhibition continues to show promise as a targeted therapy against NF1-related MPNST with dysregulated cell cycle signaling. The discovery of additive combination therapies based on identified non-CDK4/6 kinase targets highlights the translational capacity of predictive kinome profiling. Further characterization of abemaciclib combination therapies and their *in vivo* efficacy is therefore warranted.

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Combining Selumetinib with Drugs Targeting Epigenetic Regulators in Different Cell-Based MPNST Models Representing Initial and Progressed MPNSTs

Itziar Uriarte-Arrazola, MSc, *Hereditary Cancer Group, Germans Trias i Pujol Research Institute (IGTP)*

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas originating from cells derived from the neural crest (NC), with a poor prognosis. They constitute the primary cause of Neurofibromatosis Type 1 (NF1)-related mortality. Timely resection remains the only curative approach, highlighting the need for new therapeutic strategies. Developing novel *in vitro* models that faithfully replicate MPNST genetics and biology could be key.

MPNST initiation is determined by the loss of commonly inactivated tumor suppressor genes (TSGs). We have developed iPSC-derived NC-based 3D MPNST models carrying the loss of *NF1, CDKN2A* and PRC2, with or without a heterozygous mutation in *TP53* (named 3KO+ and 3KO, respectively). Our 3D model genuinely recapitulates the glial-to-mesenchymal switch observed in MPNSTs and, form MPNST-like tumors when engrafted in the sciatic nerve of nude mice. Employing these 3D MPNST models and isogenic controls (1KO *(NF1)* and 2KO *(NF1, CDKN2A)* spheroids), we conducted a high-throughput screening of ~300 epigenetic compounds present in the NCATS (National Center for Advancing Translational Sciences) compound library. We tested the impact of these compounds on cell viability, spheroid size, and cell death using the different 3D spheroid models. Three drug classes were identified: BET inhibitors (BETi), HDAC inhibitors (HDACi) and PARP inhibitors (PARPi). Notably, PARPi appear to be more effective in 3KO and 3KO+ spheroids compared to 1KO and 2KO spheroids, suggesting a specific sensitivity for cells with no PRC2 function.

To further investigate these potential treatments, we evaluated the viability of 3KO spheroids when co-treated with the MEK inhibitor (MEKi) Selumetinib. Results were validated using three independent 3KO cell lines in 3D, representing early MPNST formation, and three established MPNST cell lines in 2D, derived from progressed MPNSTs with a general gained and rearranged genome. Toxicity was also assessed in *NF1*(+/-) fibroblasts. When combined with Selumetinib, BET and HDAC inhibitors exhibited similar responses in both models, with a slightly higher sensitivity in the 3D models. However, HDACi combos showed some toxicity in *NF1*(+/-) fibroblasts, consistent with previous reports (Shi et al, 2024). Contrarily, PARPi exhibited only a strong effect in 3D models, but almost no effect on MPNST cell lines, either as a monotherapy or in combination with Selumetinib.

Currently, we are conducting *in vitro* assays to assess cell viability and apoptosis using triple-drug treatments. Additionally, we are testing drug combinations in a patient-derived xenograft (PDX) NF1 MPNST *in vivo* model.

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High-Content Microscopy for Characterizing and Predicting Drug Response in *NF1*$^{-/-}$ Schwann Cell Cultures and NF1 Patient-Derived Tumor Organoids

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Purpose: We are extending the foundation of our drug screening program for NF1 to quantify high-content microscopy readouts of cell phenotypes.

Methods: We applied the high-content, multiplex fluorescence microscopy assay called Cell Painting[1] in two-dimensional Schwann cell cultures (**Figure 1**). We collected Cell Painting images of two isogenic Schwann cell lines, one of wildtype genotype *(NF1*$^{+/+}$*)* and one of *NF1* null genotype *(NF1*$^{-/-}$*).* Separately, we also optimized a Cell Painting assay in three-dimensional NF1 patient-derived organoids.[2] This is the first time this assay has been applied in this setting. We and others have shown that Cell Painting quantifies cell health phenotypes[3], compound mechanism of action[4], toxicity[5], cancer cell resistance[6], and provides new information not captured by molecular readouts like gene expression.[7] We developed a 3D image analysis pipeline to segment individual cells, perform quality control, and quantify cell-type specific, high-content features from drug screening NF1 patient-derived organoids.

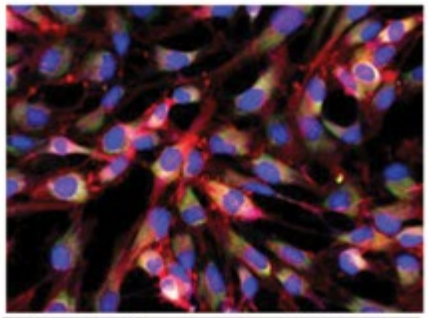

Figure 1. Representative Cell Painting images of 2D Schwann cells. Nuclei in blue, actin in red, endoplasmic reticulum in green.

Results: In 2D, we showed proof-of-concept that pairing high-content microscopy with machine learning can distinguish *NF1* genotypes in Schwann cells.[8] Our machine learning model predicted *NF1* genotype in a never-before-seen holdout test set of Schwann cells over 2x better than random (**Figure 2**). In 3D, our image analysis pipeline successfully segments and processes high-content, single-cell morphology features from organoids (**Figure 3**). We screened dozens of drugs in different NF1 patient cutaneous neurofibroma samples and quantified the phenotypic impact of drugs with different targets and mechanisms of action. All data analysis code is publicly available on GitHub to accelerate community efforts.

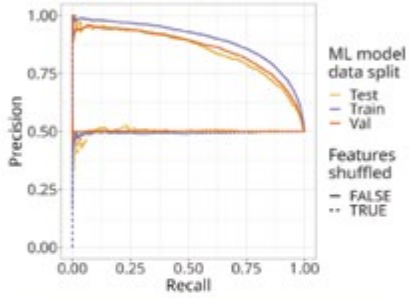

Figure 2. Precision-recall curves for predicting *NF1* genotype in Schwann cells. Each data split represents different cells. The dotted lines are models trained on randomized data.

Conclusion: The human eye can only detect large differences in fluorescence microscopy images. Instead, high-performance computing and machine learning are required to distinguish subtle but important patterns. 2D-based microscopy methods are amenable for extremely high-throughput drug screening and can identify therapeutic agents that make NF1 patient cells look healthy.[9] Our 3D-based microscopy methods can prioritize drugs in a physiologically relevant environment specific to individual NF1 patients. Combined, our phenotypic drug screening platform may help to discover new and personalized therapeutic agents for NF1 patients while avoiding drug toxicity pitfalls and side-stepping high attrition rates in traditional drug discovery pipelines.

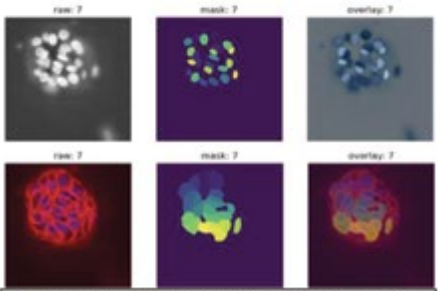

Figure 3. Example organoid segmentation. We show an example from a random z slice (z=7). Top: nuclei segmentation; bottom: whole cell segmentation.

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Disclosures: MMH and HS are employees of iNFixion Bioscience. GPW is on the scientific advisory board of iNFixion. AS is a founder and owner of Icona BioDx.

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High-Throughput Screening Identifies Polymeric Nanoparticles for Delivery of Full Length Human NF1 Gene to Schwann Cells

Cherry Gupta, *Battelle Memorial Institute*

Purpose: Polymeric nanoparticles (PNPs) are modular delivery systems that can be optimized for targeted therapeutic delivery. Advances in rational design, including high-throughput screening and computational approaches, have shifted nanoparticle development from trial-and-error methods to structured, data-driven strategies. This has significantly improved preclinical discovery pipelines for nanomaterials. Here, we employ a high-throughput design-build-test-learn platform to enhance the delivery efficiency of PNPs for the treatment of Neurofibromatosis Type 1 (NF1), a genetic disorder caused by the loss of functional neurofibromin. NF1 gene replacement therapy holds immense clinical potential but is hindered by challenges in delivering the large 8.5 kbp *NF1* gene using viral vectors. To overcome this, we utilized cationic PNPs for encapsulating and delivering the full-length human NF1 plasmid (EF1a-NF1-2A-EGFP, ~20 kbp) to human Schwann cells (SCs).

Methods and Results: Designing the perfect PNP is complex due to vast chemical diversity, biological system intricacies, and current modeling limitations. To address this, we used our High-Throughput Synthesis, Characterization, and Assessment of Nanoparticles (HIT SCAN™) platform to systematically screen over 500 different PNPs and identify the most promising "hit" candidates. The polymers were synthesized via reversible addition-fragmentation chain-transfer (RAFT) polymerization and self-assembled into PNPs. Our approach consisted of two rounds of screening.

In the first round, we screened ~450 first-generation (G1) PNPs derived from six selected monomers to assess plasmid loading, transfection efficiency (TE), and cytotoxicity in immortalized human NF1-null SCs (hTERT NF1-/- ipNF97.4). Select PNPs with specific monomer combinations emerged as promising candidates for plasmid encapsulation and delivery. **Twenty hit PNPs** demonstrated partial neurofibromin restoration in SCs. *In vivo* testing confirmed gene expression in mice and rat cortex, but G1 PNPs exhibited insufficient TE to fully restore neurofibromin function.

In the second round, a machine learning (ML)-guided approach was used to refine the design space. An ML model, trained on successful G1 candidates, identified key structural and compositional features, leading to a second-generation (G2) of 96 PNPs. G2 PNPs exhibited a **5x increase in TE in SCs**, from ~4% in G1 to ~20% in G2. Functional assays assessing biodistribution, delivery efficacy, and neurofibromin restoration in vivo are ongoing.

Conclusion: By combining our HIT SCAN™ platform with data-driven PNP evolution, we identified novel PNPs capable of efficiently delivering the large NF1 gene to SCs. The **5x improvement in TE** in G2 PNPs underscores the potential of high-throughput screening for accelerating nanomedicine development for genetic disorders like NF1.

Funding: This work is supported by a grant from the Gilbert Family Foundation Gene Therapy Initiative.

Discovery of Bicyclic Peptides that Engage Wild-Type KRAS and Block Downstream Signaling in NF1$^{(-/-)}$ Schwann Cells

Sandeep Lohan, *Virginia Commonwealth University, Richmond, VA*

Overactivation of Ras is the primary basis for neurofibromatosis type 1 (NF1) disease. Despite intense drug discovery efforts, Ras proteins have proven to be difficult targets for small molecule drug discovery. Therapies for NF1 have; therefore, resorted to targeting kinases involved in downstream signaling which are more "druggable." Macrocyclic peptides as a therapeutic modality are attracting increasing attention due to their ability to bind to targets intractable to small molecules; however, lack of cell permeability is an ongoing challenge. To find peptides that engage KRAS in cells, we used mRNA display to generate bicyclic libraries, with one cycle containing a short, cyclic cell penetrating peptide to enable cellular entry. After 7 rounds of in vitro selection, we identified putative hits from next generation sequencing (NGS) data. After scale up synthesis, surface plasmon resonance (SPR) and biolayer interferometry (BLI) data revealed Kds as low as 100-200 nM against the GDP-bound and GcP-bound conformation of WT KRAS. Treatment of IPN 97.4 NF-1$^{(-/-)}$ Schwann cells with our peptides at micromolar concentrations led to a reduction of phospho-ERK in a time and dose-dependent manner. Similar effects were observed on cancer cell lines with aberrant KRAS signaling. Together, our data highlights the power of mRNA display to find macrocyclic peptides with dual properties of cell penetration and target binding, and provides leads for future drug discovery for molecules that directly engage Ras proteins for the treatment of NF1.

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Targeting NAD Metabolism as a Therapeutic Strategy for NF1-Associated High-Grade Gliomas

Swati Dubey, *Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California Los Angeles*

Background and Purpose: NF1-associated high-grade gliomas (HGGs) frequently exhibit ATRX loss and CDKN2A deletions, leading to leading to genomic instability and altered metabolic states. Given the essential role of NAD^+ in cellular metabolism and DNA repair, we investigated NAD metabolism as a therapeutic target in NF1-associated HGGs, by inhibiting nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme in the NAD synthesis salvage pathway.

Methods: We evaluated the sensitivity of NF1-associated HGG cell lines (JHH-NF1-GBM1 and TM31) to NAMPT inhibitors FK-866 and GNE-617. Immortalized human astrocytes served as non-cancerous controls. The impact of NAMPT inhibition on temozolomide (TMZ) sensitivity and MEK/ATR inhibitor synergy was also evaluated.

Results: NF1-associated HGG cell lines exhibited high sensitivity to FK866 and GNE-617, with IC50 values range between 2-4 nM, while normal human astrocytes remained unaffected at concentrations beyond 100 nM **(Figure 1a)**. NAMPT inhibition significantly sensitized JHH-NF1-GBM1 cells to TMZ, reversing intrinsic resistance **(Figure 1b)**. Combination treatment with NAMPT and MEK/ATR inhibitors displayed strong synergistic effects **(Figure 1c)**.

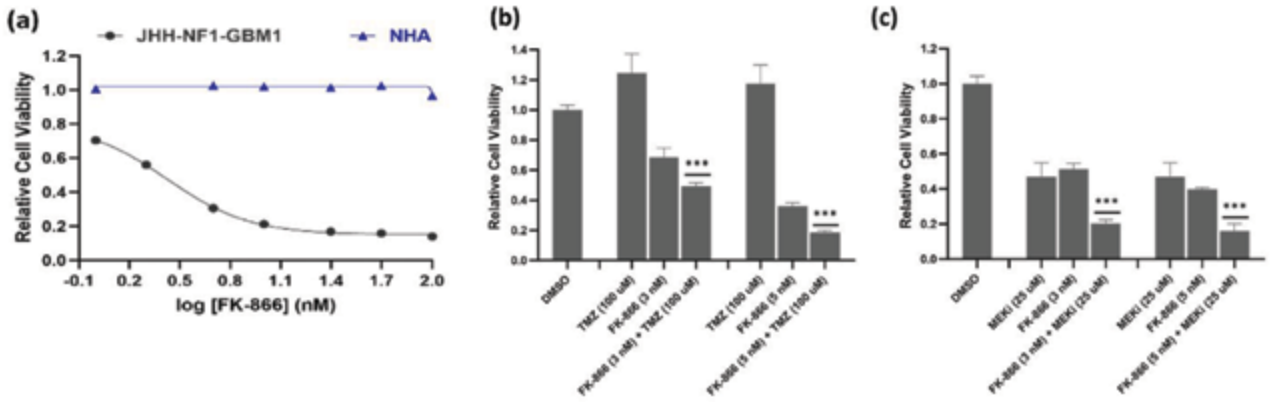

Figure 1: (a) Dose-response curves showing high sensitivity of JHH-NF1-GBM1 (NF1-associated HGG cell line) to NAMPT inhibitor FK866, with minimal effect on NHA (immortalized normal human astrocytes). NAMPT inhibition sensitizes JHH-NF1-GBM1 cells to TMZ, overcoming TMZ resistance (b) and shows strong synergy with MEK inhibitor AZD 6244 (c). (n=4, Mean±SD, Statistical significance was determined using a two-tailed Student's t-test)

Conclusion: By selectively targeting tumor cells while sparing normal astrocytes, NAMPT inhibitors provide a therapeutic approach that warrants further investigation and support the rationale for developing combinatorial treatments, integrating standard and targeted therapy with NAMPT inhibitors to enhance therapeutic efficacy in NF1-associated HGGs.

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Identification and Functional Analysis of Novel Neurofibromin-Interacting Proteins

Alex Dyson, PhD, *Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA*

The *NF1* gene encodes the protein neurofibromin which functions as a RAS GTPase-activating protein (RAS-GAP). The GAP-related domain (GRD) of neurofibromin is essential for its role in catalyzing the hydrolysis of active Ras-GTP into inactive Ras-GDP. Pathogenic missense mutations within the GRD have been characterized that affect neurofibromin's catalytic activity or ability to bind RAS.

Drosophila (fruit fly) models of NF1 display several robust neurobehavioral phenotypes resembling clinical symptoms of the disorder. High conservation between human *NF1* and its fly ortholog *(dNf1)* at the amino acid level facilitates modelling of patient-derived missense mutations in this model organism. We have modeled pathogenic GRD mutations in *Drosophila* to evaluate their functional impact at the molecular, anatomical and neurobehavioral levels.

We found that dNf1 transgenes bearing GRD mutations fail to rescue *dNf1* phenotypes including growth, sleep, and circadian rhythmicity deficits. For proteomics study using affinity purification-mass spectrometry, we selected three mutations known or predicted to affect GAP activity, either affecting the active site (R1276P) or RAS-binding regions (R1391S, K1423E). Curiously, we found that GRD mutant neurofibromin revealed significantly more binding partners than wild type. These additional proteins included known interactors of NF1 (e.g. Spred), components of the Ras signaling pathway (e.g. MEK), several small GTPases, as well as novel uncharacterized proteins. We speculate that neurofibromin with impaired GAP activity serves as a 'protein trap', allowing transient interactions between neurofibromin and its associated proteins to be captured.

Putative neurofibromin-interacting proteins were selected for functional analysis, based on a) possessing phosphatase or kinase activity, b) membrane localization, or c) being a hit in all three mutant lines. Our follow up studies use the power of *Drosophila* genetics to confirm whether these genes function in the same pathway as *dNf1,* either in concert or antagonistically, to regulate *dNf1*-dependent growth, sleep and cognition. Finally, we are testing whether novel dNf1 interactors identified in our fly "protein trap" proteomics analyses are conserved between the corresponding human orthologs of these proteins and neurofibromin.

Our approach has identified novel interacting protein partners of neurofibromin using *Drosophila* models of NF1. This work will potentially increase our understanding of the mechanisms underlying neuronal dysfunction in NF1, as well potentially identify new therapeutic targets.

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Funding: Children's Tumor Foundation Young Investigator Award (YIA).

Targeting Stem-Like Cells in Plexiform Neurofibroma: Big Data-Driven Discovery and *In Vivo* Validation of HSP90 Inhibition

Sajjad Khan, PhD, *Medical College of Wisconsin*

Background: The paucity of patients limits research in rare diseases like plexiform neurofibroma (PN). Access to biomedical big data resources, such as the NF Data Portal, enables integrated data mining, significantly expediting drug discovery for PN. This study combines single-cell RNA (scRNA) sequencing, bulk PN sequencing, cell modeling, drug screening, gene regulatory network analysis, and drug combination prediction to characterize PN tumors and identify novel treatments. HSP90 inhibitors (HSP90i) are prioritized for targeting stem-like tumor cells in PN, with SNX-2112 showing promise as a therapeutic candidate. The combination of HSP90i with Selumetinib may overcome the limited efficacy and long-term toxicity of Selumetinib monotherapy.

Purpose: This study aims to validate the vulnerability of stem-like PN tumor cells to HSP90i and assess the therapeutic potential of HSP90i alone and in combination with Selumetinib. Importantly, we extend our findings to an in vivo model to evaluate the efficacy of SNX-2112 in tumor inhibition.

Methods: scRNA sequencing was used to characterize PN tumor heterogeneity and identify stem-like populations. Gene regulatory network analyses of bulk sequencing data characterized conserved signaling pathways among PN cell lines and primary tumors. Drug screening studies were integrated with data mining to prioritize four HSP90i candidates (SNX-2112, SNX-5422, Retaspimycin, and Geldanamycin). Cell viability assays were conducted across five PN cell lines to assess the single-agent and combinatorial effects of HSP90i with Selumetinib. Synergy analysis was performed to determine the efficacy of combination therapy. To extend these findings, an in vivo PN model expressing luciferase was used to evaluate the efficacy of SNX-2112 alone and in combination with selumetinib. Bioluminescence imaging was performed to monitor tumor growth.

Results: SNX-2112 demonstrated potent inhibition of stem-like PN tumor cells. SNX-2112 treatment resulted in a marked decrease in luciferase signal in vivo compared to the control cohort, indicating significant delay in tumor growth. It also exhibited synergy with Selumetinib, significantly reducing Selumetinib dosage requirements, in vitro and in vivo.

Conclusion: Integrated data analysis with different data types is a powerful approach for identifying therapeutic targets in PN. SNX-2112 effectively targets stem-like tumor cells and, in combination with Selumetinib, reduces drug dosage requirements while enhancing tumor inhibition. Crucially, in vivo studies confirm the efficacy of SNX-2112 in suppressing PN tumor growth alone and in combination with selumetinib, supporting its potential as a therapeutic strategy.

Funding: This study is funded by Neurofibromatosis Therapeutic Acceleration Program at John Hopkins.

Selumetinib + Montelukast: Shrink Plexiform Neurofibroma with the Combination of Two FDA-Approved Drugs

Oluwatosin Aina, *Medical College of Wisconsin*

Background: Current treatment with MEK inhibitors shows limited efficacy and multiple long-term adverse effects in pediatric patients with Plexiform Neurofibroma (PN), highlighting an unmet need for novel therapies. Monocytes and macrophages comprise up to 30% of the PN tumor mass and influence tumor growth and drug response. Our award-winning integrated drug mining identified montelukast, an FDA-approved pediatric anti-asthma drug, combined with selumetinib, can significantly reduce the PN tumor size and reprogram the myeloid population within the tumor microenvironment. Montelukast is a cysteinyl leukotriene receptor-1 (CysLTR1) antagonist with a long-standing safety profile that inhibits infiltration of macrophages and monocytes into the lungs of pediatric asthmatic patients.

Purpose: We aim to repurpose montelukast for a novel combination therapy with selumetinib to improve PN inhibition and reduce the selumetinib dosage.

Methods: Employing cell viability assays, we investigated the effect of montelukast and its combination with Selumetinib on well-characterized PN and Schwann cell lines. Macrophage phenotype reprogramming was evaluated by gene expression level (RT-qPCR), and macrophage infiltration was investigated using the transwell migration assay. The effect of the drug combination *in vivo* was tested on the transplanted mouse tumor from *DhhCre;Nf1$^{fl/fl}$;Luc* model. Bioluminescence was used to monitor the tumor growth in the control, selumetinib, and combination-treated cohorts. Tumor sizes were determined at the end time point, and flow cytometric analysis was used to characterize the macrophages in the harvested tumors.

Results: Our studies show that montelukast significantly reduces the viability of PN tumor cells compared to Schwann cells, promotes an anti-tumor phenotype in macrophages, and inhibits macrophage migration. *In vivo* and *in vitro* drug combination tests show that montelukast enhances selumetinib's anti-tumor activity. While selumetinib alone (50 mg/kg/day p.o) caused 27% ($n=5$, $p=0.225$) decrease in mean tumor volume compared to the control, the combination with montelukast (40 mg/kg/day i.p. + selumetinib 40 mg/kg/day p.o.) reduced mean tumor volume by 69% ($p=0.002$). Moreover, montelukast significantly decreased the percentage of TAMs in the residual tumors.

Conclusions: These findings strongly demonstrate that montelukast can be repurposed in combination therapy with Selumetinib in clinical trials to improve treatment efficacy and reduce selumetinib dose and toxicity in PN patients. Montelukast, with its established safety profile for over two decades and its dual anti-tumor and immunomodulatory effects, holds significant promise for the early treatment of PNs before malignant transformation. More so, unlike novel drug development, our drug repurposing approach potentially offers a faster process and more cost-effective treatment for PN.

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A Novel NF1 Optic Pathway Glioma Mouse Model Reveals Visual Deficits Independent of Retinal Ganglion Cell Loss

Brent K Young, *Stanford University*

Purpose: Neurofibromatosis type-1 (NF1) is a genetic disorder caused by mutations in the neurofibromin tumor suppressor gene. 15-20% of patients with NF1 may form optic pathway gliomas (OPGs), which frequently result in visual deficits due to damage to retinal ganglion cells (RGCs) whose axons travel through the optic nerve. Current treatments for vision loss in NF1 have poor outcomes; therefore, new therapies are needed. One of the barriers to treatment development is the animal models available. While human OPGs are found throughout the optic pathway, OPGs in current mouse models are restricted to near the optic chiasm. Additionally, these models produce inconsistent (RGC) death and vision loss making treatments difficult to study. Here we describe the characteristics of a new mouse model of NF1 that presents more human-like phenotypes, including gliomas throughout the optic pathway, myelination defects, RGC loss, and visual deficits.

Methods: We crossed two available mouse lines, one that is hemizygous for NF1 ($NF1^{KO/fl}$) with another that expresses GFAP:Cre in astrocyte precursor cells at E11.5 ($NF1^{KO/fl}$; GFAP:Cre). This double knockout of NF1 in astrocyte precursors is responsible for the astrogliosis and glioma formation in the optic nerve. We then evaluated their visual defects, glioma development, and RGC loss. To evaluate vision, we used optomotor reflex, which measures visual acuity, pupillary light reflex, to evaluate light sensitivity, and looming object fear response to assess contrast detection. We measured glioma size, myelination, in nerve samples, and immunofluorescence staining was used both nerve and retina tissue to identify cell types and numbers.

Results: By two months of age, $NF1^{KO/fl}$; GFAP:Cre mice have OPG formation. Additionally, while all $NF1^{KO/fl}$; GFAP:Cre mice have gliomas, 22% of the optic nerves have OPGs outside of the area near the chiasm as is traditionally observed in NF1 mouse models **(Figure 1)**. Furthermore, 78% of nerves have severe hypomyelination compared with 0% of littermate controls **(Figure 1)**. By 4 months of age, immunofluorescence labeling of RGCs showed 16% lower RGC density with some regions of the retina having over 50% loss. Surprisingly, we found that RGC loss does not correlate with visual deficits. We found that both two- and four-month-old mice have severe visual deficits including reduced spatial frequency and looming object detection, but the younger mice have no reduction in RGC density compared to age matched controls.

Conclusions: This new hybrid NF1 mouse strain provides phenotypes similar to those in human patients including glioma formation, hypomyelination, and visual deficits. Furthermore, it appears that visual deficits in NF1KO/fl; GFAP:Cre mice are due to disruptions in neural signaling or circuitry since they are independent of the RGC loss that occurs at later ages. Therefore, effective strategies must focus on restoring normal circuit functions, such as promoting axon myelination, rather than only on tumor reduction or neuroprotection.

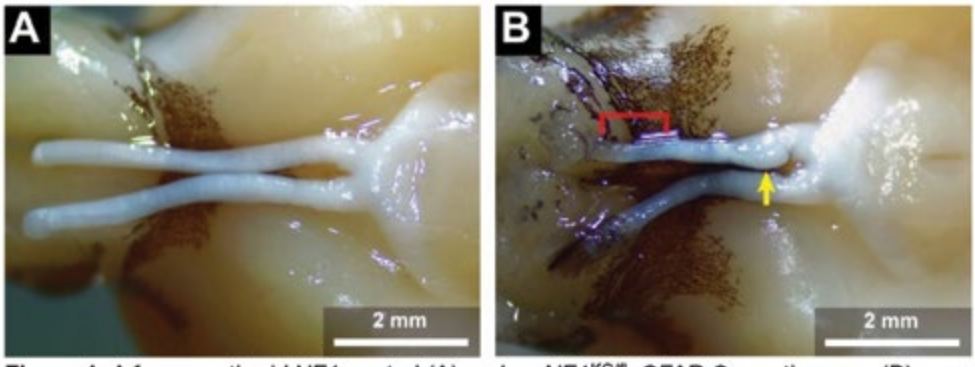

Figure 1: A four-month-old NF1 control (A) and an $NF1^{KO/fl}$; GFAP:Cre optic nerve (B) showing large glioma formation (yellow arrow) and hypomyelination (red bracket).

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Use of a p120RasGAP Glue to Inhibit KRAS in NF1-Null Cells

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder that is associated with a spectrum of pathologies including glioma, autism spectrum disorder, Lisch nodules of the iris and neurofibroma. The *NF1* gene encodes neurofibromin, a Ras GTPase-activating protein. Deletion or disabling mutations in *NF1* gene result in an absence of neurofibromin and sustained Ras activation, driving uncontrolled cell proliferation and tumorigenesis. Targeting the Ras protein directly has until recently proved challenging due to the lack of binding cavities on molecular surface and due to its high affinity for GTP/GDP.

Purpose: Our study aims to evaluate the efficacy of novel molecular glues designed to modulate Ras activity by increasing the binding of Ras to p120RasGAP, a ubiquitously expressed negative regulator of Ras that augments the GTPase activity of Ras, assisting in the conversion of active Ras-GTP into the inactive, Ras-GDP form. The goal is to use these compounds to enable p120RasGAP to functionally replace neurofibromin in *NF1*-deficient cells, restoring normal Ras signaling.

Methods: We used an artificial intelligence/machine learning (AI/ML) protocol to identify potential molecular glues that should stabilize the p120RasGAP/Ras complex. To assess the impact of these compounds, we used NF1-deficient cell line and employed Western Blot analysis to measure changes in phosphorylated ERK (p-ERK) levels as a downstream marker of Ras activity. In addition, we performed MTT assays to determine the effects on *NF1*-deficient cell proliferation. Finally, we used shRNA-mediated knockdown of the RASA1 gene, which encodes p120RasGAP protein, to verify whether the observed effects are dependent on the proposed Ras/RasGAP interaction mechanism.

Results: Preliminary data indicate a reduction in p-ERK levels upon treatment with novel compounds, suggesting partial suppression of Ras signaling. Furthermore, we achieved a functional knockdown of the *RASA1* gene and subsequent loss of the p120RasGAP protein. In knockdown cells, the molecular glues had a less potent effect in reducing p-ERK levels, suggesting that our compounds act through the proposed mechanism rather than producing off-target effects.

Conclusion: AL/ML methods can be used to design molecular glues that stabilize the interaction of p120RasGAP to Ras, providing a promising avenue for targeted NF1 therapies. Such compounds might be useful in reducing Ras activity in NF1-mutant cells.

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HLX-1502: A Novel Potential Treatment for Neurofibromatosis Type 1 Plexiform Neurofibromas

Svetlana Saveljeva, PhD, *Healx Ltd*

HLX-1502 is currently being investigated in an open-label Phase 2a trial (INSPIRE-NF1) to evaluate safety and efficacy in patients with neurofibromatosis type 1. Identified using Healx's AI-informed drug discovery approach, HLX-1502 has a novel mechanism of action, and is supported by data that suggest a favourable safety profile.

HLX-1502 significantly reduces nerve volume and tumour number in the *Postn-Cre*$^{+}$ *Nf1*$^{fl/fl}$ mouse model of plexiform neurofibroma. The level of efficacy observed in these models is equivalent to selumetinib. Notably, HLX-1502 does not inhibit the RAS/MAPK pathway, instead acting through a number of pathways affecting mitochondrial function and causing changes to cellular bioenergetic capacity. Prolonged treatment with HLX-1502 induced cellular senescence of Schwann cells, and impaired recovery of cellular viability/re-growth upon treatment washout, when compared to selumetinib. Over time, following repeat dosing, HLX-1502 accumulates in the nerve tissue, demonstrating a 45-fold higher half-life when compared to plasma. Finally, analysis of single cell transcriptional signatures from nerve tissues highlighted a number of pathways affected by HLX-1502 treatment, contributing to efficacy *in vivo.*

Efforts to further understand mechanisms behind the activity of HLX-1502 in NF1 are ongoing, as well as additional studies to determine its potential in other NF1 subindications and in tumour prevention.

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Disclosures: Simone Manso, Emma Davies, Triin Tammsalu, Svetlana Saveljeva, Meera Raja, Ivan Angulo-Herrera, Ross Mills, David Bedford, Ian Roberts, Dan Mason, Jane Brennan, Alexander Syme, Emma Tulip, Robert Wilson, Neil Thompson & Dave Brown
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Exploring the Potential of Personalised Cancer Vaccines *Aimed at Treating* NF1-Related Tumours and Premalignant Lesions

Ashwin Adrian Kallor, *Arvita, Victoria BC, Canada*

Purpose: Cancer vaccines hold immense therapeutic potential and are poised to revolutionise cancer therapy. Despite this, vaccines have been approved for only a few cancers, with rare cancers completely overlooked. To overcome this, we sought to identify potential peptide vaccine candidates for neurofibromatosis type-1 (NF1), a tumour-predisposition syndrome with birth prevalence of 1/3,000-1/4,000.

Methods: NF1 mutations arise in neural-crest–derived tissues and symptoms often manifest in childhood. Sequencing data taken from neurofibromatosis patients was analysed to identify somatic variants and aberrant splicing events. These variants were translated in silico to obtain a list of neoantigens. In parallel, an analysis of aberrant splicing in the syndrome was conducted in order to identify potential neoantigen targets in the dark proteome, searching for non-canonical neoantigens. The coding and "non-coding" genomic regions obtained from the RNA-Seq data were subjected to translation to obtain a separate list of "non-canonical" peptides.

Results: Resulting aberrant peptide lists were searched against a collection of publicly available immunopeptidomic mass spectrometry datasets, in order to prioritize aberrations in regions with broad and confirmed immune-visibility. Only those aberrant peptides that were observed to bind weakly to strongly to the Major Histocompatibility Complex Class I protein (MHC-I) were selected and further scored by population visibility, and mutation recurrence. We have developed a comprehensive list of potential neoantigens from coding and non-coding regions identified in this publicly available cohort.

Conclusion: We have developed a novel vaccine target identification system that elucidates the immunopeptide landscape of the major types of NF1 related tumours and pre-malignant lesions. We plan to advance these through pre-clinical trials to demonstrate that they are indeed presented.

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RNA Therapeutic Approaches for Neurofibromatosis Type 1

Santiago Vernia

Neurofibromatosis 1 (NF1) is an autosomal dominant disorder with a reported incidence of 1 in 3000 births. NF1 is caused by loss-of-function mutations in the *NF1* gene which encodes neurofibromin, a tumour suppressor with RAS-GTPase activity. For this reason, DNA editing, or *NF1* re-expression in Schwann cells would be plausible therapeutic options. However, delivery vectors efficiently targeting Schwann cells have not been described, limiting the applicability of such strategies.

Exosomes (EVs) are a subset of extracellular vesicles, with a diameter of 40-160 nm and characterised by the membrane enrichment of tetraspanins such as CD63.

EVs mediate delivery/uptake of different cargoes contributing to cell-to-cell communication in health and disease. For this reason, there is an increasing interest in their use as diagnostic markers and delivery vectors. However, technical limitations such as exosome´s heterogeneity and limited targeting options have hampered their clinical application.

To overcome such limitations, we have engineered a chimeric variant of CD63, designed to promote RNA loading, specific cell targeting and homogeneity of EVs, named Trispanin RNA-loading and -targeting Exosomes (T-REX).

This construct consists of an external domain displaying targeting ligands and FLAG-tag for EV tracking and purification, and an intraluminal domain containing the L7Ae protein (previously described), for the exosomal loading of C/D_{box}-containing mRNAs.

RNA and single molecule microscopy analysis confirmed that T-REX EVs are able to efficiently load the mRNA of interest. Moreover, different mRNA payloads including nLuc, Mango aptamers and NF1-GRD (GAP related domain) are transferred to Schwann cells *in vitro,* and protein expression confirms endosomal escape.

Our short-term plan is to test the biological effect of NF1 GRD-expressing exosomes in cells and mouse models of NF1, and evaluate the potential applicability of this strategy to other pathologies associated with Schwann cells.

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Extending the Life of an Acute NF1 Model Through Nonsense Suppression

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20% of Neurofibromatosis 1 (NF1) patients carry a germline nonsense mutation in the *NF1* gene, which encodes neurofibromin. This class of mutation generates a premature termination codon (PTC) in the transcribed mRNA, which typically prevents protein expression. Suppressing translation termination at the resulting PTC, a process called readthrough, can rescue partial levels of full-length, functional protein expression. Small molecules have been identified that promote readthrough of NF1-associated PTCs. To test the efficiency of readthrough compounds in an *in vivo* NF1 model, we created an acute CAGGs-CreERT2 (*Nf1*$^{Flox/R816X}$) NF1 mouse model that carries one floxed WT *Nf1* (*Nf1*^{Flox}) allele and a second *Nf1* allele bearing a PTC at codon R816 (*Nf1*^{R816X}). This model is necessary because mice homozygous for *Nf1* PTCs are inviable. The acute NF1 mice express Cre systemically; administration of tamoxifen induces transport of Cre into the nucleus to excise the WT *Nf1*^{Flox} allele, leaving expression of only the *Nf1*^{R816X} allele. Upon tamoxifen administration, these mice begin losing weight within a few days and are humanely euthanized when their initial body weight is reduced by 20% (around 2-weeks later). The purpose of the current study is to: 1) find alternative points of readout for NF1 restoration in the mouse model, and 2) determine whether small molecules can extend the lifespan of the acute NF1 mouse model *via* nonsense suppression.

We initially calculated the level of neurofibromin decrease and phospho-ERK/total-ERK increase in multiple organs (brain, kidney, heart, lung, and liver) upon tamoxifen administration to determine the feasibility of using neurofibromin and phospho-ERK as biological markers of functional neurofibromin restoration. We found at least a 50% reduction in full-length neurofibromin and up to a two-fold increase in phospho-ERK/total-ERK in tissues. The brain and the kidneys were selected for subsequent endpoint assessment due to their neurofibromin protein levels and relevance.

Two readthrough compounds have shown promise by suppressing the *NF1* R816X mutation in *in vitro* NF1 models: the synthetic aminoglycoside, ELX-02, and the eRF1 degrader, SRI-41765. We assessed the ability of these compounds to extend the lifespan of acute NF1 mice. Mice were administered 10mg/kg ELX-02 once daily, 30mg/kg SRI-417675 b.i.d., or the matching vehicle for two weeks prior to *Nf1*^{Flox} excision. Mice were then given three doses of 75mg/kg tamoxifen i.p. every other day while still receiving the readthrough drug or vehicle. The mice continued receiving the readthrough agent or vehicle until they were euthanized at 28 days post tamoxifen initiation, or when their initial body weight was reduced by 20%. Both ELX-02 and SRI-41765 significantly extended the life of the mice by at least 25% beyond that of vehicle controls. A trend toward increased full-length neurofibromin and decreased phospho-ERK/total-ERK was also seen in mice treated with either readthrough compound. The next step will be to combine the treatments to determine if we can further extend acute NF1 mouse lifespan and improve NF1 endpoints.

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BIOINFORMATICS, OMICS AND SHARING PLATFORMS

Multi-Omics Integration of Malignant Peripheral Nerve Sheath Tumors Identifies Potential Targets for Chr8q-Amplified Clones

Belinda B. Garana, PhD, *Pacific Northwest National Laboratory, Richland, Washington*

Purpose: Chromosome 8q (Chr8q) amplification in malignant peripheral nerve sheath tumors (MPNST) is associated with high-grade transformation in MPNST, which has a five-year survival rate of only 20-50%. Despite Chr8q gain being a common event in MPNST, there is not currently a treatment approach that allows for personalization based on copy number status. In this work, we employed a proteogenomic approach to interrogate the molecular differences between Chr8q-wildtype and -amplified tumors to both better understand tumor progression and identify drugs which may target vulnerabilities unique to each set of tumors.

Methods: To do so, we leveraged our growing library of fully characterized MPNST patient-derived xenografts (PDXs) and collected LC-MS/MS global and phospho-proteomics measurements for six of these samples. We then paired these data with transcriptomics and copy number data to identify specific molecular changes that corresponded with Chr8q gain across the samples. By integrating these omics measurements, we identified specific pathways that were distinctly active in either Chr8q-amplifed or wild type samples as well as potential drugs that could be uniquely sensitive in each set of tumors.

Results: In addition to changes caused by the genes on Chr8q such as over-expression of MYC, our results identified dramatic changes in transcriptional regulation and protein signaling upon Chr8q amplification that predict differences in drug sensitivity across Chr8q status. CDK4 and PLK1 were among potential targets identified for Chr8q-amplified MPNSTs whereas MEK and EGFR were predicted to be vulnerabilities in Chr8q-wildtype MPNSTs.

Conclusion: This study paves the way for future studies of MPNST treatments stratified by Chr8q status. Furthermore, these results represent a new way to target tumor heterogeneity in MPNST through the proteogenomic integration and drug sensitivity prediction in distinct tumor subpopulations.

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Multiomic Analyses at Single-Cell and Spatial Resolution Reveal Distinct Evolution Patterns and Immune Composition in PRC2-Loss Versus PRC2-Retained MPNST

Kuangying Yang, *Division of Oncology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO*

Purpose: Neurofibromatosis Type 1 (NF1) is a common neurogenetic and tumor predisposition syndrome, leading to the development of benign plexiform neurofibroma (PN) that can progress to malignant peripheral nerve sheath tumors (MPNST). Loss of function of polycomb repressive complex 2 (PRC2) which methylates histone H3 on lysine 27 (H3K27me3), is a common feature of MPNST and is associated with poorer prognosis in MPNST patients. We previously demonstrated MPNST with PRC2 loss exhibit a more immune suppressive environment. To deeply understand the role of PRC2 loss in MPNST pathogenesis to inform novel treatment options for NF1-MPNST, we performed multiomic analyses to explore how PRC2 loss impacts tumor evolution and immune composition.

Methods: Paired PN and MPNST samples from 12 patients (PRC2 loss: N = 7; PRC2 retained: N = 5) were collected to investigate the molecular events and microenvironment during tumor progression. At the genomic level, we defined clonal evolution from PN to MPNST with whole exome sequencing (WES) data by a novel pipeline developed in our laboratory: Precise DNA Variant Calling (PDVC). At the transcriptomic level, we integrated bulk and single-cell RNAseq data to determine how PRC2 loss influences meta-signatures and immune cell signaling pathways through deconvolution analyses. At the epigenetic level, we performed parallel proteomic and histone analyses to determine how PRC2 loss affects histone post-translational modifications and immune responses. Finally, at the spatial proteomic level, we utilized multiplexed Hyperion Imaging Mass Cytometry (IMC) staining to construct niche-similarity networks and validate the different tumor-stroma-immune composition in PRC2-loss vs -retained MPNST as well as their precursor lesions.

Results: Diverse clonal evolution patterns including distinct types of NF1, CDKN2A, and MTAP structural variants were identified in PRC2-loss vs -retained MPNST through PDVC-WEX pipeline. Global proteomics and transcriptomics analyses indicated MPNST with PRC2 loss have poorer antitumor immune infiltration and responses compared to those with PRC2 retained. The trial study of IMC data provided a differential landscape of morphological features, spatial cellular interactions, and niche-similarity networks in PRC2-loss vs -retained MPNST. All IMC results and supplementary single-cell RNAseq data from NCI will be available before the Material Review Process of 2025 NF Conference by CTF.

Conclusion: Our findings demonstrated that MPNST with PRC2 loss are “cold tumors” with poorer antitumor immune responses and a more malignant progression path from PN, compared to those MPNST with PRC2 retained, which can potentially offer clues to develop treatments targeting the immune system for NF1-MPNST.

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Near-Absolute Quantification of the NF1 Tumor Kinome Using a Targeted Proteomics Approach

Christine A. Berryhill, *Indiana University*

Purpose: The leading cause of death for NF1 patients remains malignant peripheral nerve sheath tumors (MPNSTs). To date, NF1 MPNSTs are treated with potentially complicated surgical resection and/or conventional chemotherapeutic agents. Several kinase inhibitors have been tested in clinical trials, but to date these trials have not been successful. There are currently no approved medical therapies for NF1 MPNST. Adaptive response of the kinase signaling network—kinome reprogramming – has been observed in response to chemotherapy in various cancers, including NF1 MPNSTs. This work aims to address the critical gaps in understanding the baseline heterogeneity of the NF1 MPNST kinome and its downstream impact on signaling networks. Recent technological advances enable the quantitative measurements of kinase protein abundances from limited tissue sample ($\sim$20 μg). By coupling this technology with other omics approaches, we can better elucidate the baseline kinome subtypes and inform future early phase clinical trials.

Methods: We used a state-of the art, quantitative proteomic method, SureQuant™ parallel response monitoring (PRM), to determine the near-absolute abundance of kinases from NF1 patient tumors using only 10 μgs of tissue (n = 15). These samples were also subjected to genomic, transcriptomic (bulk and single-cell), and multiplexed kinase inhibitor bead (MIB) affinity chromatography coupled with mass spectromety (MIB/MS) analysis. Multi-omic analysis was conducted to reveal preliminary patterns across the NF1 MPNST tumor landscape. To assess adaptive kinome response, we treated NF1 MPNST 8814 cell line with CDK4/6 inhibitors for either 24 hours or 7 days to perform SureQuant™ and integrating the data with bulk transcriptomic and functional proteomic kinome profiling, resulting in a comprehensive multi-omic analysis of the adaptive kinome.

Results: Over 150 kinase protein abundances were detected using the SureQuant™ method in both the NF1 patient tumor samples and CDK4/6i-treated 8814 cells. Preliminary multi-omics analysis reveal distinct clusters in the NF1 patient tumor kinome, highlighting the heterogeneity of the NF1 MPNST kinome and upregulation of specific kinases unique to patient subsets. SureQuant™ analysis of the CDK4/6 inhibited 8814 cells demonstrated the reproducibility of this quantification approach. Ongoing multi-omic analyses aim to elucidate the relationships between protein abundance, mRNA expression, and kinase activity.

Conclusion: This multi-faceted approach will provide valuable insight into the heterogeneity of NF1 MPNST kinome landscape and kinome adaptation. The identification of potential therapeutic targets and kinome subtypes will accelerate the development of targeted therapies and improve clinical trial design for NF1 MPNST patients.

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Development and Evaluation of Precise DNA Variant Calling (PDVC): An HPC-Based Pipeline for Comprehensive DNA Variant Analysis for Genomically Complex Tumors

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Purpose: Genomically complex tumors, including NF related tumors, pose unique challenges due to their complex and heterogeneous genomic landscapes. To advance precision medicine for these patients, we have developed Precise DNA Variant Calling (PDVC), a high-performance computing (HPC)-based automated pipeline for comprehensive DNA variant analysis. PDVC is designed to detect a broad spectrum of genomic alterations from both whole exome and whole genome sequencing data, thereby supporting accurate diagnoses and personalized treatment strategies.

Methods: Sequencing data in FASTQ format were processed using BWA, SAMtools, and GATK to generate BAM files. Germline variants were detected using GATK HaplotypeCaller for SNVs and the GATK germline copy number pipeline for structural variants. For somatic variant detection, Mutect2 and Strelka were employed to call SNVs, while the GATK somatic copy number pipeline was used to calculate read-depth ratio (RDR) and B-allele frequency (BAF) and perform segmentation, which were then applied to identify microgains/microlosses and chromosome-level copy number variants. Thirteen malignant peripheral nerve sheath tumor (MPNST) samples and their paired normals were analyzed using the PDVC-WEX pipeline for comprehensive variant calling.

Results: Analysis of 13 MPNST tumor samples revealed diverse genomic alterations. Three types of *NF1* structural variants were identified: five tumors exhibited germline *NF1* microdeletions, four tumors harbored somatic *NF1* microdeletions, and one tumor showed copy-neutral loss of heterozygosity (LOH), in which the normal sample had an *NF1* microdeletion but the tumor retained both *NF1* alleles. In addition, two types of *CDKN2A* variants were detected in 11 of 13 tumors, including homozygous deletion and partial exon deletion. Moreover, three types of *MTAP* variants were observed in 8 of 13 tumors: homozygous loss, heterozygous loss, and partial exon deletion.

Conclusion: The PDVC pipeline is an effective tool for complex variant calling, demonstrating high accuracy and reproducibility in detecting a diverse range of genomic alterations, including *NF1, CDKN2A,* and *MTAP* variants. Its comprehensive approach and integration of HPC resources support its application in both research and clinical settings, ultimately enhancing diagnostic precision, informing personalized treatment strategies, and improving outcomes in pediatric oncology.

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The Therapeutic Mechanisms of Oncolytic Viruses in the Treatment of Cutaneous Neurofibromas

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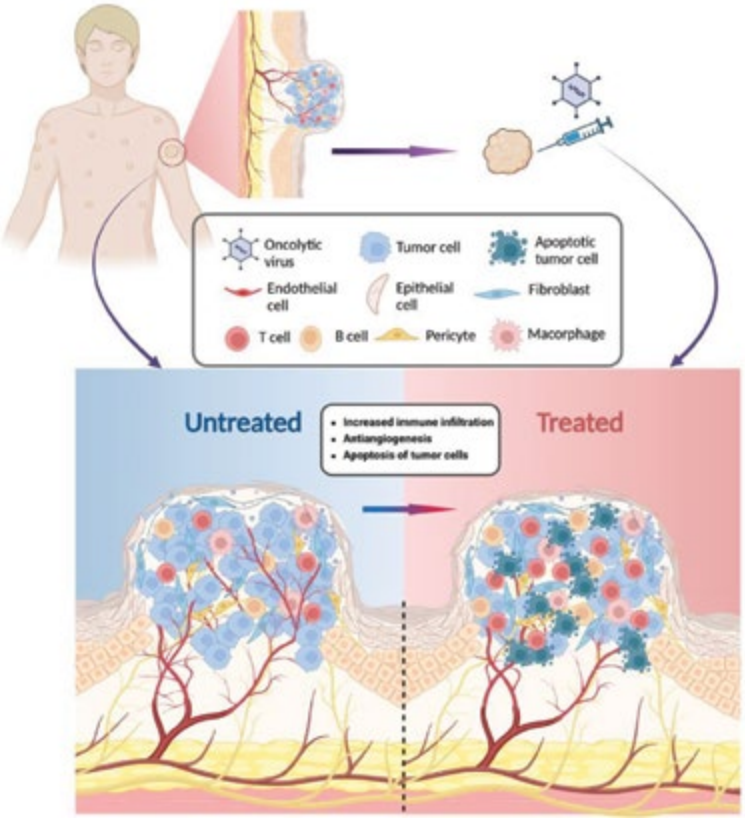
Neurofibromatosis Type 1 (NF1) frequently leads to the development of cutaneous neurofibromas (CNFs), which are benign yet debilitating tumors that currently lack effective therapeutic options and urgently need novel treatment strategies. Oncolytic viruses (OVs), a promising cancer immunotherapist class, have successfully targeted various malignancies, while their effectiveness in treating immunologically "cold" tumors like CNFs remains poorly understood. In this study, we investigated the therapeutic potential of OVs for CNFs by employing single-nucleus and single-cell RNA sequencing to analyze tumor samples before and after OV treatment. Our findings revealed post-treatment alterations in the tumor microenvironment (TME), including increased infiltration of T cells, B cells, and macrophages, alongside reduced Schwann cells (SCs), the primary tumorigenic cells in neurofibromas. We found that OVs directly induced apoptosis in SCs and inhibited angiogenesis by disrupting VEGF signaling. Furthermore, OV therapy transformed the TME from an immunologically "cold" to a "hot" state, enhancing antitumor immune responses. These findings highlight the multifaceted efficacy of OVs in treating CNFs and suggest their broader potential in modulating tumor-immune interactions.



Secretome Distinguishes Spectrum of NF1 Associated Peripheral Nerve Sheath Tumors

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Purpose: Early detection and interception of malignant transformation in Neurofibromatosis 1 (NF1) associated peripheral nerve sheath tumors (PNST) remains a significant clinical challenge. Clinical symptoms are insensitive, and standard of care imaging has limited specificity, especially during the critical window for clinical intervention when benign plexiform neurofibromas (PN) transform to pre-malignant atypical neurofibromas (AN). Furthermore, invasive tissue biopsy has limited negative predictive value given intratumoral heterogeneity and that patients frequently have multiple PNST. Novel non-invasive and tumor site-agnostic surveillance assays provide potential for early diagnosis and intervention. We hypothesize that circulating proteins in the plasma (the secretome) accurately and non-invasively distinguish the spectrum of PN, AN and malignant peripheral nerve sheath tumors (MPNST).

Methods: The secretome of 118 plasma samples with matched histologically confirmed diagnoses (Healthy $n = 10$, PN $n = 29$, AN $n = 25$, and MPNST $n = 54$) from 79 patients were analyzed using a proximity extension assay (PEA) panel for 1461 proteins *(Olink).* Samples were classified by their highest-grade lesion. Briefly, dual-recognition antibodies labelled with unique DNA oligonucleotides are brought into proximity by simultaneously binding a target protein, enabling hybridization and extension of the oligonucleotides to create a "barcode" quantifiable by Next Generation Sequencing. Unique protein signatures for each tumor state were identified using one-versus-all (OVA) comparisons Healthy-versus-all (HvA), PN-versus-all (PvA), AN-versus-all (AvA), and MPNST-versus-all (MvA) with ANOVA and post-hoc Tukey honestly significant difference (HSD) of normalized protein expression (NPX) outputs from PEA. Proteins were considered significant with a NPX difference ≥ 1.2 and p adj <0.05. Individual protein's performances were assessed using Youden's index and a receiver operating characteristic (ROC) curve. An integrated signature of all significant PN proteins was developed using a support vector machine (SVM) model.

Results: 88 proteins were significant for HvA comparisons, 114 proteins for PvA, 136 proteins for AvA, and 284 for MvA. Individual proteins' performance for HvA had a median AUC of 0.76 (interquartile range (IQR): 0.72-0.8), median sensitivity 0.60 (IQR: 0.53-0.81), median specificity 0.90 (IQR: 0.8-1.0); PvA had a median AUC of 0.64 (IQR: 0.62-0.67), median sensitivity of 0.38 (IQR: 0.33-0.47) , and median specificity of 0.97 (IQR: 0.86-1.0); AvA had a median AUC of 0.70 (IQR: 0.67-0.73), median sensitivity of 0.52 (IQR: 0.44-0.61) and median specificity of 0.84 (IQR: 0.76-0.92); MvA had a median AUC of 0.76 (IQR: 0.73-0.79), median sensitivity of 0.57 (IQR: 0.52-0.67), and median specificity of 0.92 (IQR: 0.84-0.96). Given the high prevalence of PN in NF1 and the relatively high specificity of identified proteins, we postulated that a signature predicting that all tumors are PNs would have clinical utility. To improve performance, we integrated PN associated proteins using an SVM model. SVM with leave one out cross-validation significantly improved the PN-signature's sensitivity (0.69, 20/29 PN) and specificity (0.99, one AN was misclassified as PN).

Conclusions: This pilot study demonstrates that circulating proteomics can non-invasively distinguish between disease states in NF1 and that an integrated PN protein signature predicts whether a patient's tumor burden remains PN or has transformed with high confidence (positive predictive value: 0.95, negative predictive value 0.91). Importantly, PEA uses just 40uL of plasma per sample enabling the integration of non-invasive orthogonal biomarkers, such as cell free DNA, all from one tube of blood.

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CoderData: a Python Package and Collection of Benchmark Datasets to Enable Development of Machine Learning Models for Drug Sensitivity in NF1 Organoids

Jeremy Jacobson, *Pacific Northwest National Laboratory, Seattle, WA*

Purpose: While numerous machine learning models have been published that utilize omics data for drug sensitivity prediction[1], these methods often require larger, more diverse datasets than are currently available for NF1. Enhancing drug response prediction is vital for the NF community, which currently has only has two approved NF1 therapies[2,3]. The purpose of CoderData is to provide a series of harmonized datasets[4-15]—including cell lines, ex vivo, organoid, and patient derived xenograft (PDX) data—to empower both machine learning and deep learning analytical approaches in inferring drug response in NF1.

Methods: We developed the Cancer Omics and Drug Experiment Response Data (CoderData) Python package that allows users to download, reformat, and perform balanced splits creating data ready for model integration. Our automated build pipeline 1) automates the collection of drug structure and cancer data from multiple sources including the NF Data Portal[16], 2) harmonizes drug curves, omics measurements, and mutation variant nomenclature, and 3) publishes the data on FigShare with programmatic retrieval using our Python package. We plan to benchmark drug response models using multiple cancer model types to attain better predictions than those based solely on PDXs.

Results: To date we have curated molecular measurements (genomics, transcriptomics, proteomics, copy number) from approximately 4300 unique samples across multiple model systems such as two-dimensional cell lines (1280) with multiple measurements, organoid models (388), patient-derived xenografts (25), ex vivo (1022) and tumor data (1561). This collection includes data from patient-derived NF1 tumors (malignant peripheral nerve sheath tumors and cutaneous neurofibromas). Additionally, we assembled drug response data for roughly 55,000 unique drugs across these samples, and further integration of patient-derived organoids (86) is underway.

The package is available at https://pnnl-compbio.github.io/coderdata/ .

Conclusions: The integration of harmonized data from multiple cancer model systems, particularly organoid data, may advance the power of machine learning and standard statistical models to predict drug sensitivity in rare NF1 tumors. The CoderData package not only facilitates training and benchmarking of these models through an accessible Python package but also supports cross-application between cell line/tumor data and organoid systems through data harmonization. This cross-model integration enhances the potential for robust, generalizable predictions and positions CoderData as the only benchmark dataset to combine cell line, ex vivo, and NF1 organoid data—fostering collaboration and further contributions from the research community[17,18].

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Identification of Different Malignant Peripheral Nerve Sheath Tumor Types in NF1 Patients

Eduard Serra, PhD, *Germans Trias i Pujol Research Institute (IGTP)*

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft-tissue sarcomas appearing either sporadically or in the Neurofibromatosis type 1 (NF1) context. MPNSTs tend to metastasize and have a poor prognosis. The diagnosis of MPNSTs can be challenging and heterogeneity exists, since different tumor entities may share overlapping histological characteristics. Our working model on MPNST development is as follows: MPNST initiates progression through the loss of key tumor suppressor genes (TSG) *(NF1, CDKN2A, SUZ12/EED, TP53)* in the cell of origin. Then, a general genomic rearrangement occurs, that needs to be compatible with cell viability for tumor progression. The resulting cell identity, with its expression and functional properties, will define MPNST histology and clinical behavior. The present project aimed to clarify the genomic characteristics of MPNSTs, evaluate their utility for understanding MPNST heterogeneity and identify any potential biological and clinical value.

We performed a comprehensive genomic analysis in an initial set of 20 clinically diagnosed MPNSTs that helped us identify recurrent genetic and genomic features characteristic of MPNSTs: TSG inactivation signature, low mutation burden, absence of activating mutations, global LOH status and copy-neutral (CN) LOH regions and specific copy number genomic profile. These genomic characteristics were confirmed using 50 additional MPNSTs from Cortes-Ciriano *et al.* (2023) we completely re-analyzed. With the extended set of MPNSTs and considering these genomic features, we identified three distinct types of MPNSTs present in NF1 patients, mainly defined by their combination of inactivated TSGs.

The first group of MPNSTs bears the inactivation of *NF1, CDKN2A* and PRC2, exhibits a conventional histology and loss of H3K27 trimethylation. A second group of MPNSTs bears the inactivation of *NF1, TP53* and PRC2, and associates with conventional histology with heterologous elements, loss of H3K27 trimethylation and, commonly, a complete 2n genome in LOH. The third group is composed of tumors found only in NF1 patients, bearing the inactivation of *NF1* and *CDKN2A,* highly rearranged genome, with no activating mutations, and associates with a conventional MPNST histology, maintaining the H3K27 trimethylation. Finally, we identified a highly heterogeneous group of tumors, diagnosed initially as MPNSTs, with different TSG inactivation signatures, with activating mutations and diverse histological presentations and methylation patterns. This group needs further characterization but is probably composed of many tumors not being MPNSTs.

We will present the potential biological and clinical implications of this classification of NF1-associated MPNSTs.

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Deep Learning Predicts CDKN2A/B Status from H&E-Stained Whole Slide Images in Peripheral Nerve Sheath Tumors

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Purpose: Loss of *CDKN2A/B,* a key cell cycle regulator, is a frequent and likely early initiating event in the transformation of benign plexiform neurofibroma (PN) to pre-malignant atypical neurofibromatous neoplasm of uncertain biologic potential (ANNUBP) and ultimately malignant peripheral nerve sheath tumors (MPNST)[1-4]. Recent consensus guidelines proposed that biallelic *CDKN2A/B* inactivation is sufficient for molecular diagnosis of ANNUBP, irrespective of histopathology[5]. We hypothesize that deep learning models, agnostic to pathologists' histologic annotation of ANNUBP features[6], can learn morphologic features from hematoxylin and eosin (H&E) whole slide images (WSI) that correlate with *CDKN2A/B* mutations and thereby enable genotype prediction from standard histology.

Methods: We analyzed 24 peripheral nerve sheath tumors (PNSTs), comprised of 7 PN and 17 atypical neurofibroma (AN)/ANNUBP, resected at the National Institutes of Health (NIH) between 2017-2023. Tumors were molecularly characterized using TSO 500 *(Illumina)* targeted next-generation sequencing (NGS) to assess *CDKN2A/B* status: *CDKN2A/B* wild type (wt) ($n = 12$), *CDKN2A/B* loss ($n = 8$), *CDKN2A* loss ($n = 3$), and *CDKN2B* loss ($n = 1$). Matched H&E slides were digitized into WSI using a Zeiss slide scanner, and clinical histopathologic diagnoses were confirmed by an independent pathologist using HALO *(Indica Labs).* From a training set of mutationally annotated WSI (*CDKN2A/B/AB* loss: $n = 9$; *CDKN2A/B* wt: $n = 9$), digital image patches were extracted and encoded into feature representations using UNI, a general-purpose self-supervised encoder model for pathology[7]. These features were passed into a clustering-constrained-attention multiple-instance learning (CLAM) model for slide-level classification[8]. Briefly, within the CLAM framework, an attention network ranked and pooled patches to generate slide-level representations. All 24 WSI, including six held-out samples, were divided into 4 validation splits with each set balanced for *CDKN2A/B/AB* loss ($n = 3$) and *CDKN2A/B* wt ($n = 3$) to assess performance. Attention scores were visualized as a heatmap on WSI to identify morphological patterns predictive of mutations.

Results: The trained deep learning framework accurately predicted *CDKNA2A/B* status from H&E-stained WSI, achieving a mean AUC of 0.84 (Validation Split 1: 0.78; Validation Split 2: 0.78, Validation Split 3: 0.78; Validation Split 4: 1.0) and a mean accuracy of 0.79 (Validation Split 1: 0.83 (5/6); Validation Split 2: 0.67 (4/6), Validation Split 3: 0.67 (4/6); Validation Split 4: 1.0 (6/6)).

Conclusions: This pilot study demonstrates that attention based deep learning models may predict diagnostically significant mutational profiles from routine H&E slides. Digital pathology-based molecular annotation has the potential to reduce reliance on sequencing, lower diagnostic costs, and improve accuracy, particularly in resource-limited settings. A significant limitation of this study, however, is the lack of an independent test dataset. Future work will therefore focus on expanding the validation dataset and conducting an independent, multi-institutional study to optimize model performance.

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The PRC2-Dependent and -Independent Surface Proteome of Malignant Peripheral Nerve Sheath Tumors

Christopher Stehn, BS, *University of Minnesota – Twin Cities, Minneapolis, MN*

Malignant peripheral nerve sheath tumors, or MPNST, are soft tissue sarcomas that arise from plexiform neurofibromas. These tumors develop following a series of sequential genetic alterations, primarily *NF1* loss-of-heterozygosity, deletion of *CDKN2A/B,* and biallelic inactivation of *SUZ12* or *EED* causing inactivation of polycomb repressor complex 2 (PRC2). Despite the well-defined genetic etiology of many cases, treatment options for MPNST are limited due to an immune-cold tumor microenvironment and a lack of targeted therapies. To aid discovery of viable treatment options for MPNST, our lab has undertaken surface protein profiling of several MPNST model cell lines and patient-derived xenografts (PDX) using a surface-protein-capture technique in combination with mass spectrometry. Proteins localized to the cell surface serve as the interface of the tumor cell with the surrounding extracellular matrix and tumor microenvironment as well as actualizing autocrine and paracrine signals from surrounding cells. The resulting surface proteome – or "surfaceome" – profiles reveal a variety of proteins expressed on the surface of MPNSTs. Using a model of MPNST in which we have re-expressed *SUZ12* under the control of doxycycline, we have identified changes to the cell surfaceome that appear to be driven by PRC2 activity. These changes are consistent with changes in mRNA expression within the same model system for some, but not all, proteins. We defined an MPNST consensus surfaceome by evaluating proteins that were expressed in a majority of the MPNST models assayed. In order to determine a clinically actionable set of surface markers from this consensus set, we narrowed our search to proteins within the top half of this set by expression. Remaining proteins were assessed for expression in normal human tissue using ProteomicsDB (https://www.proteomicsdb.org) where proteins expressed below a defined threshold in a majority of tissues were then checked for evidence of membrane localization in the Cell Surface Protein Atlas (https://wlab.ethz.ch/cspa). Ultimately, we have derived a list of surface proteins expressed across both tumor-derived cell lines and PDX samples. Assessment of protein abundance via flow cytometry was used to derive an average count of each target per cell. Use of antibody-drug conjugates reveals potential high efficacy in targeting a subset of these proteins – namely, MET, EGFR, HER2, and PTK7 – in our MPNST models. Lastly, we have worked in collaboration with Christine Pratilas and her group to assess the expression of these high profile targets in MPNST tissue microarrays. These efforts will aid the elucidation of mechanisms driving MPNST malignancy as well as the discovery of targeted therapies for patients with MPNST. Additionally, it will provide a resource for other researchers to mine to aid in therapeutic development.

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Advancing NF1 Research Through a Comprehensive Biorepository of Primary Tumor Specimens, Preclinical Models, Genomic and Clinical Data for NF1-Associated Tumors

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Purpose: Neurofibromatosis type 1 (NF1) is an inherited neurogenetic condition associated with increased risk of developing a variety of tumors, including cutaneous neurofibromas (cNF), plexiform neurofibromas (pNF), atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP), and malignant peripheral nerve sheath tumors (MPNST). The Johns Hopkins NF1 Biospecimen Repository was established in 2016 to enhance research for NF1 tumors via access to primary human tissues and preclinical models. It has since become a vital resource for NF1 research worldwide and continues to expand the scope of available biospecimens to address growing requests from the NF research community. In addition to the growing collection of banked tumor tissue, the biorepository has broadened its offerings to include single-cell suspensions from tumors, tissue microarrays (TMA), genetically diverse cell lines, and patient-derived xenografts (PDX).

Methods: Patients with clinically or genetically confirmed NF1 undergoing surgical resection or biopsy of NF1-associated tumors are invited to participate in this IRB-approved study for specimen collection. Specimens include blood fractions and tumor tissues. Tissue fragments are frozen, paraffin-embedded, and digested into single-cell suspensions. Cell lines and PDX are attempted with malignant tumors. For new cell lines, IncuCyte live-cell imaging is used to assess growth and response to MEK and SHP2 inhibitors. Banked specimens are genomically characterized via whole exome sequencing (WES), whole genome sequencing (WGS), and RNA sequencing (RNAseq), with data accessible through the NF Data Portal. Clinical annotations and outcomes data are made available to investigators upon scientific review and IRB approval.

Results: Since its inception, over 400 unique samples have been banked from 206 patients, including pNF (n=93), MPNST (n=70), cNF (n=124), blood fractions, and xenograft (n=5) specimens. RNAseq (n=89), WES (n=114), and WGS data (n=21) are available through the NF Data Portal. Three novel patient-derived MPNST cell lines have been generated and are sensitive to SHP2 or MEK inhibition. In addition, a new TMA panel of cNF, pNF, MPNST, and control tissues has been validated with key biomarkers and is available upon request. To date, 83 research requests have been approved by the NF1 biospecimen repository fostering collaboration and advancing NF1 research.

Conclusions: The Johns Hopkins NF1 Biospecimen Repository serves as a high-quality, clinically and genomically characterized resource supporting NF1 research. In response to evolving scientific needs, the biorepository has expanded to include single-cell suspensions, TMA blocks, and newly developed MPNST cell lines. These efforts further enhance its value for critical therapeutic advances for patients with NF1.

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Inference of Microglia Activation Cell Fate in Single-Cell Resolution on the Atlas of Healthy, Diseased, and Host-Upon-Transplantation Microglia

Emil Kriukov, *Schepens Eye Research Institute of Massachusetts Eye and Ear, Boston, MA*

Purpose: Microglia plays a crucial role in formation and progression of the gliomas within the central nervous system, including optic pathway glioma. We propose to build an atlas of mouse microglia in multiple healthy, diseased and post-transplantation conditions to study the microglia activation / deactivation in detail.

Methods: We collected scRNA-seq data for healthy developing (E10-P8), healthy adult (4-52 weeks), optic nerve crush, microbeads-induced glaucoma (MB) and host-upon-transplantation (HUT) microglia from mouse retina. HUT condition sequencing was performed upon H9-Brn3b:tdTomatoThy1.2-hESC differentiation into retinal organoids with further transplantation into Cx3cr1-GFP mice and GFP+ cells sorting and sequencing 3 days post transplantation. Other conditions were obtained from public sequencing data and include more than a 100 individual batches. Our computational pipeline includes R- and Python-dependent packages, such as Seurat, scanpy, scFates, velocyto, scVelo.

Results: We build the atlas of mouse retinal microglia. We demonstrate activation of microglia in HUT condition and profile the homeostatic (P2ry12), activated (Apoe), and transitory states. We confirm microglia activation to be bi-directional, continuous, and reversible process, according to cell fate trajectory reconstruction using RNA Velocity. We study microglia activation and observe the dynamics in percentage of activated cells between the conditions: with ~30% of activated cells out of the microglia pool in healthy adult condition, healthy developing condition is characterized with less percentage (~16%) of activated cells. ONC demonstrates increasing gradient of activated microglia up to 1-week post-ONC with ~65% of microglia being activated. 2-week post-ONC, being post-peak, is characterized by decreasing trend of activated microglia. On the other hand, MB glaucoma condition does not show major increase of microglia activation compared to healthy adult microglia.

We perform pathway analysis using the microglia activation related pathways. There, developing microglia has the lowest enrichment score of microglia activation, where HUT condition has the highest activation score. Some of the pathways were more specific to ONC (regulation of microglial cell activation) or MB-glaucoma (acute inflammatory response) than HUT condition.

Conclusion: Our multiconditional atlas of mouse retinal microglia highlights major changes happening in the process of microglia activation with age, disease, and upon contact with human neurons. We study and profile the bi-directional track of microglia activation. Transplantation tends to be the strongest stress factor for microglia activation compared to ONC or MB-glaucoma. The atlas, being publicly available, provides an opportunity for further studies of NF1 microglia in comparison to the described conditions.

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Chromosomal Alterations and Intra-Tumor Heterogeneity in MPNST Revealed by Integrated Single-Cell Multi-Omics

Yidan Pan, *The University of Texas MD Anderson Cancer Center, Houston, TX*

Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas not infrequently with heterologous intra-tumoral differentiation. ~50% of cases associated with neurofibromatosis type 1 (NF1). Despite genomic advances, most studies analyze single samples per patient, limiting insights into intra-tumor heterogeneity (ITH). We aimed to characterize ITH using integrated single-cell multi-omics to address limitations of previous approaches.

Methods: We developed a multi-omics integration pipeline for multi-platform data down to the single-cell level, including bulk Whole-Genome Sequencing (WGS), single-nucleus DNA (snDNA-seq) and RNA sequencing (snRNA-seq), and multi-regional shallow-coverage WGS derived from multiple Laser Capture Microdissection (LCM) spots within tissue sections. 63 samples spanning 17 patients were collected and processed, as summarized in **Figure 1**. Somatic mutations were called using a consensus approach (MuSE2, Strelka2, Mutect2); copy number aberrations (CNAs) were identified with the Battenberg algorithm for bulk WGS and validated across platforms.

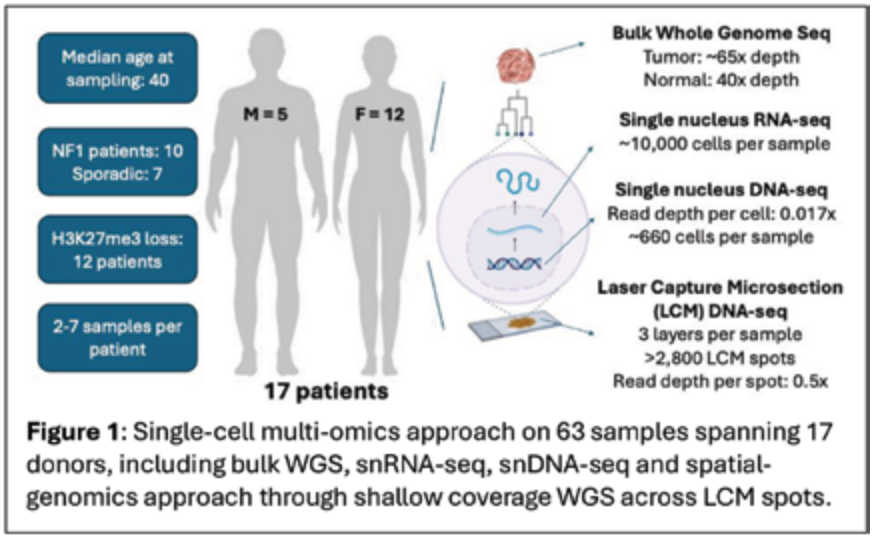

Figure 1: Single-cell multi-omics approach on 63 samples spanning 17 donors, including bulk WGS, snRNA-seq, snDNA-seq and spatial-genomics approach through shallow coverage WGS across LCM spots.

Results: MPNSTs exhibited low-to-moderate mutational burden (0.52–2.34 mutations/Mb, median: 1.23) but extensive chromosomal alterations, including frequent whole-genome doubling (47/63 samples; 14/17 patients) and widespread loss of heterozygosity (4–93%, median: 26%). Cross-platform comparison of CNA profiles across WGS, snDNA-seq (ASCAT.sc), LCM, and snRNA-seq (inferCNV) validated the bulk results and revealed subclonal CNA co-occurrence within individual cells or spots. Single-cell transcriptomic analysis distinguished malignant cells from normal mesenchymal cells by allelic imbalance evaluation and demonstrated diverse transcriptomic profiles among malignant cells across patients. To demonstrate that malignant cluster separation was not due to *in-silico* batch effects, we generated four mixed libraries from pooled nuclei, revealing the same clusters within single batches.

Conclusion: Our data suggest that chromosomal alterations are dominant drivers of MPNST, and integrating multi-platform data down to the single-cell level enables comprehensive characterization of ITH. Ongoing efforts include phylogenetic reconstruction using spatially resolved CNA profiles and integrative driver gene analysis, which together aim to unravel spatially informed tumor evolution and support the development of targeted therapeutic strategies.

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AI-Driven Machine Learning and CRISPR Guide RNA Optimization for Precision Medicine in Neurofibromatosis

Shivi Kumar

Neurofibromatosis (NF) is a genetic disorder characterized by mutations in the NF1 and NF2 genes, leading to tumor formation, nerve damage, and significant patient morbidity. Despite advances in targeted therapies, treatment responses remain highly variable, and there is a lack of predictive tools to determine which patients will benefit from specific interventions. This study presents a novel AI-powered precision medicine framework that integrates machine learning for NF1/NF2 mutation classification and deep-learning-optimized CRISPR guide RNA selection for gene therapy. Genomic data was sourced from ClinVar, HGMD, and the Leiden Open Variation Database (LOVD), with additional pathogenicity scores obtained from REVEL and PolyPhen-2. The machine learning model achieved 93% accuracy in classifying NF1/NF2 mutations and 92% precision in predicting disease severity. The AI-driven CRISPR guide RNA optimization framework demonstrated a 98% target specificity rate and a 72% reduction in off-target effects. A web-based deployment system was created using Streamlit, allowing real-time genomic analysis, mutation classification, and automated CRISPR guide RNA recommendations. These findings underscore the potential for AI-driven approaches to advance precision medicine and provide a scalable solution for neurofibromatosis treatment.

1. Introduction:

1.1 Background and Clinical Significance: Neurofibromatosis (NF) is a rare autosomal dominant genetic disorder affecting approximately 1 in 3,000 individuals. It is caused by pathogenic mutations in either the NF1 or NF2 gene, leading to widespread tumor formation along the nervous system. NF1, caused by mutations in the NF1 gene, results in dysregulation of the RAS-MAPK signaling pathway and manifests as cutaneous neurofibromas, plexiform tumors, and optic gliomas. NF2, arising from mutations in the NF2 gene, leads to a loss of function in merlin, a tumor suppressor protein, often resulting in vestibular schwannomas, meningiomas, and progressive hearing loss. Although targeted therapies such as MEK inhibitors have demonstrated some efficacy in reducing tumor burden, their effectiveness varies widely among patients. This variability highlights the urgent need for predictive tools capable of stratifying patients based on their likelihood of responding to specific treatments.

Neurofibromatosis is a highly complex disorder due to its genetic heterogeneity and diverse clinical manifestations. Patients with NF1 and NF2 can exhibit a wide range of symptoms, making it difficult to establish uniform treatment protocols. Traditional genetic testing has provided some insights into the mutations responsible for NF, but the lack of predictive biomarkers for treatment response has hindered the advancement of personalized medicine in this field. The variability in tumor progression and response to pharmacological interventions necessitates a more sophisticated approach that integrates genomic data analysis with computational modeling. This underscores the need for advanced computational techniques such as machine learning to bridge the gap between genetic insights and clinical applications.

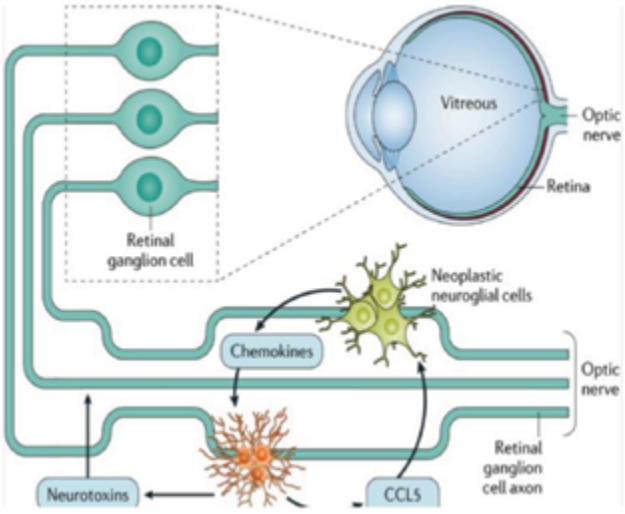

Figure 1. Pathophysiology and Molecular Mechanisms of Neurofibromatosis Type 1 and Type 2. NF1 encodes neurofibromin, a Ras-GTPase-activating protein that suppresses the Ras-MAPK pathway, preventing uncontrolled cell growth. NF2 encodes merlin, a cytoskeletal protein regulating cell adhesion and proliferation. Mutations in these genes lead to tumorigenesis, primarily affecting the peripheral and central nervous system. Adapted from **Nature Reviews Disease Primers**

1.2 Study Objectives: The primary objective of this study was to develop an AI-powered precision medicine framework that integrates machine learning and CRISPR gene editing optimization to improve treatment strategies for NF patients. Specifically, the study aimed to build a supervised machine learning model for NF1/NF2 mutation classification and disease severity prediction. Additionally, an AI-enhanced deep-learning framework was developed to optimize CRISPR guide RNA selection, ensuring high on-target efficiency and reduced off-target effects. Finally, a web-based deployment system was designed to provide clinicians and researchers with real-time genomic analysis, allowing for automated mutation classification and gene-editing recommendations. By integrating these computational tools, this research seeks to address the fundamental challenge of treatment heterogeneity in NF.

2. Methods:

2.1 Data Acquisition: Genomic and mutation effect data were obtained from publicly available databases, including ClinVar, the Human Gene Mutation Database (HGMD), and the Leiden Open Variation Database (LOVD). These sources provide curated variant classifications, including benign, likely pathogenic,

and pathogenic mutations, which were essential for model training. Pathogenicity scores were extracted from REVEL and PolyPhen-2 to enhance the stratification of variants based on their functional impact. NF1 and NF2 mutation records were further categorized by exon location, mutation type, and disease severity, ensuring a structured dataset for downstream analysis. To maintain data integrity and optimize model performance, preprocessing steps were conducted, including sequence cleaning, normalization, and variant effect annotation. Sequence cleaning involved filtering low-confidence variant calls, ensuring that only high-quality genomic alterations were retained.

Normalization corrected batch effects across datasets to prevent systematic biases in the model training phase. Variant annotation utilized multiple bioinformatics tools to cross-reference mutation impact predictions, facilitating accurate classification of disease-relevant variants. The cleaned and annotated dataset was then used for both machine learning model development and CRISPR guide RNA design. Additionally, cross-validation was performed to ensure the consistency and accuracy of annotated data, minimizing any biases that could impact downstream analyses. Feature extraction involved identifying the most informative genetic markers associated with disease progression. Various computational methods, such as Shannon entropy and k-mer frequency analysis, were used to refine the dataset and improve classification accuracy. The cleaned dataset was formatted into feature matrices suitable for input into the machine learning model, ensuring compatibility with supervised learning techniques. This structured approach facilitated high-throughput screening of NF-associated mutations, improving the model's ability to detect pathogenic variants efficiently.

2.2 Machine Learning Model Development: A supervised classification model was implemented using multi-layer perceptron (MLP) neural networks, with additional experiments conducted using XGBoost and Random Forest classifiers. The dataset was divided into an 80:20 training-validation ratio to ensure robust performance evaluation. Feature selection techniques, including principal component analysis (PCA) and Lasso regression, were applied to reduce dimensionality while preserving the most informative features for classification. Hyperparameter tuning was conducted using grid search cross-validation, optimizing the learning rate, number of hidden layers, and activation functions.

The classification model was designed to predict both NF1/NF2 mutation pathogenicity and disease severity. Accuracy, F1-score, and area under the receiver operating characteristic curve (AUC-ROC) were used as evaluation metrics. The final MLP model achieved 93% accuracy in mutation classification and 92% precision in disease severity prediction. Feature importance analysis revealed that mutations within key tumor suppressor domains of NF1 and NF2 had the highest predictive weight, confirming the model's ability to identify clinically relevant alterations. These results demonstrate the potential for AI-based approaches to enhance early diagnosis and targeted intervention in NF.

2.3 Web-Based Deployment System: A Streamlit-based web interface was developed to enable real-time genomic analysis and mutation classification. The application was designed to be accessible to both researchers and clinicians, providing an interactive platform for inputting genomic data, running AI-powered analyses, and visualizing results.

The web tool was built using Python and Streamlit, leveraging pre-trained machine learning models and CRISPR guide RNA optimization algorithms. The backend of the system was implemented using TensorFlow for neural network inference, scikit-learn for mutation classification, and Biopython for genomic sequence processing. The application integrates pandas for handling structured genomic datasets and matplotlib/seaborn for real-time data visualization.

Users can upload genomic sequences in FASTA or VCF format, which are then processed through the AI model pipeline. The system extracts relevant features, performs pathogenicity classification, and optimizes CRISPR guide RNAs. Real-time computations and API integrations allow automated retrieval of mutation annotations from ClinVar, OncoKB, and HGMD, ensuring that predictions remain up-to-date with the latest clinical findings.

The interface features:

- File Upload System: Accepts FASTA/VCF genomic sequences for analysis.
- AI-Powered Pathogenicity Prediction: Classifies mutations as benign, likely pathogenic, or pathogenic.
- CRISPR Guide RNA Optimization: Generates highly specific CRISPR editing recommendations, reducing off-target risks.
- Dynamic Data Visualization: Heatmaps and interactive plots for genomic variant impact analysis.
- Exportable Reports: Users can download results, including AI-generated mutation classifications and CRISPR guide RNA designs, in PDF and CSV formats.

For deployment, the web application is hosted on Streamlit Cloud, allowing scalable, real-time processing. The source code is managed via GitHub, enabling version control and collaborative enhancements. The full deployment is accessible at https://shivikumar.streamlit.app/, providing an intuitive and accessible resource for AI-driven CRISPR precision medicine in neurofibromatosis. A Streamlit-based web interface was developed to enable real-time genomic analysis and mutation classification. Users can upload raw FASTA or VCF files, and the AI model automatically predicts mutation pathogenicity, stratifies disease severity, and generates optimized CRISPR guide RNA sequences. API integration was implemented to facilitate future connectivity with clinical databases, including ClinVar and OncoKB. The web platform provides an intuitive dashboard that displays mutation effects, CRISPR target sites, and editing scores, enabling researchers and clinicians to make informed decisions regarding therapeutic interventions.

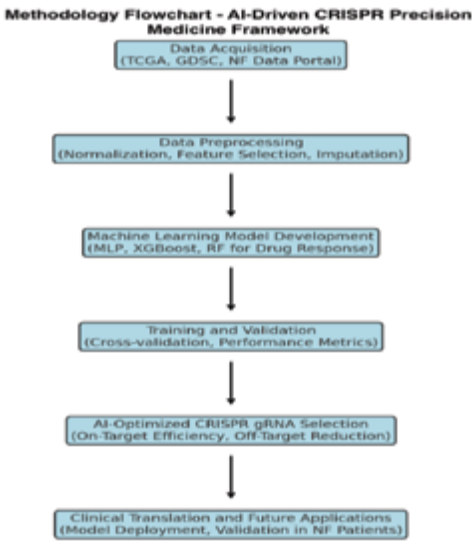

Figure 1: AI-Driven CRISPR Precision Medicine Methodology Flowchart

This flowchart outlines the structured methodology employed in the study, illustrating the sequential steps from genomic data acquisition to AI-driven CRISPR guide RNA selection and clinical translation. The framework integrates machine learning for NF1/NF2 mutation classification, deep-learning-based optimization for CRISPR targeting, and model validation for precision medicine applications in neurofibromatosis patients.

3. Results:

3.1 Machine Learning Model Performance: The machine learning model exhibited high predictive accuracy, achieving a 93% accuracy rate in NF1/NF2 mutation classification. The disease severity stratification model demonstrated 92% precision, indicating strong capability in distinguishing between mild, moderate, and severe cases.

Feature importance analysis revealed that mutations within tumor suppressor domains of NF1/NF2 had the highest predictive weight, with truncating mutations in exons 10–15 of NF1 and exons 2–7 of NF2 correlating with increased severity. The classifier's AUC-ROC score of 0.96 confirmed its high discriminatory power in mutation classification.

3.2 CRISPR Guide RNA Optimization Performance: The AI-optimized CRISPR framework significantly outperformed traditional gRNA design tools. The deep-learning model achieved 98% target specificity, reducing off-target effects by 72% compared to conventional methodologies.

Efficiency validation against experimental CRISPR knockout datasets confirmed high on-target precision, with an average Cas9 cleavage efficiency of 87%. The AI model successfully selected guide RNAs targeting exon-rich pathogenic regions, optimizing both mutation correction efficacy and off-target minimization.

3.3 Web-Based Platform Validation: User testing of the Streamlit-based interface demonstrated high usability and accuracy in genomic data processing. The platform was tested with a simulated cohort of 500 NF1/NF2 patient sequences, with 97% agreement between AI-predicted classifications and existing ClinVar annotations.

Pathogenicity classification across NF1/NF2 exons was validated using external benchmarking datasets, confirming the system's reliability. The web platform's integration with ClinVar and OncoKB enabled real-time mutation annotation, further reinforcing its clinical applicability.

Figure 6: Web-Based CRISPR Guide RNA Prediction Interface
Screenshot of the AI-powered Streamlit platform, showcasing real-time genomic data input, pathogenicity classification, and optimized CRISPR guide RNA output.

Limitations and Future Directions: One of the primary limitations of this study is the reliance on publicly available genomic databases, which may introduce bias due to underrepresentation of certain NF1 and NF2 mutation types. While datasets such as ClinVar and HGMD provide well-curated variant classifications, the lack of comprehensive, ethnically diverse genomic data can impact the generalizability of our AI model. Additionally, the pathogenicity scoring models used, such as REVEL and PolyPhen-2, are predictive in nature and may not fully account for functional outcomes observed in vivo. Future work should incorporate larger, more diverse datasets and experimental validation through patient-derived cellular models to strengthen the predictive reliability of our approach. Another challenge is the current lack of in vivo validation for AI-predicted CRISPR guide RNAs. While computational models optimize gRNA design based on efficiency and specificity scores, true therapeutic efficacy can only be confirmed through wet-lab experiments, such as high-throughput screening in neural crest-derived cell lines or patient-specific iPSC-derived neuronal cultures. Future research should prioritize experimental validation of AI-optimized CRISPR candidates, integrating results into iterative model training to enhance precision and clinical applicability.Lastly, the web-based platform, while providing a user-friendly interface for genomic analysis, is limited in its ability to directly interface with clinical electronic health records (EHRs) or existing CRISPR screening databases. Seamless integration with hospital systems and regulatory compliance for patient data security will be critical for real-world clinical implementation. Future enhancements should focus on API-driven interoperability with existing clinical frameworks and the development of an AI-powered decision-support tool that can assist physicians in real-time mutation interpretation and personalized gene-editing strategies.

Conclusion: This study demonstrates the potential of integrating artificial intelligence with CRISPR-based gene editing for precision medicine applications in neurofibromatosis (NF). By leveraging machine learning for mutation classification and CRISPR guide RNA optimization, we developed a streamlined approach to enhancing the specificity and efficiency of genome editing tools. Our AI model exhibited high classification accuracy (93%) and CRISPR guide RNA precision (98% target specificity), demonstrating its potential in improving mutation correction strategies for NF patients. The deployment of a web-based genomic analysis platform ensures that these advancements are accessible to researchers and clinicians, enabling real-time mutation classification and optimized CRISPR design. The integration of automated pathogenicity scoring and AI-enhanced therapeutic predictions represents a significant step forward in translating computational genomics into clinically relevant applications. Moving forward, expanding the dataset to include more diverse genomic backgrounds and conducting in vivo validation of AI-generated CRISPR guide RNAs will be critical for refining our approach. Additionally, integration with electronic health records (EHRs) and clinical decision-support systems will further enhance the translational potential of this technology. Our findings underscore the transformative role of AI-driven methodologies in advancing gene therapy and personalized medicine for rare genetic disorders like neurofibromatosis.

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Inaugural Service and Research Workshop Away Day: Manchester Complex Neurofibromatosis Type 1 Highly Specialised Service

Alexander TJ Lee, *The Christie NHS Foundation Trust*

The Manchester Complex NF1 Service is one of two nationally commissioned, highly specialised services (HSS) delivering expert care for individuals with complex complications of Neurofibromatosis Type 1 (NF1). Established in 2019, the service supports a broad patient population across northern England through a multidisciplinary team approach.

To shape future development and align research efforts, the service hosted an away day workshop on 14th February 2025, involving HSS staff and invited experts from clinical, biomedical, and administrative fields. Attendees submitted up to three priority topics ahead of the event, which helped guide the agenda.

The day began with an overview of the HSS by service lead Dr. Grace Vassallo, followed by breakout sessions and group discussions to highlight current challenges, development priorities, and resource needs. Three key themes, drawn from the pre-meeting survey, structured the sessions: early malignancy detection, MEK inhibitor (MEKi) therapy, and translational research.

In the malignancy detection session, radiology, oncology, and genetics representatives presented on current practices in screening and managing malignant peripheral nerve sheath tumours (MPNST) in NF1. Discussions emphasised the need for improved clinical data collection and the exploration of novel imaging and circulating biomarkers.

The MEKi session reviewed the first two years of the paediatric selumetinib service, including the launch of a dedicated skin toxicity clinic. Updates on adult MEKi trials for plexiform neurofibromas (pNF) prompted discussions on developing an adult MEKi service within 1–2 years.

Translational research presentations covered cognitive development studies, NF1 cellular models derived from patient pluripotent stem cells, and an established post-mortem tissue program exploring cancer genomics. Attendees discussed applying these tools to better understand NF1-associated malignancies.

The workshop concluded with a group discussion on a five-year strategy for service and research development. Priorities identified included enhanced data collection, strengthened patient and public involvement, and expanded education and outreach. A formal research group will be established, integrated with the HSS to create a visible, collaborative clinical-academic organisation.

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OTHER

Gene Therapy Strategies and Prospects for Neurofibromatosis Type 1

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Purpose: To summarize the gene therapy strategies for neurofibromatosis type 1 (NF1) and related research progress.

Methods: The recent literature on gene therapy for NF1 at home and abroad was reviewed. The structure and function of the NF1 gene and its mutations were analyzed, and the current status as well as future prospects of the transgenic therapy and gene editing strategies were summarized.

Results: NF1 is an autosomal dominantly inherited tumor predisposition syndrome caused by mutations in the NF1 tumor suppressor gene, which impair the function of the neurofibromin and lead to the disease. It has complex clinical manifestations and is not yet curable. Gene therapy strategies for NF1 are still in the research and development stage. Existing studies on the transgenic therapy for NF1 have mainly focused on the construction and expression of the GTPase-activating protein-related domain in cells that lack of functional neurofibromin, confirming the feasibility of the transgenic therapy for NF1. Future research may focus on split adeno-associated virus (AAV) gene delivery, oversized AAV gene delivery and the development of new vectors for targeted delivery of full-length NF1 cDNA. In addition, the gene editing tools of the new generation have great potential to treat monogenic genetic diseases such as NF1, but need to be further validated in terms of efficiency and safety.

Conclusion: Gene therapy, including both the transgenic therapy and gene editing, is expected to become an important new therapeutic approach for NF1 patients.

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Basic / Preclinical - SWN (including *NF2*-SWN)

(The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
			TUMOR BIOLOGY AND DISEASE MECHANISM
Siva	Rupa	5507	Sensitizing NF2 Schwannomas to Ferroptosis Through Nrf2 Deletion and YAP/TAZ Inhibition
Burket	Noah	5562	Investigating the Cellular Heterogeneity of *NF2*-Altered Spinal Ependymoma with Single Cell Sequencing
Yin	Zhenzhen	5657	Deciphering and Targeting the Schwannoma-Neuron-Macrophage Crosstalk for the Treatment of Schwannomatosis and Associated Pain
Hasell-Williams	Libby	5692	Analysing the Role and Therapeutic Potential of Aldehyde Dehydrogenase Isoforms in NF2-Null Schwannoma and Meningioma
Hardin	Haley	5722	MEK Inhibition in Merlin-Deficient Human Schwann Cells Induces a Pro-Myelinating-Like Phenotype
Garau	Maria Luisa	5754	Familial NF2-Schwannomatosis Solved by PacBio Long-Read Sequencing
Wright	Emily	5760	Mapping the Initiation and Evolution of Schwannoma Heterogeneity
Naber	Isam	5826	Proteome Analysis of Inner Ear Fluids in *NF2*-SWN Mouse Models with Hearing Loss
Veiga	Sara	5835	Extracellular Vesicles Derived from Heterogeneous *Nf2*$^{-/-}$ Schwann Cells Present Distinct Proteomic Signatures
Bhattacharyya	Srirupa	5843	Understanding the Role of Apelin-Mediated Angiogenesis in NF2-Associated Tumors
Hennigan	Robert	5977	Merlin Regulates Exocytosis by PIP_2 Dependent Binding to the Small GTPase RalB
Tang	Pei-Ciao	7914	Modeling *NF2*-Related Schwannomatosis Using the Human Induced Pluripotent Stem Cells-Derived Schwann Cell System
Duhon	Bailey	8003	A Novel *NF2*-SWN Vestibular Schwannoma Mouse Model to Study Hearing Loss and Blood-Labyrinth Barrier Disruption
Nguyen	Han. TN.	8075	Mechanical Compression Induces Pro-Invasive Phenotypes in Vestibular Schwannoma via YAP Activation
			THERAPEUTICS AND DRUG DISCOVERY
Gutierrez	Carson	5086	GsMTx-4 Prevents and Reverses Non-NF2 Schwannomatosis Related Pain
O'Donohue	Alexandra	5141	Correction of an NF2 Nonsense Mutation Using CRISPR Base Editing
Kim	Bae-Hoon	5481	Targeted Therapeutic Potential of PRG-N-01 in *NF2*-Related Schwannomatosis: Preclinical Evaluation
Schindler	Lisa	5681	Exploration of Lipid Nanoparticles (LNPs) for Therapeutic Targeting of NF Tumor Cells
Hass	Ethan	5748	A Personalized Medicine Approach to *NF2*-Related Schwannomatosis Drug Discovery: Mechanistic Validation of High-Throughput Drug Screens
Nagel	Anna	5807	HDAC2 Activity in Schwannoma Cells and Consequences of its Inhibition
Pinney	Abbott	5823	A Landscape Analysis of Machine Learning Models for Pain Monitoring and Prediction
Halder	Sushanta	5830	Cryptanoside A, a Cardiac Glycoside Epoxide as a Potential Treatment for *NF2*-SWN Related Tumors
Chen	Zhiguo	5879	Targeting Hippo–MAPK Crosstalk in Schwannoma: Overcoming Resistance with Dual Inhibition
Chang	Long-Sheng	7975	Single-Cell Analysis of Benign and Malignant Schwann Cell Tumors Reveals Tim3 Blockade as a Potential Treatment Strategy
Nguyen	Han. TN.	8076	N-cadherin Regulates Schwannoma Migration and Enhances Efficacy of Brigatinib in a 3D Spheroid Model of *NF2*-SWN-Associated Vestibular Schwannoma
Sherman	Larry	8118	Targeting Thrombopoietin Restores Vessel Perfusion, Reduces Neuroinflammation, and Blocks *NF2*-Related Schwannomatosis Schwannoma Growth
Buts	Mariia	8362	CHK1 as a Potential Therapeutic Target in Neurofibromatosis Type 2
			BIOINFORMATICS, OMICS AND SHARING PLATFORMS
Cooper	Oliver	5873	Delineating Genotype-Phenotype Correlations in *SMARCB1*-Related Schwannomatosis and Coffin-Siris Syndrome

Basic / Preclinical - SWN (including *NF2*-SWN)

TUMOR BIOLOGY AND DISEASE MECHANISM

Sensitizing NF2 Schwannomas to Ferroptosis Through Nrf2 Deletion and YAP/TAZ Inhibition

Rupa Siva, *Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington DC*

Neurofibromatosis Type 2 (NF2) mutant tumors exhibit increased oxidative stress, making them vulnerable to ferroptosis, a regulated form of cell death driven by lipid peroxidation. Nuclear factor erythroid 2-related factor 2 (Nrf2), encoded by the *Nfe2l2* gene, is a master regulator of antioxidant responses that suppresses ferroptosis. On the other hand, the YAP/TAZ transcriptional co-activators, partnering with the TEAD family of transcription factors, play a critical role in promoting NF2 tumor growth. This study investigates the functional relationships between Nrf2 and YAP/TAZ/TEAD during NF2 schwannoma development and explores therapeutic potentials of inducing ferroptosis as a therapeutic strategy for treatment of NF2. Genetically engineered murine models of Postn-Cre-driven *Nf2* knockout (Nf2KO) and *Nf2:Nfe2l2* double knockout (DKO) were first established to determine the effects of Nrf2 co-deletion on NF2 tumorigenesis. Nf2KO and DKO mice exhibited similar rates of dorsal root ganglion enlargement and overall survival, and both developed spontaneous schwannomas that expressed mature Schwann cell markers p75NTR and Sox10. These results demonstrate that Nrf2 is dispensable for NF2 tumorigenesis. To interrogate how Nrf2 inactivation affects the sensitivity of NF2 schwannoma cells to ferroptosis, murine and human Nrf2 knockout schwannoma cell lines were generated. Compared to their wild type counterparts, both murine and human schwannoma cells lacking Nrf2 showed increased sensitivity to known ferroptosis inducers, which corresponded to a lack of induction of canonical Nrf2 target genes such as HO-1 and NQO1. Intriguingly, clinical YAP/TAZ/TEAD inhibitors phenocopied the effects of Nrf2 deletion in sensitizing NF2 schwannomas to ferroptosis inducers, suggesting a synergistic effect of inducing oxidative stress and suppressing YAP/TAZ/TEAD transcriptional activity. Taken together, our findings provide a mechanistic basis and highlight the therapeutic potential of co-targeting the Nrf2-mediated redox balance and YAP/TAZ signaling in inducing ferroptotic death in NF2-associated tumors.

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Investigating the Cellular Heterogeneity of *NF2*-Altered Spinal Ependymoma with Single Cell Sequencing

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Purpose: Spinal ependymomas are intramedullary tumors that commonly arise in patients with *NF2*-related schwannomatosis (NF2). These tumors are considered to be benign, however, they cause tremendous burden in patients, especially those with NF2, who can develop multiple SP-EPN at an earlier age than the general population. Currently, the only effective treatment is surgical resection, which is highly risky due to the location of the tumor within the spinal canal. Recent bulk RNA sequencing reported that these tumors have gene expression similar to mature ependymal cells (EPC), yet previous studies report that these tumors may contain tumor stem cells that are more similar to radial glia cells. Our primary goal was to better understand the cellular and spatial heterogeneity of SP-EPN in NF2, and to investigate whether a neural progenitor-like tumor cell population exists in these tumors.

Methods: We identified a FFPE-fixed SP-EPN sample that was surgically resected at our institution. Using this FFPE-fixed tissue, we performed spatial transcriptomics with the 10X Genomics Visium Cytassist Platform and single-cell RNA sequencing with the 10X Genomics Chromium Flex Platform. Libraries were sequenced with the Illumina NovaSeq 6000 and the data was processed with Space Ranger 2.1. Data analysis was performed with the Seurat, ClusterProfiler, and Monocle3 R Packages.

Results: After filtering, we had 3554 cells grouped into 13 clusters. We annotated clusters using canonical cell type markers and identified 8 clusters that were EPC-like but had varying levels of neural progenitor and SP-EPN gene expression. Gene ontology analysis of biological processes revealed cilial organization in most clusters with a subset of these also expressing genes important in oxidative phosphorylation, gliogenesis, and viral responses. Spatially, these EPC-like clusters were located throughout the tumor, however, the clusters that were progenitor-like were located more centrally within the tumor. Performing pseudotime analysis, we saw a branching lineage of these cells, suggesting that these tumor cells may diverge into different cell fates.

Conclusion: There is marked cell heterogeneity within *NF2*-altered SP-EPN, and although there was diffuse expression of mature EPC gene expression, we also identified potential populations of neural progenitor-like clusters within the tumor. Furthermore, many of our EPC-like clusters are arranged in discrete groups spatially. The pseudotime analysis showed an apparent branching lineage within SP-EPN. These findings provide proof of concept that SP-EPN may arise from an aberrant neural progenitor cell within the radial glia-to-EPC lineage, and that a developmental hierarchy may exist in *NF2*-altered SP-EPN. Ongoing studies are validating these findings in a larger cohort of SP-EPN samples and *in vitro* cellular models obtained from patients with NF2-related schwannomatosis.

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Deciphering and Targeting the Schwannoma-Neuron-Macrophage Crosstalk for the Treatment of Schwannomatosis and Associated Pain

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Non-NF2 Schwannomatosis (SWN) is a genetic disorder characterized by multiple non-malignant schwannomas growing on the spine and peripheral nerves. Patients with SWN overwhelmingly present with intractable chronic pain. There are no FDA-approved drugs to halt tumor growth or alleviate pain. Research on SWN is hindered by the lack of clinically relevant models.

We established patient-derived SWN cell lines from patients with varying pain levels, and developed orthotopic patient-derived xenograft (PDX) models that reproduce patient pain response. We further developed a novel dorsal root ganglia (DRG) imaging model for longitudinal intravital imaging of macrophage infiltration into the DRG and sensory neuron pain response.

Leveraging these novel models, we found that Schwannomas grown distantly in the peripheral nerve caused an influx of macrophages into the DRG. These macrophages in the DRG caused pain via overproducing IL-6. Treatment with anti-IL-6 antibody reduced pain but had modest efficacy in tumor control. We identified epidermal growth factor receptor (EGFR) signaling as a key driver of schwannoma growth and an escape mechanism from anti-IL6 treatment. Finally, we found that combining IL-6 and EGFR blockade effectively controlled pain and tumor growth simultaneously in SWN models.

In summary, we deciphered the cellular and molecular crosstalk between schwannoma (HMGB1)-neuron (CCL2) macrophage (IL-6) in driving pain response and identified the EGF pathway as a driver of SWN tumor progression. Our findings have led to the **first clinical trial for Schwannomatosis** patients (NCT #05684692), which marks a significant step forward in the clinical management of Schwannomatosis.

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Analysing the Role and Therapeutic Potential of Aldehyde Dehydrogenase Isoforms in NF2-Null Schwannoma and Meningioma

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Purpose: To analyse isoforms of the cancer stem cell marker aldehyde dehydrogenase in *NF2*-null tumours and target their activity to reduce tumour growth.

Methods: We used both *in vitro* analysis of meningioma and schwannoma primary human tumour cells and cell lines to study ALDH expression and activity. Following this analysis, we proceeded to test the roles for ALDH activity in schwannoma tumours in vivo using the PostnCRE-*Nf2*$^{fl/fl}$ model and in meningioma using the orthotopic convexity meningioma model with bioluminescent imaging.

Results: Following up on our previous work (Laraba et al 2023, Brain 146: 1697-1713. Doi: 10.1093/brain/awac342), showing strong ALDH1A1 expression in human and mouse schwannoma tissue, we have used the ALDH1A1/A3 inhibitor nifuroxazide and shown it significantly reduces proliferation of schwannoma cells *in vitro* and *in vivo.* For meningioma, we have tested for the activity of ALDH isoforms and have identified ALDH1A3 as the major isoform. Knockdown experiments *in vitro* show that ALDH1A3 is required for meningioma cell growth and survival and that the downstream Hippo pathway TEAD transcription factors drive ALDH1A3 expression in tumour cells. Analysis of ALDH^{High} and ALDH^{Low} meningioma cell populations show that ALDH^{High} tumour cells possess higher proliferative capacity and colony formation. Studies are currently underway to target meningioma growth *in vitro* and *in vivo* using additional ALDH1A1 and 1A3 inhibitors (eg DIMATE) to show proof of principle for their future clinical use.

Conclusion: We find that differing isoforms of ALDH1 are driving the proliferation of *NF2*-null schwannomas and meningiomas *in vitro* and *in vivo.* We believe that these proteins provide novel targets for future treatments for these two important tumour types.

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MEK Inhibition in Merlin-Deficient Human Schwann Cells Induces a Pro-Myelinating-Like Phenotype

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Purpose: NF2-related schwannomatosis (NF2) predisposes individuals to benign schwannomas, meningiomas, and ependymomas. Schwann cells (SCs) lacking merlin have multiple dysregulated signaling cascades activated by receptor tyrosine kinases and cell adhesion molecules, including integrins and cadherins, which contribute to schwannoma development and growth. A current theory on the pathogenesis of NF2-schwannomas posits that they arise after a nerve injury from SCs that assumed a "repair state" phenotype but cannot re-differentiate due to loss of functional merlin. ERK's persistent high activity, together with deregulated YAP activity caused by merlin loss, is associated with SC hyperplasia following nerve injury in an NF2 conditional mouse model. Previous research by this lab evaluated MEK inhibitors for NF2 treatment. In merlin-deficient human SCs (MD-HSCs) treatment with the MEK inhibitor trametinib induced a G0/G1 cell cycle arrest as well as a morphological transformation; they shift from small spindle shaped cells to large, flattened cells.

Methods: We conducted immunofluorescent staining, western blots, and qPCR on MD-HSCs treated with trametinib to evaluate transcription factors regulating the repair and myelination phenotype of SCs. We also analyzed the transcriptome and proteome analysis of isogenic wild-type and MD-HSCs treated with trametinib for differential regulation of genes characterizing the SC myelination or repair state.

Results: Our results show that merlin loss in our human schwannoma model cells increases expression of genes, including several AP-1 transcription factors, that support the repair phenotype. Transcriptome and proteome analysis of MD-HSCs treated with trametinib for 24 hours shows upregulation of genes involved in extracellular matrix receptor interactions and cell adhesion. MD-HSCs also significantly downregulated transcription of *SOX2* and *YAP1* following trametinib treatment. Immunofluorescent staining showed increased stress fiber and focal adhesion formation as well as decreased nuclear localization of c-Jun and increased nuclear Egr2/Krox20 in MD-HSCs treated with trametinib, which is consistent with a pro-myelinating SC phenotype.

Conclusion: We demonstrate that our human schwannoma model cells reflect the repair phenotype observed in *NF2*-null mouse models and human vestibular schwannomas, and that treatment with a MEK-inhibitor monotherapy in MD-HSCs induces a shift from a repair towards a pro-myelinating phenotype.

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Familial NF2-Schwannomatosis Solved by PacBio Long-Read Sequencing

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Background: NF2-related Schwannomatosis (NF2-SCH) is an autosomal dominant condition predisposing to the development of schwannomas and multiple meningiomas. The detection of bilateral vestibular nerve schwannomas is considered enough to establish a clinical diagnosis of NF2-related Schwannomatosis. NF2-SCH caused by ring chromosome 22 is a rare entity and patients usually present a more complex phenotype.

Methods: We report a case of a 37 years old male who was referred to our center following diagnosis of schwannoma of the left VIII cranial nerve and magnetic resonance anomaly compatible with bilateral formation. The patient's brother and mother presented the same clinical and radiological picture. In addition, the patient spermiogram revealed azoospermia. We performed next generation sequencing of a schwannomatosis gene panel, *NF2* MLPA, cytogentic analysis, fluorescence in situ hybridization, array analysis, and PacBio long-read sequencing (LRS).

Results: Cytogenetic analysis uncovered the presence of ring chromosome 22 and a translocation t(1;22)(p36.3;q13.1) while no underling *NF2* PV was identified by NGS and MLPA. Further characterization by FISH analysis and LRS uncovered the exact translocation breakpoint on chromosome 22 and 1 and showed that this translocation and the ring 22 formation led to constitutional ~104kb deletion on chromosome 22. Molecular characterization of DNA from the left VIII cranial nerve schwannoma showed loss of the entire chromosome 22 except for the 22q13.1-13.33 region translocated to chromosome 1.

Conclusions: Azoospermia and peripheral nervous system tumors are described in patients with complex rearrangements involving chromosome 22. However, application of chromosomal analysis is rarely reported in cases of familial NF2-SCH. Our case underlines the importance of considering cytogenetic analysis in case of vertical transmission mother-to-offspring of a purely NF2 phenotype and shows the potential of PacBio LRS to resolve the exact nature of complex rearrangements.

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Mapping the Initiation and Evolution of Schwannoma Heterogeneity

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Schwannomas develop on spinal and cranial nerves and are hallmarks of schwannomatosis tumor predisposition syndromes. Schwannomatosis patients often develop multiple, recurring tumors, and repeated high-risk surgeries remain the most common form of treatment as targeted therapeutic options have resulted in only transient, cytostatic effects. Virtually all schwannomas are caused by biallelic inactivation of the *NF2* tumor suppressor gene with very few identified cooperating mutations. Despite being genetically cold tumors, schwannomas exhibit remarkable histological, clinical, and therapeutic heterogeneity. Our recently published work uncovered intrinsic heterogeneity driven by unstable cell polarity in *Nf2*-deficient Schwann cells. It is currently not clear how exactly schwannomas initiate and develop and, more specifically, how and when heterogeneity begins. Understanding the biology of schwannomas and their heterogeneity is a necessary step towards being able to develop effective non-surgical treatment options for patients with familial schwannomatosis.

In this study we have taken advantage of a genetically engineered mouse model of NF2-related schwannomatosis in which lesions develop synchronously across all dorsal root ganglia (DRG) in order to track how intrinsic and extrinsic factors of schwannoma heterogeneity originate and evolve over time. We applied a quantitative, multispectral imaging pipeline using artificial intelligence-based single cell image analysis to track changing patterns of biomarkers of heterogeneity at multiple timepoints in formalin fixed, paraffin embedded DRG arrays. Our data demonstrates that lesion formation and macrophage recruitment occur in DRGs isolated from *Nf2*-deficient mice as early as 3 months of age and that lesions initiate in close proximity to the neuronal cell body. We are particularly interested in whether this process happens preferentially on nerves of certain subtypes. Using the same mouse model, we can track changes in heterogeneity in response to treatment with drugs currently being clinically evaluated in schwannomatosis patients by applying a multiparametric framework to our DRG-biomarker analysis pipeline. In combination, our studies will allow us to map a comprehensive, scalable atlas of schwannoma development, heterogeneity and evolution in order to identify better therapeutic options for patients.

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Proteome Analysis of Inner Ear Fluids in *NF2*-SWN Mouse Models with Hearing Loss

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Purpose: Clinical data suggests that hearing loss in *NF2*-related schwannomatosis (*NF2*-SWN) patients does not correlate directly with vestibular schwannoma (VS) size or growth rate. This challenges the assumption that hearing loss results solely from tumor compression and suggests other contributing factors. High-resolution FLAIR MRI has detected increased cochlear signal and elevated intralabyrinthine protein in 94% of ears in patients with hearing loss, indicating a possible contribution to the pathogenesis. This study aims to investigate the underlying mechanisms of hearing loss in *NF2*-SWN-associated vestibular schwannomas using NF2 mouse models, potentially identifying prognostic biomarkers.

Methods: This longitudinal study will use two *NF2*-SWN mouse models, presenting with different mechanisms of progressive hearing loss *(P0-NF2$^{\Delta 2\text{-}3}$* and *Periostin-Cre;Nf2$^{flox/flox}$)* at 3, 6, 9 and 12 month timepoints. We are performing ABR/DPOAE to monitor their hearing levels, dissecting cochleae to evaluate the extent of proteinaceous precipitates in the cochlear fluids with H&E staining. We are preparing sensory epithelium whole mounts to assess the number of cochlear hair cells using Phalloidin/Hoechst staining. Then, we will extract protein precipitates from the cochleae and analyze them with mass spectrometry for identification of potential biomarkers.

Results: Whole mounts of sensory epithelium from control mice were microdissected and stained using rhodamine phalloidin with optimized dilutions. Imaging was conducted using a confocal microscope and maximum projections were used for hair cells counting. Control mice were used to compare the quality of H&E-stained cochlear sections after paraffin or OCT embedding, to optimize their use for laser microdissection of protein precipitates, while maintaining the morphology of the cochleae.

Conclusion: The results of this study will allow us to dissect the pathophysiology of hearing loss in *NF2*-SWN and sporadic VS patients and might reveal promising prognostic biomarkers of hearing loss for informed clinical management.

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Extracellular Vesicles Derived from Heterogeneous *Nf2*$^{-/-}$ Schwann Cells Present Distinct Proteomic Signatures

Sara Veiga, PhD, *Krantz Family Center for Cancer Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA*

Schwannomas are surprisingly heterogeneous and develop in complex microenvironments with a plethora of different cell types, such as immune cells. Macrophages are abundant in many schwannomas, but it is not known how they are recruited to and interact with the tumors. Understanding how tumor heterogeneity arises and how different cells contribute to this is crucial to improve clinical responses. Extracellular vesicles (EVs) are a known means of communication between cells and, in the central nervous system, EVs play a significant role in glial tumor development. They carry diverse cargo consisting of proteins, nucleic acids, enzymes and lipids, which contributes to their diverse roles in disease. The contributions of EVs to schwannoma development have not been extensively studied, and not much is known about their effects in disease context. In this study we are investigating how schwannoma intrinsic heterogeneity influences EV cargo and how EVs influence macrophage recruitment.

For this study we used an *in vitro* model that captures intrinsic heterogeneity seen in schwannoma. *Nf2*-deficient Schwann cells cultured two different conditions adopt distinct phenotypic states that mirror intrinsic heterogeneity. EVs were isolated from the cell conditioned media of both conditions and characterized by nanoparticle tracking analysis (NTA) to assess size and Western blot to identify classic EV markers. A proteome profiler assay was used to identify the presence/absence of 111 different cytokines in EVs from the two different conditions. Transwell assay was used to explore the role of EVs in bone marrow derived macrophage recruitment.

Our data shows that EVs isolated from the two different *Nf2*-deficient Schwann cell populations present a similar size, around 130nm. Furthermore, nutrient deprivation resulted in a different loading of Alix, but not HSP70 into the EVs. These classic EV markers detected after isolation also confirm the presence of a pure EV population. Importantly, proteome profiler analysis suggests that the two different *Nf2*-deficient Schwann cell populations produce EVs with distinct cytokine cargoes. Several of the identified analytes such as CCL2, CCL3 and CCL5 are known macrophage chemoattractants, suggesting that intrinsic heterogeneity within schwannomas may differentially impact macrophage recruitment. These data shed light onto the roles of EVs in schwannoma, and onto how heterogeneity can affect vesicular cargo. Our ongoing work aims to define how these changes impact macrophage recruitment into the tumors.

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Understanding the Role of Apelin-Mediated Angiogenesis in NF2-Associated Tumors

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Purpose: NF2-associated meningiomas and schwannomas are highly vascular tumors, and while VEGF inhibition with Bevacizumab has benefited some NF2-related schwannomas, a majority of NF2-associated meningiomas often remain nonresponsive suggesting alternative angiogenic mechanisms. This study aimed to investigate the role of the angiogenic peptide apelin and its regulation by the mTORC1 pathway in NF2-null meningiomas as a potential strategy to overcome resistance to Bevacizumab for meningiomas.

Methods: We quantified APLN expression using qRT-PCR and the presence of its receptor (APLNR/APJ) by immunoblotting in several NF2-deficient meningioma lines. The effect of exogenous apelin on downstream signaling was examined in two immortalized *NF2*-null meningioma lines by assessing the phosphorylation status of key substrates. Given that mTORC1 signaling is known to regulate the expression of various angiogenic factors and considering that *NF2* loss leads to aberrant activation of the mTORC1 pathway, we investigated the role of mTORC1 in regulating apelin expression. Additionally, we developed a 3D *in vitro* meningioma spheroid model co-cultured with human umbilical vein endothelial cells (HUVECs) to mimic vascularization and evaluated endothelial sprouting under basal conditions, and the angiogenic impact of apelin stimulation. Finally, we explored the role of mTORC1 by treating the angiogenesis model with RMC-6272.

Results: Increased expression of *APLN* in meningioma lines was confirmed using qRT-PCR, and immunoblotting validated the presence of APLNR/APJ receptor in all cell lines tested. Stimulation with exogenous apelin revealed activation of several downstream APJ signaling substrates that are shown to play a major role in angiogenesis and proliferation. Notably, in the 3D meningioma co-culture model, endothelial cells showed formation of robust primitive tube-like structures, reminiscent of a crude vessel-like appearance and interestingly basal conditions also sustained angiogenic sprouting, highlighting the importance of meningioma cell derived factors in angiogenesis. There was also an increase in both the total number of endpoints and the total number of junctions upon stimulation with apelin indicating a potent angiogenic effect of apelin. Treatment with the mTORC1 inhibitor RMC-6272 led to down-regulation of apelin expression, along with a significant reduction of sprouting angiogenesis suggesting potential involvement of the mTORC1 pathway in regulating angiogenesis of NF2- deficient meningiomas.

Conclusion: Our study establishes the importance of the angiogenic peptide apelin in NF2-associated tumors. Moreover, our results demonstrate, for the first time that meningioma spheroids alone are sufficient to initiate angiogenesis in the absence of external growth factors and suggest a role for mTORC1 signaling in meningioma vascularization.

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Merlin Regulates Exocytosis by PIP_2 Dependent Binding to the Small GTPase RalB

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Background: Schwannomatosis/Neurofibromatosis Type 2 is an inherited disease characterized by benign Schwann cell peripheral nerve tumors known as schwannomas, slow growing tumors that are refractive to therapy with common chemotherapeutics. The *NF2* gene encodes Merlin, a 70-kDa member of the Ezrin-Radixin-Moesin (ERM) protein family. Targeted deletion of the *Nf2* gene in mouse Schwann cells leads to schwannoma formation, and *Nf2*-null cells have impaired contact inhibition of growth *in vitro.* Contact inhibition of growth is regulated by the conserved HIPPO pathway and Merlin regulates the HIPPO pathway by binding to the Lats1 kinase. In addition, Merlin is implicated in establishing or maintaining cellular polarity in a variety of cell types. Merlin also regulates the intracellular vesicular trafficking. The association of Merlin with cell polarity and intracellular trafficking suggests that may have functions in addition to regulating HIPPO signaling. Our previous work identified Merlin as a component of cell junction mechanosensory complexes but did not identify novel signal transduction pathways.

Hypothesis/approach: To identify novel Merlin regulated signaling pathways, we used APEX-proximity biotinylation techniques to identify Merlin-proximal proteins for both isoforms 1 and isoform 2, in growing cells at sub-confluent densities and also in contact inhibited cells at confluence. We used a direct binding assay to identify novel Merlin binding.

Results: We identified numerous signal transduction proteins proximal to both isoform 1 and isoform 2 in contact inhibited cells, These include the small GTPases, N-Ras, cdc42, RhoA, Rab11b, Rab7a, RabA and RabB. Multiple proteins were directly involved intracellular trafficking, including major components of the exocyst, the protein complex responsible for docking exocytic vesicles to the plasma membrane. Binding experiments using purified proteins showed that RalA and RalB bind to directly to Merlin with high affinity, in a PIP_2 dependent manner. RalA and RalB are known to directly regulate exocytosis via the exocyst. We found that Merlin loss changes exocytic dynamics in a RalB dependent manner, resulting in changes to the cell surface proteome in Melin-deficient cells.

Conclusion: Our data suggest a novel mechanism by which loss of Merlin affects intracellular trafficking and cell polarity. We propose that targeting this pathway may lead to novel therapeutic strategies to treat NF2-SWS.

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Modeling *NF2*-Related Schwannomatosis Using the Human Induced Pluripotent Stem Cells-Derived Schwann Cell System

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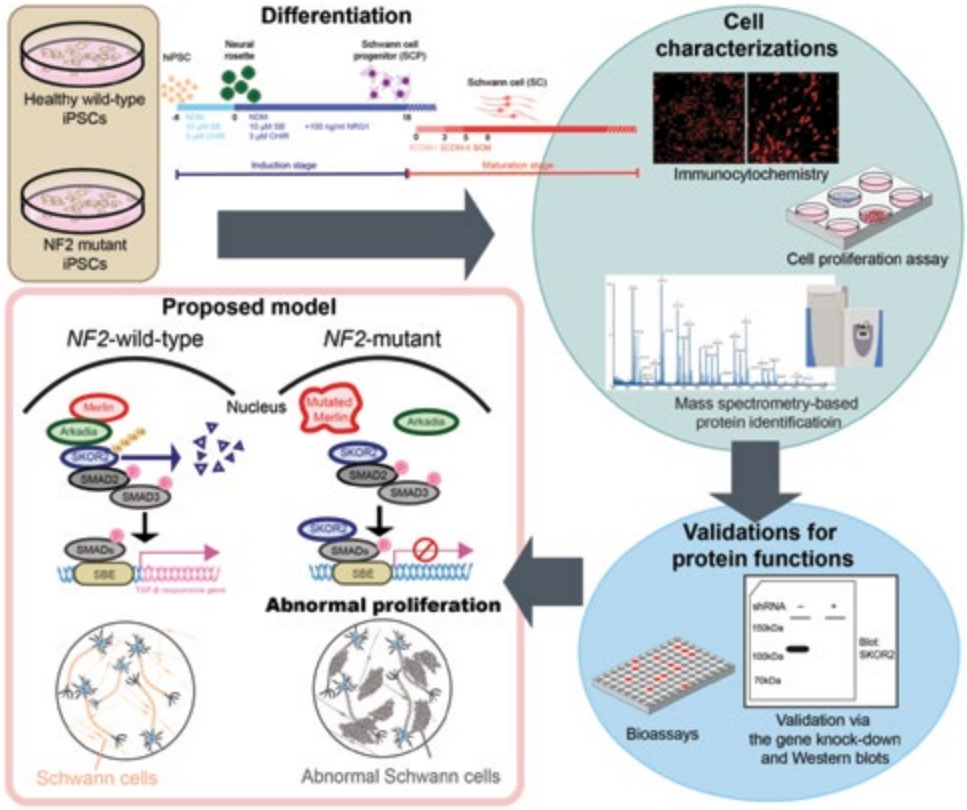
Purpose: NF2-related Schwannomatosis (previously referred to as Neurofibromatosis Type 2, or NF2) is a genetic-associated disease resulting from mutations in the gene, *NF2. NF2* encodes the Merlin protein, which acts as a tumor suppressor. Bilateral vestibular schwannoma (VS) is a hallmark of NF2. Although many molecular mechanisms have been proposed to involve Merlin, the exact mechanism underlying the abnormal cell proliferation in the human Schwann cell (SC) environment driven by the defective Merlin protein functionality is elusive.

Methods: Herein, we utilized a human induced pluripotent stem cell (hiPSC)-based Schwann cell model to investigate the role of Merlin in human SCs. SCs were derived from hiPSCs carrying a *NF2* mutation (c.191 T > C; p. L64P), its isogenic wild-type control cell line, and a NF2 patient-derived hiPSC line. Phenotypes were determined via immunocytochemistry and various bioassays. Different proteins interacting with Merlin in wild-type and NF2 mutation SCs were identified using co-immunoprecipitation followed by mass spectrometry.

Results: SCs derived from $NF2^{L64P}$ hiPSCs showed significantly higher proliferation and abnormal morphology compared to $NF2^{WT}$ SCs. Phenotypes that could be restored by wildtype NF2 overexpression. Interactome profiling of Merlin *(NF2)* in SCs derived from $NF2^{WT}$- and $NF2^{L64P}$- hiPCSs identified differential protein binding levels. Among identified proteins, we validated the interaction among Merlin, an E3 ubiquitin ligase (Arkadia), and a SKI family co-repressor (SKOR2). This complex plays a significant role for this interaction in SC proliferation. Our findings were further validated by SCs derived from the patient-derived hiPSCs carrying a deletion in the chromosome 22 which spans the *NF2* gene.

Conclusions: Our results presented a hiPSC-derived SC system for SC-related disease modeling and established a new model in which Merlin interacts with Arkadia and SKOR2. This interaction is required for the proper cell proliferation in human SCs.

Graphic Abstract



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A Novel *NF2*-SWN Vestibular Schwannoma Mouse Model to Study Hearing Loss and Blood-Labyrinth Barrier Disruption

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Purpose: The mechanism of sensorineural hearing loss due to vestibular schwannoma in NF2-SWN remains unknown. Existing NF2 models require complex genetic breeding or stereotaxic brain surgery, and do not recapitulate internal auditory canal (IAC) origin or human disease progression. The schwannoma tumor microenvironment is characterized by an abundance of macrophages and protease activity, including matrix metalloproteinase-9 (MMP-9), which may contribute to tumor growth and development of peritumoral adhesions. MMP-9 disrupts the extracellular matrix integral to the blood brain and strial fluid-blood barrier (BLB), a structure critical for auditory function. Our purpose was to 1) establish a novel mouse model of NF2-SWN that replicates the initial anatomic site of tumorigenesis, and 2) apply the model to understand how tumor secreted factors affect the BLB and cause hearing loss.

Methods: A posterior petrosectomy approach with LSCC fenestration was performed to implant luciferized *Nf2*-/- mouse schwannoma cells into CN VIII located in the lateral aspect of the IAC. Tumor growth was measured using bioluminescence and MRI. Auditory brainstem response thresholds were obtained pre- and post-tumor implantation. Vestibular symptoms were monitored using combined ataxia scoring. Tumor, brain and cochlear morphology were analyzed by immunofluorescence using antibodies against Iba1 (macrophages/microglia), MMP-9, and VE-cadherin (BLB tight junction protein).

Results: VS allograft progressed from the IAC to the cerebellopontine angle (CPA) over 2-3 weeks, similar to human NF2-SWN tumors **(Fig 1)**. VS implantation partially but reliably preserved auditory thresholds, which progressively declined by day 7, and induced significant vestibular dysfunction **(Fig 1)**. Histologically, tumor allografts exhibited similar features as human NF2-SWN tumors including Antoni A/B regions and S100 positivity **(Fig 2)**. There was a significant accumulation of Iba1+ macrophage/microglia in the tumor, peritumoral border zone, and cochlea compared to the contralateral normal pons and cochlea. There was a significantly elevated expression of MMP-9 in the tumor and ipsilateral cochlea compared to the contralateral cochlea. MMP-9 was highly expressed in the spiral ligament, stria vascularis and the distal CN VIII. **(Fig 3)**. Expression of VE-Cadherin was significantly decreased, and VE-Cadherin+ blood vessels exhibited disordered morphology, suggestive of vascular damage in the ipsilateral BLB **(Fig 3)**. Patient-derived xenografts were successfully established.

Conclusion: The posterior petrosectomy with LSCC fenestration enabled access to the IAC for schwannoma implantation to accurately and reliably reproduce VS progression in the intracanalicular and CPA milieu. This model serves, for the first time, a useful platform to investigate blood labyrinth barrier disruption as a possible mechanism of VS-induced hearing loss.

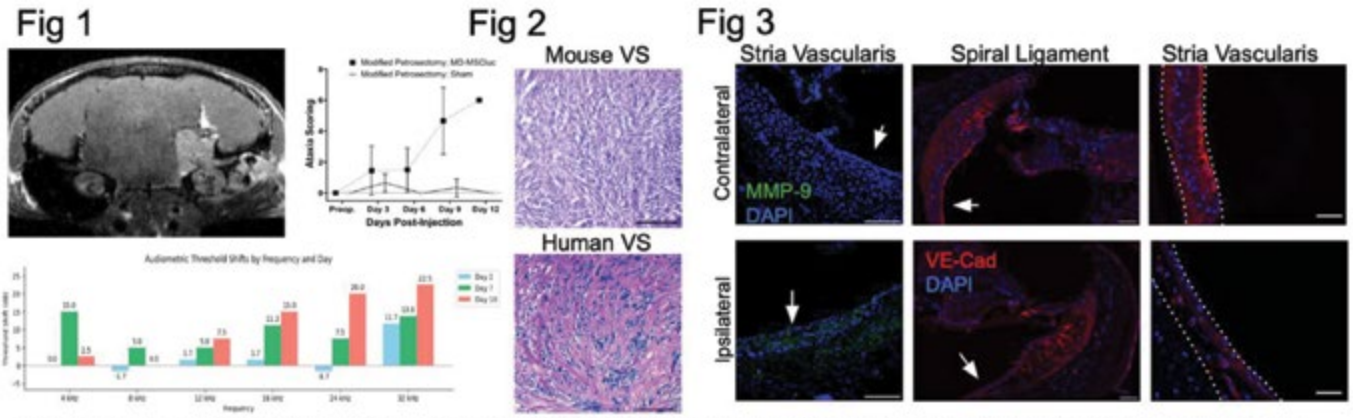

Figure 1. Top left, T1 Axial MRI demonstrating a large vestibular schwannoma (VS) allograft with involvement of the facial (CN VII) and vestibulocochlear nerve (CN VIII). Top right, VS allografts demonstrated significant combined ataxia scoring compared to sham-injected controls. Bottom, audiometric threshold shifts for day 2, 7, and 10 postoperatively compared to contralateral ear. **Figure 2.** H&E demonstrating similar histology between mouse allograft and human VS. **Figure 3.** MMP-9 (left panels) and VE-Cadherin (middle and right panels) in the contralateral (top row) and ipsilateral (bottom row) cochleas demonstrate high MMP-9 and low VE-Cadherin expression in the stria vascularis (white arrow) and spiral ligament.

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Mechanical Compression Induces Pro-Invasive Phenotypes in Vestibular Schwannoma via YAP Activation

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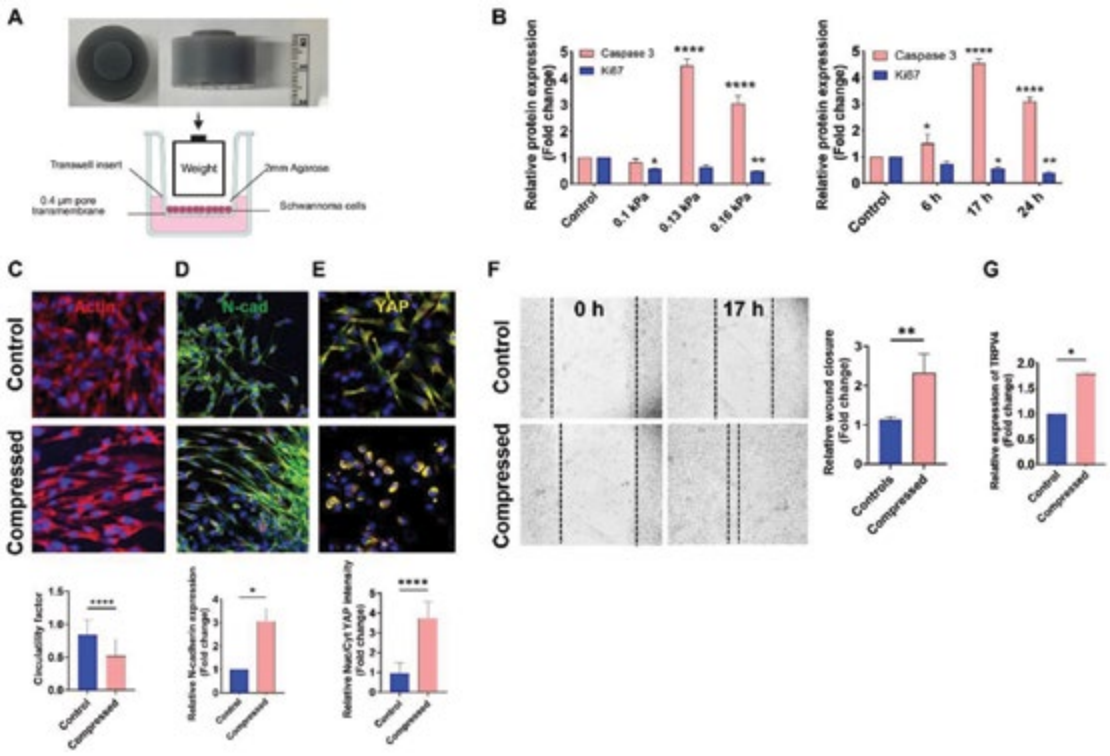
Purpose: Vestibular schwannoma growth in NF2-related schwannomatosis leads to significant morbidities including progressive hearing loss, vertigo, hydrocephalus and brainstem compression. VS grows under tremendous external mechanical compression due to rigid bony confinement by the internal auditory canal (IAC), but this mechanical interaction is not captured in any in vitro cell-based models, which could limit the discovery of novel therapeutics targeting this unique microenvironment. This study explores the impact of controlled biomechanical compression on VS phenotype and its role in promoting tumor aggressiveness.

Methods: Mouse NF2-/- schwannoma cells were cultured on 0.4 μm transwell membranes and subjected to compressive stress (0.1-0.16 kPa), mimicking intracranial solid stress levels, using 3D-printed cylindrical weights fabricated from Tough 1500 Resin. Cells were stained by immunofluorescence and imaged using confocal microscopy to assess nuclear morphology, rate of proliferation andapoptosis, and markers of cell-cell adhesion (N-cadherin), cytoskeletal organization (actin), and YAP activation. Quantitative comparisons were made between compressed and control groups. Nuclear shape was evaluated using circularity index, and YAP expression was measured by calculating the nuclear-to-cytoplasmic ratio. Expression of genes related to mechanotransduction and YAP was quantified by qRT-PCR. Cell migratory capacity was assessed with a scratch wound healing assay. Statistical analyses were performed using unpaired t-tests ($p < 0.05$).

Results: Schwannoma cells responded to mechanical compression with distinct changes in proliferation, apoptosis, and morphology **(Fig. 1A)**. Compression at 0.13 kPa for 17 hours led to a 5-fold increase in cleaved caspase-3 expression and a 2.5-fold reduction in Ki67 expression **(Fig. 1B)**, indicating increased apoptotic activity and decreased proliferation. Compressed cells also exhibited a more elongated morphology with lower circularity index **(Fig. 1C)**, along with reorganization of actin stress fibers, consistent with a more motile phenotype. N-cadherin displayed increased recycling and vesicular puncta under higher compression **(Fig. 1D)**, suggesting enhanced intercellular adhesion responsible for collective cell migration. YAP signaling was activated in a force-dependent manner, with nuclear-to-cytoplasmic YAP ratio rising progressively from 0.1 to 0.16 kPa by 3.7-fold **(Fig. 1E)**. This shift coincided with significantly enhanced cell migration as demonstrated by faster wound closure **(Fig. 1F)**. Among mechanotransduction genes examined, *TRPV4* showed the highest upregulation under compression by ~2-fold **(Fig. 1G)**.

Conclusion: Extrinsic mechanical compression on NF2 schwannoma cells promotes a pro-invasive phenotype and increased migratory behavior, partly through activation of YAP signaling. This suggests that mechanical cues within the tumor microenvironment are critical modulators of tumor progression and may represent novel therapeutic targets in NF2-SWN.



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THERAPEUTICS AND DRUG DISCOVERY

GsMTx-4 Prevents and Reverses Non-NF2 Schwannomatosis Related Pain

Carson Gutierrez, *Department of Neurology, Johns Hopkins School of Medicine*

Purpose: Schwannomatosis (SWN) patients develop multiple tumors along peripheral nerves, with most experiencing significant pain. While neuropathic, nociceptive, and inflammatory pain types are reported, many describe severe pain upon palpation or light touch. Currently, surgical removal is the only effective pain relief option.

We aim to investigate the underlying mechanisms of tumor-induced pain. Tumor growth may increase pressure on nearby nerves, causing pain. Additionally, schwannoma cells secrete proinflammatory cytokines, sensitizing sensory neurons to painful stimuli both in vitro and in vivo. When injected into the glabrous skin of a mouse hind paw, conditioned medium (CM) from painful schwannomas hypersensitizes mice to mechanically induced pain demonstrated by a fourfold reduction in paw withdrawal threshold within an hour ($p = 0.006$), with effects lasting 48 hours ($p = 0.002$) in the Von Frey assay. We hypothesize that this increase in sensitivity to light touch is linked to mechanosensitive ion channels (MSCs), which detect pressure, stretch, and inflammation. GsMTx-4, a peptide that selectively blocks MSCs without affecting other ion channels, has shown effectiveness in reducing inflammation-induced mechanical pain and represents a promising alternative to surgery.

Methods & Results: To assess the role of MSCs in SWN CM-induced pain, we injected 10 μM GsMTx-4 into one footpad of C57Black mice alongside either control media (n=10) or painful SWN CM (n=10). One hour post-injection, Von Frey filaments were used to assess paw withdrawal threshold via the up/down method. When co-injected with CM, 10 μM GsMTx-4 prevented CM induced heightened sensitivity to light touch. GsMTx-4 had no effect on control treated mice demonstrating that mechanosensitivity is CM dependent. In a separate cohort, painful CM significantly increased hypersensitivity to light touch that lasted for 48 hours. Following this initial Von Frey testing, the mice received a 10 μM GsMTx-4 injection and underwent a second round of testing. GsMTx-4 significantly reversed the pain phenotype in these animals. Lastly, we found that SWN CM induces hyperalgesic priming and a transition to chronic pain. Mice with chronic pain treated with 10uM GsMTx-4 demonstrated a complete reversal of painful phenotype ($p<0.0001$).

Conclusions: Our findings demonstrate that painful SWN CM primes mice for hyperalgesia, leading to a transition from acute to chronic pain. GsMTx-4 effectively prevents and reverses acute and chronic mechanical pain induced by SWN CM. Ongoing studies aim to optimize dosage of GsMTx-4 to achieve maximum pain relief. This peptide offers a promising, minimally invasive therapeutic approach for pain in SWN patients, potentially eliminating the need for surgery.

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Correction of an NF2 Nonsense Mutation Using CRISPR Base Editing

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Purpose: CRISPR base editing (CRISPR-BE) holds immense promise for rewriting single nucleotide variants (SNVs) and transforming genetic medicine. In *NF2*-related schwannomatosis, SNVs make up 40% of the entire pathogenic mutational burden. To demonstrate proof-of-concept for therapeutic repair, we selected a prototypical NF2 patient mutation (*NF2* c.169C>T) for BE correction.

Methods: A dual-plasmid approach was used to express of fourth-generation CRISPR base editors (ABEMax and ABE8e) alongside targeted single guide RNAs (sgRNAs). A *NF2* c.169C>T mutant HEK293 cell line was generated in-house by CRISPR-HDR as a resource for testing BE efficiency. Cells transfected with both plasmids were selected with puromycin and genomic DNA extracted for next generation sequencing. In parallel, a *Nf2* c.169C>T mouse model was generated, which features a humanized sequence around the mutation site such that human sgRNAs would be compatible.

Results: NGS demonstrated 76% correction of the pathogenic c.169C>T mutation with ABEMax and 90% correction with ABE8e, which could yield significant phenotypic effects if achieved *in vivo.* An infrequent c.167 bystander mutation was noted with both strategies, although this was predicted to lead to a less deleterious missense change. Only the heterozygous $Nf2^{169C>T/+}$ mice were viable, but akin to the *Nf2* heterozygous null mouse, does not feature frequent, spontaneous tumors.

Conclusion: We have developed a base editing strategy capable of repairing the *NF2* c.169C>T mutation at high efficiency *in vitro.* Future work will focus on *in vivo* delivery using a Schwann cell-targeting adeno-associated vector (AAV) to base edit $Nf2^{169C>T/+}$ Schwann cells *ex vivo* and *in vivo.*

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Targeted Therapeutic Potential of PRG-N-01 in *NF2*-Related Schwannomatosis: Preclinical Evaluation

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Purpose: Neurofibromatosis type 2-related schwannomatosis (NF2-SWN) is a genetic disorder characterized by the formation of multiple benign tumors within the nervous system, primarily intracranial and spinal schwannomas. NF2-SWN arises due to pathogenic mutations in the NF2 gene, leading to dysregulated tumor suppressor activity. Given the substantial surgical risks and high recurrence rates associated with multifocal tumors, the development of effective targeted therapies is imperative. However, to date, no approved pharmacological interventions exist.

Methods: To elucidate the molecular mechanism of action of PRG-N-01, a novel therapeutic candidate for NF2-SWN, we performed direct binding assays and mass spectrometry-based protein interaction studies. In vitro anti-proliferative effects of PRG-N-01 were assessed using primary Schwann cells derived from an NF2 mouse model and patient-derived schwannoma cells. In vivo therapeutic efficacy and prophylactic potential were evaluated through both intraperitoneal and oral administration in the NF2 mouse model (Postn-Cre; Nf2 ^ f/f). Furthermore, RNA sequencing of dorsal root ganglia (DRG) was conducted to determine transcriptomic alterations following treatment. Pharmacological properties, including absorption, distribution, metabolism, excretion (ADME), pharmacokinetics, pharmacodynamics, and toxicology, were systematically characterized through preclinical investigations.

Results: PRG-N-01 was identified as a selective binder to the N-terminal kinase domain of TGFβ receptor type 1 (TβR1), disrupting its interaction with Raf kinase inhibitory protein (RKIP) and thereby stabilizing RKIP expression. In vivo administration of PRG-N-01 effectively suppressed schwannoma growth in the DRG, while early oral treatment demonstrated prophylactic benefits in NF2-SWN progression. Gene expression profiling revealed that PRG-N-01 downregulated tumor-promoting pathways while promoting transcriptional programs associated with Schwann cell differentiation and normal cellular metabolism. Preclinical evaluations confirmed that PRG-N-01 exhibits favorable drug-like properties, supporting its potential as a viable therapeutic agent.

Conclusions: Our findings provide compelling preclinical evidence supporting PRG-N-01 as a promising targeted therapy for NF2-SWN. These results establish a strong rationale for advancing PRG-N-01 into clinical trials, underscoring its potential as a novel therapeutic strategy for NF2-SWN patients.

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Exploration of Lipid Nanoparticles (LNPs) for Therapeutic Targeting of NF Tumor Cells

Lisa Schindler, PhD, *Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany*

Purpose: NF is a group of genetic conditions causing tumor growth on nerves throughout the body. While subtypes differ in clinical presentation and underlying pathogenic variants, a unifying feature is the central role of **Schwann cells (SCs) in tumor formation.** Current treatments, e.g. surgery, radiotherapy, and systemic small-molecule drugs, face challenges such as nerve damage, off-target toxicity, and limited efficacy. The ability to directly target SCs holds great potential to address these.

Adeno-Associated Viruses (AAV) have been explored for targeting, and CRISPR has been investigated to restore gene function. However, two factors appear limiting: (1) Low gene editing efficiency, necessitating repeated therapy, (2) AAV immunogenicity preventing such re-dosing, thus limiting therapy success.

Recently, **Lipid Nanoparticles (LNPs)** have gained notoriety as delivery vehicles due to their employment for COVID-19 mRNA-based vaccines. LNPs do not exhibit notable immunogenicity, enabling repeated therapy. LNP composition is highly customizable and shapes both specificity and efficacy towards target cells. Finally, LNPs can easily be mass-produced and hold a variety of cargo, including mRNA, peptides and small-molecule drugs.

Therefore, in a pilot study we explored the potential of LNPs for targeting of NF tumor cells, choosing mRNA-delivery to *NF2*-SWN tumor cells as initial use case.

Method: In vitro specificity and efficacy testing was performed by automated microscopy-based high-content screening. Wild type and *NF2*-deficient SCs (mouse and human) were treated with increasing concentrations of 6 EGFP-mRNA-loaded LNPs.

In vivo targeting was investigated in an *NF2*-SWN mouse model comparing 5 LNPs and 3 injection routes:

1. Intratumoral – direct delivery into the tumor
2. Intrathecal – systemic delivery bypassing the blood-brain/nerve barrier
3. Intravenous – systemic delivery requiring blood-brain/nerve barrier penetration

Tissue sections of sciatic schwannomas, trigeminal ganglia, and brains were analyzed for EGFP expression. Co-labeling identified Schwann, meningeal, and ependymal cells—the primary *NF2*-SWN tumor-forming cells.

Results: In vitro analysis demonstrated dosage-dependent targeting, identified several potential SC-targeting candidates with one clear lead. Strong expression was observed in >75% of cells. In vivo analysis demonstrated SC-targeting for all routes, with differences between LNPs.

Conclusion: Initial results indicate the suitability of LNPs for SC-targeted delivery of mRNA in vitro and in vivo, justifying further refinement and evolution of methodology. Currently ongoing proof-of-concept studies delivering LNP-encapsulated *NF2*-mRNA will further reveal the potential to support novel *NF2*-SWN treatment options. Beyond *NF2*-SWN, application to related SC diseases, including NF1, appears possible due to LNPs' superior cargo and loading capacity flexibility.

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A Personalized Medicine Approach to *NF2*-Related Schwannomatosis Drug Discovery: Mechanistic Validation of High-Throughput Drug Screens

Ethan W Hass, BS, *University of Central Florida College of Medicine, Orlando FL*

Purpose: Patients with *NF2*-related schwannomatosis (*NF2*-SWN) develop bilateral vestibular schwannomas as well as paraspinal schwannomas. Our studies sought to confirm results of high-throughput drug screens conducted on primary schwannoma cells isolated from a debulked paraspinal schwannoma resected from a severe pediatric patient.

Methods: Previous time lapse drug sensitivity screens identified three pharmacologics out of 20 tested that produced a 50% decrease in well confluence compared to DMSO controls at 100nM over 96 hours. These were: the dual PI3K/HDAC inhibitor CUDC-907, the Src family kinase inhibitor dasatinib, and the combination of a MEK inhibitor, trametinib with a bromodomain and extraterminal protein (BET) inhibitor, BMS-986158. Follow-up flow cytometry studies evaluated the ability of each treatment to cause cell cycle arrest (EdU incorporation) or cell death (violet ratiometric assays). Drug target modulation was validated using automated capillary-based immunoblotting.

Results: Trametinib and dasatinib both promoted a G1 cell cycle arrest, whereas BMS-986158 promoted a G2 cell cycle arrest. CUDC-907 and the combination of trametinib with BMS-986158 were the only treatments that promoted apoptotic cell death. Trametinib and BMS-986158 were ineffective alone. By 6 hours of treatment, dasatinib completely inhibited FAK phosphorylation at tyrosine 576 (Y576) required for catalytic activity and caused proliferation arrest. Brigatinib, an ALK inhibitor that also inhibits FAK, did not reduce proliferation in the drug screens at the doses tested; however, it effectively reduced total FAK levels by 30% with detectable Y576 phosphorylation at 6 hours of treatment. FAK Y576 phosphorylation by SRC promotes its activity. Dasatinib dramatically increased total SRC; phosphorylation of tyrosine 527, a site of negative regulation by CSK, was absent. This is consistent with a cellular compensation mechanism against SRC inhibition.

Conclusion: Of the 20 therapeutics tested on primary paraspinal schwannoma cells, we identified three effective candidates. Dasatinib was the most cytostatic drug tested and caused SRC-dependent inhibition of FAK. Comparatively, the FAK inhibitor in clinical trials for *NF2*-SWN, brigatinib reduced total FAK levels but failed to arrest proliferation. CUDC-907 and trametinib in combination with BMS-986158 both promoted cell death. This data supports the use of limited patient samples for mechanistic drug studies and personalized medicine to advance the standard of care for *NF2*-SWN.

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HDAC2 Activity in Schwannoma Cells and Consequences of its Inhibition

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Purpose: NF2-related schwannomatosis is a genetic condition characterized by the growth of bilateral vestibular schwannomas. Ongoing research focuses on finding an effective pharmacological therapy, unfortunately clinically tested drugs control tumor growth temporarily in less than 15% of trial patients. Targeting many expected pathways like phosphoinositide 3-kinase (PI3K) or receptor tyrosine kinases (TRKs) pathway have a cytostatic effect allowing cells to adapt to treatment. We are investigating compounds that have a cytotoxic effect as potential treatments and to discover underlying molecular mechanisms leading that lead to cell death.

Methods: We tested our model merlin-deficient human schwannoma cells (HS02 and HS05), and patient derived NF2 primary schwannoma cells (NCH1) for response to a dual pan-HDAC/PI3K inhibitor, CUDC-907 at 100 nM concentration, unless stated differently. We analyzed basal HDACs protein levels, and their potential fluctuations during the treatment. We explored apoptosis induction and cell cycle progression using flow cytometry and apoptosis protein arrays (T=16 and 24h). We performed transcriptome analysis of CUDC-907 treated HS02 and HS05 cells. We tested two other HDAC inhibitors: givinostat, a recent (2024) FDA-approved drug for Duchenne's muscle dystrophy used to minimize sarcopenia that has low toxicity and romidepsin, a HDAC1/2 inhibitor used to treat cutaneous T-cell lymphoma.

Results: All model merlin deficient human Schwann cells (MD-HSCs) and patient-derived schwannoma cells responded to CUDC-907 with IG50s between 0.3 and 6 nM. 100 nM CUDC-907 reduced proliferation of model MD-HSC by 73-80%, patient derived vestibular schwannoma cells by 58%, and a patient-derived spinal schwannoma cells by 40% (NCH1). All cell lines responded with apoptosis after 24-72 hours of CUDC-907 treatment. We found that schwannoma cells express HDAC2, however the levels varied from sample to sample with the lowest expression observed in NCH1 cells that had the lowest treatment response compared to HS02 or HS05. The apoptosis protein arrays showed a dramatic elevation of p21 protein (cyclin-dependent kinase inhibitor 1 (CDKN1A)) as reported for other HDAC inhibition studies. P21 is a potent cell cycle inhibitor, and cell cycle analyses confirmed a significant cell cycle arrest in both HS02 and HS05 treated with CUDC-907. The transcriptome analysis showed downregulated genes were enriched in cell cycle and cell proliferation processes. When testing givinostat and romidepsin, we found that pan-HDAC inhibitor, givinostat was less potent and required $>1\ \mu$M concentration to have a cytostatic effect. However, romidepsin showed a very strong response profile similar to CUDC-907. This finding together with the expression profile of schwannoma cells indicates that HDAC2 is a good drug target for schwannomas.

Conclusion: We found that schwannoma cells preferentially express HDAC2, which when inhibited induces cell cycle arrest and apoptosis. It can serve as a specific target for pharmacological intervention of HDAC activity to inhibit schwannoma growth and potentially promote its regression.

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A Landscape Analysis of Machine Learning Models for Pain Monitoring and Prediction

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Purpose: Pain is an inherently subjective experience that complicates effective diagnosis and treatment, particularly in patients with schwannomatosis, where pain is often severe and underreported. Advances in machine learning and continuous physiological monitoring now offer promising avenues for objective pain assessment. This project conducts a scoping review of existing pain prediction models—examining machine learning models trained on physiological data—to identify research gaps and promising methodologies.

Methods: A comprehensive search of publications in the PubMed database was conducted using variations of query terms "machine learning," "artificial intelligence," "deep learning," "pain," "analgesic," "prediction," "classification," and "estimation." 26 publications that introduce at least one machine learning model capable of predicting instantaneous or long-term pain were included in the survey. Information such as the model architecture (e.g., SVMs, CNNs, RNNs), its training dataset (e.g., UNBC-McMaster Shoulder Pain Expression Archive Database, Infant COPE database), and the modality of the training data (heart rate, skin conductance, EEG, EMG), was extracted from each included paper to identify trends and gaps in pain prediction models.

Results: Within our survey, we have identified two main types of pain monitoring machine learning models: instantaneous and long-term. We found that these models usually fall into another two categories, being pain intensity prediction and pain/no pain classification. We have identified functional magnetic resonance imaging (fMRI) and electroencephalogram (EEG) data as being the leading two data modalities used to identify pain signatures. Finally, we found that the majority of the models in the survey use original datasets which are unique to the studies in which the models are introduced.

Conclusion: Our review identified several trends in pain monitoring machine learning models. The labelling of most models into either pain intensity prediction or pain/no pain classification provides a useful framework for discussion of autonomous pain monitoring techniques. The recognition of a trend towards using neurological signatures for monitoring highlights both the potential of EEG and fMRI as well as the relative lack of other data modalities which might be more convenient and practical for those living with chronic or recurring acute pain. Finally, the identified lack of a common dataset for training, validation, and testing among the models gathered in this survey limits the feasibility of comparing their reported accuracies and other metrics. This emphasizes the need for a large-scale, accessible pain recognition dataset for more objective comparison between pain recognition models.

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Cryptanoside A, a Cardiac Glycoside Epoxide as a Potential Treatment for *NF2*-SWN Related Tumors

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Cardiac glycosides, such as digoxin and digitalis, are Na^+/K^+-ATPase (NKA) antagonists used to treat heart failure and exhibit anti-tumor effects. Cryptanoside A is a naturally occurring cardiac glycoside epoxide isolated from the plant *Cryptolepis dubia* (Burm.f.). Unlike digoxin and digitalis, cryptanoside A has distinct structural variations, contributing to a comparatively lower binding affinity to NKA and potentially lower side effects. It has also been shown to be cytotoxic to colon, breast, and ovarian cancer, and melanoma cell lines. We aim to evaluate the growth inhibitory and anti-tumor activities of cryptanoside A in neurofibromatosis type 2-related schwannomatosis (NF2-SWN) tumors and its mechanism of action. We extracted cryptanoside A from *Cryptolepis dubia.* Its purity was confirmed via high-performance liquid chromatography and mass spectroscopy, and its structural identity verified using nuclear magnetic resonance. Cryptanoside A effectively inhibited the growth of *NF2*-deficient benign meningioma Ben-Men-1 and AG-NF2-Men cells and malignant meningioma KT21-MG1 cell lines with the IC_{50} values of 101, 104, and 131 nM, respectively. Similarly, schwannoma cells were vulnerable to growth inhibition by cryptanoside A. Treatment of AG-NF2-Men cells with cryptanoside A at 1x IC_{50} led to both G_1 and G_2/M arrest, and at 2x and 5x IC_{50} led to G_2M arrest after one-day treatment. Incucyte Caspase-3/7 Green Apoptosis Assay demonstrated that cryptanoside A induces caspase cleavage, indicating apoptosis. Western blots confirmed PARP cleavage in cryptanoside A-treated cells. Additionally, cryptanoside A increased the levels of the phosphorylated stress activated protein kinase p-$p38^{SAPK}$ and the DNA damage response marker γH2A.X. Experiments are in progress to evaluate the anti-tumor activity of cryptanoside A in the orthotopic AG-NF2-Men xenograft model. Also, we are performing transcriptomic analysis to examine the signaling pathways affected by cryptanoside A. Our preclinical findings support further development of cryptanoside A as a potential treatment for NF2-SWN associated tumors.

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Targeting Hippo–MAPK Crosstalk in Schwannoma: Overcoming Resistance with Dual Inhibition

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Schwannoma is the predominant tumor type associated with neurofibromatosis type 2 (NF2) and schwannomatosis. Our previous studies demonstrated that Hippo pathway dysregulation, particularly YAP/TAZ activation, is essential for schwannoma development. However, crosstalk between the Hippo and other pathways, including MAPK pathway, enables tumor cells to evade YAP/TAZ-TEAD-targeted therapies, leading to limited therapeutic efficacy and duration of response with single-pathway inhibition. This underscores the urgent need for novel combination therapeutic strategies. In this study, we evaluated the efficacy of a novel TEAD inhibitor, SW-682 in combination with the MAPK pathway (MEK1/2) inhibitor mirdametinib in a preclinical schwannoma model carrying Lats1/2 deletion in Schwann cells. Our *in vitro* experiments revealed that mirdametinib sensitizes schwannoma cells to TEAD inhibition, resulting in enhanced suppression of cell proliferation. Mechanistically, Western blot and RT-PCR analysis demonstrated compensatory interactions between the Hippo and MAPK pathways, with modulation of YAP/TAZ-dependent gene expression and ERK1/2 signaling activity. Furthermore, *in vivo* combination of SW-682 with mirdametinib resulted in a significantly enhanced tumor growth inhibition as compared to SW-682 or mirdametinib alone in this schwannoma mouse model. These findings highlight the importance of targeting pathway crosstalk to overcome therapeutic resistance in schwannoma and provide a strong rationale for future clinical evaluation of dual inhibition of the YAP/TAZ-TEAD and MAPK pathways.

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Single-Cell Analysis of Benign and Malignant Schwann Cell Tumors Reveals Tim3 Blockade as a Potential Treatment Strategy

Long-Sheng Chang, PhD, *Nationwide Children's Hospital and The Ohio State University*

Objective: Vestibular schwannomas (VS) are benign Schwann cell (SC) tumors commonly seen in patients with neurofibromatosis type 2-related schwannomatosis (abbreviated NF2 in short). Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive SC-derived sarcomas and can arise from benign plexiform neurofibromas (PNFs) frequently found in patients with NF1. Presently, an effective FDA-approved drug to treat these SC tumors is not available. Our goals are to comprehensively decode SC heterogeneity and the tumor microenvironment (TME) in these benign and malignant SC tumors and to identify novel therapeutic targets.

Methods: We profiled the single-cell transcriptomes of NF2-related and sporadic VS and compared them with those of PNFs and MPNSTs. Immunohistochemistry was performed to confirm specific protein expression in tumor sections. The *Lats1/2* genetically-engineered mouse (GEM) model of MPNST and volumetric MRI were used to evaluate the anti-tumor efficacy of anti-Tim3 monoclonal antibody.

Results: Single-cell RNA-sequencing analysis confirmed that schwannomas, irrespective of being NF2-related or sporadic VS or non-VS, harbored tumor SC subpopulations resembling those of repair SCs in peripheral nerve injury. This contrasts with the presence of a nestin-negative stem-like mesenchymal neural-crest subpopulation in MPNST. Various immune cell subpopulations were identified within the TME of VS, PNF, and MPNST. Macrophages, both M1 and M2, are highly represented along with a small population of T and NK cells in VS. Also, macrophages displaying robust pro-tumorigenic M2 signatures were found in PNFs and MPNSTs. Intriguingly, all three SC tumors display dysfunctional immune microenvironment, as reflected by little expression of the immunostimulatory IL-2 but high levels of TNFalpha, TGFbeta, and interferon-gamma in the immune cell subsets. Both MHC class I/II antigens are readily detected in all three SC tumors along with expression of several immune-checkpoint receptors, including TIM3 in macrophage and T cell subsets and VISTA and LAG3 in T cells within the TME. Robust expression of the two common Tim3 ligands, galectin 9 and HMGB1, are also detected. Immunohistochemistry confirmed Tim3 protein expression in VS and MPNSTs. Importantly, anti-Tim3 monoclonal antibody treatment suppresses MPNST growth in the *Lats1/2* GEM model.

Conclusions: Neurofibromatoses are a group of genetic diseases that manifests both benign and malignant SC tumors, which cause significant morbidity and mortality. Our study suggests that immune checkpoint inhibition, such as Tim3 blockade, may have therapeutic potential for treating these tumors.

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N-cadherin Regulates Schwannoma Migration and Enhances Efficacy of Brigatinib in a 3D Spheroid Model of *NF2*-SWN-Associated Vestibular Schwannoma

Han. TN. Nguyen, PhD, *The Ohio State University Wexner Medical Center, Columbus, OH*

Purpose: Current treatment options for NF2-SWN associated tumors, including vestibular schwannoma (VS), remain limited. Targeted therapies using tyrosine kinase inhibitors (TKIs), such as brigatinib and dasatinib, offer benefit in <10% of treated patients and have cumulative dose toxicity. We focus on N-cadherin, a key cell adhesion molecule that regulates collective migration and survival signaling in cancer. N-cadherin activates both RTK non-RTK pathways, modulate downstream including STAT3[1], PI3K/AKT-mTOR[2], and NF-κB[3]. We hypothesized that enhanced N-cadherin signaling promotes VS survival through these parallel pathways, and that combining N-cadherin inhibition with TKI could synergistically enhance their therapeutic response. Using primary VS cultures and a novel 3-dimensional (3D) NF2 schwannoma spheroid model, we examined N-cadherin expression and inhibition impact schwannoma behavior and TKI efficacy.

Methods: N-cadherin expression was examined using immunofluorescence. N-cadherin knockdown (shNCad) was achieved in human NF2 schwannoma cells using lentiviral-shRNA. A 3D schwannoma spheroid model in extracellular matrix or in co-culture with astrocytes was used to assess schwannoma migration via confocal microscopy. Primary VS cultures were evaluated for N-cadherin vesicle localization and collective migration. IL-6 was used to induce stemness-associated genes (OCT3/4, SOX2, KLF4, cMYC) and quantified by qRT-PCR. Therapeutic efficacy of dasatinib and brigatinib, alone or in synergy with N-cadherin inhibition by a small molecule (BZA) and neutralizing antibody (GC-4), was evaluated using MTT viability assay. Protein expression of AKT, SRC, and FAK was analyzed by western blot.

Results: Primary VS tumors showed heterogeneous N-cadherin expression, with enriched intracellular vesicle staining in leader cell subpopulations from tumors located in the internal auditory canal. shNcad increased schwannoma migration on ECM but reduced it in astrocyte co-culture, indicating microenvironment-specific effects. In 3D models, N-cadherin vesicle localization stabilized leader-follower dynamics in collective migration. IL-6 induced by over 4-fold increases in stemness markers, which was blocked by shNcad **(Fig 1)**. shNCad cells exhibited significantly enhanced sensitivity to TKIs, with >64-68% reduction of IC_{50} values for dasatinib (0.57 μM vs. 1.58 μM) and brigatinib (0.19 μM vs. 0.60 μM). Combination treatment with TKIs and an N-cadherin inhibitor suppressed phosphorylation of key cell survival pathways including AKT, SRC, and FAK. These effects were consistently validated across three independent primary VS cultures **(Fig 2)**.

Conclusion: N-cadherin regulates structural cohesion, collective migration, and stemness in VS cells. Its inhibition enhances sensitivity to TKIs and suppresses multiple survival signaling pathways. Targeting N-cadherin represents a novel therapeutic strategy to improve drug responsiveness and overcome resistance in NF2-SWN.

Figure 1

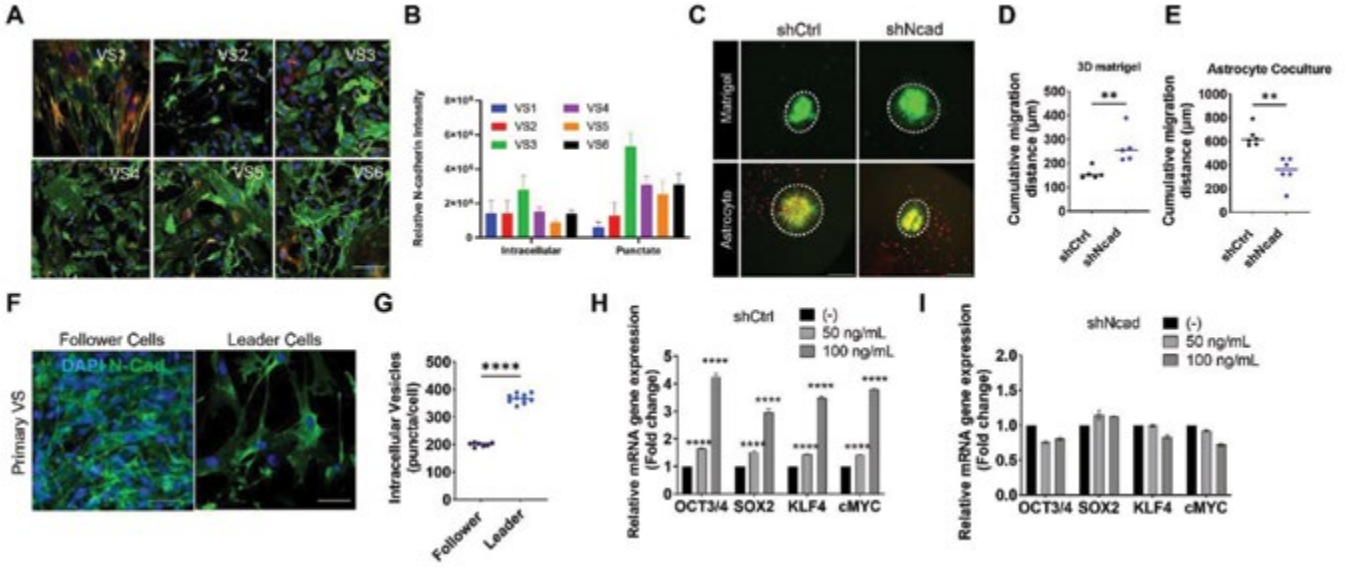
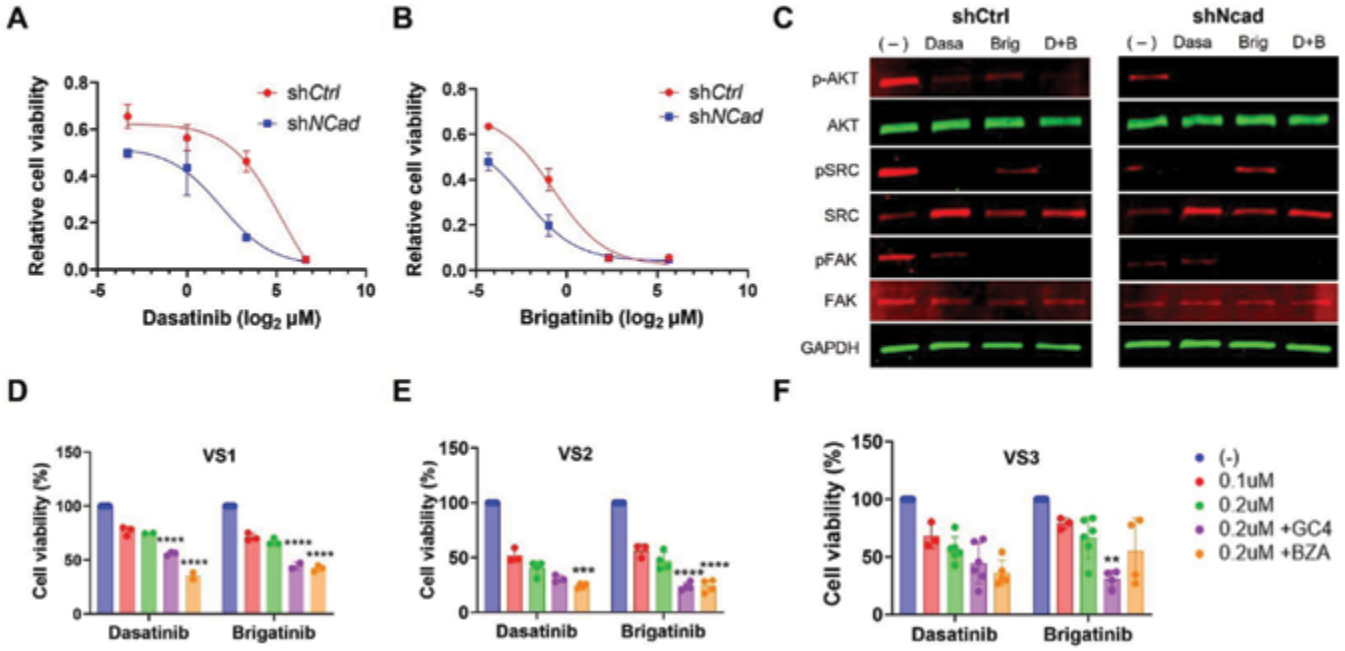


Figure 2



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References:
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Targeting Thrombopoietin Restores Vessel Perfusion, Reduces Neuroinflammation, and Blocks *NF2*-Related Schwannomatosis Schwannoma Growth

Larry S. Sherman, PhD, *Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR*

Purpose: Surgical interventions for *NF2*-related schwannomatosis (NF2) vestibular schwannomas carry the risk of permanent deafness and can exacerbate vestibular and facial nerve complications. Although several drugs have been tested in NF2 patients, they have demonstrated only limited efficacy and significant side effects. We previously reported that losartan, an angiotensin receptor blocker that blocks fibrogenic and inflammatory angiotensin signaling, could block schwannoma growth and prevent schwannoma-induced hearing loss in a mouse model of vestibular schwannoma. However, losartan can also have significant side effects. Losartan has antithrombotic functions, inhibiting platelet activity and coagulation factors while increasing fibrinolytic activity in a dose- and time-dependent manner. Thrombopoietin (TPO) is the major physiological regulator of platelet production and can stimulate platelet aggregation. Here, we tested whether a Gal-NAc_3-conjugated 20 nucleotide TPO antisense oligonucleotide (TPO-ASO-AB062) could be a safer and more effective agent to inhibit schwannoma growth.

Methods: We pretreated non-tumor bearing mice with TPO-ASO-AB062 for 4 weeks then implanted *Nf2*$^{-/-}$ tumors in the sciatic nerve. When the tumor reached 2-3 mm in diameter, mice were randomized into treatment groups. To examine if TPO-ASO-AB062 treatment affected tumor vessel perfusion, we injected FITC-lectin (2 mg/kg) i.v. to identify perfused vessels. We also screened tumor tissues for chemokine expression related to neuroinflammation.

Results: We found that TPO-ASO-AB062 treatment significantly delayed tumor growth but did not cause systemic toxicity as evaluated by body weight loss. TPO-ASO-AB062 treatment reduced platelet counts in mice, but not to severe levels that would cause hemorrhage. TPO-ASO-AB062-treated tumors had more blood vessels with an open lumen and labeled with FITC-lectin, suggesting increased vessel perfusion. Finally, we observed reduced expression of the macrophage chemokine, CCL2, and the neutrophil chemokine, CXCL2 in TPO-ASO-AB062-treated tumors, indicating that TPO-ASO-AB062 treatment reduced inflammatory cell recruitment into the tumor microenvironment.

Conclusion: Our data indicate that TPO-ASO-AB062 can delay tumor growth, normalize vessel perfusion, and reduce inflammatory signaling that contributes to NF2-related schwannomatosis morbidity. TPO-ASO-AB062 or similar agents are therefore potential candidates as therapies for NF2 schwannomas.

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Disclosures: Dr. Norah Green Verbout is a scientist affiliated with the company that generated TPO-ASO-AB062

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CHK1 as a Potential Therapeutic Target in Neurofibromatosis Type 2

Mariia Buts, *Medical Faculty No. 1, Bogomolets National Medical University, Kyiv, Ukraine; Cancer Signaling & Microenvironment Program, Fox Chase Cancer Center, Philadelphia, PA*

Neurofibromatosis type 2 (NF2) is a genetic tumor predisposition syndrome that causes bilateral vestibular schwannomas, meningiomas, and peripheral nerve tumors. The hallmark mutations of the disease occur in the *NF2* gene, leading to inactivation or loss of its gene product, the tumor suppressor protein Merlin. Merlin activates the Hippo tumor suppressor pathway by promoting YAP degradation and suppressing the expression of genes involved in cell proliferation and survival.

Purpose: Given the limited number of therapeutic intervention targets within the Hippo signaling pathway, we aimed to explore alternative vulnerabilities in *NF2*-mutant cells such as the DNA damage response (DDR) pathways. DNA damage repair is an essential part of normal cell function and failure to activate the DDR pathway typically results in cell death. DNA breaks are sensed by protein kinases such as ATM, ATR, CHK1 and CHK2.

Methods: *NF2-null* Schwann cells were plated in 96-well plates for high-throughput kinome drug screening. After 24h we added 1 μM of each drug of the Cayman kinase inhibitor library and incubated for 72h. We then measured cell viability using Alamar Blue and found CHK1 as a possible target. CRISPR knockout and SiRNA of CHK1 were performed to further confirm the possible use of CHK1 as a therapeutic target in *NF2-null* Schwann cells.

Results: We found through an unbiased high-throughput kinome drug screen that *NF2*-null Schwann cells exhibited a selective sensitivity to CHK1 inhibitors, including LY2606368 (Prexasertib). Knockdown or knockout of CHK1 in *NF2*-null cells was also associated with an increased sensitivity to Prexasertib. Further pharmacological inhibition of upstream (ATR) and downstream (WEE1) effectors of CHK1, and other CHK1 inhibitors (AZD7762, CHIR-124) also caused a differential growth sensitivity in *NF2*-null cells. We further demonstrated that *NF2*-null cells displayed elevated levels of DNA damage, and they are especially vulnerable to combined drug treatment with Prexasertib and a second inhibitor of DNA-damage repair such as the PARP inhibitor Olaparib or hydroxyurea.

Conclusion: *NF2*-null Schwannomas cells were sensitive to DNA damaging agents and CHK1 inhibitors. Our data shows that the combination of drugs against these therapeutic targets can be a plausible therapeutic strategy for *NF2*-null Schwannomas.

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BIOINFORMATICS, OMICS AND SHARING PLATFORMS

Delineating Genotype-Phenotype Correlations in *SMARCB1*-Related Schwannomatosis and Coffin-Siris Syndrome

Oliver Cooper, *Division of Evolution, Infection and Genomics, School of Biological Sciences, The University of Manchester, Manchester, UK; Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester, UK*

Purpose: The Switch/Sucrose Non-Fermentable (SWI/SNF) complex modulates access to regulatory regions by dynamically repositioning histone proteins. This regulates gene expression and is particularly important for cellular differentiation programmes. Variants in genes encoding the constitutive subunits are implicated in various disease states. SWI/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily B, member 1 (*SMARCB1,* OMIM *601607) is a core subunit of the SWI/SNF complex, tethering the complex at the nucleosome while remodelling activity is performed. Germline variants in *SMARCB1* have been associated with schwannomatosis (OMIM #162091), rhabdoid tumour predisposition syndrome (RTPS1, OMIM #609322), and Coffin-Siris syndrome (OMIM #614608), a congenital neurodevelopmental disorder. We have previously demonstrated a significant difference in the type and location of germline pathogenic variants found in schwannomatosis compared to RTPS1. In this study, we now compare *SMARCB1* variants associated with schwannomatosis and Coffin-Siris syndrome.

Methods: To this end, a semi-systematic literature review has been performed, curating a comprehensive dataset of currently published pathogenic or likely pathogenic SMARCB1 germline variants in individuals diagnosed with schwannomatosis or Coffin-
Siris syndrome. This dataset includes an update on cases from the Manchester Centre for Genomic Medicine, including novel SMARCB1 variants. The final dataset was used to compare both the type and position of variants causative of each condition.

Results: Compared to Coffin-Siris syndrome, schwannomatosis has a more complex mutational spectrum regarding both variant type and position. Schwannomatosis-associated variants predominantly cluster at either end of the gene, particularly in exons encoding the DNA binding domain or in the 3' untranslated region (3'UTR). Most of these variants are hypomorphic, affecting splicing or reducing transcript stability, respectively. In contrast, most Coffin-Siris syndrome-associated variants are found in exons encoding the C-terminal domain, although recent reports of novel variants outside of this domain may represent subgroups with unique phenotypes and distinct mechanisms. These variants are almost invariably missense and in-frame deletions, with no protein truncating variants observed.

Conclusion: Comparative analysis of the mutational spectra has reinforced the established genotype-phenotype correlations for schwannomatosis and expanded those for Coffin-Siris syndrome. This work provides a basis for our future mechanistic studies, which will characterise how recurrent pathogenic SMARCB1 variants are involved in pathogenesis of schwannomatosis and Coffin-Siris syndrome. The overall aim of this research is to inform novel condition-specific therapeutic strategies and improve the accuracy of genetic diagnoses.

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Clinical - NF1 (The Poster Sessions are a Non-CME Activity)

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Zhang	Song	5565	The Silent Growth: Unraveling the Malignant Transformation in Pediatric Neurofibromatosis
Sheth	A.	5644	CENPF as a Biomarker and Therapeutic Target for MPNST in NF1 Patients
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Lucas	Alyssa	5805	Role of MEKi in Spinal Cord Astrocytoma and Malignant Peripheral Nerve Sheath Tumor: A Case Report
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			PLEXIFORM NEUROFIBROMA
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Armstrong	Amy	5237	Lack of Correlation of FDG-PET/CT SUV Maximum and Malignant Transformation in Neurofibromatosis Type 1-Related Plexiform Neurofibromas
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Nghiemphu	Phioanh	5542	Healthcare Providers' (HCPs') Perspectives on the Management of Adults with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibroma (PN): Insights from a US Survey
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Hu	Xiaojie	5578	Luvometinib (FCN-159) in Pediatric Participants with Neurofibromatosis Type 1: An Updated Report on the Efficacy and Safety of a Multi-Center, Open-Label, Single-Arm Phase II Study

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Kiaei	Dorsa	5667	TRAM-01: A Phase 2 Study of Trametinib for Pediatric Patients with Neurofibromatosis Type 1 and Plexiform Neurofibromas
Wang	Shengcai	5676	Real-World Outcomes of Selumetinib in Chinese Pediatric Patients with Inoperable Neurofibromatosis Type 1-Associated Plexiform Neurofibroma (NF1-PN): Interim Analysis of Baseline Characteristics from the PEDIA Study
Nishida	Yoshihiro	5682	Characteristics of Patients with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Japan and Real-World Safety and Effectiveness of Selumetinib: A 1-Year Interim Analysis of an All-Case, Postmarketing Surveillance Study
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Clinical - NF1 (The Poster Sessions are a Non-CME Activity)

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Jia	Wangcun	5460	Emergence and Development of Nascent Cutaneous Neurofibromas in Pediatric NF1 Patients
Verma	Hannah	5622	Results of a Phase 1 Trial of Topical Immunotherapy Diphencyprone for Cutaneous Neurofibromas in Neurofibromatosis Type 1
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Clinical - NF1 (The Poster Sessions are a Non-CME Activity)

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Kaplan	Gamze	5764	*"Flipping a coin to whether or not they had the condition and then rolling a dice to decide how severe it was going to be":* Pregnancy Experiences of Expectant Parents with Neurofibromatosis Type 1 (NF1)
Aldossari	Gada	5770	A Tertiary Care Center Experience on Prevalence, Characteristics, and Outcomes of Moyamoya Syndrome in Neurofibromatosis Type 1 (NF1)
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Yogaratnam	Arjun	5900	Dietary Considerations for People with NF1: A Systematic Review of the Literature
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MPNST

When Symptoms Confuse: Diagnosing MPNST in a 3-Year-Old

Xin Zhang, MD, *Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

Purpose: This case study highlights the diagnosis and management of a malignant triton tumor (MTT) in a 3-year-old girl with neurofibromatosis type I (NF1). The case underscores the importance of accurate diagnosis and treatment strategies for pediatric patients with complex tumor presentations associated with NF1.

Methods: The patient was referred to the pediatric oncology department due to a recurrent mass in her left wrist. Initial imaging studies, including ultrasound and MRI, revealed a soft tissue mass consistent with a neurofibroma. A repeat excision was performed to evaluate the tumor's nature, followed by pathological analysis and genetic testing. Immunohistochemical staining was conducted to assess tumor markers. The patient subsequently received six cycles of Ifosfamide/Doxorubicin chemotherapy. After chemotherapy, a second surgical excision was performed, and her response was monitored through PET/CT scans.

Results: Genetic sequencing identified a germline mutation in the NF1 gene, consistent with the patient's diagnosis of neurofibromatosis type I (NF1). Additionally, somatic mutations were detected within the tumor tissue, including a heterozygous mutation in NF1 and deletions in CDKN2A/B. Pathological examination of the excised mass revealed features characteristic of MTT, with positive immunohistochemical markers such as Desmin, MyoG, and MyoD1, while P16, SOX10, S100, and H3K27me3 were negative. Following chemotherapy, pathological analysis of the second surgical excision confirmed the presence of active tumor cells, indicating persistent disease. PET/CT scans demonstrated reduced metabolic activity surrounding the tumor site, suggesting a partial response to treatment, with no evidence of systemic metastasis.

Conclusions: The case exemplifies the challenging nature of diagnosing MPNST and its subtypes in pediatric patients, particularly in the context of NF1. MTT, a rare and aggressive form of MPNST, requires a multifaceted treatment approach, including surgical resection and chemotherapy. The detection of active tumor cells after chemotherapy highlights the need for continued vigilance in monitoring and treating residual disease. Despite high recurrence rates and poor prognosis, this patient exhibited no tumor growth or metastasis one year post-diagnosis, suggesting that early surgical intervention combined with targeted therapy may enhance outcomes. Further research into the efficacy of MEK inhibitors and combination therapies is warranted to improve management strategies for NF1-associated tumors. This case emphasizes the need for comprehensive genetic analysis and molecular profiling to facilitate tailored treatment options for pediatric sarcomas with ambiguous clinical presentations.

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Multifunctional Chemoreactive Nanosonosensitizers Exert Antitumoral, Antibacterial and Wound Healing Effects on Malignant Peripheral Nerve Sheath Tumors

Wei Wang, *Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

Purpose: Currently, surgery is the only available and effective clinical strategy for treating malignant peripheral nerve sheath tumors. However, even with complete surgical resection, poor physical health or cachexia would cause wound delay or infection. To solve this critical issue, DSF-CuO_2@DMSNs are rationally designed.

Methods: The nanosonosensitizers are engineered to exert high performance and synergistic sonodynamic/chemodynamic/chemotherapeutic tumor treatment under the stimulation of ultrasound by producing reactive oxygen species and dithiocarbamate–copper complexes *in situ* in response to the tumor-specific acidic and hypoxic microenvironment.

Results: Significant inhibition in cell viability, increment in cell apoptosis and death, and tumor cell-xenograft experiments all demonstrate the antitumoral capability of DSF-CuO_2@DMSNs in combination with ultrasound stimulation. The upregulated expression of caspase-3 and cleaved caspase-3 in both cell and resected xenograft protein lysates indicated the increment of cell apoptosis. Moreover, DSF-CuO_2@DMSNs also induce strong antibacterial effects and wound healing abilities under the activation of ultrasound both *in vivo* and *in vitro,* with histological examination results of quick wound closure, absence of bacterial infection, and new blood vessel formation.

Conclusion: Altogether, the multifunctional DSF-CuO_2@DMSNs are highly desirable chemoreactive nanoagents that can provide a distinct postoperative therapeutic strategy for both tumor inhibition and wound recovery.

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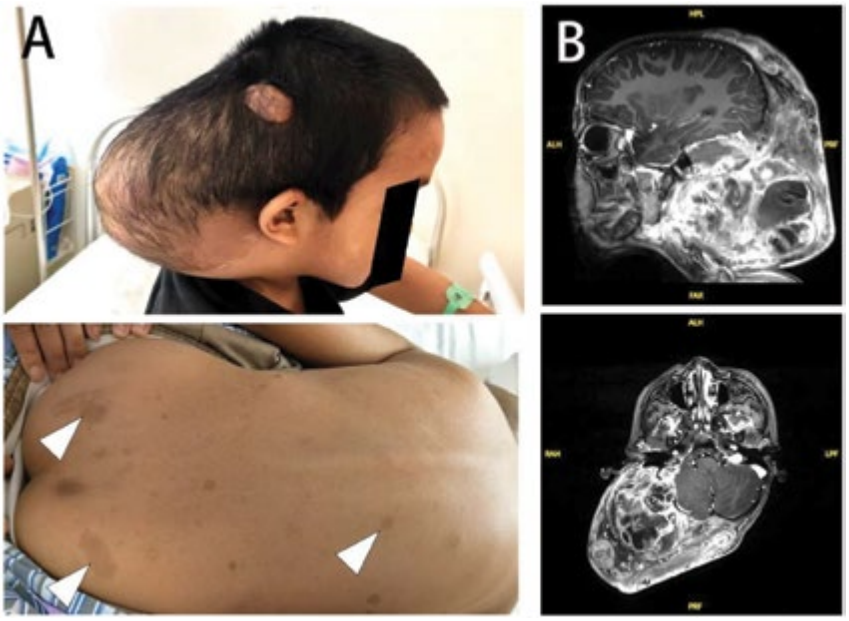
The Silent Growth: Unraveling the Malignant Transformation in Pediatric Neurofibromatosis

Song Zhang, MD

A 10-year-old boy diagnosed with neurofibromatosis presented with an 8-year history of a right temporal scalp lump that had progressively enlarged over three years and worsened over the past six months. When he was 2 years old, the lump was flesh-red, the size of a soybean, protruding from the skin surface, and painless. As he grew older, the lump gradually increased in size to that of an egg. In March 2019, following two and a half years of rapamycin treatment and a diagnosis of hemangioma, the scalp lump continued to gradually increase to the size of a fist. Since February 2024, the mass began to grow rapidly, spreading from the right temporal region to the occipital region and neck, eventually becoming half the size of the head. Upon presentation, physical examination revealed a large scalp mass in the right temporal, parietal, occipital, and cervical regions, approximately 40 cm in diameter, with a hard texture, elevated temperature, tenderness, and immobility. Pigmentation was observed on the frontal and temporal scalp, as well as multiple café-au-lait macules (CALMs) (>6) on the chest, abdomen, back, and buttocks **(Figure A)**. Cranial magnetic resonance imaging conducted subsequently confirmed these findings **(Figure B)**. In August 2024, he underwent excision of head tumor. Postoperative pathology showed malignant peripheral nerve sheath tumor (MPNST). Unfortunately, he succumbed to a grand mal seizure approximately one month postoperatively. Although malignant transformation of plexiform neurofibromatosis is rare, rapid tumor growth or significant local pain within a short timeframe may indicate its occurrence. This child was misdiagnosed as a hemangioma at an early stage, which led to a poor prognosis to a certain extent. Currently, the use of MEK inhibitors offers hope for patients with plexiform neurofibromatosis type 1.



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CENPF as a Biomarker and Therapeutic Target for MPNST in NF1 Patients

A. Sheth, PhD, *Department of Pediatrics, Herman B Wells Center for Pediatric Research, IUSM Indianapolis, IN*

Purpose: Individuals with Neurofibromatosis Type 1 (NF1) are at an increased risk for developing benign peripheral nerve sheath tumors known as plexiform neurofibromas (PNF), which may transform into malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs are highly aggressive, treatment-refractory form of sarcoma and are the leading cause of death in NF1 patients. There is a critical need for predictive biomarkers to identify PNFs with malignant potential, as well as molecular targets for developing effective therapies for MPNSTs. Profiling of gene expression changes associated with PNF progression from to MPNST identified *CENPF,* which encodes centromere protein F (CENPF), as the most significantly upregulated gene in MPNST compared to precursor lesions, suggesting a role for this gene in the malignant transformation of PNF. Overexpression of CENPF has been implicated in the progression and poor prognosis of various cancers but has not been interrogated in MPNST.

Methods: To assess CENPF expression across the PNF-MPNST continuum we utilized immunohistochemistry (IHC) to quantify CENPF protein levels.

To assess CENPF as a therapeutic target for MPNST, we performed *in vitro* cell viability, apoptosis, and colony formation assays in MPNST cell lines following genetic depletion of CENPF. *In vivo* therapeutic efficacy of CENPF depletion on tumor growth was assessed via implantation of MPNST cell lines with stable expression of CENPF-targeting or scrambled shRNA in immunodeficient NRG mice.

To assess the effect of CENPF loss on the functional kinome, we performed MIB/MS on MPNST cell lines following CENPF depletion.

To determine whether progression of PNF triggers the production of CENPF autoantibodies that may be used as a serological marker of malignancy, we performed enzyme-linked immunosorbent (ELISA) assays of plasma collected from $Nf1^{flox/flox};Cdkn2a^{flox/flox};PostnCre+$ mice, which recapitulate the spontaneous development of PNF that progress to MPNST, to detect and quantify CENPF-specific antibodies in MPNST-bearing mice.

Results: Quantitative detection of CENPF protein in human tumor samples via IHC confirmed significantly increased CENPF levels in MPNST compared to precursor lesions. We found that genetic depletion of CENPF significantly abrogated growth of human MPNST cell lines *in vitro* and *in vivo.* Kinome profiling revealed downregulation of MET upon CENPF depletion *in vitro.* ELISAs confirmed CENPF-specific autoantibodies in the plasma of tumor-bearing $Nf1^{flox/flox};Cdkn2a^{flox/flox};PostnCre+$ mice.

Conclusions: Our results implicate CENPF as a predictive biomarker for the diagnosis of MPNST in NF1 patients, either through immunohistochemical analysis of biopsy samples or non-invasive serological detection of CENPF autoantibodies. Further, CENPF may represent a promising therapeutic target for MPNST treatment.

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Elizabeth Sierra Potchanant: NIH DHART SPORE Career Enhancement Program Award; DOD New Investigator Award

Clinical Presentation, Risk Factors and Survival in 132 Patients with Neurofibromatosis 1 Associated Malignant Peripheral Nerve Sheath Tumor, 2009–2025

Rosalie E Ferner, MD, *Neurofibromatosis Service, Department of Neurology, Guy's and St. Thomas' NHS Foundation Trust, London UK*

Purpose: There is a 15.8% lifetime risk of developing neurofibromatosis 1 (NF1) associated malignant peripheral nerve sheath tumor (MPNST). Diagnosis and treatment are challenging, high-grade tumors metastasize widely and many have a poor outcome.

We evaluated clinical presentation, risk factors and survival in individuals with NF1 associated MPNST in a single national neurofibromatosis center.

Method: We carried out a retrospective case record study of 132 NF1 patients with histologically proven MPNST, who attended our national NF1 Center between 2009-2025. We evaluated: gender, NF1 family history, age at diagnosis, presenting symptoms, MPNST location, and size. Metastatic, new or recurrent MPNST was noted. We also documented prior radiotherapy, atypical neurofibromatous neoplasm of uncertain biologic potential (ANNUBP), *NF1* microdeletion, disease burden, NF1 neuropathy, other malignancy and family history of MPNST. Survival at five years and ten years post diagnosis was calculated.

Results: 66 males and 66 females were assessed, age range 9-74 years, mean age 31 years, (SD 13.1) at diagnosis. Seven patients had clinically segmental NF1; 57/127 (44.8%) had an NF1 family history; Predominant symptoms were pain 111/127 (87.4%), tumor growth 98/113 (86.7%), hard texture 52/74 (70.3%), neurological deficit 57/114 (50%). Weight loss was detected in 24/97 (24.7%), n varied due to missing / not evaluable data. Tumor size was 5-230 mm, mean 94.0 (SD 46.7). The commonest MPNST locations were lower limb 36/132, (27.3%) and abdomen/pelvis 29/132 (22%). Metastases were diagnosed in 43/132 (32.6%) and new or recurrent MPNST in 13/132 (9.8%). Radiotherapy was given prior to MPNST diagnosis in 9/121 (7.4%); ANNUBP was present in 13/132, (9.8%); *NF1* microdeletion was detected in 7/74 (9.5%); a heavy internal disease burden was detected in 61/99 (61.6%); NF1 neuropathy was diagnosed on neurophysiology in 8/17 (47%) and other malignancy was found in 31/131 (23.7%) patients. A family history of MPNST was present in 11/126 (8.7%). Weight loss ($p = .002$), pain ($p = .041$) and MPNST size ($p = .046$) were associated with a worse outcome. Five year survival was 62% and ten year survival was 58.5%; median survival time was 20.1 years.

Conclusion: Our large, single center cohort of 132 patients with NF1-MPNST had a high frequency of known presenting manifestations and risk factors. Weight loss, pain and tumor size were significantly associated with a worse outcome. Our current survival data show improved survival, compared with historical studies, underscoring the importance of coordinated neurofibromatosis centers and patient and clinician education.

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Neurofibromatosis Type 1 in Identical Twins with Development of a Malignant Tumor of Peripheral Nerves in One Twin

Idrisova Zhannat, *Kazakh National Medical University named after S.D. Asfendiyarov, Department of Nervous Diseases, Scientific Research Institute of Fundamental Medicine named after B. Atchabarov*

Purpose: Clinical case study of neurofibromatosis phenotype spectrum in identical twins with NF1 diagnosis

Methods: Acquisition of clinical data of NF1 (neurofibromatosis 1) patients with observations, magnetic resonance imagining (MRI), specialty consultations, medical-genetic testing.

Results: Both boys, 11 years old, presented with multiple café au lait spots from 1 to 3 cm, up to 10 spots in in the axillary area. Diagnosis was verified by genetic testing – nonsense mutation in NF1 gene chr17:31206297C>T, c.1318C>T, p.Arg440Ter in both twins.

First twin had small plexiform neurofibroma above m. sternocleidomastoideus on the left, below the corner of the lower jaw on the left, measuring 1.5 by 1 cm, bulging 0.5 cm out the skin surface. Second twin had from 2019 (age of 6 years old) a large neurofibroma in the left inguinal-iliac region on the left, emanating from the roots and plexus at the level of S1-S5, dimensions 7 cm by 4 cm and by 2 cm; according to MRI in 2024 in STIR regime the tumor had sized 10cm by 9 cm by 4.5 cm. In 2022, first biopsy confirmed NF1, in 2024, the tumor started to enlarge **(see Figure 1)** with volume 13 cm by 10 cm and by 6cm, additionally the boy started complaining the pain appeared during walking and at rest. Same year, biopsy of pelvic tumor masses at the Korean clinic was performed. Biopsy results: increase cellularity marked, nuclear pleomorphism marked, necrosis present, mitosis 2/10 HPF; suggestive of malignant peripheral nerve sheath tumor. Patient underwent 4 courses of chemotherapy, tumor decreased in volume, pain almost gone, treatment continues.

Conclusion: Identical twins with the same mutation in NF1 gene have a significant difference in the clinical picture (phenotype): one child has classic Neurofibromatosis type 1 with multiple (>10) coffee-with-milk spots and one small plexiform neurofibroma, and the other twin has a malignant tumor of the membranes of peripheral nerves of the inguinal-iliac region.

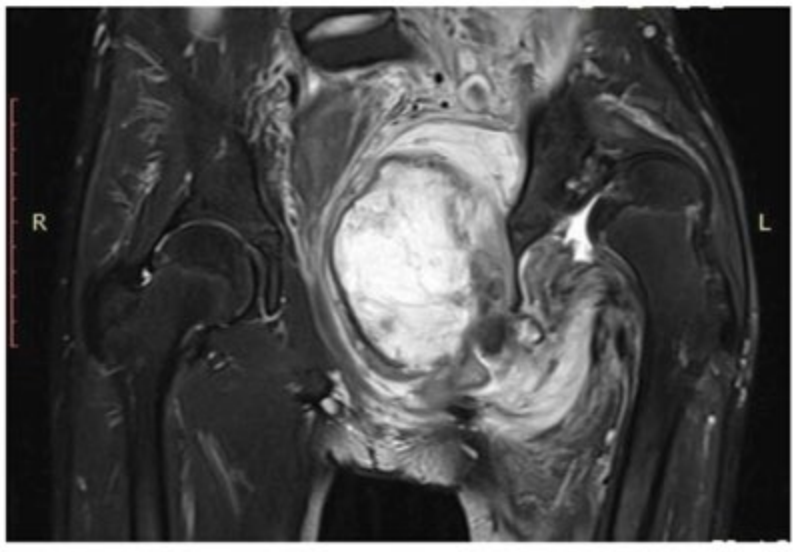

Figure 1. Massive pelvic neurofibroma of 11 years old boy (one from identical twin pair).

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Role of MEKi in Spinal Cord Astrocytoma and Malignant Peripheral Nerve Sheath Tumor: A Case Report

Alyssa Lucas, MD, *Clinical Neuro-Oncology Fellow, Rose Ella Burkhardt Brain Tumor & Neuro-Oncology Center, Cleveland Clinic Foundation, Cleveland, OH*

Introduction: Malignant peripheral nerve sheath tumor (MPNST) is a form of soft tissue sarcoma (STS) arising from nerve sheaths with increased incidence in individuals with neurofibromatosis type 1 (NF1). Given the correlation of MPNST with somatic and germline NF1 mutations, the lack of the NF1 gene product neurofibromin is thought to play a role in tumorigenesis of MPNST. However, it is actually a complex interplay of many different mutations that lead to over-activation of the RAS signaling pathway and tumor growth[1,2]. Therefore, selectively inhibiting any single mutation with a targeted drug is likely to fail[1,3].

Case Presentation: A 44-year-old woman with a history of NF1 with prior MPNST in the retroperitoneum that had received radiation and surgical resection presented with right leg weakness and was subsequently found to have an intramedullary mass at T4-T5 on MRI imaging. Pathology of the lesion was consistent with high grade glioma, suspected high-grade astrocytoma with piloid features, IDH-wildtype, MGMT not hypermethylated, WHO Grade 3. She underwent 54 Gy in 30 fractions of radiation therapy with concurrent temozolomide (75 mg/m2 daily), followed by off-label treatment with the MEK inhibitor selumetinib. She completed 12 cycles of selumetinib with dose reductions due to rash when a plexiform neurofibroma in her right elbow significantly increased in size. MRI of the right upper extremity revealed a multicystic lesion measuring 6.5 x 5.6 cm with central necrosis; FDG-PET CT demonstrated increased metabolic uptake (SUV 8.3), consistent with neoplasm. The mass was resected with negative margins, pathology consistent with MPNST. She received 60 Gy in 30 fractions of radiation to the right elbow. Post-treatment PET CT demonstrated decreased FDG uptake, and her thoracic cord glioma remained stable on MR imaging.

Discussion: This case demonstrates several points, including that treatment with MEK inhibitor monotherapy is insufficient to treat MPNST or prevent malignant transformation of plexiform neurofibroma in patients with NF1, though tumorigenesis is mediated in part by the RAS pathway[1,2]. In addition, FDG-PET imaging should be considered for surveillance of plexiform neurofibromas given increased sensitivity compared to MRI, especially in high-risk individuals with personal or family history of MPNST[4]. Finally, in addition to utility in NF1-mutant low-grade gliomas, MEK inhibitor therapy may be a beneficial alternative post-radiation treatment with MGMT-unmethylated high grade gliomas in particular[3,5,6]. Developing additional treatment options remains imperative given the significant morbidity and mortality of MPNST.

Figures:

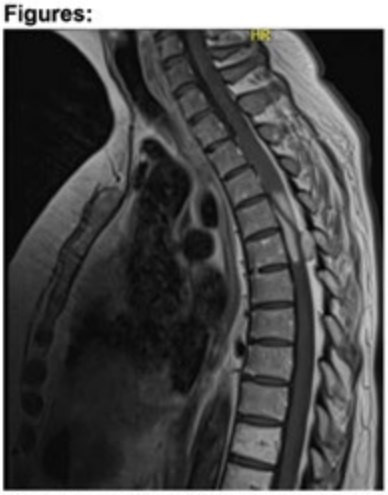

Figure 1. Initial sagittal post-contrast MRI demonstrating intramedullary T4-T5 mass, in addition to extradural neurofibroma at the same level.

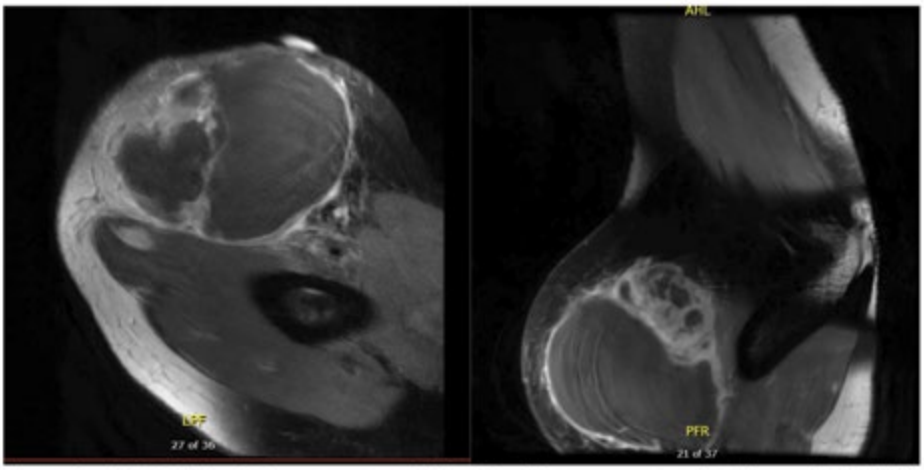

Figure 2a (left): initial axial post-contrast MRI of the right upper extremity; Figure 2b (right) is a sagittal view of the same scan.

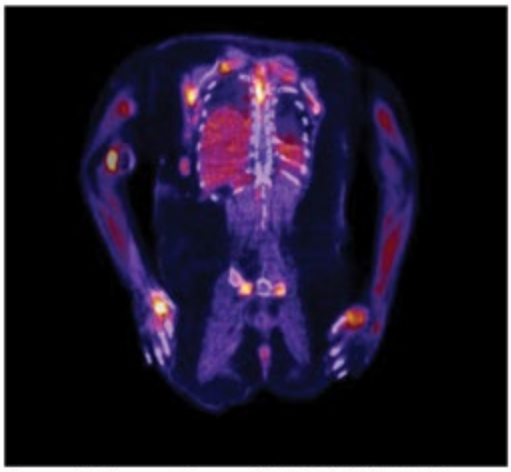

Figure 3. Coronal view of FDG-PET CT with increased uptake demonstrated in the right elbow in the location of the mass.

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NF2 is Recurrently Mutated and Regulates Hippo Signaling in *NF1* Mutant Peripheral Nervous System Tumors

Aaron Wright, BS, *University of California San Francisco*

Purpose: Mutations in both *NF1* and *NF2* cause peripheral nervous system (PNS) tumors, yet a mechanistic link connecting these tumor suppressors remains elusive. Here, we interrogate the role of NF2 in *NF1* mutant peripheral nervous system (PNS) tumors.

Methods: Human patient malignant peripheral nerve sheath tumors (MPNSTs) were analyzed using whole exome sequencing (WES) and methylation profiling to connect NF2 status to histopathologic signatures. Stable CRISPR interference (CRISPRi) *sgNF1* or *sgNF2* knockdown cells were generated in human immortalized peripheral nerve (iPN), neurofibroma, and MPNST (ST88-14) cells. Bulk RNA-sequencing was performed on parental and CRISPRi modified cell lines. Both control and *sgNF2* iPN, neurofibroma, and MPNST cells were analyzed by functional kinome profiling and immunoblots. Co-immunoprecipitation (co-IP) assays were performed to measure NF2 complex formation.

Results: *NF2* mutation or deletion of chromosome 22q harboring the *NF2* locus was observed in 19/36 (53%) human MPNSTs, and *NF2* alteration was associated with significantly increased Ki-67 index ($p=0.03$, t-test) and histopathologic grade ($p<0.001$, Chi-square). Consistently, *sgNF2* repression in neurofibroma or MPNST cells significantly promoted both cell growth and drug resistance. Functional kinome profiling revealed that *sgNF2* repression in *NF1* mutant MPNST cells modulated Hippo signaling through regulation of STE20-like protein kinase 1/2 (MST1/2) activity and loss of serine-127 YAP phosphorylation, a key Hippo pathway transcriptional effector. In contrast, *sgNF2* repression in *NF1* intact iPN cells did not modulate Hippo signaling but instead resulted in MEK/ERK activation and cell cycle regulation consistent with context specific NF2 roles in the PNS. Co-immunoprecipitation of endogenous NF2 revealed increased association with LATS1 and angiomotin in MPNST cells and *sgNF1* iPNs compared to control sgNTC iPNs.

Conclusion: In sum, our data suggest *NF1* loss modifies NF2 function in the PNS and converges on the Hippo signaling pathway as being selectively perturbed in *NF1* deficient MPNST cells.

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Development of a Genomics-Based Nanopore Sequencing Test for the Differential Diagnostics of MPNSTs and ANNUBPs

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Malignant peripheral nerve sheath tumors (MPNST) are soft tissue sarcomas with bad prognosis and the leading cause of Neurofibromatosis Type 1 (NF1)-related mortality. In the NF1 context, MPNSTs usually arise from preexisting benign plexiform neurofibromas (pNF) through a premalignant state called atypical neurofibromatous neoplasm with unknown biological potential (ANNUBP), although it is currently not possible to determine if a given ANNUBP will ever progress into an MPNST. Surgery is currently the only curative treatment for these tumors. However, differentiating between these tumors radiologically and histologically, especially in the transition phases, can be difficult.

This project aims to develop a new molecular test for the differential diagnosis of ANNUBPs and MPNSTs, including the detection of ANNUBPs in the early steps of MPNST transformation, based on the recurrent alterations found in MPNSTsz. The test is based on nanopore sequencing and takes advantage of the selective sequencing capabilities these sequencers have to perform in-silico enrichment and their ability to determine nucleotide sequence and DNA methylation simultaneously.

We performed a detailed genomic characterization of a set of MPNSTs using WGS, RNA-seq and methylation arrays and identified recurrent alterations seen on subgroups of MPNSTs at the genetic, genomic (copy-number, loss of heterozygosity, and chromosomal rearrangements) and epigenomic level. We designed an enrichment panel targeting those regions and are currently testing its performance in surgical specimens and minimally invasive biopsies. We are also developing a custom data analysis pipeline to process the generated data, extract all key features and produce a reasoned and data-backed classification of the samples.

In conclusion, we are developing a new genomics-based tool for better ANNUBP and MPNST diagnostics, with a potential impact on patient's management, survival and quality of life.

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Multi-Parametric Biomarker Development to Predict Malignant Conversion in Patients with Neurofibromatosis Type 1

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Background and Purpose: Atypical neurofibromas (aNF)/ atypical neurofibromatous neoplasm of unknown biologic potential (ANNUBPs) have been identified as pre-malignant precursors of malignant peripheral nerve sheath tumors (MPNSTs) in patients with neurofibromatosis type 1 (NF1) and described as "distinct nodular lesions" (DNLs) on imaging. We prospectively evaluated the frequency, multi-parametric whole body magnetic resonance imaging (WB-MRI) features, and natural history of DNLs in people with NF1 with clinico-genetic features deemed "high-risk" for malignancy.

Methods: This prospective single-center study enrolled consecutive participants with NF1 considered "high-risk" for MPNST development, based on genetics (i.e. NF1 microdeletion), personal or family history of ANNUBP, aNFs or MPNST, large pNF burden (defined as having multiple pNFs or one >3 cm), or prior radiation treatment. Participants undergo an annual non-contrast whole-body MRI for five years using parallel imaging and total imaging matrix per standard protocol (comprised of isotropic volumetric T2 short tau inversion recovery (STIR) and diffusion weighted imaging (DWI) and apparent diffusion coefficient (ADC) mapping) at 3.0 Tesla. Two musculoskeletal radiologists record the presence and imaging features of DNLs (defined as largest diameter > 3cm and absent "target" sign) at baseline and subsequent serial WB-MRI for years 1 through 4. Histological correlation for all lesions sampled or clinical stability > 12 months for benign DNLs served as reference standard. Descriptive statistics are reported.

Results: We prospectively enrolled 83 eligible participants and acquired WB-MRIs in 81 (median age(years): 31 (interquartile age (IQR): 24, 41); 33 (40.7%) males. "High-risk" criteria for enrolled participants was: large internal plexiform neurofibroma (n=68/81 (84%)), microdeletion syndrome (n=11/81 (13.6%)), prior radiation therapy (n=4/81 (4.9%)) and personal history of ANNUBP/aNF (n=1/81 (1.2%)) or MPNST (n=20/81 (24.7%)). At baseline WB-MRI (n=81), 58 DNLs were detected in 21 individuals (frequency = 26%) and characterized based on their imaging features or histology as MPNST (5/58, 9%), ANNUBP (1/58, 4%), or benign (52/58, 90%). Malignant DNLs tended to exhibit restricted diffusion (mean ADCmin=0.07 X10-3 mm2/s, $p<0.01$). At year 1 WB-MRI (n=69), two new DNLs were detected and one DNL exhibited growth and decreasing ADC values; ultimately all three were confirmed as MPNST based on histology. At year 2 WB-MRI (n=41) and year 3 WB-MRI (n=13), no change in pre-existing DNLs or new DNLs were detected.

Conclusion: Although DNLs are detected with high frequency (26%) on baseline WB-MRI in "high-risk" individuals with NF1, the majority (90%) have followed a benign course. A total of 8 MPNST diagnoses were made in 81 participants over 3 years in this patient population. Restricted diffusion on WB-MRI (ADCmin<1.0 X10-3 mm2/s) is a potential marker of malignancy within DNLs. Growth or development of new DNLs in this population is rare (~4%) but has a high predictive value for malignant conversion.

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PLEXIFORM NEUROFIBROMA

Detection of Superficial and Deep Plexiform Neurofibromas in Patients with Neurofibromatosis 1 Using Whole-Body Magnetic Resonance Imaging

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Purpose: This retrospective study aimed to assess the prevalence and distribution of superficial and deep-seated plexiform neurofibromas (PNs) in patients with neurofibromatosis 1 (NF1) using whole-body magnetic resonance imaging (WB-MRI).

Methods: We conducted a retrospective analysis of NF1 patients who underwent WB-MRI in 2024 and 2025 at the Division of Dermatology, Tottori University Hospital, as part of the 12-member NF1 Japan Clinical Network.

Results: A total of 35 patients (22 females and 13 males) who met the 2021 diagnostic criteria underwent WB-MRI, regardless of the presence or absence of clinically recognized PNs. The median age at the time of WB-MRI was 37.5 years (range: 9-76 years). A total of 42 PNs larger than 4 cm were identified, with an average of 1.2 tumors per patient. Among the 35 patients, 22 (62.9%) had at least one superficial or deep-seated PN **(Figure 1)**: 15 patients (42.9%) had superficial PNs, 12 patients (34.3%) had deep-seated PNs, and 2 patients (5.7%) exhibited a superficial PN contiguous with deep-seated PNs. Among the 19 superficial PNs, 8 (42.1%) were located in the limbs, 6 in the trunk, and 5 in the head and neck. In contrast, among the 21 deep-seated PNs, 8 (38.1%) were found in peripheral nerves of the limbs, 7 in deep anatomical structures (paraspinal regions, mediastinum, and retroperitoneum), 4 in the trunk, and 2 in the head and neck.

Conclusion: In this study, 62.9% of NF1 patients had either superficial or deep-seated PNs, and 11 of 35 patients (31.4%) exhibited multiple PNs in various anatomical locations. These findings underscore the utility of WB-MRI in the comprehensive detection and characterization of both superficial and deep-seated PNs in patients with NF1.

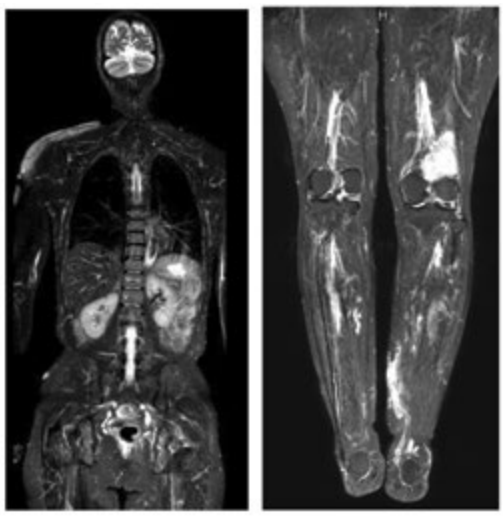

Figure 1. Whole-body magnetic resonance imaging revealed multiple superficial plexiform neurofibromas located in the right shoulder and left lower leg, as well as a deep-seated plexiform neurofibroma in the popliteal fossa.

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Luvometinib (FCN-159) in Adult NF1 Patients: Efficacy and Safety Outcomes from a Multi-Center, Open-Label, Single-Arm Phase II Trial

Xiaojie Hu, MD, *Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China*

Purpose: Neurofibromatosis type 1 (NF1) is a genetic disorder caused by autosomal dominant mutations with constitutive MAPK pathway activation. Plexiform neurofibromas (PN) occur commonly in NF1 patients and are associated with severe morbidity and risk of malignant transformation. Luvometinib, a novel MEK1/2 inhibitor with high selectivity was evaluated in the Phase II trial.

Methods: This multi-center, open-label, single-arm Phase II trial enrolled patients with NF1-related PN deemed inoperable, incompletely resected, or recurrent post-surgery. In the adult cohort, patients received continuous 8 mg luvometinib once daily (RP2D from Phase I) in 28-day cycles. Primary endpoint was investigator-assessed and confirmed objective response rate (ORR) per REiNS criteria. Key secondary endpoints included clinical benefit rate (CBR), disease control rate (DCR), progression-free survival (PFS), duration of response (DOR), and safety. Here we present the efficacy and safety results in the adult cohort.

Results: As of September 23 2024, 63 adult patients were enrolled and treated with luvometinib. With a median follow-up of 29.3 months, the investigator-assessed and confirmed overall response rate (ORR) reached 42.9% (27/63, 95% CI: 30.5-56.0), all partial responses. The CBR was 84.1% (53/63, 95% CI: 72.7-92.1) and the DCR was 95.2% (60/63, 95% CI: 86.7-99.0). The median PFS was 31.4 months (95% CI: 31.38-NE), with 1-year and 2-year PFS rates of 91.0% and 75.2%, respectively. The median DOR was 25.8 months (95% CI: 20.27-NE), with 1-year and 2-year DOR of 95.8 % and 69.6% respectively. Pain reduction was notable: 78.3% (18/23) patients with baseline tumor pain (NRS$\geq$2) achieved $\geq$2 points decrease. Regarding safety, treatment-related adverse events (TRAEs) were observed in 63 patients, with majority were grade 1-2. Grade $\geq$3 TRAEs occurred in 24 (38.1%) patients, with folliculitis (25.4%), paronychia (6.3%), elevated creatine phosphokinase (3.2%), and hypertension (3.2%) occurred in more than 1 patient. Serious TRAEs occurred in 2 (3.2%) patients. TRAEs-induced dose interruption and discontinuation occurred in 41 (65.1%) and 9 (14.3%) patients, respectively. No TRAEs led to dose reduction or death.

Conclusion: Luvometinib demonstrates notable clinical activity and a favorable tolerability profile in adults with NF1-related PN. ClinicalTrials.gov: NCT04954001.

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Prevalence and MRI-Based Characteristics of Distinct Nodular Lesions in Patients with NF1 on Whole-Body MRI

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Background and Purpose: A subset of patients with neurofibromatosis type 1 (NF1) exhibits plexiform neurofibromas (PNF), and a large tumor burden is a predictor for the transition into either pre-malignant atypical neurofibromas (ANF/ANNUBP) or malignant peripheral nerve sheath tumors (MPNST). Distinct nodular lesions (DNL) describe PNF with a characteristic appearance on magnetic resonance imaging (MRI) as well-demarcated, encapsulated-appearing lesions of ≥ 3 cm in size that can be present within or outside of a PNF. Previous studies showed that some of these DNLs had atypical features on the histologic evaluation and/or a loss of CDKN2A/B, indicating that DNL might play a role as precursor lesions in the process of malignant transformation. Whole body MRI (WB-MRI) is the imaging method of choice for the detection and follow-up of DNL. However, little is known about the prevalence and MRI-based characteristics of DNL in NF1 patients. Therefore, we aimed to retrospectively review WB-MRI scans of 291 NF1 patients regarding the prevalence of DNL and to characterize their morphological as well as topographical appearance.

Methods: We retrospectively reviewed 291 WB-MRI scans (between 2007 – 2022) of 291 NF1 patients (median age 29.0 years [IQR 12.8 - 45.2]; 51.6% male). WB-MRI data were evaluated by two radiologists regarding the presence, location, and maximum diameter of DNL based on coronal and axial STIR images. We also determined whether the DNL was located in a surrounding PNF or not.

Results: Among the 291 patients, 127 (43.6%) had at least one DNL on their MRI examination of whom 69 (54.3%) were male. Maximum DNL diameters ranged from 3 to 17 cm (mean 4.7 cm [SD ± 1.9 cm]), and most DNL were located paravertebrally at the nerves (22%) and along the sciatic nerves (36%). Patients with at least one DNL showed an average of 3.0 DNL on WB-MRI. In our cohort, 12 NF1 patients had a history of MPNST, and among these, 6 had DNL in their most recent MRI examination (range 1 - 10 DNL per patient).

Conclusion: Our results provide an assessment of the prevalence and the analysis of DNL in NF1 patients, considering their morphological as well as topographical appearance. Longitudinal studies are needed to better understand the growth characteristics of DNL in NF1 patients. In addition to the use of exclusively morphological characteristics, quantitative imaging biomarkers are also required to improve the risk stratification of these lesions.

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Figures

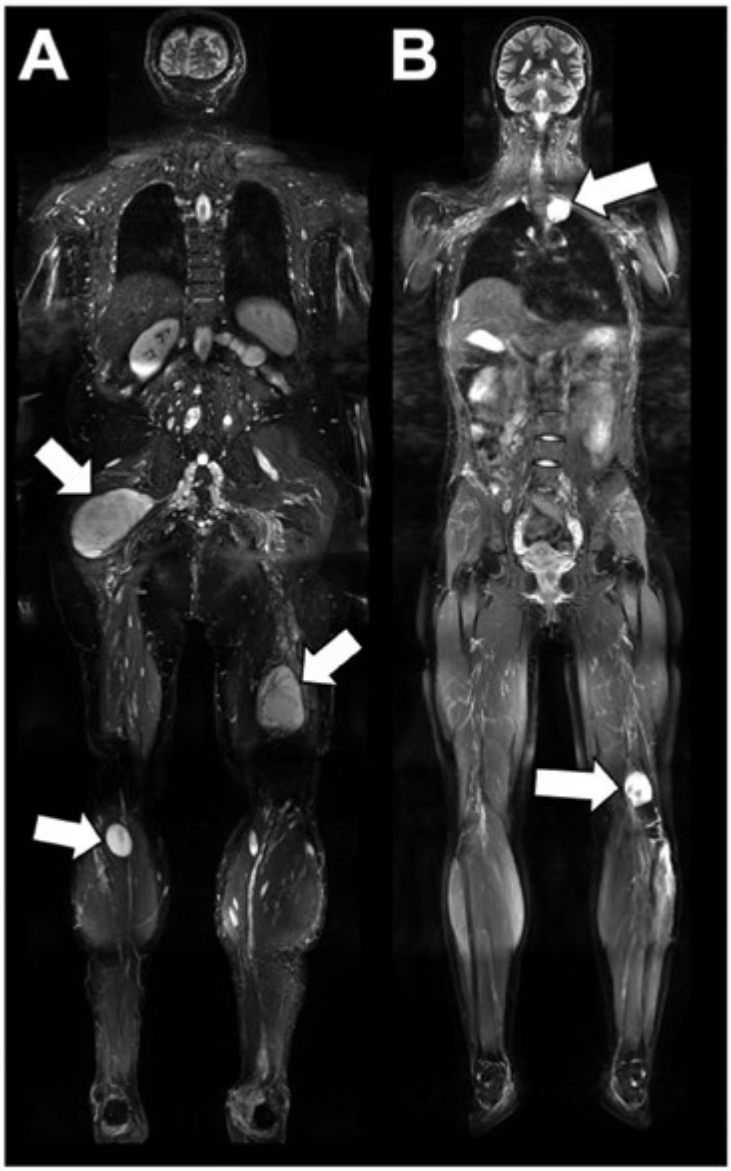

Figure: Images of coronal T2-weighted, fat-saturated magnetic resonance imaging sequences of two patients acquired at 3T:

(A) Patient 1: Large DNL of the right buttock, the left thigh, and the right lower leg in a 42-year-old man (arrows).

(B) Patient 2: DNL of the left upper thoracic aperture and the left knee (arrows) in a 27-year-old woman.

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Lack of Correlation of FDG-PET/CT SUV Maximum and Malignant Transformation in Neurofibromatosis Type 1-Related Plexiform Neurofibromas

Amy Armstrong, MD, *Washington University in St. Louis School of Medicine*

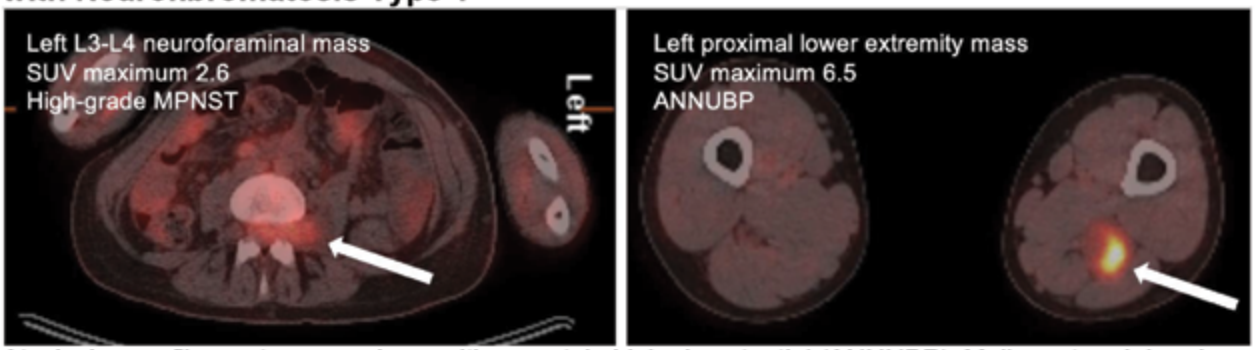
Objective: Optimal management of malignant peripheral nerve sheath tumor (MPNST) relies on early diagnosis of malignant transformation in predisposing plexiform neurofibromas (PN) in patients with Neurofibromatosis Type 1 (NF-1). Intratumor heterogeneity is a known occurrence in PNs where malignant transformation to MPNST can occur in part, but not all, of the underlying PN. Efforts have been made to standardize imaging guidelines for lesions suspicious for MPNST and FDG-PET/CT is routinely performed. Higher standardized uptake value (SUV) raises possibility for malignancy with studies using a SUV maximum (SUVmax) cut-off of 3.2-4.5, however no ideal value has been substantiated. We assessed correlation between SUVmax and malignant pathology in NF1 patients with PN.

Methods: We evaluated patients with NF1 enrolled on the Tissue and Blood Acquisition for Genomic Analysis and Collection of Health Information for Patients with Bone and Soft Tissue Sarcoma: The Sarcoma Oncology Group at Washington University in St. Louis who underwent FDG-PET/CT imaging. Those patients who had subsequent biopsy or resection due to suspected malignant transformation based on FDG-PET/CT findings were reviewed.

Results: We identified eight patients with FDG-PET/CT imaging showing a lack of correlation between high SUVmax and malignant pathology. Median age of this group was 22 years (range 11-59 years). Lesions with SUVmax range of 6.1-13.3 were determined to be either neurofibroma or atypical neurofibromatous neoplasm with uncertain biologic potential (ANNUBP), but not MPNST. One patient had two lesions evaluated due to findings on FDG-PET/CT with discordant correlation between SUVmax and pathology (see below). Furthermore, one patient's initial biopsy of a mass with SUVmax of 7.7 revealed neurofibroma, but subsequent resection identified ANNUBP.

Conclusions: Sampling of suspicious lesions in NF1-related PN is critical as wide resection of non-metastatic MPNST can be curative. FDG-PET/CT is a non-invasive tool to assess for malignant transformation in patients; however, we caution that higher SUVmax can be seen in benign neurofibromas and pre-malignant ANNUBPs, the latter which does carry a risk of transformation to MPNST. Additionally, SUVmax did not correlate with malignant pathology in the same patient. We suggest using SUVmax to direct biopsy or resection while understanding the limitations of this modality. In the future, we hope other non-invasive testing such as detecting circulating tumor DNA or other more sensitive radiotracers can aid in diagnostic work-up for this high-risk population and studies are underway.

Figure 1. Lack of Association of SUV maximum and Malignant Pathology in Patient with Neurofibromatosis Type 1



Atypical neurofibromatous neoplasm with uncertain biologic potential (ANNUBP); Malignant peripheral nerve sheath tumor (MPNST)

Development of MRI-Based Deep Learning Models for Whole Body Tumor Identification in Patients with Neurofibromatosis Type 1: A Multicenter Study

Cheng-Jiang Wei, MD, *Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

Purpose: Early differentiation between malignant peripheral nerve sheath tumors (MPNSTs) and plexiform neurofibromas (PNFs) is essential for improving the prognosis of patients with neurofibromatosis type 1 (NF1). Radiological imaging provides a noninvasive method for MPNST screening; however, current manual identification requires substantial expertise from radiologists and is time-consuming. Deep learning models have shown promise in differentiating between benign and malignant lesions. However, traditional deep learning methods face challenges in this context due to interference from the heterogeneity of whole-body backgrounds and limited data availability. This study aimed to develop highly accurate MRI-based deep-learning models for the early automated screening of MPNSTs against complex whole-body background **(Figure 1)**.

Methods: In this study, we collected a Chinese seven-center cohort to address the challenge of limited data, comprising 347 subjects (251 PNF, 96 MPNST) with multiparametric MRI data. A total of 3150 T2-weighted MRI images with typical tumor lesions were selected (2141 PNF, 1009 MPNST). Furthermore, our one-step model incorporated eight normal tissue/organ labels to provide contextual information, offering a solution for tumors embedded in complex backgrounds. To address privacy concerns, we employed a lightweight deep neural network suitable for deployment in hospital settings.

Results: This model demonstrated excellent performance in simultaneous area detection and differential diagnosis (accuracy: 85.71%, threshold: 85%) while requiring less computational power compared to other classical U-Net- and ResNet-based models. Additionally, compared to another one-step model trained exclusively on PNF/MPNST labels, incorporating information from surrounding normal organs/tissues improved diagnostic accuracy from 61.90% to 79.37% for MPNST and from 46.67% to 70% for PNF **(Figure 2)**.

Conclusion: This advancement could represent a significant milestone in the early clinical screening of MPNST and provide a novel approach for the 'fully automated' identification of whole-body background tumors, such as metastatic cancers.

Figure 1. A flowchart of the study process of our MRI-based model development

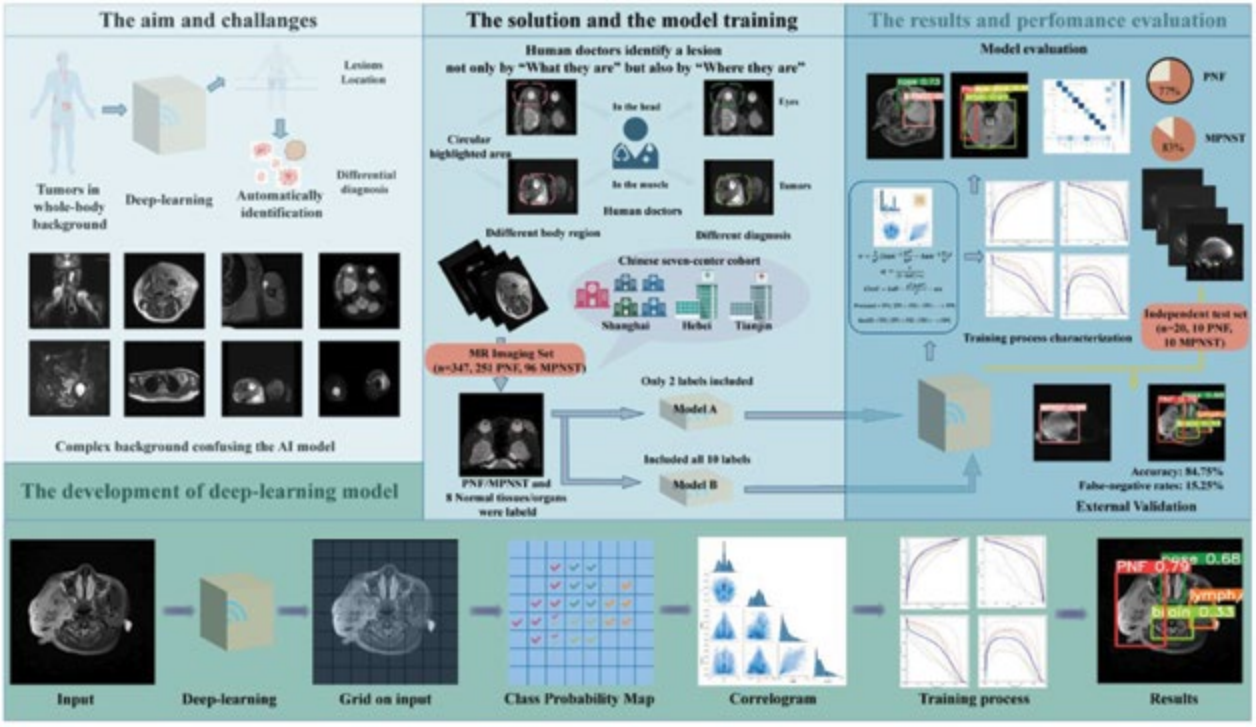


Figure 1 legend. We developed automatic segmentation and classification deep-learning AI models for the differential diagnosis of MPNST from benign PNF. In the training process, we introduced the surrounding information to augment their performance. The Figure 1 was created with Biorender.com.

Figure 2. The results classified by the one-step whole-body tumor identification deep learning model

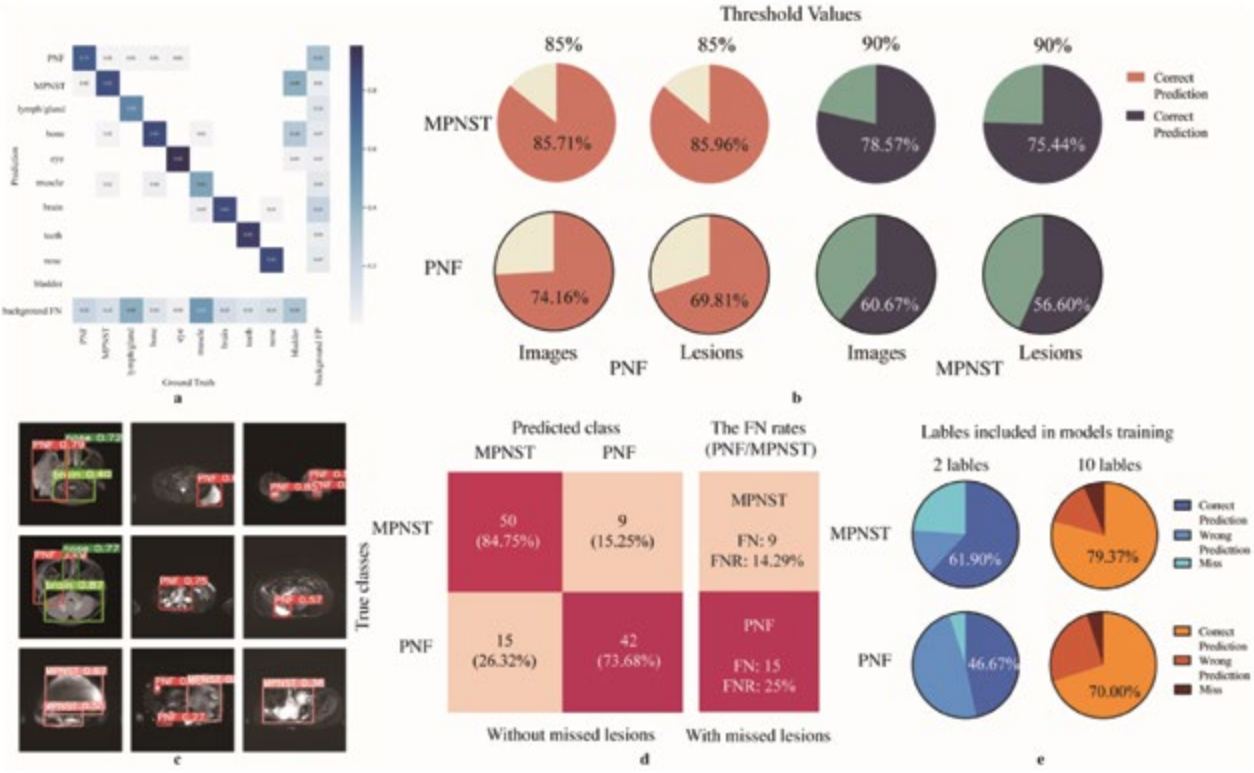


Figure 2 legend. (a) The confusion matrix of the training process. (b) The correct prediction percentages in the test set. An image might include several tumor lesions, and the percentage was calculated both along the number of images and tumor lesions at threshold values as 85%/90%. (c) Examples of the PNF images in the test set classified by the model. (d) The confusion matrix of the results of independent test set 2. The model showed an accuracy of 84.75% and a false negative rate of 15.25% in this independent test set. (e) Model performance comparison between the deep learning model trained on PNF/MPNST labels only and one that includes surrounding normal organ/tissue information. The model trained with information from surrounding normal organs/tissues enhanced diagnostic accuracy from 61.90% to 79.37% for MPNST and from 46.67% to 70% for PNF, affirming our hypothesis.

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Funding: National Natural Science Foundation of China (82472579; 82102344; 82172228)

Evaluating the Durability of Selumetinib Treatment in Children with Symptomatic Plexiform Neurofibromas Associated with NF1

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Purpose: Selumetinib, a MEK inhibitor, has been approved in China for treating symptomatic, unresectable plexiform neurofibromas (PN) in children aged 3 years and older with neurofibromatosis type 1 (NF1). This Phase I trial (CTR20200357) aims to evaluate the long-term efficacy and safety of Selumetinib in Chinese pediatric patients with NF1-associated PN, presenting updated findings from follow-up assessments conducted over a period of up to 4 years.

Methods: From December 2020 to February 2025, 16 pediatric patients with symptomatic/progressive PN received Selumetinib (25 mg/m^2 BID) until disease progression or intolerable adverse events (AEs). Efficacy endpoints included PN volume reduction (REiNS criteria), café-au-lait spots, health-related quality of life (HRQoL), pain, and physical function. Safety assessments included AEs and AEs of special interest (AESIs). MRI and clinical evaluations were performed every 4 cycles for the first 24 cycles, and every 6 cycles thereafter, with 14 patients completing over 42 cycles (median follow-up: 44 cycles).

Results: The cohort comprised 16 pediatric patients (median age: 11 years; range: 4-16). Patients received a median of 44 cycles of Selumetinib (range: 20-53). One patient discontinued treatment due to disease progression after the 20th cycle. At the initial data cutoff (DCO) (August 15, 2023), the overall response rate (ORR) was 81.3% (95% CI: 54.4%, 96.0%), with no complete responses (CR), 13 confirmed partial responses (cPR), 2 unconfirmed partial responses (uPR), and 1 stable disease (SD). Independent central review (ICR) reported an ORR of 62.5% (95% CI: 35.4%, 84.8%). All patients exhibited a reduction in target PN volume compared to baseline, with a mean reduction of -42.30% (±15.95%) by investigator assessment and -29.56% (±22.62%) by ICR. At the final DCO (February 28, 2025), 14 patients (87.5%) had a median volume reduction of 45.6% (range: 20.1%-61.2%). Clinical assessments indicated significant improvements in pain management, physical function, HRQoL, and symptom severity. Treatment-related AEs were reported by all patients, predominantly fever (38%) and rash (50%), all graded as 1 or 2 severity per CTCAE 5.0.

Conclusions: Selumetinib demonstrates durable efficacy, with a median response duration of 3.5 years, along with sustained functional benefits and a favorable safety profile in pediatric patients with NF1-associated PN. These findings support its long-term use with proactive management of AEs. Discrepancies between investigator assessments and independent central review highlight potential bias in subjective imaging evaluations, emphasizing the need for emerging AI imaging technologies to enhance objectivity in clinical trial assessments.

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Indirect Treatment Comparison (ITC) of Mirdametinib and Selumetinib for the Treatment of Children with Neurofibromatosis Type 1-Associated Plexiform Neurofibromas (NF1-PN)

Isha Mol, MS, *Cytel, Rotterdam, Netherlands*

Purpose: Mirdametinib is the first FDA-approved MEK1/2 inhibitor for both adult and pediatric patients 2 years of age and older with NF1 who have symptomatic PN not amenable to complete resection. We report efficacy and safety outcomes utilizing ITC in children with NF1-PN treated with mirdametinib vs selumetinib.

Methods: Unanchored matching-adjusted indirect comparison (MAIC) and simulated treatment comparison (STC) analyses evaluated outcomes of mirdametinib (ReNeu; NCT03962543) vs selumetinib (SPRINT; NCT01362803), where feasible.

Results: Mean best percent reduction from baseline in target PN volume by blinded independent central review (BICR) was significantly greater with mirdametinib vs selumetinib (MAIC: -36.0% vs -22.8%; STC: -36.2% vs -22.8%). The mean best percent reduction from baseline difference (95% CI) in the MAIC was -13.2% (-22.4%, -4.1%; $P=.005$; effective sample size [ESS]=54); in the STC was -13.4% (-22.2%, -4.4%; $P=.004$; N=54). In the MAIC and/or STC, all-grade treatment-related adverse event (TRAE) rates were significantly lower with mirdametinib vs selumetinib for dermatitis acneiform, diarrhea, nausea, vomiting, fatigue, blood creatinine phosphokinase increase, dry skin, pruritus, constipation, abdominal pain, stomatitis, hair color change, headache, and neutrophil count decrease ($P<.05$). Rate of dose reductions due to TRAEs was significantly lower with mirdametinib vs selumetinib (MAIC: 12% vs 28%, odds ratio [OR]=.355, $P=.048$; STC: 11% vs 28%, OR=.309, $P=.028$). Differences in confirmed objective response rate by BICR (MAIC: 51.3% vs 44%, ESS=21; STC: 55.5% vs 44%; N=56) and rates of all-grade paronychia (MAIC/STC: 30% vs 42%), white blood cell count decrease (MAIC: 6% vs 14%; STC: 5% vs 14%), alopecia (MAIC/STC: 13% vs 8%), and asymptomatic ejection fraction decrease (MAIC: 20% vs 14%; STC: 18% vs 14%) were not significantly different between mirdametinib vs selumetinib. Study limitations include possible confounding of unmeasured treatment effect modifiers.

Conclusions: Mirdametinib demonstrated a significantly greater mean best percent reduction from baseline in target PN volume, lower rate of dose reductions due to TRAEs, and lower rates of most TRAEs vs selumetinib in children with NF1-PN. None of the analyses conducted significantly favored selumetinib.

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Anatomy-Informed Dynamic UNet-Based Whole-Body MRI Approach for Automated Neurofibroma Segmentation in Patients with Neurofibromatosis Type 1

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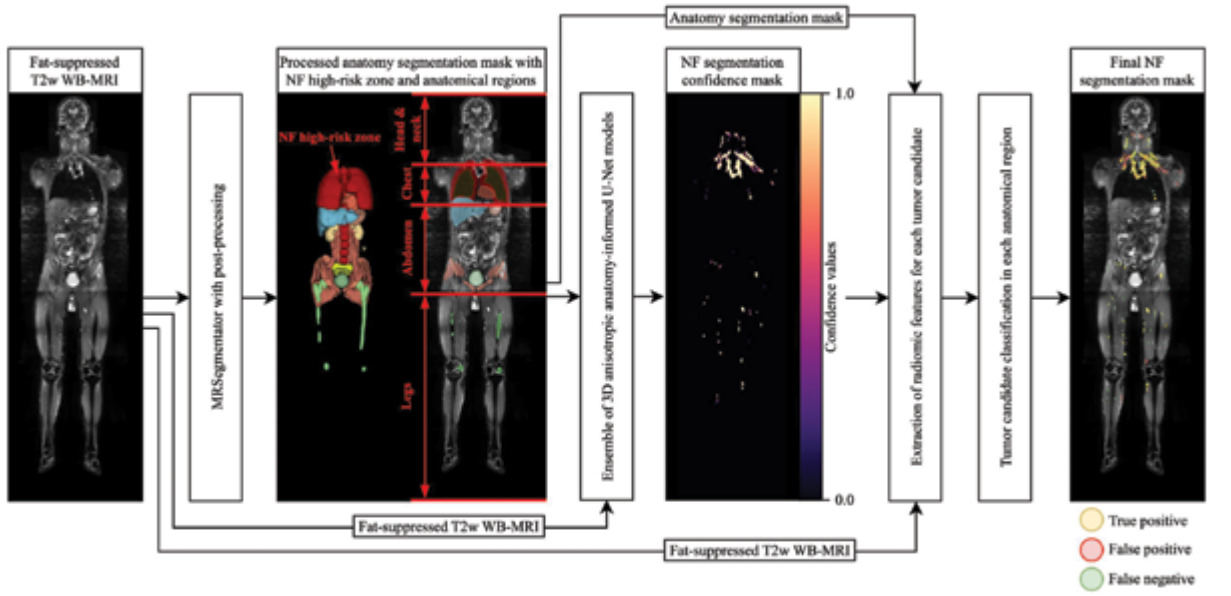
Purpose: Accurate segmentation of neurofibromas (NF) in whole-body magnetic resonance imaging (WB-MRI) is crucial to assess tumor burden and monitor disease progression in patients with neurofibromatosis type 1 (NF1). However, automated tumor quantification has so far performed poorly, with a reported nnU-Net-based Dice similarity coefficient (DSC) of only 25%. We therefore evaluated an anatomy-informed dynamic UNet-based whole-body MRI (WB-MRI) approach to improve automatic neurofibroma (NF) segmentation.

Methods: WB-MRI data included T2-weighted fat-suppressed scans of 109 NF1 patients from 2006 – 2020 acquired at 1.5 or 3T. NFs were manually segmented using ITK-SNAP 3.8.0. MRSegmentator was applied to generate an anatomy segmentation mask, extended with a high-risk zone for NFs. The second stage employed an ensemble of 3D anisotropic anatomy-informed U-Nets to generate an NF segmentation confidence mask. Finally, tumor candidates were extracted from the confidence mask and classified based on radiomic features to distinguish tumors from non-tumor lesions. The pipeline was trained on a cohort of 63 MRIs with a median tumor burden of 318 ml and evaluated on three test sets representing different conditions: in-domain data validation (13 MRIs, median tumor burden 1311 ml), varying imaging protocols and field strength (11 MRIs, median tumor burden 442 ml), and low tumor burden cases (22 MRIs, median tumor burden 6 ml).

Results: The anatomy-informed NF segmentation pipeline is visualized in **Figure 1.** Experimental results show a 68% improvement in per-scan DSC, a 21% increase in per-tumor DSC, and a two-fold improvement in F1 score for tumor detection in high tumor burden cases by integrating anatomy information. The method is integrated into the 3D Slicer platform for practical clinical use, with the code publicly accessible.

Conclusion: In this study, we present a fully automated pipeline for NF segmentation in fat-suppressed T2-weighted WB-MRI, including anatomy segmentation, NF segmentation, and tumor candidate classification. The proposed anatomy-informed dynamic UNet-based approach has the potential to significantly improve WB-MRI NF segmentation. Future integration into interactive workflows may facilitate integration into clinical routine, hereby contributing to individualized risk stratification.

Figure 1:



Supporting Figure: Anatomy-informed NF segmentation pipeline. The input T2-weighted whole-body MRI (T2w WB-MRI) data are segmented using the MRSegmentator model, followed by post-processing to refine the anatomy segmentation mask of four anatomical zones. 3D anisotropic anatomy-informed U-Net models are employed to generate an NF segmentation confidence mask. Each zone is segmented with a dedicated DynUnet to obtain respective NFs masks. The NF masks are then merged to form the final segmentation mask.

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Adult Patient and Caregiver Perspectives on the Impact of Neurofibromatosis Type 1-Associated Plexiform Neurofibroma (NF1-PN): Insights from a US Qualitative Survey

Phioanh L. Nghiemphu, MD, *David Geffen School of Medicine, University of California, Los Angeles, CA*

Purpose: This study aimed to improve understanding of the disease burden, healthcare experiences, and unmet needs of adults with NF1-PN in the USA, from the perspective of patients and caregivers.

Methods: Individual, 45-minute, double-blind telephone interviews were conducted in the USA (July 27–August 4, 2023). Participants (≥18 years) had NF1-PN or were caregivers of adults with NF1-PN. All participants were required to be involved in NF1-PN treatment decisions. Caregivers who participated were not associated with patients who participated. Interviewers followed discussion guides, which contained questions covering patient background and NF1-PN diagnosis, treatment journey, relationship with healthcare providers, and unmet needs. All participants were recruited through physician referrals, patient organizations, and patient databases.

Results: The study included 11 adult patients with NF1-PN and two caregivers of adult patients with NF1-PN. Overall, 62% of patients were aged ≥35 years. Most patients (85%; n=11/13) had been diagnosed in childhood. Additionally, patients had multiple other NF1-associated conditions, including pain-related disorders (n=10), psychiatric disorders (n=7), and chronic migraine (n=6). NF1-PN affected most aspects of patients' lives, including overall quality of life, mental health, personal relationships, and participation in school/work. Of patients diagnosed in childhood, 82% (n=9/11) had been transitioned to an adult practitioner. Transition to adult care occurred at 18/19 years for most patients and was typically driven by the pediatric care team (n=6/9). The transition process was uncomplicated for most patients (56%; n=5/9); the remainder experienced challenges, including finding an appropriate provider (n=3/9). Most patients (77%; n=10/13) reported receiving routine healthcare annually; patients with lapses in care (n=3/13) provided reasons including lack of local physicians (n=1/13), lack of healthcare insurance (n=2/13), and the perception that there is nothing that can treat/cure NF1-PN (n=3/13). NF1-PN medication management for adults included pain medications over-the-counter (n=9) and prescription (n=3), vitamin supplements (n=1), and skin creams/oils (n=2). When asked what could be improved in the management of NF1-PN, patients and caregivers cited more informed care and disease state information (n=7) and improved treatments for NF1-PN (n=13), including medications that would slow/stop PN growth (n=6), reduce PN size (n=8), improve pain management (n=5), and were covered by insurance (n=2).

Conclusion: This US-based study of adults with NF1-PN and caregivers demonstrated the high disease burden on patients despite variability in manifestations and highlighted the need for more informed care and improved treatment options.

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Healthcare Providers' (HCPs') Perspectives on the Management of Adults with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibroma (PN): Insights from a US Survey

Phioanh L. Nghiemphu, MD, *David Geffen School of Medicine, University of California, Los Angeles, CA*

Purpose: The aim of this study was to better understand experiences, approaches, and challenges in treating and managing adults with NF1-PN from the perspective of US physician HCPs.

Methods: Quantitative data were collected through 25-minute online surveys (July 27–August 23, 2023). HCPs were asked for their perspectives on unmet needs in the diagnosis, management, and treatment of adults with NF1-PN. A subset of participants participated in quali-quant interviews to provide additional context to their responses. HCPs must have been in practice for 3–35 years, spent ≥50% of their time in direct patient care, and managed ≥100 patients in the last 12 months for any condition, including ≥3 adults (aged ≥18 years) with NF1-PN.

Results: Overall, 41 HCPs completed the survey; 4/41 participated in quali-quant interviews. HCPs reported the most common manifestation in patients prior to NF1-PN diagnosis as neurofibromas (66% adults). The most burdensome manifestations reported for adult patients were brain stem gliomas (80%), visual impairment (78%), neuropathic pain (76%), other (non-neuropathic) pain (73%), and bladder/bowel dysfunction (68%). HCPs generally reported follow-up appointments with most adults every 2–3 months (68%). Physical exam of the skin (92%) and diagnostic imaging (79%) were the most common PN monitoring methods. The most common treatments for adult PN-related issues included physiotherapy (76%) and pain medication (90%). Types of pain medication included pregabalin (76%), gabapentin (73%), analgesics (66%), and non-steroidal anti-inflammatory drugs (61%). While selumetinib was FDA approved for the treatment of pediatric patients with symptomatic, inoperable NF1-PN during the study period, 46% of physicians reported use of selumetinib in adult patients and 50% of physicians reported use in pediatric patients. Over two-thirds of HCPs highlighted both the limited and lack of effective treatment options as the greatest challenges in the management of adult NF1-PN. With regard to continuity of care, HCPs reported that 17% of patients fall out of care when transitioning from pediatric to adult care and attribute this to lack of transition guidelines/standards as a key challenge.

Conclusion: From the perspectives of HCPs, adult patients with NF1-PN experience a considerable number of issues, including high rates of reported pain. Additionally, HCPs generally reported that adults have a high overall burden of disease, face many challenges in their transition from pediatric to adult care, and could benefit from more effective treatment options to manage manifestations of NF1-PN.

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A Phase 1/2 Study of the Pharmacokinetics and Safety of the Selumetinib Granule Formulation in Children Aged ≥1 to <7 Years with Neurofibromatosis Type 1-Related Plexiform Neurofibroma (NF1-PN): Primary Analysis of the SPRINKLE study (NCT05309668)

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Purpose: NF1-PN symptoms can substantially impact quality of life. While the selumetinib (ARRY-142886, AZD6244) capsule formulation is approved for pediatric patients with symptomatic, inoperable NF1-PN (aged ≥2/3 years; region-dependent), young children may have difficulty swallowing capsules. SPRINKLE aimed to identify a dosing regimen for the selumetinib granule formulation in children aged ≥1–<7 years with symptomatic, inoperable NF1-PN.

Methods: Participants were enrolled into Global Cohort (GC)1 (aged ≥4–<7 years), GC2 (aged ≥1–<4 years), or the Japan Cohort (JC). An initial dose-finding phase confirmed the recommended initial dose of 25mg/m^2 twice daily every 12 hours (28-day cycles). Primary objectives: assess selumetinib single-dose pharmacokinetic exposure (area under concentration–time curve from 0 to 12 hours [AUC_{0-12}]) and safety. Primary endpoint was pre-defined as the 95% confidence intervals falling within 60–140% of the geometric mean AUC_{0-12} (2009h*ng/mL) achieved with capsule formulation in SPRINT. Secondary objectives: assess selumetinib and N-desmethyl selumetinib pharmacokinetics (Cycle[C]1 Day[D]1: single-dose; C2D1: multiple-dose) and palatability. At first data cut-off (April 8, 2024), all participants completed ≥3 cycles. Pharmacokinetics analyses were separate for GCs and JC.

Results: Of 36 eligible participants (GC1: n=15; GC2: n=17; JC: n=4), 61.1% were male; median age was 3.9 years (range 1.1–7.0). Geometric mean AUC_{0-12} (95% confidence interval) at C1D1 in GC1 (n=13), GC2 (n=15), and overall GC (n=28) was 1902 (1647, 2197), 1699 (1436, 2009), and 1790h*ng/mL (1609, 1993), respectively; therefore, the primary pharmacokinetics endpoint was met. Median time to reach maximum observed concentration post-administration was ~2 hours (C1D1, C2D1). Selumetinib accumulation was observed at C2D1. Overall (N=36), median duration of exposure was 10.8 months (range 2.7–25.3). All participants had ≥1 adverse event (AE); 97.2% had ≥1 treatment-related AE. The most common AEs were pyrexia and dry skin (47.2% each), with paronychia (44.4%) the most common treatment-related AE. Two (5.6%) participants experienced serious AEs (not treatment-related). No Grade ≥4 AEs were reported. No AEs led to dose reduction or discontinuation. 30.6% of participants had AEs (most commonly pyrexia and vomiting; 11.1% each) leading to dose interruption. No new safety concerns were identified. Most participants (82.4%) reported swallowing their medication without problems.

Conclusion: Selumetinib granule formulation (25mg/m^2 dose-equivalent twice daily) has comparable exposure to the capsule formulation. No new safety concerns were identified, demonstrating that the granule formulation is suitable for children with NF1-PN who cannot swallow capsules and/or are younger than current approval age.

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MEK Inhibitor Therapy in Infants and Very Young Children

Nikolas Hillenbrand, MD, *Pediatric Neurology, University of Frankfurt/Germany*

Purpose: Plexiform neurofibromas (PN) are the hallmark of neurofibromatosis type 1 (NF1) and frequently are inoperable. For these patients the MEK inhibitor selumetinib provides a non-invasive therapeutic alternative which reliably reduces PN size. However, selumetinib has only been approved for NF1-patients aged two years and older (three years and older in the EU) so that younger children cannot benefit from this therapy. Here, we present our experience with 4 NF1-patients aged 4 – 14 months with inoperable, symptomatic PN who received off-label MEKi therapy.

Keywords: NF1-associated plexiform neurofibromas, MEK-inhibitor therapy, early treatment

Methods: Case 1: MRI of a 14-month-old boy with genetically confirmed NF1 revealed a large intra-abdominal mass with retroperitoneal, intraspinal, and subcutaneous involvement. The intraspinal component of the histologically confirmed neurofibroma was surgically removed, leaving a significant portion of the intra-abdominal tumor mass intact which warranted MEKi therapy.

Case 2: A 4-month-old girl with genetically confirmed NF1 presented with left-sided exophthalmos and a cervical mass at birth. MRI revealed a large tumor involving the orbit, the acoustic meatus, and parts of the pharynx. A biopsy confirmed the diagnosis of PN. Vision was unaffected, but a BERA showed left-sided hearing loss. Based on these findings, MEKi therapy was indicated.

Case 3: A 7-month-old boy with genetically confirmed NF1 presented with a swelling extending from the left cheek to the parotid region. MRI revealed sphenoid wing dysplasia and a partially lobulated tumor with a volume of 42.1 ml, suggestive of PN. Follow-up examination showed a 16% increase in tumor volume. The progressive tumor growth and increasing dystrophy of the patient warranted the initiation of MEKi therapy.

Case 4: A 4-week-old boy with genetically confirmed NF1 presented with sphenoid wing dysplasia, right exophthalmos, right temporal arachnoid cyst, PN of the right trigeminal nerve, and structural epilepsy. After 4 months the facial swelling had markedly increased and volumetric MRI demonstrated an increase in tumor size from 3.72 ml to 14.37 ml so that MEKi therapy was initiated.

Results: Since very young children are unable to swallow capsules MEKi therapy was started off label with trametinib that is available as oral solution. We could demonstrate that MEK inhibitors can reliably be administered in this age group and did not result in adverse effects. Effects on PN size will be assessed by volumetric MRI.

Conclusion: MEKi therapy is feasible in very young children, is well tolerated, can reduce PN growth and prevent PN-related morbidity.

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Efficacy Observation of Selumetinib in the Treatment of Neurofibromatosis Type 1 with Plexiform Neurofibromas in Children from Western China

Zhi Wang, *Chengdu Women's and Children's Central Hospital*

Objective: To investigate the clinical efficacy and safety of selumetinib in children with neurofibromatosis type 1 (NF1) and symptomatic plexiform neurofibromas (PN) in Western China.

Methods: Seventeen children diagnosed with NF1 and PN (symptomatic, inoperable tumors causing pain or craniofacial deformity) from January 2023 to December 2023 were enrolled. They were divided into an experimental group (n=10, treated with oral selumetinib at 25 mg/m^2 twice daily + regular follow-up) and a control group (n=7, clinical observation only). The treatment period was 12 months.

Evaluation criteria:
1. Lightening of café-au-lait spots (≥1-grade reduction on a visual assessment scale) and ≥20% reduction in diameter.
2. MRI-assessed volume reduction of the largest PN (≥1/3 reduction in 3D volume).
3. Clinical and imaging assessments at baseline, 4 months, and 8 months.

Results:
1. In the experimental group, café-au-lait spot lightening efficacy was 85.7% (6/7), and diameter reduction efficacy was 71.4% (5/7); no improvement was observed in the control group (*P*<0.05).
2. MRI showed an average PN volume reduction of 28.6% (maximum 33%) in the experimental group, while the control group showed no significant changes (*P*<0.01).
3. Mild adverse events (2 cases of diarrhea, 1 case of rash) occurred in the experimental group, but none led to treatment discontinuation. No drug-related adverse events were reported in the control group.

Conclusion: Selumetinib significantly improves cutaneous lesions and reduces tumor volume in children with NF1 and PN in Western China, with manageable safety. Early application of targeted therapy is recommended to improve prognosis.

Brief Discussion: NF1 with PN is an autosomal dominant disorder lacking effective treatments. Selumetinib, a MEK inhibitor, inhibits RAS/MAPK pathway activation to slow tumor growth[1]. This study aligns with previous findings[1], demonstrating PN volume reduction and skin lesion improvement. However, the small sample size warrants further validation[2]. Long-term efficacy and resistance mechanisms require future investigation to optimize therapeutic strategies[5].

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Long-Term Efficacy and Predictors Analysis of Selumetinib in Chinese Adult Patients with Neurofibromatosis Type 1 and Inoperable Plexiform Neurofibromas

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Purpose: Selumetinib, the first targeted therapy approved for children with inoperable neurofibromatosis type 1 associated plexiform neurofibroma (NF1-PN), normally required long-term treatment to maintain response. However, its long-term outcomes in adults remains understudied. In addition, given that prolonged treatment burdened adults financially and psychologically, identifying patients who are most likely to benefit from selumetinib could help alleviate these challenges. This study aimed to evaluate the long-term efficacy, safety and patient-reported outcomes (PROs) of selumetinib in adult NF1 patients and explore potential predictors for therapy outcomes.

Methods: This study included adult patients from a Chinese phase I study (NCT04590235), with enrollment between December 2020 and September 2021, and follow-up through September 2024. Data included baseline demographics, volumetric responses, adverse events (AEs), PROs, and blood examinations. The long-term objective response rate of this Chinese adult cohort was calculated and compared to the short-term outcomes, and their common AEs were reported. Mixed effect models were used to compare PROs across response groups. Clinical features and imaging parameters were selected by correlation analysis and univariate logistic regression. Radiomics features were selected by the least absolute shrinkage and selection operator (LASSO) method. And a radiomics score (Rad-score) for per lesion was calculated by selected radiomics features and their weighted coefficients. Decision tree was utilized to assess the predictive value of these features, evaluated by the receiver operating characteristics (ROC) area under curve (AUC).

Results: Among 16 patients (median age 24.5 [18-51] years) participating in this study, 11 (69%) achieved confirmed partial response (cPR), 4 (25%) had stable disease, and 1 (6%) was progressive, with a median follow-up of 40 (10-47) cycles. AEs were mild. Results of PROs demonstrated improvement in pain interference of PR patients throughout the treatment duration, while the quality of life presented no significant difference. For predictors analysis, the radiomics score (Rad-score) -based model performed the best (AUC, 0.9), followed by the age and lactate dehydrogenase (LDH)-based model (AUC, 0.89).

Conclusion: This study confirmed that selumetinib was well-tolerated and effective for long-term treatment in NF1 adult patients. Age, LDH level and Rad-score were observed as potential predictors for selumetinib efficacy, warranting larger randomized controlled trials for further validation.

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Luvometinib (FCN-159) in Pediatric Participants with Neurofibromatosis Type 1: An Updated Report on the Efficacy and Safety of a Multi-Center, Open-Label, Single-Arm Phase II Study

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Purpose: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disease characterized by multiple progressive tumor and non-tumor manifestations, with abnormal activating MAPK pathway. Plexiform neurofibromas (PN) presents in 20-50% of NF1 patients. It can lead to clinical symptoms with a high rate of disability and teratogenesis and has the risk for malignant transformation. Luvometinib is a highly potent and selective inhibitor of MEK1/2. The aim of current study is to evaluate the safety and efficacy of luvometinib in pediatric patients with NF1-related PN. The preliminary findings were disclosed at ASCO 2024. Here, we present the updated results of pediatric cohort.

Methods: This is a multi-center, open-label phase II clinical trial. The primary endpoint was the objective response rate (ORR) evaluated by investigators per REiNS criteria. Key secondary endpoints included the ORR evaluated by a blinded independent review committee (BIRC), duration of response (DOR), progression-free survival (PFS) and safety.

Results: As of September 23, 2024, 46 pediatric patients were enrolled and treated with luvometinib at a dose of 5 mg/m^2 (the RP2D from phase I study). The median follow-up was 25.1 months. The investigators-evaluated ORR was 60.5% (26/43, 95% CI: 44.4-75.0, $P<0.0001$) with partial response in 26 patients. The BIRC-evaluated ORR was 44.2%(19/43, 95% CI: 29.1-60.1, $P<0.0001$, with partial response in 19 patients. The median PFS were not reached, with 1-year PFS rates of 95.3% (investigator) and 100% (BIRC), respectively. The median DOR were not reached, with the 1-year DOR rates of 87.6% (investigator) and 92.3% (BIRC), respectively. Pain reduction was notable: 78.6% (11/14) of patients with baseline tumor pain (NRS$\geq$2) achieved complete pain resolution (NRS=0). Treatment-related adverse events (TRAEs) occurred in 45 patients (97.8%), with Grade $\geq$3 TRAEs in only 10 patients (21.7%), including folliculitis (4.3%), dermatitis acneiform (4.3%), increased blood creatine phosphokinase (4.3%), decreased ejection fraction (2.2%), upper respiratory tract infection (2.2%), pneumonia (2.2%), anemia (2.2%), and gastrointestinal disorders (2.2%). Serious TRAEs were reported in 2 (4.3%) patients. TRAEs led to dose interruption in 14 (30.4%) patients. No TRAEs led to dose reduction, discontinuation, or death. No new safety signals were observed.

Conclusion: Luvometinib showed promising efficacy and manageable safety profile without dose reduction nor discontinuation due to TRAE in pediatric patients with NF1-related PN. The study of luvometinib with long-term follow-up is ongoing. Clinical trial information: NCT04954001.

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Burying the Golden Thread: Traditional Medicine Meets Modern Science in NF1 Treatment

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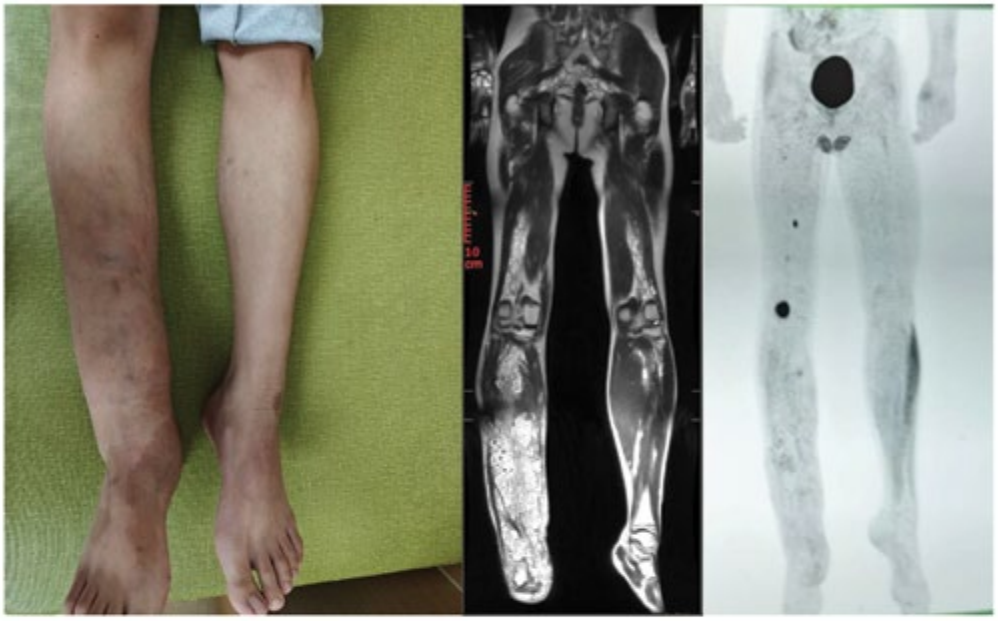
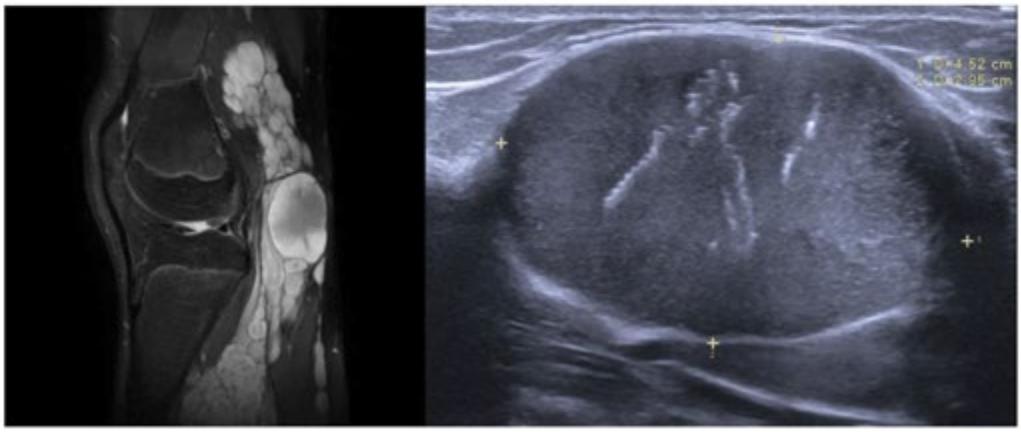
A 14-year-old male with neurofibromatosis type 1 (NF1) diagnostic criteria-fulfilling features presented with two cardinal manifestations: (1) ≥6 café-au-lait macules (CALMs) with axillary freckling noted since infancy, and (2) progressive right lower limb hypertrophy persisting for 11 years. The hypertrophic limb exhibited intact overlying skin without erythema, ulceration or tenderness. Intermittent paresthesia localized to the right plantar region was reported. Previous therapeutic interventions included polidocanol sclerotherapy and intralesional bleomycin injections. In February 2024, the patient underwent an unproven treatment known as "Burying the Golden Thread" at a traditional medicine clinic in South Korea. A whole-body 1.5T MRI revealed disseminated T2-hyperintense multiple nodular lesions throughout his body, predominantly in the right lower extremity. PET-MRI fusion demonstrated a 2.1×2.1cm nodule (SUV max 12.9) in the right popliteal intermuscular space. Ultrasound-guided core needle biopsy histopathology confirmed plexiform neurofibroma without evidence of cytological atypia. Central pathology review by NF1 specialists at Shanghai Ninth People's Hospital confirmed there is no necrosis and no mitotic figures the tissue specimen. Initiated selumetinib at 40 mg/m^2 BSA twice daily, calculated using the Mosteller formula (√[height(cm)×weight(kg)/3600]). In the most recent follow-up at 18-week after starting the medication, the patient's right lower limb had significantly thinned, and tumor shrinkage is ongoing. No significant treatment-related toxicity (CTCAE v5.0) were observed during the treatment.





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Retrospective Review of Mitogen Activated Protein Kinase (MEK) Inhibitor Treatment for Children with Inoperable Plexiform Neurofibroma (PN) at Guy's National NF1 Centre from July 2016 – November 2024

Mandy Myers, RN, *National Complex Neurofibromatosis 1 Service, Guy's and St. Thomas' NHS Foundation Trust*

Background: MEK inhibitors (MEKi) have been used for the treatment of inoperable and symptomatic plexiform neurofibromas (PN) in children with neurofibromatosis type 1 (NF1) in the UK since 2016. In May 2022 NICE approved Selumetinib for use on the NHS and prior to this MEK inhibitors (Selumetinib and Trametinib) were used on named patient/ compassionate basis and as part of clinical trials[1, 2]

Methods: We retrospectively reviewed the notes of all children discussed in our national MEK Multi-Disciplinary Meetings (MDM) since 2017. A total of 46 cases from Guy's Hospital complex NF1 service were reviewed up until November 24.

Results: 36 patients were approved for treatment but only 28 patients went on to receive treatment. Primary indications for starting MEKi included disfigurement (10), impairment (3) or potential threat to function (6) and pain (9). Location of the target PN for the 28 patients treated included craniofacial (8), extremity (7), neck (6), spinal/ paraspinal (1) and other (7). Age range at initiation of therapy was 11 months to 18 years; mean 9 years 5 months. Duration of treatment 1 month-8 years 7 months; mean 2 years 3 months. 5 patients with neck/ craniofacial PN have had additional surgery either before or during treatment. 10 patients (45% of our cohort) have stopped; 8 because of lack of benefit, 1 because of intolerable skin side effects, 1 because of severe sepsis. To date benefit has been reported in 14 patients (50%) of whom 11 remain on treatment with 8 reporting improvement in pain symptoms, 4 reporting slowing of growth, 1 reduction in size of PN and 1 showed an improvement in her cardiorespiratory sleep study. Of the 3 patients who reported benefit but stopped treatment, 1 was because of intolerable side effects, 1 took a natural treatment break after almost 3 years and decided not to continue and 1 who reported improvement in pain symptoms, experienced growth of PN.

Conclusion: MEK inhibitors can benefit some but not all patients with inoperable symptomatic PN. They can be used in conjunction with surgical approaches i.e. debulking. Treatment was generally well tolerated; however, patients require frequent re-evaluation to assess response to treatment and side effects.

Thus far 50% of patients report benefit and over half of these report reduction in pain symptoms. 55% remain on treatment. Once a paediatric formulation becomes available, we anticipate a shift in indication to start Selumetinib to younger patients with plexiform neurofibromas.

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1. Intermittent Dosing Of Selumetinib In Childhood NF1 Associated Tumours (INSPECT)
2. GSK-MEK116540: An Open-Label, Dose-Escalation, Phase I/II Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics, and Clinical Activity of the MEK Inhibitor Trametinib in Children and Adolescents Subjects with Cancer or Plexiform Neurofibromas and Trametinib in Combination with Dabrafenib in Children and Adolescents with Cancers Harboring V600 Mutations

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Characteristics, Cycle of First Onset, and Time to Resolution of Commonly Reported (≥15%) Treatment-Related Adverse Events (TRAEs) in the ReNeu Trial of Mirdametinib in Children and Adults with Neurofibromatosis Type 1 (NF1)-Associated Plexiform Neurofibroma (PN)

Laura J. Klesse, MD, PhD, *University of Texas Southwestern, Dallas, TX*

Background: Mirdametinib is the first FDA-approved MEK1/2 inhibitor for both adults and children (≥2y) with symptomatic NF1-PN not amenable to complete resection. This analysis evaluated cycle of first onset and time to resolution of commonly reported TRAEs in the pivotal ReNeu trial, where patient- and parent proxy-reported outcome improvements and PN volume reductions were demonstrated.

Methods: Safety results are reported as of the data cutoff (September 20, 2023). Median cycle (1-cycle=28 days) of first onset and time to resolution (days) for common TRAEs (≥15%) were evaluated for prepubescents (2 to <12y, n=32), postpubescents (12 to <18y, n=24), young adults (18 to ≤50y, n=48), and older adults (>50y, n=10). Time to resolution was calculated from the start of the first event to the end of the last event. The protocol provided strategies for AE supportive care, but no AE prophylaxis was mandated at treatment initiation.

Results: The most frequent cycle of onset for TRAEs was Cycle 1 (81%). Common TRAEs were mostly grades 1 to 2 (83%, **Table 1**) and did not occur with greater frequency or severity over time. Median cycle of onset (**Table 2**) and time to resolution (**Figure**) for common TRAEs are reported. In prepubescents, median cycle of onset was Cycle 1 for vomiting; Cycle 3 for dermatitis acneiform; and Cycle 5 for paronychia, diarrhea, and nausea. For postpubescents, median cycle of onset was Cycle 1 for dermatitis acneiform, diarrhea, nausea, and vomiting; and Cycle 4 for paronychia. In young adults, median cycle of onset was Cycle 1 for dermatitis acneiform, diarrhea, and nausea; and Cycle 2 for vomiting. For older adults, median cycle of onset was Cycle 1 for dermatitis acneiform, diarrhea, nausea, and vomiting. Time to resolution was variable between TRAEs and among patients in all age groups. Eighteen patients had a TRAE of asymptomatic ejection fraction (EF) decrease, median (range) time to onset was 127 (41, 1361) days, and all except one prepubescent patient experienced resolution. No patients experienced a symptomatic EF decrease.

Conclusions: In ReNeu, symptomatic improvements and PN volume reductions were observed at the earliest measured timepoint (Cycles 3 and 5, respectively) and were sustained over time. In all age groups, common TRAEs were detected earlier than treatment benefits and were generally resoluble. It is important to manage patients' expectations and proactively manage TRAEs to improve treatment experience and adherence.

Table 1. TRAEs (Incidence of ≥15%) in the ReNeu Trial

	Prepubescent (Ages 2 to <12 years) (n=32)		Postpubescent (Ages 12 to <18 years) (n=24)		Young Adult (Ages 18 to ≤50 years) (n=48)		Older Adult (Age >50 years) (n=10)		Total (N=114)	
TRAEs, n (%)	Grades 1 to 2	Grades ≥3	Grades 1 to 2	Grades ≥3	Grades 1 to 2	Grades ≥3	Grades 1 to 2	Grades ≥3	Grades 1 to 2	Grades ≥3
Overall TRAEs	23 (72)	0 (0)	20 (83)	3 (12)	43 (90)	5 (10)	9 (90)	0 (0)	95 (83)	8 (7)
Dermatitis acneiform	5 (16)	0 (0)	19 (79)	1 (4)	37 (77)	5 (10)	8 (80)	0 (0)	69 (61)	6 (5)
Diarrhea	13 (41)	0 (0)	8 (33)	1 (4)	21 (44)	0 (0)	7 (70)	0 (0)	49 (43)	1 (1)
Nausea	7 (22)	0 (0)	5 (21)	0 (0)	19 (40)	0 (0)	2 (20)	0 (0)	33 (29)	0 (0)
Vomiting	4 (12)	0 (0)	4 (17)	0 (0)	14 (29)	0 (0)	2 (20)	0 (0)	24 (21)	0 (0)
Asymptomatic ejection fraction decrease	8 (25)	0 (0)	2 (8)	1 (4)	5 (10)	0 (0)	2 (20)	0 (0)	17 (15)	1 (1)
Paronychia	11 (34)	0 (0)	6 (25)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	18 (16)	0 (0)

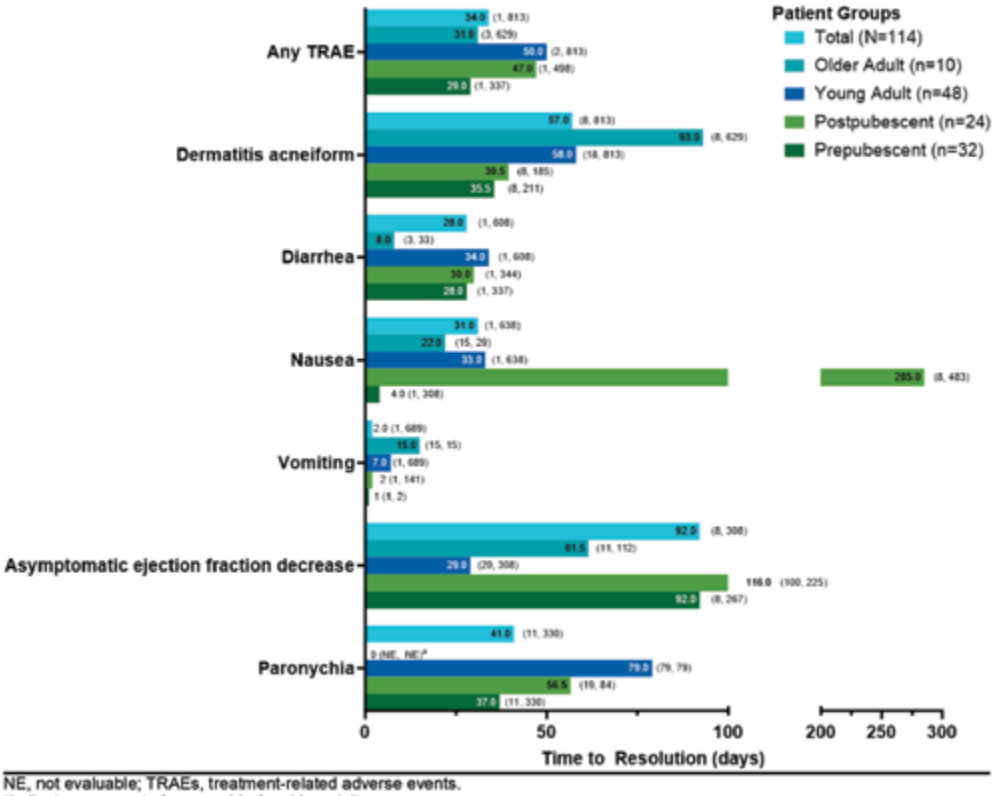
TRAEs, treatment-related adverse events.
TRAEs have been reported in order of decreasing incidence.

Table 2. Median (Range) Cycle of TRAE (Incidence of ≥15%) First Onset in the ReNeu Trial

Cycle of TRAE Onset, Median (Range)	Prepubescent (Ages 2 to <12 years) (n=32)	Postpubescent (Ages 12 to <18 years) (n=24)	Young Adult (Ages 18 to ≤50 years) (n=48)	Older Adult (Age >50 years) (n=10)	Total (N=114)
Any TRAE	1.0 (1, 19)	1.0 (1, 8)	1.0 (1, 2)	1.0 (1, 2)	1.0 (1, 19)
Dermatitis acneiform	3.0 (1, 14)	1.0 (1, 9)	1.0 (1, 1)	1.0 (1, 2)	1.0 (1, 14)
Diarrhea	5.0 (1, 24)	1.0 (1, 16)	1.0 (1, 3)	1.0 (1, 11)	1.0 (1, 24)
Nausea	5.0 (1, 24)	1.0 (1, 21)	1.0 (1, 13)	1.0 (1, 1)	1.0 (1, 24)
Vomiting	1.0 (1, 10)	1.0 (1, 13)	2.0 (1, 10)	1.0 (1, 1)	1.0 (1, 13)
Asymptomatic ejection fraction decrease	5.0 (2, 28)	9.0 (5, 24)	2.0 (2, 49)	4.0 (3, 5)	5.0 (2, 49)
Paronychia	5.0 (2, 28)	4.0 (2, 9)	6.0 (6, 6)	-	5.0 (2, 28)

TRAEs, treatment-related adverse events.
TRAEs have been reported in order of decreasing incidence.

Figure. Median (Range) Time to TRAE (Incidence of ≥15%) Resolution in the ReNeu Trial



NE, not evaluable; TRAEs, treatment-related adverse events.
[a]Indicates no event of paronychia for older adults
Time to resolution was calculated as the start date of the first event of the preferred term to the end date of the last event of the preferred term. If the last event of the preferred term was ongoing, then the event was considered unresolved and was not included in the summary statistics.

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LJK: Uncompensated relationships with Alexion Pharmaceuticals;
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PA: Honoraria with SpringWorks Therapeutics, SERVIER; Consulting or Advisory Role with SpringWorks Therapeutics, SERVIER; Patents, Royalties, Other Intellectual Property for Morpholino oligonucleotides useful in cancer treatment; Patent number: 11679121; Abstract: Disclosed are morpholino oligonucleotides that can be used to silence expression of MGMT, pharmaceutical compositions that include said morpholino oligonucleotides, and methods of using said morpholino oligonucleotides in the treatment of cancer, particularly methods that involve the use of radiation to deliver said morpholino oligonucleotides. Type: Grant Filed: December 7, 2020; Date of Patent: June 20, 2023;
SM: Consulting or advisory role with SpringWorks Therapeutics;
PN: Honoraria with Alexion Pharmaceuticals; Consulting or Advisory Role with SpringWorks Therapeutics; Research Funding with Chimerix (Inst), Recursion Pharmaceuticals (Inst), NCCN (Inst), SpringWorks Therapeutics (Inst), Millennium (Inst), Erasca, Inc (Inst), Global Coalition for Adaptive Research (Inst), Children's Tumor Foundation (Inst);
ZS: Employment with Adjuvant Behavioral Health; Leadership with Adjuvant Behavioral Health; Speakers' Bureau with Alexion Pharmaceuticals;
DS: Consultancy/advisory role with Orbus Therapeutics, PRA, Anheart Therapeutics, Servier (I), SymBio Pharmaceuticals; royalties for submissions to UpToDate;
MN, MDW, AL: employment with SpringWorks Therapeutics, Inc; equity interest in SpringWorks Therapeutics, Inc;
SS: No relevant disclosures;
TW: Consulting or Advisory Role with Novocure, Alexion Pharmaceuticals, SpringWorks Therapeutics, SERVIER, Anheart Therapeutics; Travel, Accommodations, Expenses with SERVIER;
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RA: Consultancy/advisory role with Alexion Pharmaceuticals; honoraria from Alexion Pharmaceuticals;
KN: Consultancy/advisory role with Servier Pharmaceuticals, royalties for editorial role with Oxford University Press;
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Congenital Bilateral Plexiform Neurofibromas of the Cavernous Sinuses Presenting as Buphthalmos at Birth

Aaina Kochhar, MD, *Children's Hospital Colorado, Department of Pediatrics, Division of Genetics and Metabolism*

Purpose: We describe a term newborn presenting with congenital glaucoma, buphthalmos and poor vision, who was found to have bilateral cavernous sinus plexiform neurofibromas extending through the skull base foramina into the left, greater than right, orbits and face. Treatment with a MEK inhibitor was initiated given progressive vision impairment.

Method: A full term newborn female infant presented with left-sided buphthalmos and congenital glaucoma. Brain MRI showed bilateral enhancing cavernous sinus masses, extending along both trigeminal nerves, and nodular enhancement of right oculomotor nerve. These lesions were initially suspected to be atypical intracranial hemangiomas or plexiform neurofibromas. The patient had multiple cafe au lait macules on exam, but did not meet clinical diagnostic criterion for Neurofibromatosis type 1 (NF1) in the newborn period. She received medical treatment for glaucoma. Genetic testing and follow up imaging were done.

Results: Whole exome sequencing showed a heterozygous denovo pathogenic variant in the NF1 gene c.1756-1759del, p.(Thr586ValfsTer18). Follow up MRI brain, orbits and face at 3 months of age, showed increasing size of bilateral plexiform neurofibromas expanding the cavernous sinuses and extending through skull base foramina into left greater than right orbit and face. Given the growth in size of the masses, impact on vision and potential other cranial nerve functions, therapy with the MEK inhibitor, Trametinib, was recommended.

Conclusion: We describe aggressive bilateral cavernous sinus plexiform neurofibromas with intraorbital extension and vision loss. It is an unusual clinical manifestation of NF1 and we offered treatment with a MEK inhibitor in this scenario, to stabilize/prevent further growth of the plexiform neurofibroma.

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TRAM-01: A Phase 2 Study of Trametinib for Pediatric Patients with Neurofibromatosis Type 1 and Plexiform Neurofibromas

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Purpose: Plexiform neurofibromas (PNs) are observed in up to 50% of patients with neurofibromatosis type 1 (NF1). Trametinib has been used widely to treat PNs but limited data has been reported on its efficacy within a clinical trial. We conducted a phase 2 trial with trametinib for patients with NF1 and inoperable PNs.

Patients and Methods: Patients with NF1 and inoperable PNs aged ≥ 1 month to ≤ 25 years when starting trametinib were eligible. Patients received trametinib once daily orally continuously for a maximum of 18 cycles each 28 days long. Patients completed patient-reported outcome measures. Response evaluations were performed after three cycles for 6 months and then every 6 months. The PN volumes were centrally quantified using a semi-automatic 3D segmentation method.

Results: Forty-six patients were recruited. The median age was 11.1 years (range 0.7-19.8). The median baseline tumor volume was 104.6 ml (range 16.3 to 650.1 ml). All patients underwent volume analysis demonstrating an overall response rate (volume decrease of ≥ 20%) of 47.8% (22/46 patients). Median volume change was -19.3% (range -80.5 to 3.5). Forty-one patients (89.1%) completed the planned treatment. The 3 years progression free survival and the event free survival were respectively 73.1% (95% CI 53.3%-85.5%) and 47.1% (95% CI 0.29-0.63). Fifteen patients (32.6%) had a dose reduction due to adverse events and most were related to cutaneous toxicity. Three patients (6.5%) discontinued treatment due to adverse events. Analyses of the PedsQL™ revealed significant improvements in the cognitive problems, pain & hurt, procedural anxiety, and school functioning subscales at cycle 13 compared to baseline.

Conclusion: We report outcomes and volumetric response of PNs treated with trametinib within a phase 2 trial. Based on these results, trametinib is effective, overall well tolerated and offers durable response during treatment.

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EB participated to advisory board with Alexion and Novartis.
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Real-World Outcomes of Selumetinib in Chinese Pediatric Patients with Inoperable Neurofibromatosis Type 1-Associated Plexiform Neurofibroma (NF1-PN): Interim Analysis of Baseline Characteristics from the PEDIA Study

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Purpose: PEDIA was a prospective, real-world study that investigated the effectiveness and safety of selumetinib (ARRY-142886, AZD6244) in Chinese pediatric patients with symptomatic, inoperable NF1-PN.

Methods: This multicenter observational study was conducted across more than 40 centers in China, enrolling pediatric patients aged 3–16 years with symptomatic, inoperable NF1-PN. Participants received treatment with selumetinib 25 mg/m^2 twice daily and were followed for up to 24 months. Key outcomes included baseline demographic and disease characteristics, treatment profiles, and the effectiveness and safety of selumetinib.

Results: The first interim analysis reported the baseline demographic and disease characteristics. A total of 228 patients signed the informed consent form (ICF), with 207 included in the full analysis set. Among these, 59.9% (n=124) were male. Median (Q1, Q3) age was 9 years (6, 12), and median (Q1, Q3) body surface area was 1.045 m^2 (0.85, 1.27). Median (Q1, Q3) durations of NF1 and PN were 9.1 months (0.70, 37.90) and 0.2 months (0.10, 2.15), respectively. PN diagnosis was primarily based on imaging (89.9%), with PN most frequently located in the trunk (40%), head (36.6%), and limbs (25.9%). Prior PN treatment included surgery (31.4%), drug therapy (1.0%, one patient received bleomycin A5 and one with traditional herbal medicine), and radiotherapy (0.5%). The most common PN-related morbidity was pain, affecting 45.1% of patients. Among these, 33.3% had Numerical Rating Scale scores $\geq$3, with a mean score of 4.5 (standard deviation 1.4). Other morbidities included disfigurement (29.6%), decreased range of motion (6.8%), facial motor dysfunction (3.4%), vision loss (2.9%), motor weakness (1.5%), sensory deficits (2.4%), difficulty swallowing (1.0%), and respiratory compromise (1.5%). Café-au-lait macules were present in 99.0% of patients, with 86.3% having more than 10 macules. Scoliosis was observed in 36.4% of patients, and 19.9% had cutaneous neurofibromas. Optic pathway gliomas were identified in 1.0% of patients. Slit lamp examination was conducted for 110 patients, revealing abnormal results (primarily due to Lisch nodules) in 34 cases.

Conclusion: This interim analysis demonstrates the severe disease burden in pediatric patients with NF1-PN, with pain and disfigurement reported as major PN morbidities. PN is a disease affecting multiple organs, highlighting the importance of a thorough and comprehensive examination for accurate diagnosis.

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Characteristics of Patients with Neurofibromatosis Type 1 and Plexiform Neurofibromas in Japan and Real-World Safety and Effectiveness of Selumetinib: A 1-Year Interim Analysis of an All-Case, Postmarketing Surveillance Study

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Purpose: This postmarketing surveillance study aimed to assess the characteristics of patients with neurofibromatosis type 1 (NF1) and plexiform neurofibromas (PN) treated with selumetinib and the real-world safety and effectiveness of selumetinib in Japan.

Methods: All patients who were prescribed selumetinib on or after the drug launch date in Japan (November 16, 2022) were enrolled in this study. The observation period was set to 3 years from drug initiation, and patient data were captured using case report forms (data cutoff for 1-year analysis: October 9, 2024). Safety was assessed through the incidence of adverse drug reactions (ADRs), and the effectiveness (overall patient evaluation of PN and target PN size on imaging) was assessed based on physician's global assessment.

Results: In total, 52 patients were included in the analysis (median [range] age, 13.0 [5–20] years; age <19 years, 49 [94.2%] patients; female, 28 [53.8%] patients; dermatological, neurological, and bone manifestations [DNB: severity classification created by the Neurocutaneous Syndrome Research Group in Japan, wherein stage 5 is the most severe] classification stage 5, 30 [57.7%]; median disease duration [n=49], 118.0 months). Target PN lesions were most commonly observed in the head and neck (30 [57.7%] patients), followed by the trunk (20 [38.5%] patients). The median (range) treatment duration was 52.1 (21.0–65.1) weeks, and 46 (88.5%) patients continued selumetinib for 1 year. ADRs and serious ADRs were observed in 46 (88.5%) and 9 (17.3%) patients, respectively. The most common ADR was diarrhoea (15 [28.8%] patients), followed by dermatitis acneiform (14 [26.9%] patients). No fatal ADRs were reported. At 1 year, 30 (61.2%) of 49 evaluable patients showed improvement in PN, and 16 (41.0%) of 39 evaluable patients showed a decrease in the target PN size on imaging.

Conclusion: The safety and effectiveness profile of selumetinib was generally consistent with the findings of the phase 2 SPRINT and Japanese phase 1 trials. To date, no new safety concerns have been identified. We continue to assess the safety and effectiveness of selumetinib in patients with NF1 and PN in Japan.

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Phase IIa Trial of Tunlametinib in Adults with Inoperable Neurofibromatosis Type 1-Associated Plexiform Neurofibromas: Clinical Outcomes and Exploratory Analyses

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Background: Plexiform neurofibromas (PN) present a significant clinical challenge, with a notable unmet need for effective and well-tolerated therapies, particularly for the adult population.

Methods: A single-arm, open-label, Phase IIa trial of tunlametinib was conducted to assess its efficacy and safety in adults with inoperable, radiologically measurable PN. Patients received tunlametinib at a dose of 9 mg twice daily, following a continuous 21-day cycle regimen. The primary endpoint was the confirmed objective response rate (ORR), and secondary endpoints included disease control rate (DCR), duration of response (DoR), progression-free survival (PFS), and improvements in patient-reported outcomes (PROs) including quality of life, pain, physical function, and other PN-related symptoms compared to baseline.

Results: Of 15 adults (10 males, 5 females; median age, 27 years, range 18–45), 8 patients (53.3%, 95% CI, 26.69–78.73) achieved a confirmed partial response (PR), with a median neurofibroma volume reduction of -23.5% (range 1.6% to -49.1%). The median time to initial response was 19.6 months (range, 2.7 to 31.1 months), and the median time to best response was 22.4 months (range, 11.2 to 31.7 months). Significant improvements in multiple PRO domains were observed. All patients experienced at least one treatment-related adverse event (AE), the majority of which were grade 1 or 2. Notably, a marked age-related difference in treatment response and tumor heterogeneity were observed.

Conclusion: Tunlametinib demonstrated promising efficacy and an acceptable safety profile in adults with PN. The majority of patients experienced sustained tumor shrinkage and clinically meaningful improvements. The manageable toxicity profile, along with the absence of cumulative toxic effects, supports its potential for long-term treatment. Further exploratory analysis revealed the heterogeneity of responses, emphasizing the need for personalized therapeutic approaches.

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The Development and Evaluation of a Patient and Carer Guide for Selumetinib Treatment in Neurofibromatosis Type 1 in the UK

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Background: Neurofibromatosis Type 1 (NF1) is an autosomal dominant genetic condition with a birth incidence of 1 in 2500 to 1 in 3000[1] and can lead to the development of benign tumours called plexiform neurofibromas in 20-50% of patients.[3-7] Selumetinib, an oral MEK inhibitor, has been approved for treating symptomatic and inoperable plexiform neurofibromas in children aged 3-18 in the UK.[2]

To support patients and carers, a comprehensive treatment guide was developed and distributed across two national NF centres in the UK.

Method: The guide was developed by two clinical nurse specialists from both National UK NF1 centres and reviewed by both patient advocacy groups with the support and funding of Alexion, AstraZeneca Rare Disease. The guide aims to ensure NF1 patients are supported in their treatment journey and to aid communication between healthcare providers including the National NF1 Centre, paediatric oncology shared care units (POSCUs) and local paediatric care. The guide is comprehensive but needs to be clear and include picture guidance to support children and those with learning difficulties. Coloured tabbed sections covered various aspects, such as how to take Selumetinib, daily oral and skin care, possible side effects, upcoming medical appointments and support groups. Feedback was collected after 3 months from nine different patients or carers, eight of whom were already undergoing Selumetinib treatment. The age of the patients was not recorded.

Results: A scale of 1-5 was used. High feedback scores, indicated that the guide was user-friendly (8/9 – scoring 5/5 or 4/5), easy to navigate (8/9 – scoring 5/5 or 4/5) and provided reassurance (8/9 – scoring 5/5 or 4/5), with visual aids enhancing comprehension. Users found the sections on daily care routines and what to expect particularly helpful. The guide has facilitated communication with schools and extended family members, supporting a wider support network for the patient. Additionally, it assisted in managing side effects and keeping track of appointments. One patient valued the importance of the treatment guide for NF1 by comparing it to the family-held oncology records used in cancer care. One respondent suggested integration of a section on mental health will allow users to document their emotional well-being.

Conclusion: The patient and carer guide for Selumetinib treatment enhances understanding and management of the treatment process and has proven to be a valuable resource. Future developments will explore the feasibility of converting the guide into a mobile application, providing a more accessible and interactive platform.

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Successful Resection of Selumetinib Resistant Superficial Facial Plexiform Neurofibromas in Two Pediatric Patients with Neurofibromatosis Type 1

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Purpose: Neurofibromatosis type 1 is a tumor predisposition syndrome characterized by peripheral nerve sheath tumors, including histologically benign plexiform neurofibromas (PN). PN occur in several variants including typical fascicular/multi-nodular tumors, diffuse appearing tumors without clear internal structure and distinct nodular lesions. Preliminary analyses suggest that response to MEK inhibitor (MEKi) treatment may vary between these PN types. For example, distinct nodular lesions appear to respond less frequently than typical appearing PN and may grow or develop despite ongoing therapy. Here, we describe two participants in the phase I/II trial of the MEKi selumetinib, who underwent successful surgical resection of a skin infiltrating superficial PN, that demonstrated growth during selumetinib treatment, while shrinkage was maintained in the underlying, deeper target PN.

Methods: Two participants in the Phase I/II trial of selumetinib for children with NF1 and inoperable PN (SPRINT) (NCT01362803) are presented here. Information was extracted from the medical record and study database.

Results: Participant 1 was 6 years old when starting selumetinib treatment for a right facial submandibular PN. Her target tumor was classified as typical multi-nodular/fascicular PN, localized predominantly in the deep facial structures but extending into subcutaneous/cutaneous layers, and interdigitating with surrounding tissues. Approximately 1 year into selumetinib treatment, a new area of diffuse superficial tumor developed over the right mandible adjacent to the target PN. She remained on treatment for approximately 7 years at which time her target PN volume was -32.1% from baseline while the superficial PN continued to grow. She underwent plastic surgery to remove the superficial lesion, which was a PN with diffuse intra- and extra-neural components; there were no surgical complications. She continues on selumetinib post-operatively and has not had any regrowth of the superficial tumor in 43 months of observation.

Participant 2 was 7 years old at the time of starting selumetinib treatment for her right facial and periorbital PN, which had similar structural features to the PN of participant 1. At baseline, there was a small area of superficial PN visible on imaging over the right mandible adjacent to the target PN which shrank slightly during the first year of selumetinib treatment, but then began growing markedly over the next 6 years while her target PN volume was -48.8% from baseline. Due to worsening appearance concerns, the growing superficial portion of the tumor was resected by plastic surgery, with pathology showing cutaneous-subcutaneous neurofibroma with a small intraneural neurofibroma component; there were no surgical complications. She continues on selumetinib post-operatively and has not had any regrowth of the superficial tumor in 12 months of observation.

Conclusions: We present here two children treated with selumetinib for facial PN who had partial response in their deep, multi-nodular target PN, but experienced some growth in their diffuse superficial skin infiltrating PN while on selumetinib treatment. In both cases, the superficial tumors were safely resected by plastic surgery with good cosmetic outcome and no regrowth has been observed. These cases suggest that superficial and diffuse PN may respond differently to MEKi therapy than typical multi-nodular/fascicular PN. In these cases, plastic surgery may be a treatment option to safely improve appearance and selumetinib treatment may prevent regrowth. Additional prospective evaluations will be needed to determine if structural PN differences influence response to MEKi treatment and to further assess the role of surgery on superficial PN.

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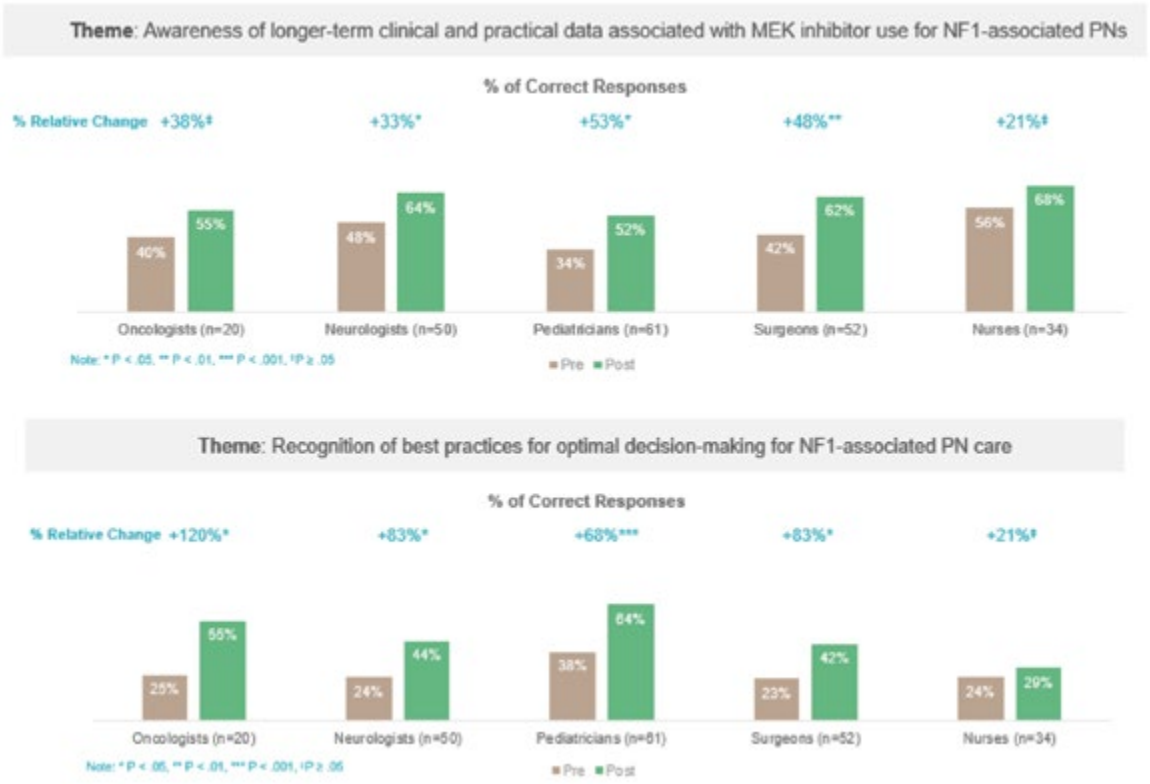
Addressing and Identifying Knowledge Gaps in NF1: The Impact of Online CME/CE on Multidisciplinary Clinician Understanding

Michelle Worst

Purpose: Neurofibromatosis type 1 (NF1) is an uncommon genetic disorder associated with plexiform neurofibromas (PNs). Therapeutic options have historically been limited. However, recent clinical data have shown expanded and improved outcomes with the use of MEK inhibitors. The purpose of this study was to assess the educational impact of a continuing medical education (CME) activity on the multidisciplinary (MDT) clinicians' understanding of ongoing data and application to care.

Methods: The educational intervention consisted of a text-based CME/CE-certified expert interview. Educational impact was measured with a pre-/post-education assessment including multiple-choice knowledge questions. Data from relevant learners who completed pre- and/or post-education assessments were included. Relative changes in percentage of correct responses were used to measure improvement in knowledge. A McNemar test assessed significant levels of changes reported, with P values $< .05$ considered statistically significant. The activity launched Nov. 21, 2024. Data were collected through Feb. 2025.

Results: Improvements were seen after education across clinician groups by learning theme, with statistical improvements observed throughout for neurologists, pediatricians, and surgeons. Nurses did not see significant gains in knowledge.



Conclusions: The study demonstrates the overall success of an online CME activity in enhancing MDT members' understanding of the impact of evolving clinical evidence in the care of patients with NF1-associated PNs. Despite improvements, it also underscores the critical remaining gaps collectively and by specialty. Additional educational activities and analyses are warranted to further clarify the link between education and patient care improvements, particularly as data continue to shape standards of care.

Cytokine Analysis in Children with NF1 and Unresectable Plexiform Neurofibromas Treated with Pegylated Interferon Alfa-2b

Allison Dockman, BS, *Pediatric Oncology Branch, National Cancer Institute*

Purpose: We performed a retrospective analysis of peripheral blood samples collected on a phase II, open-label trial of pegylated interferon alfa-2b (Peg-IFN) in children with neurofibromatosis type 1 (NF1)-associated plexiform neurofibromas (PNs). We analyzed cytokines before and during treatment and assessed the relationship to response.

Methods: Patients (n=82) with inoperable NF1-associated PNs received weekly subcutaneous Peg-IFN injections.[1] Four patients experienced a partial response (PN volume decrease ≥20% on MRI). In children with progressive PN at trial entry, Peg-IFN more than doubled the time to progression (TTP) compared to a historical placebo control group. Concentrations of 36 cytokines were measured in serum using electrochemiluminescence immunoassays (Human V-plex assay, MesoScale Diagnostics) at baseline (n=32) and after 4 cycles of Peg-IFN treatment (n=22), as well as for healthy adult volunteers (n=8). Significance was determined by a nonparametric Wilcoxon test, either paired or unpaired, and P-values were adjusted for multiple comparisons by the Benjamini-Hochberg method.

Results: Compared to healthy adult volunteers, at baseline pediatric patients with NF1 PN demonstrated significantly elevated levels of many cytokines, including IFN-gamma, IL12/IL23p40, IL8, MCP1, MIP1-alpha, MIP1-beta, and TNF-alpha; only IL15 levels were significantly lower in the patient population. After 4 cycles of Peg-IFN, levels of IL12/IL23p40, IL15, MCP1, and IP10 rose significantly, while MIP1-beta and TARC levels fell **(Fig 1)**. An analysis of the association of cytokine changes while on Peg-IFN and response by MRI demonstrated only weak correlations, and additional studies in a larger cohort would be necessary to determine any meaningful significance.

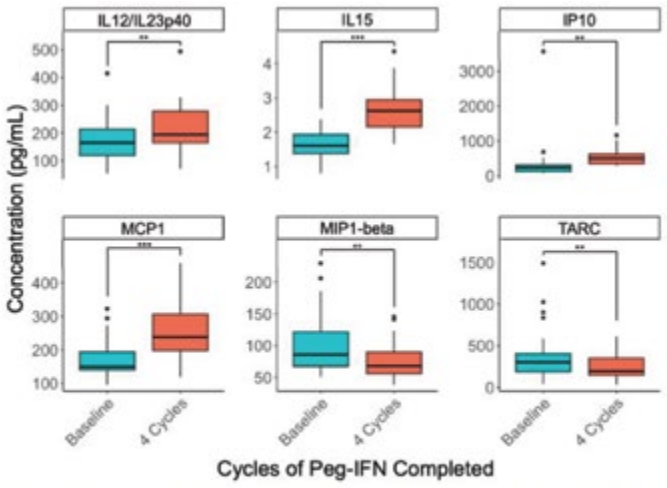


*Fig 1: Cytokine levels at baseline (blue) and after 4 cycles of Peg-IFN treatment (n=22). Mann Whitney U * p<0.05; ** p<0.01; *** p<0.001*

Conclusions: At baseline, children with NF1 PNs had higher levels of several inflammatory markers compared to healthy adult controls. The high levels of IFN-gamma, IL12/IL23p40, and TNF-alpha suggest Th1 cell activity in patients at baseline, indicative of an active cell-mediated immune response to the PNs. Only IL15, which supports NK and CD8 T cell activity, was lower at baseline in this patient population compared to healthy controls. Following treatment with Peg-IFN, increases of IL12/IL23p40, IL15 and IP10 may be suggestive of immune cell activation and migration in response to Peg-IFN. These findings suggest that Peg-IFN has immunomodulatory effects in children with PN that may contribute to the increased TTP observed in the phase II trial. Our findings require validation using a pediatric control group, which we plan to perform in a future study.

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A Rare Case of a Large Cardiac Mass in a Patient with Neurofibromatosis Type 1 (NF1)

Gurcharanjeet Kaur, MD, *Columbia University Irving Medical Center*

Purpose: Neurofibromatosis type I (NF1) is a cancer predisposition with multisystem involvement. Cardiac neurofibromas in the setting of NF1 are extremely rare with sporadic case reports.

Methods: Herein, we present a 15-year-old female with NF1 (*NF1* c.3306delA) who was found to have a large right atrial mass on echocardiogram further confirmed on cardiac MRI as a part of work-up for palpitations that started several years prior. Cardiac examination revealed a regular rate and rhythm. Auscultation revealed a normal S2. There were no clicks, rubs or gallops and the precordium was quiet. There were no murmurs.

Results: A 15 lead EKG demonstrated sinus rhythm with occasional premature atrial complexes (PACs) and a heart rate of 72 bpm. The corrected QT interval was 450 msec. An echocardiogram demonstrated a large echogenic mass in the posterior region of the right atrium near the right AV groove that appeared well circumscribed. There was otherwise normal cardiac anatomy with normal biventricular size and function. These findings were further confirmed with a cardiac MRI revealing a large, non-obstructive mass in the inferior right atrium measuring 6.0 X 3.3 X 3.2 cm. This mass appeared iso to hypointense on T1 weighted images, hyperintense on T2 weighted images with no contrast enhancement. Radiographic differentials included a neurofibroma versus an atrial myxoma. A seven-day cardiac monitor revealed episodes of supraventricular tachycardia (SVT), with max rate 245 beats per minute, coinciding with symptom onset, for which the patient has since been started on Atenolol. The patient's presenting symptom of palpitations is consistent with these episodes of SVT seen on cardiac monitor, which are likely secondary to the presence of this right atrial mass. The patient is now on selumetinib, an oral MEK inhibitor.

Conclusion: The cardiovascular system in NF1 patients has recently received significant attention leading to our knowledge about higher-than-expected frequency of congenital heart defects, however, cardiac masses are a rare occurrence without much literature on the characteristic radiographic findings. This case highlights the need for deeper understanding of the complex molecular pathways that lead to the development of cardiac masses in patients with NF1.

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The Relationship Between Quality of Life and Pain Among Adults on a Clinical Trial of Selumetinib: A Longitudinal Analysis

Staci Martin, PhD, *Pediatric Oncology Branch, National Cancer Institute*

Purpose: Plexiform neurofibromas (PNs) and other physical manifestations of NF1 can result in significant pain, which can negatively affect an individual's quality of life (QoL). An open-label, phase 2 trial of the MEK inhibitor selumetinib demonstrated improvements in pain and health-related QoL in children with NF1 and inoperable PNs (SPRINT, NCT01362803). The NCI phase 2 trial of selumetinib in adult participants also showed reduced pain (NCT02407405), but the QoL results from this study have not yet been reported. The aims of this analysis were to (1) examine QoL and its relationship to pain intensity and pain interference, and (2) describe longitudinal changes in QoL from baseline through pre-cycle 13 (pc13) in adults treated with selumetinib for inoperable NF1 related PNs.

Methods: Participants were administered self-report patient-reported outcome (PRO) measures at baseline and pre-cycles 5, 9, and 13. PROs assessed quality of life (PedsQL NF1 Module; higher=better QOL), target PN pain intensity (Numeric Rating Scale; NRS-11; 0=no tumor pain to 10=worst tumor pain), and the extent to which pain interferes with daily functioning (Pain Interference Index; PII; 0=not at all to 6=completely). Nonparametric statistics were used.

Results: A total of 33 participants (22 males, 75.8% white) had baseline PRO data and 28 had pc13 data. The mean age at baseline was 34.3 years (SD=10.9, range 18.3-60.2) and average years of education was 14.86 years (SD=2.1, range 12-20). Lower (worse) baseline mean PedsQL Fatigue scores were related to higher (worse) baseline NRS-11 PN pain intensity scores ($r=-0.375$, $p=0.03$), and better PedsQL Physical Functioning scores were marginally associated with better NRS-11 pain intensity scores ($r=-0.342$, $p=0.051$). Baseline PII scores were significantly correlated with PedsQL subscales assessing physical functioning, fatigue, paresthesias, social functioning, and emotional functioning (all $ps<0.05$), all in the expected directions. Baseline to pre-cycle 13 improvements were seen on several PedsQL subscales, including Pain, Fatigue, Physical Functioning, Paresthesias, Movement, Social Functioning, Emotional Functioning, and Worry ($ps<0.05$). Many of these changes occurred by pre-cycle 5. No differences were seen on PedsQL subscales assessing appearance concerns, skin sensations, skin irritation, or treatment anxiety from baseline to pc13 (all $ps>0.05$).

Conclusions: This study provides the first in-depth exploration of quality of life among adults with NF1 enrolled on a clinical trial of selumetinib. At baseline, tumor pain intensity and pain interference in daily life were related to aspects of QoL. Improvements with selumetinib were documented as early as pc5 in areas of physical, social, and emotional functioning. These results are largely consistent with results from the SPRINT trial in children.

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Long-Term Hematologic Effects of Selumetinib Treatment in Children with Inoperable Plexiform Neurofibromas

Alan H. Siegel, *Children's National Hospital, Washington, DC*

Purpose: Selumetinib, a mitogen-activated protein kinase (MEK) inhibitor, is the first FDA-approved treatment for children with neurofibromatosis type 1 (NF1)-associated inoperable plexiform neurofibromas. In the phase 1/2 clinical trial of selumetinib for children with inoperable plexiform neurofibromas (SPRINT, NCT01362803), treatment was associated with mild and reversible leukopenia (14%), neutropenia (22%), anemia (36%), and thrombocytopenia (12%). To maintain a durable tumor response, prolonged treatment is necessary; therefore, it is important to understand any potential long-term adverse effects. This study characterizes hematologic changes associated with long-term use of selumetinib in pediatric patients.

Methods: Data was obtained from the study database for the SPRINT trial. Comprehensive blood count (CBC) with differentials were collected per protocol at baseline and before cycles 2, 3, 4, 5, 7, 9, 11, 13, 17, 21, 25, then every 6 cycles (1 cycle = 28 days). Patient enrollment began in 2011 (Phase 1) and 2015 (Phase 2) and data was collected through February 7, 2025 and included all CBCs obtained for the duration of treatment including periods of drug hold. Parameters evaluated included white blood cell count (WBC, k/μL), hemoglobin (Hgb, g/dL), platelets (Plts, k/μL) and absolute neutrophil count (ANC, cells/μL). Simple linear regressions and descriptive statistics were calculated with GraphPad Prism version 10.4.1.

Results: There were 98 participants (59 male, median age at enrollment 10.6 years, range 3-18.5) treated for a median 67.1 cycles per person (range 2.7 to 143.5) with a total 2597 CBCs obtained (median 24 per participant, range 3 to 78). Across all CBCs, the WBC, ANC, Hgb, and Plts were within the normal range 91%, 88%, 77% and 91% of the time, respectively. The minimum values for these cell lines at any timepoint were WBC 2.02 K/uL, ANC 690 cells/μL, Hgb 6.9 g/dL, and Plts 95 k/μL. No participants required a dose reduction or drug hold for a hematologic adverse event. There was no statistically significant change over time for 73% (n=72), 87% (n=85), 59% (n=58) and 74% (n=73) of participants for WBC, ANC, Hgb and Plts, respectively. For those with a statistically significant change over time ($p<0.05$), the median slopes were -0.0266 (range -0.1333 to 0.0624) for WBC, -0.0149 (range -0.0729 to 0.2743) for ANC, 0.0133 (range -0.5793 to 0.0777) for Hgb, and -0.3802 (range -5.352 to 18.03) for Plts.

Conclusion: The data reported here is the longest follow-up to date for pediatric patients with NF1 on selumetinib. No evidence of clinically meaningful changes in hematologic parameters was observed. Though small statistically significant changes were seen in some participants, there was no clear trend (positive or negative) in any parameter. Hgb had the highest proportion of patients with a significant trend (41%) however it was a positive trend and not consistent with previously reported anemia. Notably, in all instances where the minimum values were below normal, the decreases were transient and often associated with intercurrent illness. These results are preliminary and additional analyses are ongoing.

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Application of Mek Inhibitor in Patients with NF

Xu Chen

Introduction: NF is classified into three main types: NF1, NF2, and schwannomatosis. NF1, the most prevalent form, is associated with mutations in the NF1 gene and often presents with cutaneous neurofibromas and plexiform neurofibromas. NF2, caused by mutations in the NF2 gene, typically involves bilateral vestibular schwannomas. Despite advances in surgical techniques, the treatment of NF remains challenging. Recent therapeutic developments have introduced MEK inhibitors as a promising treatment for plexiform neurofibromas in NF1.

Case Presentation: We present 3 patients with NF using Mek inhibitor profiles, 2 NF1 and 1 NF2.

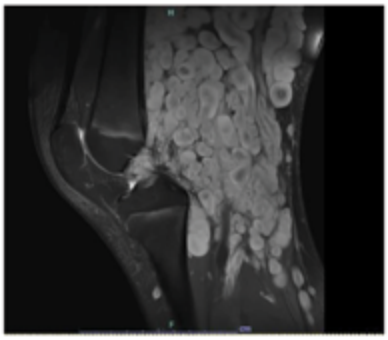
Case 1: 14 year old boy diagnosed with NF1. Four years ago a right popliteal fossa swelling was found, which grew gradually, affecting joint mobility and limping with pain. MRI suggested neurofibromatosis **(Fig. 1)**. After 1 week of treatment with Mek inhibitors, he developed a rash on the face and chest, posterior chest, and a rash on the trunk with pruritus. Dermatology consultation suggested the possibility of facial thrush and the trunk rash was a possible drug rash. After 4 months of continued medication, the tumor began to soften and the pain was reduced.

Case 2: A 10 year old boy was diagnosed with NF1. His mother had a history of NF1. 3 years ago the child presented with a subcutaneous mass on the right side of the neck, which gradually increased in size and was often associated with pain. MRI suggested plexiform neurofibromatosis **(Fig. 2)**. After 4 months of treatment with Mek inhibitor, the texture of the right neck mass became softer. The pain disappeared.

Case 3: A 19-year-old male underwent 3 surgeries for intracranial multiple meningiomas and intravertebral ventricular meningiomas. Molecular testing suggested NF2 p.Tyr150Ter mutation, and MRI showed multiple enhancing nodules in the bilateral internal auditory canal and Meckel's cavity, which led to the diagnosis of NF 2 **(Fig. 3)**. The patient voluntarily requested to take Mek inhibitor, and after 8 months of medication, the patient complained of hearing improvement without other side effects or complications, and is still under further follow-up.

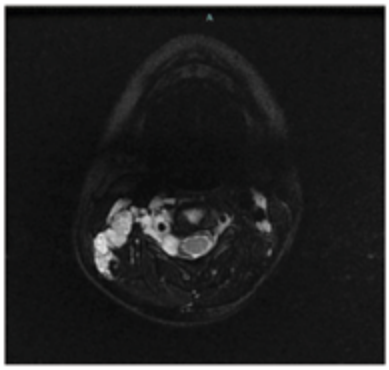
These inhibitors have shown efficacy in reducing tumor volume and improving patient outcomes. However, the long-term efficacy and safety of MEK inhibitors in NF1 patients remain under investigation. Additionally, the potential application of MEK inhibitors in NF2 is still unexplored, warranting further research to determine their effectiveness in this context. This highlights the need for continued investigation into targeted therapies to improve the management of NF.

Fig.1



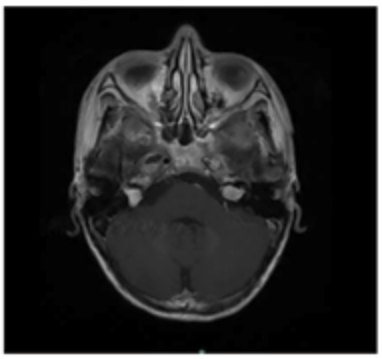
MRI of Case 1: right knee joint subcutaneous and soft tissues multiple abnormal enhancement foci, combined with the history of the disease, consider neurofibromatosis.

Fig.2



MRI of Case 2 showed multiple irregular nodules on the right side of the cervical musculature, under the sternocleidomastoid muscle, and in the right axilla. The tumor extends along the intervertebral foramina into the spinal canal, the right intervertebral space is enlarged. Combined with the medical and family history, plexiform neurofibromatosis was considered.

Fig.3



MRI of Case 3 showed multiple enhancing nodules in the bilateral internal auditory canal and Meckel's cavity, which led to the diagnosis of neurofibromatosis type 2 (NF2).

Incidental Vascular Aneurysm in Two Patients Treated with a MEK Inhibitor for Inoperable Plexiform Neurofibromas

Cecilia Tibery, PA-C, *Clinical Research Directorate (CRD), Frederick National Laboratory for Cancer Research*

Introduction: People with neurofibromatosis Type 1 (NF1) and inoperable symptomatic plexiform neurofibromas (PN), may benefit from treatment with a MEK inhibitor (MEKi). An increased risk for vascular anomalies such as arteriovenous fistulas and arterial aneurysms has been described in NF1[1], however to our knowledge these have not been reported as new findings in patients on MEKi. Here we report on 2 patients with NF1 and PN who developed aneurysms while on selumetinib.

Methods: We performed a retrospective review of two NF1 patients who developed aneurysms during treatment with selumetinib on a single arm phase 2 trial (NCT02407405). Relevant patient information, including prior history of vascular anomalies, medical/surgical history, selumetinib treatment history, selected adverse events including hypertension, and interventions were extracted from the electronic medical records. Selumetinib was administered on a continuous dosing schedule at 50 mg twice daily (1 cycle=28 days).

Results: Patient 1, a 30 year old with a left head and neck PN, had new neck swelling during his restaging evaluation during cycle 31 (~2.3 years) of selumetinib. The restaging non-contrast MRI of his PN showed an internal left carotid aneurysm (1.5x1.6 cm) that was not present on MRI 6 months prior. He underwent vascular stent placement approximately 1 week after the aneurysm was discovered. Past medical history was significant for radiation therapy for a pilocytic astrocytoma at 6 years old and for a probable high grade glioma in the left cerebellar vermis at 25 years old. Patient 2, a 41 year old with a left neck and chest PN, had a restaging non-contrast MRI during cycle 67 (~5.1 years) of treatment that identified a new aymptomatic left vertebral artery aneurysm (2.5x2.9 cm) with an arteriovenous fistula that was not seen on the restaging MRI 6 months prior. He underwent a coil embolization 1 week after diagnosis. Past medical history was significant for hypertension. Both patients recovered uneventfully.

Conclusions: People with NF1 are known to be at increased risk for developing vascular anomalies such as aneurysms[1]. To our knowledge, this is the first report of the development of aneuryms in NF1 patients receiving a MEKi for inoperable PN. Both aneurysms were identified on non-contrast MRI as part of a research protocol, highlighting the need for careful review of any imaging obtained for these patients. While the aneurysms developed on treatment with selumetinib, both patients had known risk factors such as prior radiation therapy or hypertension, therefore a relationship between selumetinib and aneurysm was considered unlikely by the study team. However, given the increasing use of MEKi for people with NF1, careful monitoring for the development of vascular anomalies will be important and provide additional information regarding any potential relationship.

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Preliminary Phase 1 Outcomes of Next Generation MEK1/2 Inhibitor PAS-004 in Adults with Inoperable Plexiform Neurofibromas

Rebecca Brown, MD, PhD, *The University of Alabama at Birmingham*

Purpose: Existing FDA-approved mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) ((MEK)) inhibitor pharmacotherapies for the treatment of plexiform neurofibromas (PNs) in patients with neurofibromatosis type 1 (NF1) can be effective, but compliance at a therapeutic dose is limited by an adverse event profile including gastrointestinal, dermatologic, musculoskeletal, renal, cardiac, ophthalmologic, and constitutional symptoms. PAS-004 is a next-generation multicyclic MEK 1/2 inhibitor with highly stable and predictable serum pharmacokinetics (PK) that can avoid undesirable toxic-range peaks while engaging and inhibiting MEK.

Methods: We will discuss the design, PK/pharmacodynamics (PD) and early safety/efficacy results from the multi-institutional international Phase 1/1b open label clinical trial of PAS-004 on inoperable PNs in adults ≥ 18 years of age. The Phase 1 dose-finding portion of the trial (n ≤24) follows a modified 3+3 design to determine 2 doses at or below the maximum tolerated dose (MTD) to test in the Phase 1b safety expansion arm (n ≤24). Efficacy endpoints include PN volumetric measures, adverse events (AEs), and quality of life (QOL) metrics as per Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) guidance. Exploratory endpoints will include PK and PD, size, and transcriptional, immunohistochemical, and proteomic outcomes from cutaneous neurofibromas.

Results: Low rates of toxicity were observed for PAS-004 with notably no dermatological, or other major organ AEs.

Conclusion: Early results confirm attainment of PK and PD goals in serum and low toxicity at potentially therapeutic doses.

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Disclosures: Dr. Brown is a paid scientific advisor for Pasithea Therapeutics; Dr. Athar has been contracted by Pasithea Therapeutics to perform molecular exploratory assays and biobanking

Funding: Pasithea Therapeutics

Illness Experience, Treatment, and Quality of Life in Chinese Patients with Neurofibromatosis Type 1: Insights from a National Patient Survey

Linguo Li, *Senior Researcher, Chinese Organization for Rare Diseases*

Purpose: To characterize diagnostic pathways, treatment patterns, and quality of life in Chinese NF1 patients, utilizing the largest patient-led dataset to date.

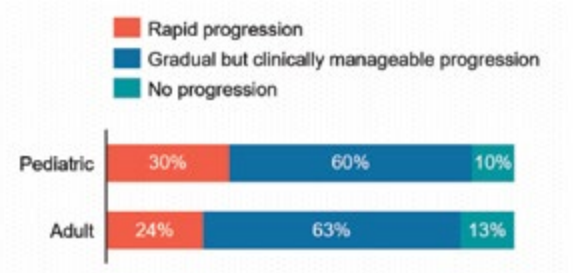
Methods: A mixed-methods approach was employed, including an online survey of 451 NF1 patients and interviews with 7 patients/caregivers and 4 clinical experts, followed by data analysis on clinical symptoms, treatment utilization, and quality of life measured by EQ-5D.

Results: Among Chinese NF1 patients, CALMs are nearly universal (94% children, 91% adults). Plexiform neurofibromas (PNs) are reported by 58% of pediatric and 42% of adult patients. Skeletal abnormalities, mainly scoliosis, affected 25% of patients.

The average time from symptom onset to consultation is 4.3 years (children) and 10 (adults), with one-third misdiagnosed. Disease progression over the past three years occurred in 90% of pediatric and 87% of adult PN patients, with ~30% experiencing rapid progression.

Figure 1: ~90% patients reported progression of PN over the past three years

% of patients by progression of PN



Surgical resection remains the dominant treatment among adult PN patients, with 36% having undergone two or more surgeries and a recurrence rate of 58–64%. In contrast, 60% of pediatric PN patients have received MEK inhibitors, primarily selumetinib, although access is limited for adults due to regulatory and financial barriers.

Quality of life is significantly impaired (VAS: 67.7 children, 61.2 adults), with pain, anxiety, and discrimination prevalent.

Conclusion: Our findings provide a comprehensive profile of NF1 phenotypes and treatment patterns in China, highlighting diagnostic bottlenecks, age-dependent treatment disparities, and a severe impact on quality of life. These results can inform future studies on MEK inhibitor efficacy, natural history modeling, and cross-regional care harmonization.

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Funding: This study was initiated and funded by the Neurofibromatosis Shenzhen Care Center (NFCC), with project support from the Chinese Organization for Rare Diseases (CORD). Additional funding was provided by Fosun Pharma.

Figure 2: PN surgery is often deferred in children due to risk, and in adults due to cost

% of PN patients by reported reasons for not receiving surgical treatment

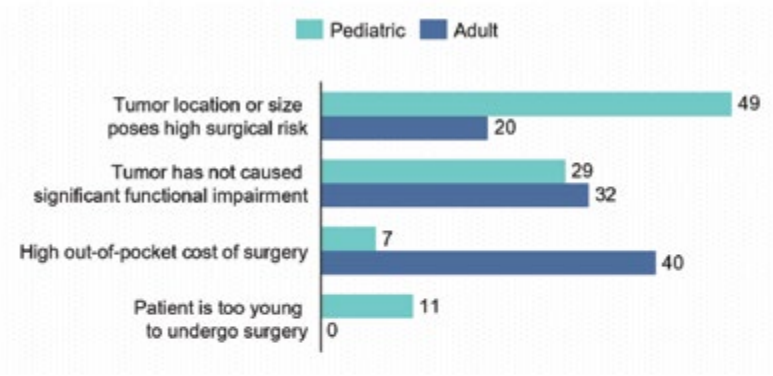
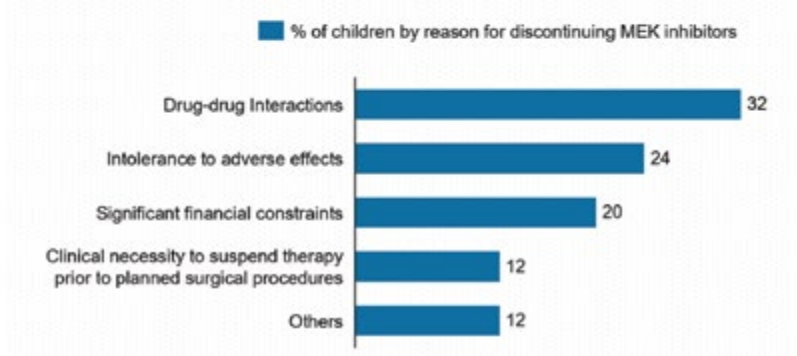


Figure 3: Side effects and drug interactions are the main reasons children discontinue MEK inhibitors



Selumetinib for Symptomatic Inoperable Plexiforms of the Foot in Children

Abinaya Seenivasan, BA, DCH, DNB, MRCPCH, Trainee, *Royal Manchester Children's Hospital, Manchester Foundation Trust, UK*

Purpose: Plexiforms of the foot can be extremely painful, lead to loss of mobility and cause dystrophic changes in nearby bone. Surgery for debulking is ineffective due to recurrence, negative effect on pain and secondary joint issues. Patients rely on pain medication and mobility aids with some considering limb amputation.

In 2009 NHS England commissioned two Highly Specialised Services (HSS) for complex Neurofibromatosis (NF) based in Manchester and London. In May 2022, NICE approved the use of Selumetinib for symptomatic inoperable plexiform neurofibromas in children aged 3 years and over in England. We describe favourable outcomes in three children with painful plexiforms of the sole of the foot who were started on Selumetinib.

Methods: We identified three children from the Manchester HSS service with plexiform NF of the sole of the foot. These were approved for the use of Selumetinib as per National pathway (2023 - 2025) for England. The main indicator for treatment and approval was pain.

Case Series: Three children aged 11, 12, and 15 years had extensive lower limb plexiform neurofibromas causing pain, deformity, and mobility issues. Their average pain scale was 6-8 on the faces scale. Of the three children, one needed a Taxi to go to school, one started using an electric wheelchair and the third was only able to walk with a crutch. He had debulking surgery a couple of years earlier but had not improved his pain and ended up with regrowth of plexiform. The team including the patient were keen to explore the option of amputation. All had pain on ambulation which was unresponsive to medication and surgery was not felt to be an option after extensive complex NF1 multi-disciplinary team (MDT) discussions and were started on Selumetinib. Follow-up at 6 months, 5 months (2 patients) and 2 years showed complete pain resolution in all three patients with significant lesion reduction clinically and radiologically in the patient who has been on for two years. All are now normally ambulant.

Conclusion: Surgery for painful plexiform neurofibromas of the foot is not typically successful and may be detrimental to overall function not only because of the potential for regrowth but also because of secondary scarring and fibrosis which are often painful in their own right. We have demonstrated the successful use of MEK inhibitors (Selumetinib) in this group of children which suggests that it should be considered earlier rather than later.

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COGNITION, BEHAVIOR AND LEARNING

Specifying a New Treatment Theory of Rehabilitation of Functional Neurological Disorder Symptoms Within a Neurofibromatosis Type 1 Paediatric Patient, Using the Rehabilitation Treatment Specification System

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Purpose: Functional Neurological Disorders (FND) are one of the most common diagnoses made in Neurology clinics[1]. FND refers to symptoms of altered motor or sensory function with no organic cause, resulting in symptoms such as limb weakness, numbness, pain, and seizures[2]. Recent studies have shown that the incidence of FND within paediatrics is estimated at 1.3 to 6.0 per 100,000, and 10% of all FND diagnosis are made alongside a pre-existing neurological condition[2]. Whilst consensus on how to rehabilitate adults with FND has been documented[3], there is no guidance on how to rehabilitate paediatric patients with additional conditions such as Neurofibromatosis Type 1 (NF1).

Designing, testing and implementation of nascent treatment approaches are often blurred by insufficient reporting of treatment theory[4], that is, how active ingredients of a treatment lead to change in aspects of a patients' function[5]. The rehabilitation treatment specification system (RTSS) provides a countermeasure to this by challenging the clinician to specify treatment theory through its common language and tripartite structure[6]. We sought to utilise the RTSS to aid reasoning and specification of the treatment theory of a rehabilitation programme for a paediatric NF1 patient living with FND.

Methods: Case report presentation of the physical therapy rehabilitation of a 17-year-old female with NF1. She presented with medical complications including an extensive optic pathway glioma treated with chemotherapy, shunted hydrocephalus and post-operative right-sided cerebral haemorrhage. Her presenting issues included a five-year history of right sided functional weakness resulting in wheelchair mobility, with a background of anorexia and an unexpected family bereavement. The RTSS was applied a priori to reason and specify treatment theory of the rehabilitation programme.

Results: Treatment components included: improving muscular endurance via hypertrophic change and increasing muscle unit activation; and improving selective trunk control by using distraction ingredients to override functional pathways and aid in neuroplastic change. Distraction ingredients included therapeutic finger painting, popping bubbles and catching objects out of her base of support. All task ingredients encouraged trunk flexion, side-flexion and rotation; tasks which she was unable to perform without distraction ingredients. Over several sessions she was able to progress through her ingredient's difficulty, leading to increased postural control. Volitional components encouraged independent performance of mindfulness.

Conclusion: This case report demonstrates a specified rehabilitation for FND in an NF1 paediatric patient, through use of the RTSS, a reasoned rehabilitation intervention for this population is available for replication, modification and testing.

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Social Cognition in Individuals with Neurofibromatosis Type 1: A Systematic Review

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Objective: The purpose of this article was to conduct a systematic review of the literature pertaining social cognition in patients with Neurofibromatosis type 1 (NF1). We analyzed social cognition (SC) which encompasses a wide set of neurobiological and social processes that enable individuals to perceive, identify, and assess social events. We selected articles that evaluated empathy, social emotions, theory of mind and moral judgement. All of these, are fundamental to understand the mechanisms that underlie social functioning and has often been found to be altered in NF1 patients.

Method: We conducted a systematic review of the literature. Articles were found using two electronic databases (Scopus and PubMed) and manual searching.

The research question that guided this systematic review was addressed using a PIO (Population, Control, and Outcome) structure. We thoroughly applied criteria that allowed us to select studies which evaluated our selected outcomes in NF1 population. These included patients with NF1 and no other genetic diagnosis, the use of a control group to compare SC performance and the use of objective measurements for results.

Results: When compared with unaffected controls, individuals with NF1 (children and adults) exhibit SC dysfunction in ToM, empathetic pain perception, and social emotion recognition. No articles evaluated moral judgment.

Conclusion: Further studies are needed, and they must evaluate specific SC domains using appropriate validated tests, and assessing changes in SC in patients as they age.

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Nighttime Challenges, Daytime Consequences: Sleep and Cognition in NF1

Emily J Carlson, PhD, *Children's National Hospital*

Purpose: Neurofibromatosis Type 1 (NF1) is associated with cognitive challenges and sleep concerns. A recent NF1 caregiver survey conducted by this group identified high levels of sleep disturbance (e.g. difficulty falling asleep) and impairment (e.g., problems caused by disrupted sleep) among children with NF1, with greater sleep concerns endorsed for children with comorbid attention-deficit/hyperactivity disorder. This study builds on these findings by examining the relationship between sleep difficulties and cognitive functioning in children with NF1 using performance-based cognitive tests and norm-based parent questionnaires.

Methods: Thirty-two children with NF1 (ages 6-16) completed a cognitive battery evaluating attention, executive functioning (EF), and learning and memory. Measures included working memory subtests from the Wechsler Intelligence Scales, the California Verbal Learning Test (CVLT), and subtests from the NEPSY, second edition (NEPSY-II). Caregiver ratings of attention, hyperactivity, and EF were obtained using the Behavior Rating Inventory of Executive Function, Second Edition (BRIEF-2) and the Behavior Assessment System for Children, Third Edition (BASC-3). Sleep disturbance and sleep impairment were measured using parent-proxy PROMIS measures. Additional sleep factors were assessed using the Child and Adolescent Sleep Checklist (CASC). Data were analyzed using Pearson/Spearman correlations and group comparisons (t-tests and Mann-Whitney U tests).

Results: Participants (M_{age}=10.28, *SD*=2.85) reported an average of 8.78 hours of sleep on weeknights (*SD*=1.5); 41% (n=13) had sleep disturbance and 38% (n=12) had sleep impairment. On average, participants with sleep disturbance and impairment had 1-1.5 less hours of sleep/night than those without. Sleep disturbance was negatively correlated with attention, EF, and both verbal and visual learning and memory performance. Sleep impairment was negatively correlated with all of these factors except for verbal learning/memory. Sleep disturbance and impairment were associated with caregiver expressed attention and EF concerns. Group comparisons revealed that participants with sleep disturbance and impairment exhibited more pronounced attention, EF, and visual learning problems than those without. Additionally, individuals with sleep impairment specifically demonstrated greater memory difficulties.

Table 1. Test statistics and significance levels for correlational and group comparison analyses

		Correlations		Group Comparisons	
Domain	Variable	Sleep Disturbance	Sleep Impairment	Sleep Disturbance	Sleep Impairment
Attention	CVLT Trial 1 Learning	***r* = -.35 (*p* = .026)**	*r* = -.22 (*p* =.120)	***t* = -1.96 (*p* = .030)**	***t* = -1.77 (*p* = .043)**
	Digit Span Forward	***r* = -.36 (*p* = .024)**	***r* = -.34 (*p* = .031)**	z = 0.91(*p* = .183)	**z = 1.93 (*p* =.027)**
	BASC Attention Problems	***r* = .60 (*p* < .001)**	***r* = .50 (*p* = .002)**	**z = -2.73 (*p* = .003)**	***t* = 3.04 (*p* = .003)**
Executive Functioning	NEPSY-II Animal Sorting	*r* = -.25 (*p* = .11)	***r* = -.39 (*p* = .019)**	*t* = -0.42 (*p* = .340)	***t* = -1.80 (*p* = .042)**
	Picture Span	***r* = -.37 (*p* = .020)**	***r* = -.34 (*p* = .028)**	*t* = -1.22 (*p* = .117)	*t* = -1.27 (*p* = .107)
	BRIEF Global Executive Composite	***r* = .62 (*p* < .001)**	***r* = .58 (*p* < .001)**	**z = -2.92 (*p* = .002)**	***t* = 3.31 (*p* = .001)**
Learning and Memory	CVLT Total Learning	***r* = -45 (*p* = .006)**	*r* = -.30 (*p* = .054)	*t* = -1.57 (*p* = .064)	*t* = -1.65 (*p* = .055)
	CVLT Long Delay Free Recall	***r* = -.31 (*p* = .045)**	*r* = -.13 (*p* = .249)	z = 1.02 (*p* = .155)	z = 0.97 (*p* =.167)
	Memory for Designs	***r* = -.32 (*p* = .042)**	***r* = -.44 (*p* = .008)**	*t* = -0.52(*p* = .304)	***t* = -2.20 (*p* = .018)**
	Memory for Designs Delayed	***r* = -.34 (*p* = .038)**	***r* = -.50 (*p* = .003)**	z = 0.36 (*p* =.359)	**z = 1.79 (*p* = .037)**

Note. Parametric and non-parametric tests were used. Variables with non-significant results are not listed to maintain focus on key findings. Significant results are bolded.

Conclusion: This study provides further evidence for the presence of sleep challenges in NF1. While children with NF1 are generally at risk for cognitive challenges, current results indicate that those with comorbid sleep problems are at even greater risk for problems with attention, EF, and learning and memory. Clinicians should monitor NF1 patients for sleep problems and be prepared to provide recommendations regarding sleep hygiene and interventions. Researchers investigating treatments aimed at improving sleep among NF1 patients should examine potential cognitive improvements associated with better sleep.

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Neurological Manifestations in Neurofibromatosis Type 1: Experience of a Tertiary Center with a Cohort of 203 Argentine Children

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Introduction: NF1 is an autosomal dominant genetic disorder with expression variability, it is due to mutation in the neurofibromin gene. It can be associated with epilepsy, neurodevelopmental disorders and cerebrovascular disease.

Objectives: To describe the most prevalent neurological manifestations in a series of pediatric patients with NF1 seen at Hospital Garrahan in Argentina in the last 10 years.

Population And Methods: We performed a descriptive, retrospective analysis of the medical records of patients with clinical criteria for NF1 with an age range of zero to 15 years, evaluated by neurology at Hospital Garrahan in the last 10 years.

Results: We registered 203 patients, 99 females with a mean age of 3 years. 133 patients reported neurodevelopmental disorders with learning difficulties in 54%, psychopedagogy was the most indicated therapy (85%). 22 (11.3%) presented focal and generalized epilepsy not always associated with structural lesions, 12 of them (54%) with pathological electroencephalogram. The most indicated drug was clobazam. 83 patients 22 (11.3%) presented focal and generalized epilepsy not always associated with structural lesions, 12 of them (54%) with pathological electroencephalogram. The most indicated drug was clobazam.83 patients (50%) had hamartomatous lesions in the cerebellum, 2 patients reported Moya Moya syndrome.

Conclusion: Our study shows that neurodevelopmental disorders are frequent in this population, among them the most diagnosed was learning disorder. Epilepsy is more common than in the general population, and focal seizures are more frequent, requiring prolonged antiepileptic drugs but with good control. In agreement with previous series, bright lesions in cerebellum were frequently observed.

Academic Achievement of Children and Adolescents with Neurofibromatosis Type 1: A Systematic Review and Meta-Analysis

Liyan Yu, PhD, *Florida State University*

Purpose: Children/adolescents with Neurofibromatosis type 1 (NF1) have higher risk for academic difficulties, compared to typically developing peers. However, findings regarding group differences in academic achievement between individuals with and without NF1 are inconsistent, potentially due to variations in study and sample characteristics. This meta-analysis aims to estimate of academic disparities between children/adolescents with and without NF1 and examine potential moderators of variations in group disparities.

Methods: This study is part of a series of meta-analyses on the neurobehavioral functions of individuals with NF1. Literature searches were conducted in Scopus, PsycINFO, Web of Science, PubMed, and ProQuest with NF1-related and neurobehavioral functioning terms (e.g., reading and writing) on March 26, 2024. Thirty-eight studies, involving 2,141 individuals with NF1 (ages 3.35–15.5), met inclusion criteria. Group differences (Hedges' g) were synthesized using robust standard error estimation and random effect models. Meta-regression was employed to examine potential moderators.

Results: Children/adolescents with NF1 exhibited significantly poorer performance in reading ($n = 38$, $k = 79$, $g = -0.82$, 95% CI [-0.96, -0.68], $p < .001$), writing ($n = 24$, $k = 31$, $g = -0.79$, 95% CI [-0.93, -0.64], $p < .001$), and math ($n = 24$, $k = 31$, $g = -0.77$, 95% CI [-0.90, -0.63], $p < .001$; **Table 1**), with medium-to-large effect sizes even after adjusting for publication bias ($g = -0.80$ to -0.52, **Table 2**). Considerable heterogeneity was observed ($I^2 =$ 71.29%–72.68%), partially explained by study and sample characteristics (**Table 3**). Specifically, greater deficits were obsevered in pseudoword reading than reading fluency ($\beta = 0.70$, $p = .025$). Lower full-scale IQ ($\beta = 0.04$, $p = .007$), verbal IQ ($\beta = 0.07$, $p < .001$), and performance IQ ($\beta = 0.03$, $p = .025$) were associated with larger group differences in writing but not in reading or math. Greater reading deficits were found when NF1 groups were compared with unaffected siblings than normative data ($\beta = 0.41$, $p = .001$), and greater writing deficits emerged when compared with siblings versus community controls ($\beta = 0.47$, $p = .017$). Sex, NF1 inheritance, ADHD, sampling methods, and measures were not significant moderators.

Conclusion: Findings highlight the need for targeted academic support and interventions for children/adolescents with NF1, especially in pseudoword reading and for those with low IQs. The substantial heterogeneity underscores the importance of using individual-level data with large sample sizes to examine predictors of academic outcomes in NF1.

Table 1. Summary of Mean Effect Size across Studies

	Hedge g	LL	UL	SE	df	p-value	n	k	Tau^2	I^2 (%)
Reading Achievement										
Overall Reading achievement	-0.82	-0.96	-0.68	0.07	35.46	<.001	38	79	0.13	71.29
Reading subskills										
Letter reading	-0.99	-1.51	-0.47	0.23	8.93	.002	10	10	0.42	83.83
Pseudoword reading	-1.43	-1.98	-0.89	0.23	6.93	<.001	8	9	0.30	79.28
Reading fluency	-0.71	-1.08	-0.33	0.15	5.83	.004	7	12	0.13	70.24
Word reading	-0.96	-1.24	-0.67	0.13	12.61	<.001	14	18	0.16	75.19
Reading comprehension	-0.89	-1.17	-0.61	0.13	11.67	<.001	13	15	0.13	62.55
Control group types										
Community healthy controls	-0.89	-1.44	-0.34	0.23	6.95	.007	8	19	0.38	83.34
Unaffected siblings as controls	-1.11	-1.28	-0.94	0.07	6.10	<.001	10	17	0.00	0.00
Normative data as controls	-0.69	-0.85	-0.53	0.08	18.69	<.001	21	43	0.09	66.89
Sampling method										
Recruited from clinic center	-0.76	-0.94	-0.59	0.08	21.25	<.001	23	41	0.13	72.04
Recruited from community	-1.05	-1.47	-0.63	0.19	8.95	<.001	10	29	0.29	72.59
Measures										
Wechsler Individual Achievement Test	-0.91	-1.15	-0.67	0.11	9.85	<.001	12	16	0.07	59.19
Woodcock-Johnson Achievement Test	-0.87	-1.45	-0.29	0.25	7.90	.009	9	14	0.37	83.55
Alouette-R Reading Test	-0.60	-0.86	-0.34	0.08	2.92	.005	4	7	0.03	45.25
Wide Range Achievement Test	-0.64	-1.06	-0.22	0.11	2.34	.020	5	5	0.01	7.17
Writing Achievement										
Writing achievement overall	-0.79	-0.93	-0.64	0.07	21.23	<.001	24	31	0.09	72.68
Writing subskills										
Spelling	-0.85	-1.01	-0.70	0.07	16.09	<.001	19	20	0.05	50.05
Written expression	-0.62	-0.99	-0.25	0.12	3.41	.011	5	8	0.06	68.01
Control group types										
Community healthy controls	-0.53	-1.00	-0.06	0.13	2.34	.039	4	4	0.03	46.78
Unaffected siblings as controls	-0.98	-1.17	-0.80	0.07	4.48	<.001	7	10	0.00	0.00
Normative data as controls	-0.78	-0.99	-0.58	0.09	11.67	<.001	14	17	0.08	65.84
Sampling method										
Recruited from clinic center	-0.85	-1.00	-0.70	0.07	12.30	<.001	16	16	0.02	28.16
Recruited from community	-0.64	-1.22	-0.05	0.16	2.43	.042	4	8	0.06	53.86
Measures										
Wechsler Individual Achievement Test	-0.91	-1.12	-0.70	0.09	8.65	<.001	11	12	0.04	47.32
Wide Range Achievement Test	-0.87	-1.22	-0.51	0.13	3.89	.003	6	6	0.03	28.99
Math Achievement										
Math achievement overall	-0.77	-0.90	-0.63	0.06	21.06	<.001	24	31	0.09	71.70
Control group types										
Unaffected siblings as controls	-0.91	-1.08	-0.73	0.07	5.38	<.001	9	12	0.00	0.00
Normative data as controls	-0.74	-0.91	-0.57	0.08	10.76	<.001	14	15	0.04	48.81
Sampling method										
Recruited from clinic center	-0.74	-0.84	-0.64	0.04	9.50	<.001	15	18	0.00	1.29
Recruited from community	-0.86	-1.34	-0.39	0.18	4.79	.006	6	9	0.19	77.16
Measures										
Wechsler Individual Achievement Test	-0.80	-1.03	-0.57	0.10	6.80	<.001	9	11	0.04	49.16
Woodcock-Johnson Achievement Test	-0.90	-1.46	-0.33	0.20	3.81	.012	5	7	0.13	63.52
Wide Range Achievement Test	-0.81	-1.23	-0.39	0.16	4.51	.005	6	6	0.07	50.53

Notes. LL = lower limit of 95% confidence interval; *UL* = upper limit of 95% confidence interval; *SE* = standard error; *df* = degrees of freedom; *n* = number of studies; *k* = number of effect sizes; Tau^2 = Tau-square; I^2 = I-squared.

Results were reported when there were at least four samples in each subgroup.

Table 2. Publication Bias Tests Results

	Funnel Plot Symmetry Test		Trim and Fill Analysis						Robu Meta-Regression				
	Egger's Test												
	Z	*p*	*N* of trimmed studies	Filled *N*	Fill *d* ES	*p*	95% CI	*t*	*β*	*SE*	*p*	*n*	*k*
Reading Achievement	-1.31	.189	39	1	-0.80	< .001	[-0.95, -0.63]	-10.04	-1.32	1.02	.215	38	79
Writing Achievement	-0.40	.693	34	10	-0.54	<.001	[-0.70, -0.37]	-6.53	-0.30	0.77	.713	24	31
Math Achievement	-1.03	.304	34	10	-0.52	<.001	[-0.69, -0.34]	-6.04	-0.64	0.93	.507	24	31

Note. Z = standard normal distribution score; *p* = probability value; filled N = number of filled studies; filled ES = effect size after filling the hypothetical unpublished studies; *t* = t-value; CI = confidence interval; *B* = estimated regression coefficient; *SE* = standard error; *n* = number of studies; *k* = number of effect sizes.

Table 3. Meta-regression Results

Moderator	*n*	*k*	*β*	*SE*	*LL*	*UL*	*df*	*p*-value
Reading vs. Writing	40	141	0.04	0.11	-0.18	0.26	27.84	.700
Reading vs. Math	40	141	0.06	0.12	-0.18	0.30	24.61	.618
Writing vs. Math	40	141	0.02	0.07	-0.12	0.15	22.40	.807
Reading Achievement								
Reading subskills:								
Letter (vs. pseudoword)	**38**	**79**	**-0.67**	**0.30**	**-1.36**	**0.01**	**9.61**	**.052**
Letter (vs. fluency)	38	79	0.02	0.26	-0.56	0.60	9.41	.935
Letter (vs. word)	38	79	-0.28	0.25	-0.82	0.26	12.55	.285
Letter (vs. comprehension)	38	79	-0.16	0.24	-0.69	0.36	12.22	.516
Pseudoword (vs. fluency)	**38**	**79**	**0.70**	**0.26**	**0.11**	**1.28**	**8.34**	**.025**
Pseudoword (vs. word)	38	79	0.40	0.21	-0.09	0.88	8.12	.095
Pseudoword (vs. comprehension)	38	79	0.51	0.27	-0.08	1.11	10.01	.084
Fluency (vs. word)	38	79	-0.30	0.15	-0.64	0.04	8.07	.073
Fluency (vs. comprehension)	38	79	-0.18	0.16	-0.54	0.17	9.10	.275
Word (vs. comprehension)	38	79	0.12	0.16	-0.21	0.45	16.13	.462
Mean age	34	75	-0.04	0.04	-0.14	0.07	5.29	.396
% Girl	32	69	0.00	0.01	-0.02	0.02	8.51	.783
% familial NF1	19	41	0.00	0.01	-0.02	0.02	2.62	.638
IQ:								
Mean full-scale IQ	37	76	0.02	0.01	-0.01	0.05	15.11	.112
Mean verbal IQ	33	69	0.04	0.02	0.00	0.08	11.12	.078
Mean performance IQ	32	68	0.01	0.01	-0.03	0.04	11.01	.684
% ADHD diagnosis	19	40	0.00	0.01	-0.01	0.02	5.90	.529
Sampling method:								
Recruited from clinic center (vs. community)	38	79	-0.28	0.20	-0.70	0.14	15.45	.182
Control group type:								
Community (vs. siblings)	38	79	-0.25	0.24	-0.77	0.26	13.73	.310
Community (vs. normative data)	38	79	0.16	0.24	-0.38	0.69	11.13	.536
Siblings (vs. normative data)	**38**	**79**	**0.41**	**0.10**	**0.19**	**0.63**	**15.32**	**.001**
Measures:								
WIAT (vs. WJAT)	38	79	0.14	0.24	-0.37	0.66	14.77	.562
WIAT (vs. ARRT)	38	79	0.28	0.12	-0.03	0.60	4.39	.069
WIAT (vs. WRAT)	38	79	0.15	0.15	-0.21	0.51	6.15	.347
WJAT (vs. ARRT)	38	79	0.14	0.24	-0.48	0.75	4.91	.583
WJAT (vs. WRAT)	38	79	0.01	0.26	-0.61	0.62	6.67	.975
ARRT (vs. WRAT)	38	79	-0.13	0.12	-0.43	0.17	6.20	.324
Writing Achievement								
Writing subskills:								
Spelling (vs. written expression)	24	31	0.24	0.17	-0.19	0.67	4.87	.214
Mean age	20	27	0.05	0.04	-0.06	0.17	3.09	.240
% Girl	18	25	0.00	0.01	-0.03	0.02	3.71	.772
% familial NF1	13	20	0.01	0.02	-0.04	0.07	3.80	.471
IQ:								
Mean full-scale IQ	**22**	**26**	**0.04**	**0.01**	**0.02**	**0.06**	**5.37**	**.007**
Mean verbal IQ	**21**	**28**	**0.07**	**0.01**	**0.05**	**0.09**	**5.30**	**<.001**
Mean performance IQ	**20**	**27**	**0.03**	**0.01**	**0.01**	**0.05**	**5.81**	**.025**
% ADHD diagnosis	12	16	-0.01	0.00	-0.02	0.01	2.71	.180
Sampling method:								
Recruited from clinic center (vs. community)	24	31	0.18	0.17	-0.30	0.67	4.05	.352
Control group type:								
Community (vs. siblings)	**24**	**31**	**-0.47**	**0.14**	**-0.81**	**-0.12**	**5.21**	**.017**
Community (vs. normative data)	24	31	-0.29	0.15	-0.70	0.13	3.81	.128
Siblings (vs. normative data)	24	31	0.18	0.12	-0.08	0.45	10.06	.152
Measures:								
WIAT (vs. WRAT)	24	31	0.05	0.17	-0.37	0.46	6.32	.790
Math Achievement								
Mean age	**20**	**27**	**0.10**	**0.02**	**0.03**	**0.18**	**2.41**	**.022**
% Girl	18	23	0.01	0.01	-0.02	0.03	3.73	.406
% familial NF1	9	11	0.00	0.02	-0.07	0.07	2.75	.854
IQ:								
Mean full-scale IQ	23	30	0.02	0.02	-0.03	0.06	4.37	.387
Mean verbal IQ	21	26	0.03	0.02	-0.01	0.06	6.21	.128
Mean performance IQ	20	25	0.01	0.02	-0.03	0.05	5.55	.550
% ADHD diagnosis	10	13	-0.01	0.00	-0.02	0.01	2.58	.186
Sampling method:								
Recruited from clinic center (vs. community)	24	31	-0.07	0.22	-0.58	0.45	7.42	.764
Control group type:								
Siblings (vs. normative data)	24	31	0.23	0.11	-0.01	0.46	12.42	.056
Measures:								
WIAT (vs. WJAT)	24	31	-0.07	0.26	-0.71	0.57	5.69	.798
WIAT (vs. WRAT)	24	31	-0.08	0.15	-0.43	0.26	8.35	.603
WJAT (vs. WRAT)	24	31	-0.01	0.26	-0.62	0.59	7.15	.965

Note. *n* = number of studies; *k* = number of effect size; *β* = estimated regression coefficient; *SE* = standard error; *LL* = Lower limit of 95% confidence interval; *UL* = Upper limit of 95% confidence interval; *df* = degrees of freedom; *p* = probability value. letter = letter reading; pseudoword = pseudoword reading; fluency = reading fluency; word = word reading; irregular = irregular word reading; mixed = regular & irregular word reading; comprehension = reading comprehension; community = community healthy controls; siblings = unaffected siblings as controls; normative = normative data as controls; WIAT = Wechsler Individual Achievement Test; WJAT = Woodcock-Johnson Achievement Test; ARRT = Alouette-R Reading Test; WRAT = Wide Range Achievement Test.
We only reported results when there were at least six samples for continuous variables and at least four samples in each subgroup of a categorical variable.

Figure 1. Flow Diagram for Inclusion and Exclusion in Meta-analysis

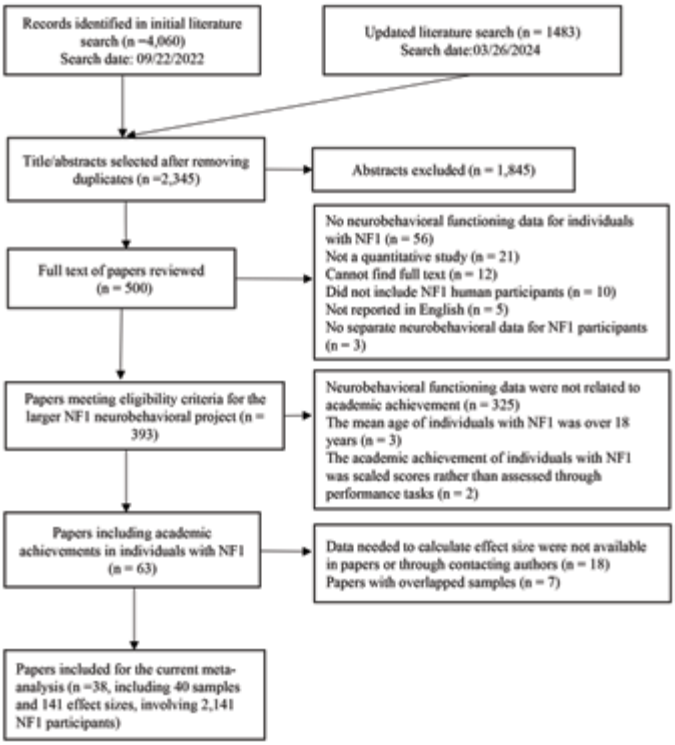
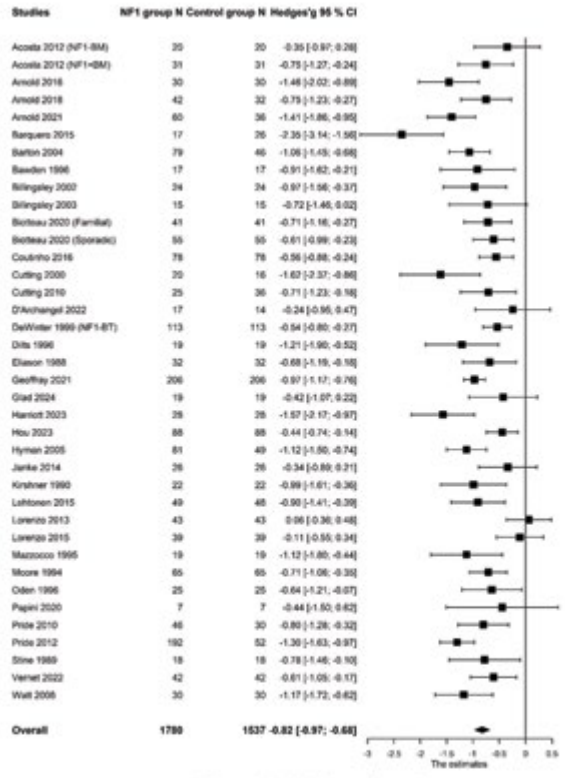
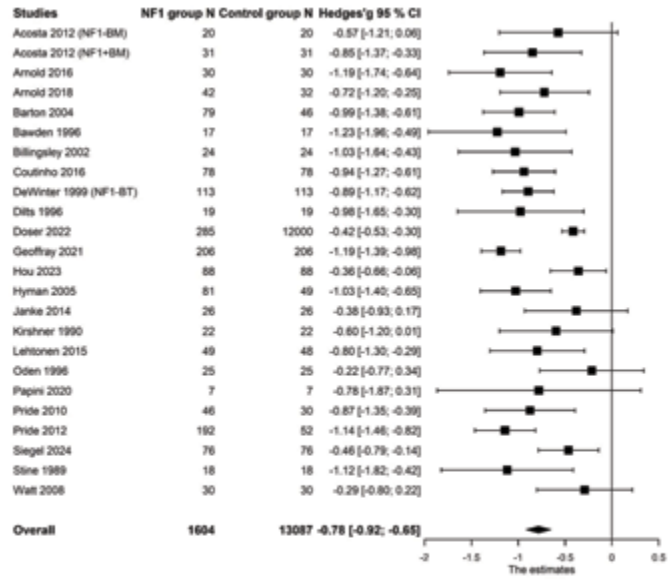


Figure 2. Forest Plots for Effect Sizes of Reading



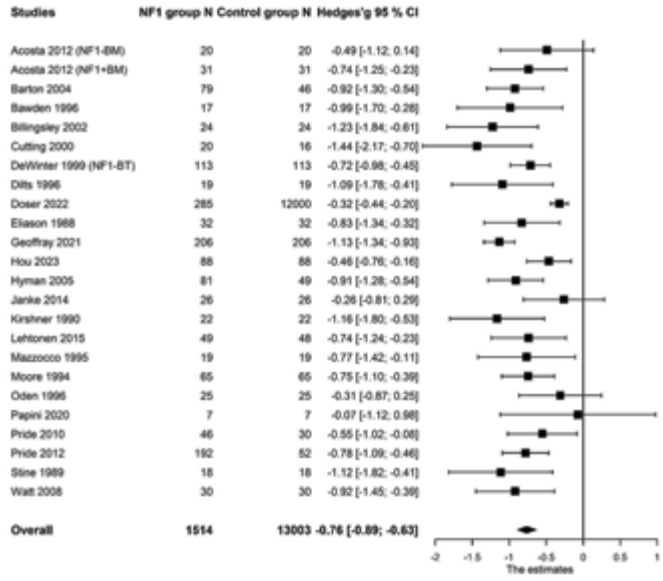
Note. NF1+BM = NF1 sample diagnosed with brain malformations, NF1-BM = NF1 sample was not diagnosed with brain malformations, Sporadic = Sporadic NF1 sample, Familial = Familial NF1 sample, and NF1-BT = NF1 sample was not diagnosed with brain tumor.
The slight differences between results in this figure and those in Table 1 are due to different methodological approaches employed.

Figure 3. Forest Plots for Effect Sizes of Writing



Note. NF1+BM = NF1 sample diagnosed with brain malformations, NF1-BM = NF1 sample was not diagnosed with brain malformations, and NF1-BT = NF1 sample was not diagnosed with brain tumor. The slight differences between results in this figure and those in Table 1 are due to different methodological approaches employed.

Figure 4. Forest Plots for Effect Sizes of Math



Note. NF1+BM = NF1 sample diagnosed with brain malformations, NF1-BM = NF1 sample was not diagnosed with brain malformations, and NF1-BT = NF1 sample was not diagnosed with brain tumor. The slight differences between results in this figure and those in Table 1 are due to different methodological approaches employed.

Full List of Authors: Liyan Yu, PhD., Florida State University, USA; Dan Liu, PhD., Florida State University, USA; Jonathan M. Payne, Psy.D., Murdoch Children's Research Institute, Australia; Xian Wu, PhD., University of Kentucky, USA; Heather L. Thompson, PhD., California State University, USA; Julia Moreira, BS., Florida State University, USA; Lauren Morey, BS., Florida State University, USA; Yang Hou, PhD., Florida State University, USA;

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Oral Language Skills of Children and Adolescents with Neurofibromatosis Type 1: A Systematic Review and Meta-Analysis

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Purpose: Previous studies have yielded inconsistent findings regarding the extent of oral language delays among individuals with Neurofibromatosis type 1 (NF1) compared with the general population. This study aims to provide a robust estimate of mean differences in oral language skills between NF1 and control groups and examine potential moderators contributing to variability in group differences.

Methods: Comprehensive literature searches were conducted across Scopus, PsycINFO, Web of Science, PubMed, and ProQuest using NF1-related and neurobehavioral function-related terms (e.g., language) on March 26, 2024. Studies assessing oral language skills in children/adolescents with NF1 and including control groups or standardized scores were analyzed. Hedges' g was calculated to quantify group difference. The robust standard error estimation and random-effects model were used to estimate the mean effect size. Meta-regression and subgroup analyses were conducted to explore potential moderators of group differences.

Results: Forty-three studies involving 2,360 children/adolescents with NF1, ranging from 0.53 to 15.5, met the inclusion criteria **(Figure 1)**. We found that children/adolescents with (versus without) NF1 performed significantly worse in overall oral language skills (n = 43, k = 122, g = -0.65, 95% CI [-0.77, -0.53], $p < .001$, I^2 = 82.01%) and in subskills **(Table 1)**, including core language skills (n = 16, k = 20, g = -0.78, 95% CI [-0.98, -0.59], $p < .001$), expressive language (n = 23, k = 35, g = -0.67, 95% CI [-0.83, -0.51], $p < .001$), receptive language (n = 21, k = 28, g = -0.56, 95% CI [-0.74, -0.37], $p < .001$), and phonological awareness (n = 11, k = 20, g = -0.72, 95% CI [-0.93, -0.52], $p < .001$), but not in pragmatic language (n = 4, k = 17, g = -0.45, 95% CI [-1.09, 0.19], p = .109). Significant publication bias was found in receptive language, and group differences remained significant after adjusting for publication bias **(Table 2)**. Overall group differences were not significantly moderated by age, sex composition, NF1 inheritance mode, ADHD diagnosis, learning disability diagnosis, intelligence, working memory, measure type, comparison group type, sampling resources, or measurement instruments **(Table 3)**.

Conclusions: Children/adolescents with NF1 exhibit significant deficits in oral language skills, particularly in core language, expressive language, receptive language, and phonological awareness, highlighting the need for targeted interventions. Substantial between-study heterogeneity in effect sizes highlights the importance to further investigate factors contributing to group differences using individual-level data and large sample size.

Table 1. Summary of Mean Effect Size across Studies

	Hedge g	LL	UL	SE	df	p-value	n	k	Tau²	I² (%)
Overall Oral Language Skills	-0.65	-0.77	-0.53	0.06	41.04	<.001	43	122	0.19	82.01
Oral language subkills										
Core language skills	-0.78	-0.98	-0.59	0.09	14.54	<.001	16	20	0.22	87.88
Overall expressive language	-0.67	-0.83	-0.51	0.08	21.47	<.001	23	35	0.12	66.10
Overall receptive language	-0.56	-0.74	-0.37	0.09	19.54	<.001	21	28	0.12	71.52
Overall phonological awareness	-0.72	-0.93	-0.52	0.09	9.07	<.001	11	20	0.05	44.17
Pragmatic language	-0.45	-1.09	0.19	0.20	3.00	.109	4	17	0.23	76.06
Age group										
Children	-0.61	-0.73	-0.48	0.06	34.01	<.001	36	104	0.13	69.63
Adolescents	-0.88	-1.48	-0.28	0.22	3.96	.016	5	15	0.48	96.32
Measure type										
Performance	-0.62	-0.73	-0.50	0.06	36.24	<.001	39	103	0.10	70.07
Report	-0.90	-1.68	-0.13	0.24	2.98	.034	4	19	0.43	90.19
Comparison group type										
Community healthy controls	-0.75	-0.95	-0.54	0.10	16.66	<.001	18	49	0.15	77.41
Unaffected siblings as controls	-0.48	-0.72	-0.23	0.09	4.58	.005	7	16	0.00	4.41
Normative data as controls	-0.61	-0.83	-0.40	0.10	17.67	<.001	19	53	0.29	87.04
Sampling resources										
Recruited from clinic center	-0.66	-0.81	-0.52	0.07	29.47	<.001	31	83	0.22	83.29
Recruited from community	-0.65	-0.89	-0.42	0.10	7.22	<.001	9	36	0.09	60.91
Measures										
Clinical evaluation of language fundamentals	-0.58	-0.94	-0.23	0.13	4.79	.008	6	17	0.05	36.99
Comprehensive test of phonological processing	-0.84	-1.20	-0.47	0.11	2.87	.006	4	10	0.04	32.52
Peabody picture vocabulary test	-0.55	-1.02	-0.08	0.20	6.96	.027	8	8	0.31	86.00
Wechsler intelligence scale for children	-0.76	-1.00	-0.53	0.09	4.72	.001	8	8	0.00	4.25
Wechsler preschool and primary scale of intelligence	-0.85	-1.15	-0.54	0.10	2.99	.003	4	13	0.03	34.10

Notes. *LL* = lower limit of 95% confidence interval; *UL* = upper limit of 95% confidence interval; *SE* = standard error; *df* = degrees of freedom; *n* = number of studies; *k* = number of effect sizes; Tau² = Tau-square; *I²* = I-squared.

Results were reported when there were at least four samples in each subgroup.

Table 2. Publication Bias Tests Results

	Funnel Plot Symmetry Test		Robu Meta-Regression					Trim and Fill Analysis[a]					
	Egger's Test												
	Z	*p*	*β*	*SE*	*p*	*n*	*k*	*N* of trimmed studies	Filled *N*	Fill *g* ES	*p*	95% CI	*t*
Overall Oral Language Skills	0.30	.762	-0.03	0.79	.971	43	122	-	-	-	-	-	-
Oral Language Subskills													
Core language skills	0.36	.719	0.14	1.09	.907	16	20	-	-	-	-	-	-
Overall expressive language	0.002	.998	-0.37	0.84	.667	23	35	-	-	-	-	-	-
Overall receptive language	-2.07	.038	-2.98	1.23	.034	21	28	21	7	-0.38	<.001	[-0.57, -0.18]	-3.98
Overall phonological awareness	0.60	.548	0.66	1.5	.689	11	20	-	-	-	-	-	-
Pragmatic language	0.87	.382	2.12	13.46	.890	4	17	-	-	-	-	-	-

Note. *Z* = standard normal distribution score; *p* = probability value; filled *N* = number of filled studies; filled *ES* = effect size after filling the hypothetical unpublished studies; *t* = t-value; *CI* = confidence interval; *B* = estimated regression coefficient; *SE* = standard error; *n* = number of studies; *k* = number of effect sizes.
[a] The Trim and Fill analysis was conducted only when the results of Egger's test were significant.

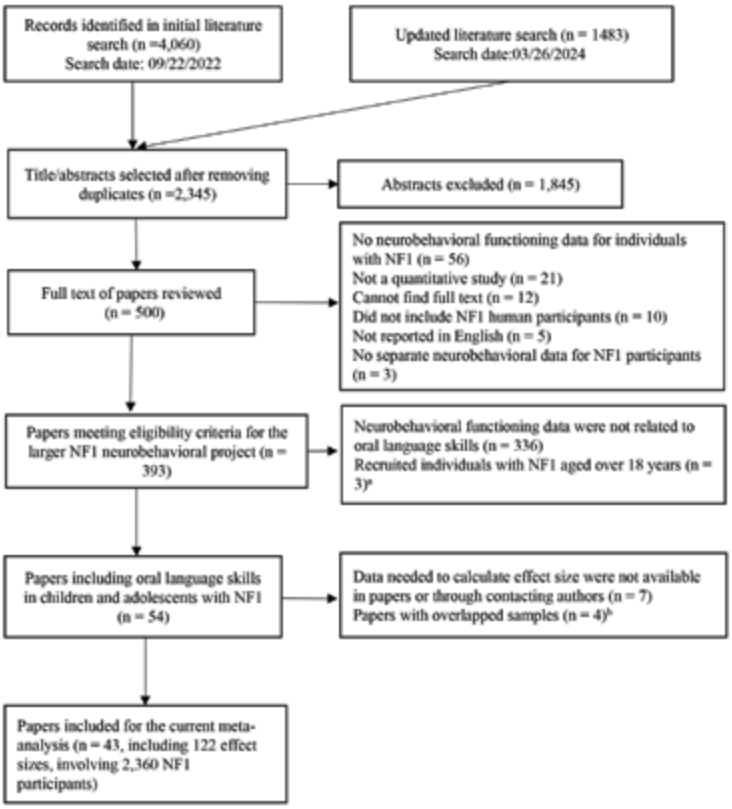
Table 3. Meta-regression Results

	n	*k*	*β*	*SE*	*LL*	*UL*	*df*	*p*-value
Mean age	41	119	0.01	0.01	-0.02	0.04	12.52	.677
Age group:								
Children (vs. adolescents)	41	119	-0.28	0.24	-0.89	0.34	5.07	.301
% Girl	37	112	0.00	0.00	0.00	0.01	8.55	.311
% familial NF1	20	57	-0.01	0.01	-0.04	0.02	6.90	.655
IQ:								
Mean full-scale IQ	33	94	0.01	0.02	-0.03	0.05	11.79	.470
Mean verbal IQ	28	85	0.02	0.02	-0.04	0.08	5.40	.495
Mean performance IQ	26	80	0.02	0.02	-0.03	0.06	8.15	.368
Mean working memory	15	43	0.79	0.61	-1.10	2.67	3.20	.285
% ADHD diagnosis	15	37	0.00	0.01	-0.03	0.02	2.84	.600
% Learning disability diagnosis	10	30	0.00	0.01	-0.02	0.01	4.60	.712
Measure type:								
Performance (vs. report)	43	122	-0.34	0.27	-1.13	0.45	3.49	.285
Sampling resources:								
Clinic center (vs. community)	43	122	0.00	0.12	-0.26	0.25	12.22	.970
Comparison group type:								
Community (vs. siblings)	43	122	0.21	0.13	-0.07	0.50	10.21	.130
Community (vs. normative data)	43	122	0.09	0.14	-0.20	0.38	32.90	.526
Siblings (vs. normative data)	43	122	-0.12	0.14	-0.43	0.19	10.03	.407
Measures:								
CELF (vs. CTPP)	43	122	-0.27	0.18	-0.78	0.23	3.70	.203
CELF (vs. PPVT)	43	122	-0.23	0.36	-1.05	0.60	7.98	.543
CELF (vs. WISC)	43	122	-0.18	0.19	-0.62	0.25	9.12	.372
CELF (vs. WPPSI)	43	122	-0.29	0.20	-0.76	0.18	6.98	.192
CTPP (vs. PPVT)	43	122	0.05	0.33	-0.85	0.94	4.20	.894
CTPP (vs. WISC)	43	122	0.09	0.14	-0.29	0.48	3.83	.535
CTPP (vs. WPPSI)	43	122	-0.01	0.14	-0.42	0.39	3.88	.924
PPVT (vs. WISC)	43	122	0.05	0.33	-0.70	0.79	8.71	.892
PPVT (vs. WPPSI)	43	122	-0.06	0.33	-0.83	0.71	7.11	.856
WISC (vs. WPPSI)	43	122	-0.11	0.13	-0.40	0.19	7.13	.424

Note. *n* = number of studies; *k* = number of effect size; *β* = estimated regression coefficient; *SE* = standard error; *LL* = Lower limit of 95% confidence interval; *UL* = Upper limit of 95% confidence interval; *df* = degrees of freedom; *p* = probability value; CELF = Clinical evaluation of language fundamentals; CTPP = Comprehensive test of phonological processing; PPVT = Peabody picture vocabulary test; WISC = Wechsler intelligence scale for children; WPPSI = Wechsler preschool and primary scale of intelligence.

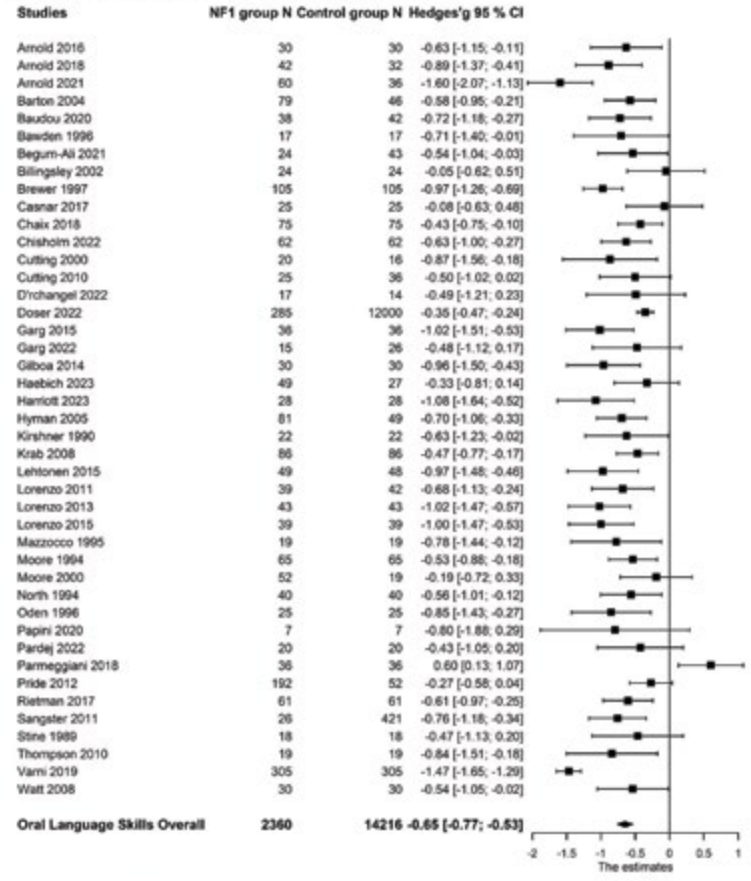
We only reported results when there were at least six samples for continuous variables and at least four samples in each subgroup of a categorical variable.

Figure 1. Flow Diagram for Inclusion and Exclusion in Meta-analysis



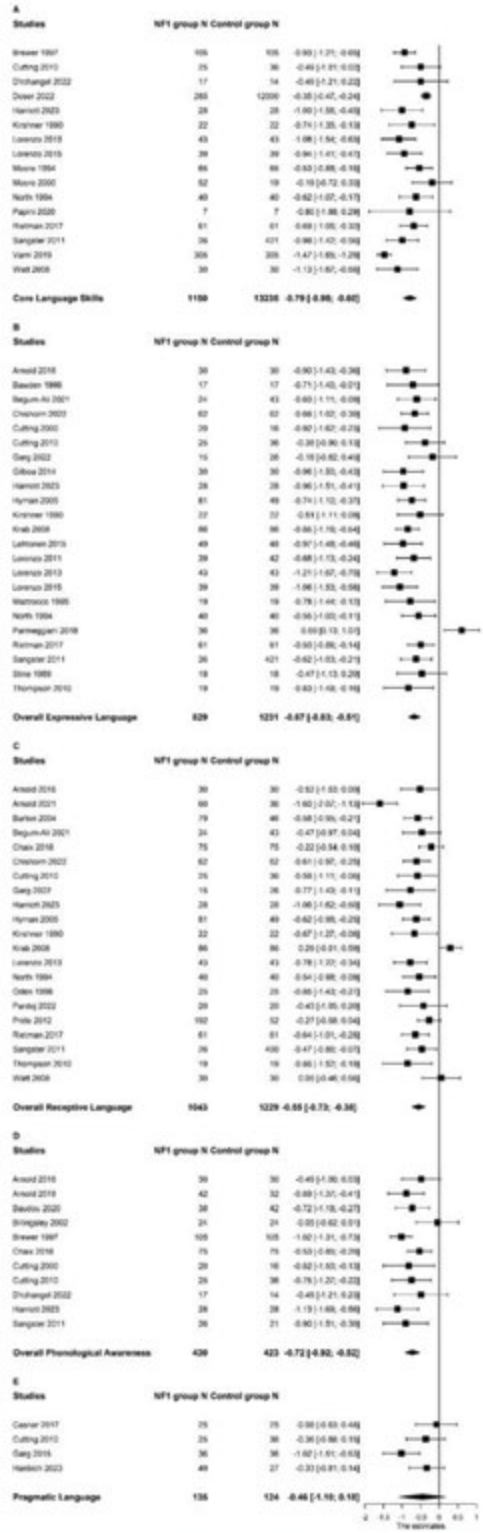
Note. [a] Three studies were excluded for recruiting individuals with NF1 aged over 18 years (Batista et al., 2014; Ferner et al., 1996; Pavol et al., 2006).
[b] Four studies were excluded due to overlapping samples with included studies (Biotteau et al., 2020; Biotteau et al., 2021; Biotteau et al., 2019; North et al., 1995).

Figure 2. Forest Plots for Effect Sizes of Oral Language Skills Overall



Note. The slight differences between results in this figure and those in Table 1 are due to different methodological approaches employed.

Figure 3. Forest Plots for Effect Sizes of Oral Language Subskills



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Slow but Sustained Head Growth Drives Macrocephaly in NF1

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Purpose: NF1 is characterized by over-activation of the RAS pathway, driving a wide range of clinical manifestations including plexiform neurofibromas, low grade gliomas, cognitive issues, and macrocephaly. While macrocephaly and relatedly, megalencephaly, has been demonstrated at birth and later life in NF1, little is known about timing of head growth for these patients compared to typical development. We aimed to clarify the timing of macrocephaly in NF1 in early life which may inform the appropriate timing of interventions to address neurocognitive manifestations of the disease.

Methods: Using electronic health records from the Children's Hospital of Philadelphia, we constructed growth charts of head circumference from age 0 to 3 years in NF1 and clinical control cohorts seen at well-child visits. Demographic information and sample sizes of available data are shown in Table 1. Using standardized (Z) scores from well-child visit growth charts, we statistically compared head circumference between NF1 patients and a left-out group of clinical controls at each well-child visit timepoint.

Results: Consistent with prior reports, head circumference around the time of birth appears increased in NF1 compared to controls **(Figure 1 A&B)**. However, head circumference rate-of-growth is slower for NF1 patients compared to controls over the first four months of life **(Figure 1C)**. After that timepoint, head circumference in NF1 outpaces controls until approximately two years of age. Differences in head circumference between NF1 and controls are statistically significant at all well-child visit timepoints starting at six months **(Figure 2 A&B)**. These patterns are recapitulated when using Center for Disease Control charts **(Figure 2C)**.

Conclusions: We identify a time window between four months and two years of age in which head circumference rate-of-growth is increased in NF1. Further research is needed in quantitative neuroimaging in the first years of life to determine the neurological correlates of accelerated head growth in this population and how it relates to cognitive outcomes.

Characteristic	N	Female Controls N = 48,774[1]	Female NF1 N = 988[1]	Male Controls N = 56,311[1]	Male NF1 N = 1,278[1]
Age (years)	107,351	0.83 (0.34, 1.51)	1.33 (0.66, 2.03)	0.82 (0.34, 1.51)	1.34 (0.68, 2.05)
Gestational Age at Birth (weeks)	100,065	39 (37, 40)	39 (38, 40)	39 (37, 40)	39 (37, 40)
Birth Weight (kg)	102,448	3.19 (2.78, 3.57)	3.42 (2.88, 3.86)	3.29 (2.86, 3.68)	3.40 (2.95, 3.77)
Race	107,351				
American Indian or Alaska Native		1 (<0.1%)	0 (0%)	49 (<0.1%)	0 (0%)
Asian		1,851 (3.8%)	55 (5.6%)	2,255 (4.0%)	33 (2.6%)
Black or African American		12,863 (26%)	263 (27%)	15,265 (27%)	180 (14%)
Multiple Races		2,560 (5.2%)	55 (5.6%)	3,209 (5.7%)	101 (7.9%)
Native Hawaiian or Other Pacific Islander		15 (<0.1%)	0 (0%)	35 (<0.1%)	0 (0%)
Other		4,353 (8.9%)	82 (8.3%)	5,040 (9.0%)	75 (5.9%)
Refused		182 (0.4%)	1 (0.1%)	211 (0.4%)	0 (0%)
Unknown		83 (0.2%)	8 (0.8%)	112 (0.2%)	0 (0%)
White		26,866 (55%)	524 (53%)	30,135 (54%)	889 (70%)
# of Observations per Subject	107,351	8 (6, 9)	10 (5, 14)	8 (6, 9)	11 (5, 14)

[1] Median (Q1, Q3); n (%)

Table 1. Demographics for the NF1 and clinical control cohorts. N refers to the total number of observations in each category, of which there were many longitudinal measurements per subject.

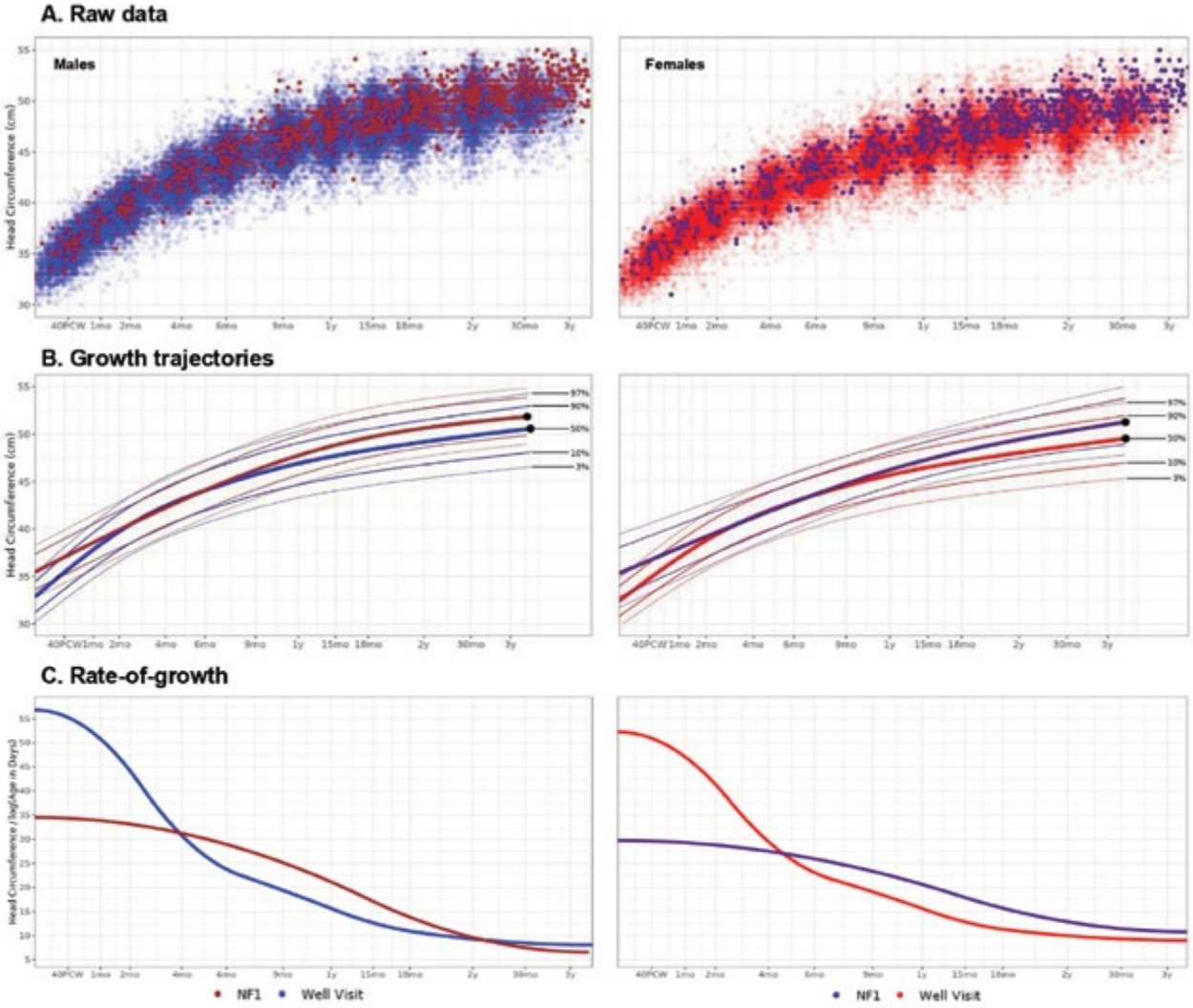

Figure 1. Head circumference growth charts for NF1 and clinical controls. (A) Raw head circumference data. Age is represented on a log-scale and is adjusted for gestational age at birth. PCW = Post-conceptional weeks. (B) Growth curves representing the 3rd, 10th, 50th, 90th, and 97th percentile. (C) Rate-of-growth curves computed by calculating the first derivative of the median trajectories. For males, NF1 cases are shown in red, and well-child visits of children without NF1 are shown in blue. For females, NF1 cases are shown in blue, and well child visits of children without NF1 are shown in red.

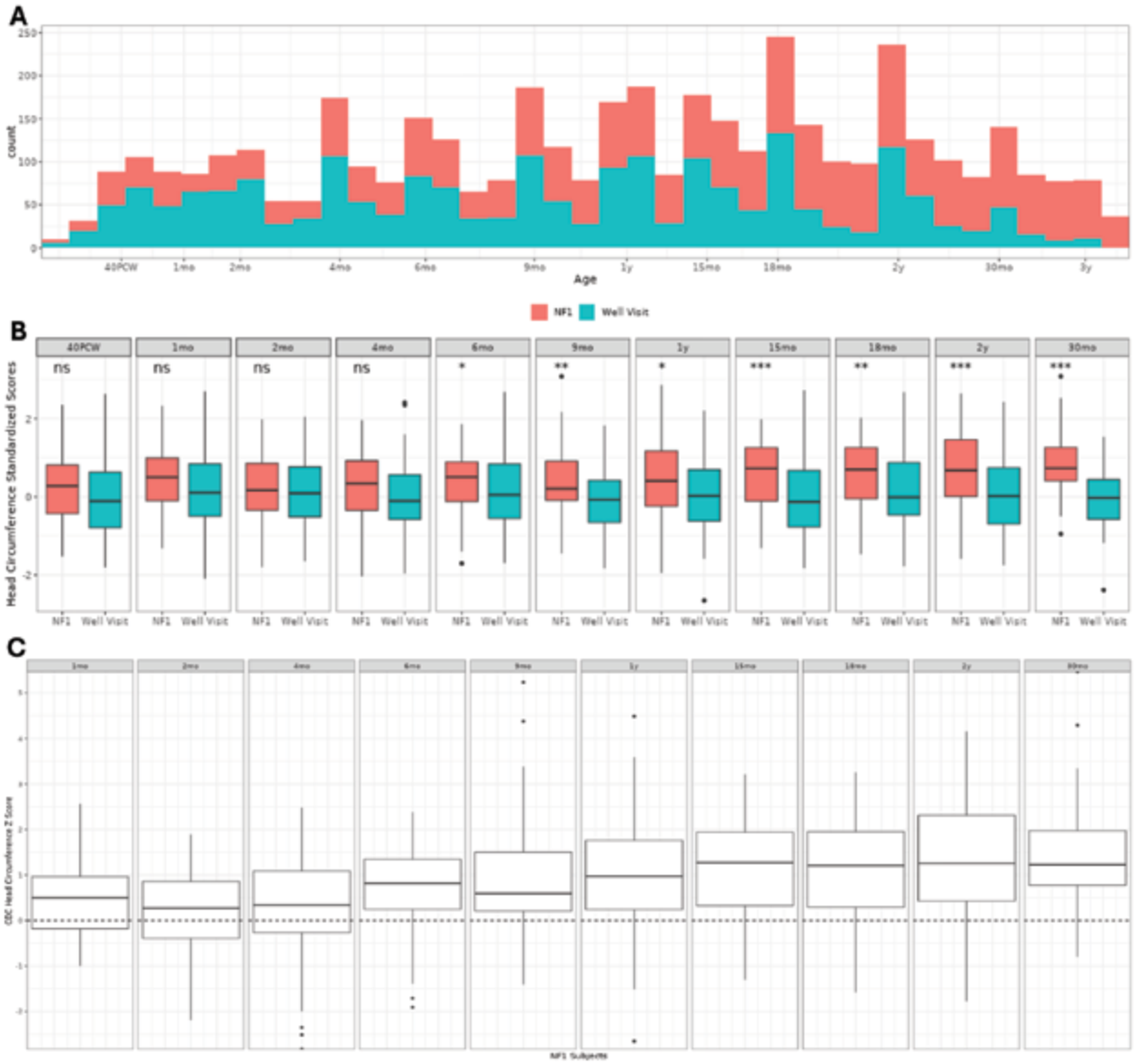

Figure 2. Statistical comparison of head circumference differences in NF1 versus clinical control. (A) Histogram of ages at head circumference measurements for NF1 and a left-out clinical control group shows highest density of data at well-child visits. (B) Comparison of standardized scores between NF1 and left-out clinical controls at each well-child visit timepoint. *P* values from pairwise t-tests were adjusted for multiple comparisons using false discovery rate (FDR) correction. *** indicates $P < 0.001$; ** indicates $P < 0.01$; * indicates $P < 0.05$; *ns* indicates $P > 0.05$. (C) Head circumference Z scores computed using CDC growth charts at each well-child visit timepoint for NF1 subjects.

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Cognitive Function in Middle-Aged and Older Adults with Neurofibromatosis Type 1: Exploring Psychosocial Predictors

Matthew R. Jamnik, PhD, *Florida State University College of Medicine*

Purpose: Neurofibromatosis type 1 (NF1) is a single-gene disorder that is associated with significant neurobehavioral challenges, including an elevated risk for cognitive impairments. While cognitive difficulties are well-documented in youth with NF1, relatively limited research has investigated cognitive function in this population during middle and older adulthood. Furthermore, although the importance of psychosocial factors for cognitive aging in the general population is well-supported, no studies have directly examined their influence in adults with NF1. Thus, the current study addresses this gap by exploring relevant psychosocial predictors of cognitive function in middle-aged and older adults with NF1.

Methods: Data were drawn from a larger, ongoing study of cognitive function in middle-aged and older adults with NF1. The current sample includes 165 individuals (27.6% males), aged 40 to 81 years old (M = 53.89, SD = 10.55). Cognitive function was measured objectively, via the modified Telephone Interview of Cognitive Status and verbal fluency (i.e., 60-second naming task), and by use of a questionnaire battery to collect self-reported ratings of subjective cognition ("memory" and "ability to think quickly"), cognitive impairment (12 items), and inattention (6 items). Psychosocial factors included personality (conscientiousness), purpose in life, loneliness (3-item University of California, Los Angeles Loneliness Scale) and NF1-related discrimination ("treated unfairly because of your NF1"). We also assessed relevant demographic characteristics (age, sex [male = 0; female = 1], and education level).

Multiple regression analyses were conducted to examine how conscientiousness, purpose in life, loneliness, and NF1-related discrimination were related to cognitive outcomes, controlling for demographics. Separate regression models were run for each psychosocial and cognitive variable.

Results: Regression coefficients for each predictor are presented in Table 1. Results demonstrate that, while not associated with objective cognition, conscientiousness and purpose in life both significantly predicted higher levels of subjective cognition and lower self-reported cognitive difficulties (impairment; inattention), whereas an opposite pattern appeared for loneliness (i.e., lower subjective cognition and increased cognitive difficulties). NF1-related discrimination significantly predicted greater self-reported cognitive impairment and inattention, as well as lower objective cognition.

Conclusion: Our study demonstrates that psychosocial factors, including personality (conscientiousness), purpose in life, loneliness, and discrimination significantly predict cognition in adults with NF1, even after accounting for sex, age, and educational differences. Thus, for interventions aimed at preventing or mitigating cognitive difficulties in this population, psychosocial factors may serve as potentially modifiable targets, although future longitudinal and experimental studies are needed for causal inference. Furthermore, when studying the complex cognitive profile of NF1, given that psychosocial factors were more salient for subjective than objective measures, it is crucial to employ multimethodological approaches.

Table 1. Regression coefficients of all predictors on cognitive variables

	Objective Cognition		Verbal Fluency		Subjective Cognition		Cognitive Impairment		Inattention	
	β	S.E.	β	S.E.	β	S.E.	β	S.E.	β	S.E.
Age	-0.01	0.02	-0.08	0.05	0.01	0.01	-0.02	0.02	-0.01	0.04
Sex	0.15+	0.55	**0.17***	**1.16**	**-0.19****	**0.15**	**0.18***	**0.56**	0.12+	1.01
Education Level	**0.31*****	**0.18**	**0.35*****	**0.39**	**0.31*****	**0.05**	**-0.25*****	**0.19**	-0.05	0.33
Conscientiousness	-0.02	0.34	-0.13	0.72	**0.43*****	**0.09**	**-0.45*****	**0.35**	**-0.64*****	**0.63**
Age	-0.03	0.02	-0.09	0.05	0.01	0.01	-0.02	0.02	-0.02	0.05
Sex	0.15+	0.53	0.15+	1.15	-0.07	0.14	0.06	0.55	-0.04	1.12
Education Level	**0.28****	**0.19**	**0.36*****	**0.41**	**0.18***	**0.05**	-0.13	0.20	0.08	0.40
Purpose in Life	0.12	0.32	-0.04	0.69	**0.45*****	**0.09**	**-0.46*****	**0.33**	**-0.48*****	**0.67**
Age	-0.01	0.02	-0.08	0.05	0.03	0.01	-0.02	0.03	-0.03	0.05
Sex	0.14+	0.53	0.14+	1.14	-0.09	0.16	0.08	0.60	-0.02	1.22
Education Level	**0.31*****	**0.18**	**0.36*****	**0.39**	**0.30*****	**0.05**	**-0.24****	**0.20**	-0.04	0.42
Loneliness	0.05	0.38	0.10	0.80	**-0.16***	**0.11**	**0.26****	**0.42**	**0.26****	**0.86**
Age	-0.02	0.02	-0.10	0.05	0.05	0.01	-0.07	0.03	-0.07	0.05
Sex	**0.16***	**0.53**	0.15+	1.15	-0.09	0.16	0.07	0.62	-0.03	1.23
Education Level	**0.30*****	**0.18**	**0.35*****	**0.39**	**0.30*****	**0.05**	**-0.24****	**0.21**	-0.04	0.42
NF1-related Discrimination	**-0.18***	**0.16**	-0.03	0.35	-0.13	0.05	**0.27****	**0.18**	**0.24****	**0.37**

$^{+}p < .08$; * $p < .05$; ** $p < .01$; *** $p < .001$

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Sleep Disturbances and Insomnia in Children with Neurofibromatosis Type 1: Associations with Cognitive, Behavioral and Emotional Outcomes

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Purpose: To characterize sleep difficulties and disorders in children with NF1 and examine their associations with cognition, behavior and emotional well-being.

Methods: This cross-sectional study comprehensively assessed sleep in children with NF1 and typically developing controls (ages 6–15 years) using validated sleep questionnaires, a well-established sleep disorder screener (Pediatric Sleep Questionnaire), detailed sleep and medical histories, and seven nights of actigraphy with sleep diaries.

Neuropsychological assessments and questionnaires of emotional well-being, behavior, and quality of life (QoL) were also collected. Insomnia was classified using established research criteria, and group differences in sleep parameters, cognition, behavior, and well-being were analyzed.

Results: Children with NF1 ($n = 107$) exhibited significantly greater sleep disturbance ($t = 5.25$, $p < 0.001$) and poorer sleep quality ($t = 6.69$, $p < 0.001$) compared to controls ($n = 53$). The most commonly reported sleep problems were difficulty initiating sleep and a restless sleep. Insomnia was present in 36% of children with NF1, a prevalence 4.5 times higher than the general population estimate of 7.86%.

Within the NF1 group, children with insomnia had significantly lower verbal memory ($p = 0.04$), poorer executive functioning, lower adaptive functioning, and lower quality of life (all $p < 0.001$). They also exhibited higher levels of depressive symptoms ($p = 0.001$) and fatigue ($p < 0.001$) and were more likely to have ADHD. No significant group differences were observed in age, sex, intellectual functioning, anxiety symptoms, sleep hygiene, or academic achievement.

Notably, screeners indicated that 28% and 39% of children with NF1 met clinical thresholds for periodic limb movement disorder (PLMD) and sleep disordered breathing (SDB), respectively, compared to 6% and 4% of controls. Overall, 13% of children with NF1 met criteria for insomnia while also surpassing thresholds for SDB and PLMD suggesting multiple sleep disorders.

Conclusion: Sleep difficulties and disturbances are highly prevalent in children with NF1, with over one third meeting criteria for insomnia – a rate nearly five times that observed in the general population. Insomnia in NF1 was associated with significant cognitive, behavioral and emotional difficulties, highlighting the broader impact of sleep disruption on daily functioning and QoL. Furthermore, a substantial portion of children with NF1 screened positive for PLMD and SDB underscoring the need for future research involving polysomnography to better refine our understanding of sleep in this population.

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Funding: US Army Medical Research and Materiel Command, Department of Defense Neurofibromatosis Research Program, award number W81XWH-19-1-0254; Johns Hopkins University via the Neurofibromatosis Therapeutic Acceleration Program (NTAP) with funds provided by Grant Agreement from Bloomberg Philanthropies.

Characterizing Autism and Neurodevelopmental Challenges in NF1: Final Results from the PANDA Study

Jonathan M Payne, PsyD, *Murdoch Children's Research Institute, Australia; Department of Paediatrics, University of Melbourne, Australia; The Royal Children's Hospital, Australia*

Purpose: To determine autism prevalence in a large NF1 cohort and examine associations between autism and other neurodevelopmental challenges.

Methods: This international prospective cross-sectional study assessed 191 children with NF1 unselected for neurocognitive deficits (3–15 years old). Participants underwent neuropsychological evaluation including parent-reported autistic traits (SRS-2). Children with elevated SRS-2 scores ($T \geq 60$) received comprehensive autism assessments, including the Autism Diagnostic Observation Schedule-2 (ADOS-2) and Autism Diagnostic Interview-Revised (ADI-R). A multidisciplinary expert panel determined DSM-5-TR autism diagnoses. Participants were categorized as: (1) no autistic traits, (2) broad autism phenotype (subthreshold symptoms), or (3) autism diagnosis.

Results: Ninety-five (50%) participants with NF1 exhibited no autistic traits, 42 (22%) demonstrated a broader autism phenotype, and 54 met DSM-5-TR diagnostic criteria for autism (prevalence: 28%; 95%CI, 21.8%-34.9%). Compared to the general population (~2%), children with NF1 were 14.1 times more likely to be diagnosed with autism. Autism prevalence did not differ between males (28.6%; 95%CI, 20.2%-38.2%; $n=105$) and females (27.3%; 95%CI, 18.3%-37.8%, $n=88$) nor between *de novo* (26.3%; 95%CI, 18.6%-35.2%, $n=118$) versus familial (29.7%; 95%CI, 19.7%-41.5%, $n=74$) NF1 inheritance ($LR=0.27$, $p=0.60$).

On intelligence testing, children with NF1+ASD and the broader autism phenotype exhibited significantly lower verbal IQ than those without autistic traits (both, $p<0.01$), but there were no group differences on nonverbal IQ measures (all, $p>0.16$). Academic achievement in reading, spelling and math were also comparable across NF1 groups (all, $p>0.29$).

Both the broader autism and NF1+ASD groups exhibited significantly elevated symptoms of ADHD, depression, anxiety, and executive behavioral problems compared to the NF1 group without autistic traits ($p<0.01$).

Adaptive functioning, however, differed across all groups, with the NF1+ASD group exhibiting the lowest scores, followed by the broader autism phenotype group, and the NF1 group without autistic traits showing the highest adaptive functioning ($p<0.01$).

Conclusions: Findings highlight the high prevalence of autism in children with NF1 and the associated impact of autism on broader neurodevelopmental outcomes in this population. Compared to those without autistic traits, children with NF1+ASD and a broader autism phenotype demonstrated significant challenges in verbal intelligence, ADHD symptoms, emotional difficulties, executive behavior, and adaptive functioning. Notably, a diagnosis of autism was uniquely associated with poorer adaptive functioning. These findings advance our understanding of the cognitive, behavioral, and adaptive difficulties faced by children with NF1 and further reinforce NF1 as a monogenic syndrome that confers a high risk for autism, emphasizing the need for targeted assessment, intervention, and monitoring.

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Funding: US Army Medical Research and Materiel Command, Department of Defense Neurofibromatosis Research Program, award number W81XWH-15-1-0619; MCRI Clinician-Scientist Fellowship, awarded to JMP.

Sleep Problems and Circadian Functioning in Children and Adolescents with Neurofibromatosis Type 1

Natalie A. Pride, PhD, *Kids Neuroscience Centre, The Children's Hospital at Westmead, Australia; Sydney Medical School, The University of Sydney, Australia*

Purpose: Drosophila models of NF1 highlight the role of the *NF1* gene in regulating the sleep-wake cycle, with reduced sleep duration, increased fragmentation, and impaired sleep depth documented. To further understand how *NF1* disrupts sleep and circadian regulation this study compared parent reported sleep patterns, actigraphy sleep measures, and nocturnal melatonin in children and adolescents with NF1 to typically developing (TD) controls aged 6-16 years of age.

Methods: Actigraphy data and sleep diaries were recorded in 101 children and adolescents with NF1 over seven days and compared to 43 TD controls. Actigraphy variables included sleep onset latency, sleep efficiency, total sleep time, and wake after sleep onset. Indicators of circadian phase, sleep midpoint and variability in sleep timing between weekday and weekend sleeps, were also analyzed. Parent and self-report measures of fatigue and quality of life (QoL) were collected. Nocturnal urinary excretion of 6-sulphatoxymelatonin (aMT6s) was measured by radioimmunoassay to estimate melatonin secretion in a subset of children with NF1 ($n = 54$) and controls ($n = 43$) matched on age, sex, and stage of puberty.

Results: Compared to controls, children with NF1 exhibited lower aMT6s levels ($Z = 2.65$, $p = 0.008$), a longer sleep onset ($t = 5.09$, $p < 0.001$), lower sleep efficiency ($t = -3.89$, $p < 0.001$) a later midpoint of sleep ($t = 2.18$, $p = 0.032$) and a greater variability in sleep timing ($t = 2.40$, $p = 0.018$). In children with NF1, lower aMT6s levels was associated with a later sleep midpoint ($p = 0.02$). Reduced sleep efficiency, longer sleep onset and greater variability in sleep timing was associated with increased fatigue (all $p < 0.02$). Longer sleep onset and reduced sleep efficiency was additionally correlated with reduced QoL in children with NF1 (all $p \leq 0.004$).

Conclusions: Children with NF1 show differences in sleep and circadian rhythms compared to TD children, including alterations in nocturnal melatonin levels, delayed sleep onset and timing, and increased sleep timing variability, commonly known as social jet lag. A greater social jetlag – which can indicate a misalignment between biological rhythms and social determined sleep schedules – may contribute to sleep disturbance and increased fatigue in NF1. These findings significantly advance our understanding of sleep disturbance in NF1. Given the potential impact of poor sleep and circadian disturbance on brain development and function, as well as overall-wellbeing, targeted interventions to improve sleep could be beneficial for children with NF1.

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Language Skills and Their Association with Psychosocial Symptoms in Children with NF1: Insights from a Multisite Study

Kristina M Haebich, PsyD, *Murdoch Children's Research Institute, Australia; Department of Paediatrics, University of Melbourne, Australia*

Purpose: The language phenotype of children with NF1 and its relationship with psychosocial comorbidities are understudied. In contrast, research in the general population have established links between language problems and the presence and development of internalizing and externalizing problems. This study aimed to characterize the nature of language skills in children with NF1 and typically developing (TD) controls, and examine associations between language, internalizing, and externalizing symptoms in NF1.

Methods: This international prospective cross-sectional study assessed 192 children with NF1 and 102 TD controls aged 4–15 years. Caregivers rated participants' language skills including speech, syntax, semantics, and coherence, using the Children's Communication Checklist, Second Edition (CCC-2), and the presence of internalizing (anxiety/depression, social withdrawal, somatic complaints) and externalizing (attention problems, aggression) symptoms on the Child Behavior Checklist (CBCL).

Results: Impaired language skills (≤1 SD below population mean) were reported for 64% of children with NF1, compared to 15% of TD controls ($p < 0.001$), with difficulties observed across all assessed language domains (all, $p < 0.001$; Cohen's $d = -1.1$ to -1.4). Children with NF1 also demonstrated significantly higher levels of internalizing and externalizing symptoms than TD controls across all symptom scales (all $p < 0.001$, Cohen's $d = 0.5$ to 0.9).

Within the NF1 group, children with impaired language skills (64%) demonstrated significantly greater psychosocial symptoms compared to those with typical language abilities (36%). This included greater social withdrawal and attention problems (both $p < 0.001$, Cohen's $d = 0.7$ and 0.9, respectively), increased somatic complaints and aggressive behavior (both $p < 0.01$, Cohen's $d = 0.4$ and 0.5, respectively), as well as elevated anxiety/depression symptoms ($p < 0.05$, Cohen's $d = 0.4$).

Logistic regression indicated that language impairment in children with NF1 was associated with a higher likelihood of anxiety/depression ($p < 0.001$, $OR = 1.11$, 95% CI = 1.0 - 1.2) and attention difficulties ($p = 0.001$, $OR = 0.9$, 95% CI = 0.8 – 0.9). This relationship persisted after adjustment for sex, social risk, age, nonverbal IQ and correction for multiple comparisons.

Conclusions: Language difficulties are a significant concern in children with NF1 and associated with psychosocial difficulties, particularly anxiety, depression, and inattention. These findings highlight the complex interplay between communication challenges and mental health in NF1, underscoring the need for targeted language interventions. Since the cross-sectional nature of this study limits causal conclusions, future longitudinal research is needed to clarify these relationships.

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Early Motor Activity Patterns as Developmental Markers for ADHD Traits in Neurofibromatosis Type 1: A Longitudinal Study

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Background: Neurofibromatosis type 1 (NF1) is a monogenic neurodevelopmental condition associated with a higher prevalence of attention deficit hyperactivity disorder (ADHD). Emerging research suggests ADHD in NF1 differs from idiopathic ADHD. Identifying early differences may clarify neurodevelopmental pathways and inform personalised treatments. This study examined early motor activity in infants with NF1 compared to those with a family history of idiopathic ADHD. The primary aim was to assess whether early motor activity differences emerge among NF1, elevated-likelihood ADHD (EL-ADHD), and typically developing (TD) infants. It was hypothesised that group differences will emerge between the first and second year of life when activity-related differences become apparent.

Methods: Data were collected as part of the Early Development in Neurofibromatosis Type 1 (EDEN) project, a longitudinal study on early functional development in NF1. Infants at 10 and 14 months across three cohorts (NF1, EL-ADHD, TD) were assessed using accelerometery, behavioural video coding, and standardised developmental scales. Group differences were analysed via ANOVA and Kruskal-Wallis tests in the statistical software R.

Results: Accelerometery data showed no group differences at 10 months. However, by 14 months, motion entropy differed between NF1 and EL-ADHD cohorts ($p<0.05$), whilst motion intensity differed between NF1 and both EL-ADHD and TD groups ($p<0.05$). Total activity levels did not differ across groups. Likewise, video coding analyses revealed that at both timepoints, time spent in stillness and motion were similar across all three groups. However, NF1 infants spent significantly less time in locomotion than TD ($p=0.0287$) and EL-ADHD infants ($p=0.0412$) and more time in non-locomotor positions than TD infants ($p=0.0489$). By 14 months, time spent in non-locomotor positions, and locomotion was comparable across groups. However, NF1 infants performed more simple locomotion ($p=0.000227$) and less complex locomotion ($p=0.0174$) than EL-ADHD infants, although no differences were observed when compared to TD infants.

Conclusion: Our findings indicate that infants with NF1 show early motor differences at as early as 10 months of age. Total activity levels were similar across groups, but NF1 infants displayed differences in movement complexity, engaging in more simple locomotion and less in complex locomotion compared to EL-ADHD infants. The similar activity levels between NF1 and EL-ADHD infants may reflect emerging ADHD traits in NF1. These findings highlight the potential for early motor differences as markers for neurodevelopmental outcomes and underscore the need for targeted early interventions in NF1 to support motor development.

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Evaluation of Emotional Intelligence Rehabilitation Programs in Adolescents and Young Adults with Neurofibromatosis Type 1 (NF1): A Prospective Randomized Study

Francina Lombardi, *Neuropediatrics Department, FLENI, Buenos Aires, Argentina*

Introduction: Neurofibromatosis Type 1 (NF1) is associated with significant neurocognitive and emotional challenges. Emotional intelligence (EI) refers to the ability to recognize, understand, manage, and regulate both one's own emotions and those of others. Deficits in EI may impact social interactions, quality of life, and psychological well-being. This study aims to assess the effectiveness of different rehabilitation strategies in improving EI in NF1 patients using a structured intervention framework, contributing to the identification of personalized, evidence-based treatments.

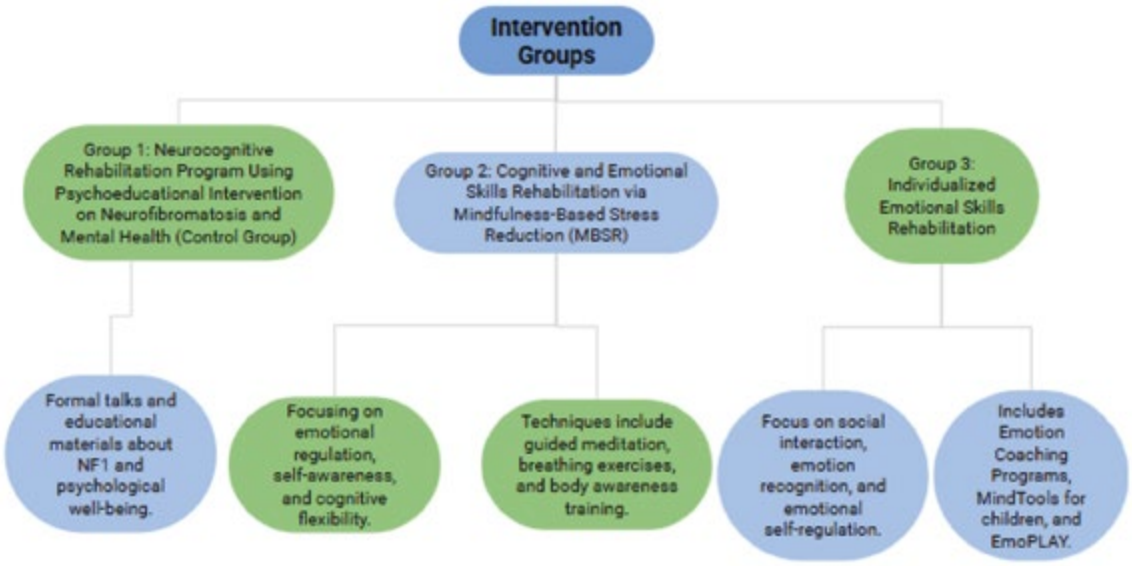
Objectives:
***Primary:** To evaluate the impact of three different rehabilitation programs on EI and regulation in NF1 patients.
***Secondary:**

- To compare the effectiveness of interventions in improving quality of life and emotional regulation.
- To assess changes in clinical symptoms, including pain management, following rehabilitation.
- To determine the feasibility and acceptability of different intervention strategies.

Study Design: This is a prospective, randomized, interventional clinical study. The sample will include 60 patients diagnosed with NF1, aged 16-24 years. Intervention duration is 24 weeks. Patients will be randomized into three intervention groups. Pre- and post-intervention comparisons will be made using repeated-measures ANOVA. Qualitative feedback from participants will be analyzed to evaluate program acceptability.
Baseline and Follow-up Assessments:
1- Baseline Evaluation: EI and regulation will be assessed using:

- **Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT)** (objective measure of EI as a cognitive ability).
- **Difficulties in Emotion Regulation Scale (DERS)** (self-report measure of emotional regulation deficits).
- **Behavior Assessment System for Children, Third Edition (BASC-3)** (assesses emotional, behavioral, and social functioning in real-life contexts).
- Quality of life and pain perception measures will also be collected.

2- The same assessments will be repeated after 24 weeks to measure intervention outcomes.



Outcome Measures: Primary outcomes will include changes in baseline scores. Secondary outcomes will include changes in quality of life, improvement in pain perception and management, and participant adherence and satisfaction with the interventions.

Conclusion: Emotional intelligence is considered a fundamental skill for all individuals, and rehabilitating these aspects in children with NF1 could significantly impact their ability to manage emotions, improve interpersonal relationships, and cope with challenging situations. This study aims to provide critical insights into the most effective intervention strategies for improving EI and emotional regulation in NF1 patients. The findings could guide the development of targeted rehabilitation programs, enhancing social and emotional functioning in this population, leading to a positive effect on their quality of life and self-esteem.

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Adaptive Functioning in NF1: The Influence of Cognition, Executive Functioning, and Social Determinants of Health in Children and Young Adults

Tina Thomas, PhD, *Children's National Hospital*

Purpose: Adaptive functioning (AF) is a crucial set of skills necessary for building independence and preparing for transition to adulthood. These abilities are often underdeveloped in individuals with Neurofibromatosis Type 1 (NF1), for whom transition planning is of vital importance given the range of medical, cognitive, and psychosocial challenges they face. We aimed to understand the impact of executive functioning (EF), intelligence (IQ), and social determinants of health (SDOH) on AF in the NF1 population, as an important step in developing interventions to support transition readiness. We hypothesized that IQ, EF, and SDOH would be associated with AF.

Methods: We extracted data from a large retrospective clinical neurocognitive dataset and medical record review. Inclusion criteria were NF1 patients who were clinically administered an adaptive measure. Neurocognitive data included IQ (Weschler scales), EF (Delis-Kaplan Executive Function System [D-KEFS] and parent-proxy reported Brief Rating Inventory of Executive Function [BRIEF]), and AF data (parent-proxy reported Adaptive Behavior Assessment System [ABAS]). The Child Opportunity Index 3.0 (COI) was extracted as a measure of SDOH. Impairment was classified as 1.5 SD below the mean. Statistical methods included parametric and non-parametric correlations and multiple regressions.

Results: Participants (N=62) were 12.34 years of age (SD=3.66, Range=8-23) and 47.7% female. Average IQ was 80.95 (SD=16.21, Range=47-115) with 36.9% classified as impaired. Adaptive data had a mean of 83.52 (SD=17.43, Range=50-120) with 29.2% classified as impaired. AF was significantly correlated with parent-reported EF (N=62, r =-0.68, $p<0.001$) and DKEFS Verbal Fluency Switching (N=32, r=0.42, p=0.016), but not IQ or COI. In regression, parent-reported EF strongly predicted AF (β=-0.57, $p<0.001$), although DKEFS Switching did not (β=0.27, p=0.070). When subdomains of parent-reported EF were examined in regression, cognitive regulation strongly predicted AF (β=-0.73, p=0.001) but emotional and behavioral regulation did not. When EF predictors were regressed on AF subdomains, cognitive regulation remained most predictive: conceptual (β=-0.64, p=0.007), social (β=-0.49, p=0.044), and practical (β=-0.61, p=0.007).

Conclusion: Consistent with previous literature, EF, specifically cognitive regulation, significantly predicted AF in our sample of children and young adults with NF1. IQ and COI were not related to AF. Small sample size was a limitation of this study. Future research may consider further analysis of medical risk factors as well as individual and family factors on AF. Overall, the current findings suggest that interventions targeted towards "real world" application of EF factors, particularly cognitive regulation skills, may help to facilitate AF development in the NF1 population.

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Not-So-Sweet Dreams: Sleep and Emotion Regulation Problems in Children with NF1

Jarrod J. Sotos, PhD, *Children's National Hospital*

Purpose: Children with Neurofibromatosis Type 1 (NF1) face well-documented cognitive and attentional challenges and are also at heightened risk for internalizing difficulties (e.g., anxiety, depression, somatization), externalizing behaviors (e.g., impulsivity, aggression, oppositionality), and emotion regulation deficits. A recent caregiver survey conducted by this group highlighted significant sleep disturbances (e.g., trouble falling asleep, frequent nighttime awakenings) and impairments (e.g., daytime sleepiness, attention difficulties, irritability) in children with NF1. Despite these findings, research on sleep in NF1 and its connection to emotion regulation and internalizing/externalizing (I/E) behaviors remains limited. This study expands on our previous work by investigating the relationship between sleep difficulties and emotion regulation in children with NF1 using norm-referenced parent questionnaires.

Methods: Caregivers of 31 children with NF1 (ages 6-16) provided ratings of their child's emotion regulation and I/E behaviors using the Behavior Rating Inventory of Executive Function - Second Edition (BRIEF-2) and the Behavior Assessment System for Children – Third Edition (BASC-3). Sleep disturbance and sleep impairment were measured using parent-proxy Patient Reported Outcomes Measurement Information System (PROMIS) measures. Additional sleep factors were assessed using the Child and Adolescent Sleep Checklist (CASC). Data were analyzed using Pearson correlations and independent samples t-tests.

Results: Participants (M_{age} = 10.83 years, SD = 2.95) averaged 8.78 hours of sleep on weeknights (SD = 1.51), with 39% (n = 12) reporting sleep disturbance and impairment, respectively. Those with sleep difficulties slept 1 to 1.5 hours less per night than their peers. Sleep disturbance and impairment were significantly associated with greater emotion regulation difficulties, internalizing behaviors, and externalizing behaviors. T-tests indicated that children with sleep disturbance and impairment exhibited more pronounced emotion regulation challenges and higher levels of internalizing symptoms, along with lower adaptability, social skills, leadership, daily living skills, and communication abilities compared to those without sleep difficulties. However, no significant differences emerged between groups in externalizing behaviors. Exploratory analyses of specific internalizing behaviors revealed that those with reported sleep disturbance had reported greater somatization symptoms, and participants with sleep impairment reported greater challenges with mood.

Table 1. Test statistics and significance levels for correlational and group comparison analyses

		Pearson Correlations		Independent Samples T-Tests	
Domain	Variable	Sleep Disturbance	Sleep Impairment	Sleep Disturbance	Sleep Impairment
Emotional Regulation	Shift	$r = .62$ ($p < .001$)	$r = .54$ ($p < .001$)	$t = 2.84$ ($p = .004$)	$t = 2.51$ ($p = .009$)
	Emotional Control	$r = .50$ ($p = .024$)	$r = .52$ ($p < .001$)	$t = 1.96$ ($p = .030$)	$t = 2.86$ ($p = .004$)
	BRIEF Emotion Regulation	$r = .59$ ($p < .001$)	$r = .58$ ($p < .001$)	$t = 2.59$ ($p = .007$)	$t = 3.07$ ($p = .002$)
Internalizing Behaviors	Anxiety	$r = .59$ ($p < .001$)	$r = .51$ ($p = .002$)	$t = 2.36$ ($p = .012$)	$t = 2.77$ ($p = .005$)
	Depression	$r = .55$ ($p < .001$)	$r = .54$ ($p < .001$)	$t = 2.37$ ($p = .012$)	$t = 3.50$ ($p < .001$)
	Somatization	$r = .53$ ($p < .001$)	$r = .49$ ($p = .005$)	$t = 2.72$ ($p = .005$)	$t = 2.25$ ($p = .016$)
	BASC-3 Internalizing Index	$r = .63$ ($p < .001$)	$r = .57$ ($p < .001$)	$t = 2.98$ ($p = .003$)	$t = 3.28$ ($p = .001$)
Externalizing Behaviors	Hyperactivity	$r = .42$ ($p = .009$)	$r = .33$ ($p = .036$)	$t = 1.52$ ($p = .070$)	$t = 1.52$ ($p = .070$)
	BASC-3 Externalizing Index	$r = .38$ ($p = .017$)	$r = .34$ ($p = .031$)	$t = 1.36$ ($p = .092$)	$t = 1.68$ ($p = .052$)
Additional BASC-3 Subscales	Adaptability	$r = -.50$ ($p = .002$)	$r = -.52$ ($p = .001$)	$t = -1.46$ ($p = .077$)	$t = -2.76$ ($p = .005$)
	Social Skills	$r = -.39$ ($p = .014$)	$r = -.45$ ($p = .006$)	$t = -2.01$ ($p = .027$)	$t = -2.51$ ($p = .009$)
	Leadership	$r = -.44$ ($p = .001$)	$r = -.60$ ($p < .001$)	$t = -1.62$ ($p = .058$)	$t = -3.95$ ($p < .001$)
	Daily Living Skills	$r = -.53$ ($p = .001$)	$r = -.68$ ($p < .001$)	$t = -2.77$ ($p = .005$)	$t = -5.17$ ($p < .001$)
	Communication	$r = -.43$ ($p = .008$)	$r = -.49$ ($p = .003$)	$t = -1.43$ ($p = .082$)	$t = -1.86$ ($p = .037$)

Note. Variables with non-significant results are not listed to maintain focus on key findings.

Conclusions: This study highlights the significant impact of sleep disturbance and impairment on emotion and behavior functioning in children with NF1. Those with disrupted sleep show greater emotion dysregulation and increased internalizing symptoms, particularly somatization and mood issues. Clinicians should assess sleep routinely and implement targeted interventions to improve sleep quality, reducing emotional distress and enhancing well-being.

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Quantitative Myelin Differences in Neurofibromatosis -1 Using T1W/T2W Ratio MRI Study

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It has been hypothesised that children with NF1 show an aberrant brain-behaviour relationship linked to Autism Spectrum Conditions (30%) and Attention Deficit Hyperactivity Disorder (50%) (Garg et al., 2013). Previous studies have shown T2-white matter hyperintensities (in the thalamus and basal ganglia) and widespread white matter microstructure differences (diffusion MRI) in NF1 suggesting aberrant myelination (Karlsgodt et al., 2012, Feldmann et al., 2010). However, no studies have investigated quantitative myelin in NF1 yet. Objectives: (i) Compare quantitative myelin levels across the grey and white matter at whole brain and lobar level between NF1 and neurotypicals, and determine (ii) Relationship between estimated myelin levels and working memory (WM) performance in children with NF1.

Participants include forty-eight children and adolescents with NF1 (age: 11-18 years; gender: 25 males and 23 females). T1-weighted and T2-weighted structural images were acquired using a Philips 3T MRI scanner. One hundred and sixty-eight (age and gender-matched) control structural MRI images were collected from the Human Connectome Project. Visuospatial n- back tasks were used to ascertain WM performance. The image preprocessing pipeline includes bias-field correction, coregistration, calculation of T1W/T2W maps, segmentation, spatial normalisation and calculation of GM/WM metrics. ANCOVA and Pearson's partial correlation was used to test the whole brain and lobar level GM/WM myelin in the case-control and correlation analysis in NF1 respectively, while the effects of age and gender were controlled. In addition, age and sex-related myelin differences were tested between NF1 and controls.

Our results showed significantly reduced grey matter/white matter at whole brain level ($F=28.165$, $p<0.001$), and the frontal ($F=6.906$, $p<0.009$), temporal ($F=27.548$, $p<0.001$), parietal ($F=7.440$, $p<0.007$) and occipital ($F=28.906$, $p<0.001$) lobes in NF1 relative to neurotypical controls. Stratified age- and sex-related myelin differences were found significantly low in NF1 than the controls at whole brain level. However, no significant correlation was found between myelin and WM performance either at whole brain or lobar level in NF1.

Our findings show that quantitative GM/WM myelin differences are consistent with previous animal model studies in NF1 (López-Juárez et al., 2017). Reduced GM/WM myelin in the whole brain and the frontal, temporal, parietal and occipital lobes suggest NF1 gene mutation may affect oligodendrocytes producing myelin in NF1. Low quantitative myelin may manifest as a consequence of loss of neurofibromin in NF1 leading to reduction in mature oligodendrocytes/ decompaction of myelin (Mayes et al., 2013). T1W/T2W ratio is a useful neuroimaging biomarker to identify quantitative myelin differences in NF1.

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Ethical Requirements: This study was conducted after receiving approval from the NHS research ethics committee.

Systematic Review: Biopsychosocial Factors Related to Attention-Deficit/Hyperactivity Disorder in Children and Adolescents with Neurofibromatosis Type 1

Yang Hou, PhD, *Florida State University*

Purpose: The risk for Attention-Deficit/Hyperactivity Disorder (ADHD) is about nine times greater for individuals with neurofibromatosis type 1 (NF1), compared to the general population. Understanding what factors may contribute to ADHD (symptoms or diagnosis) in individuals with NF1 can inform future management and intervention development to improve their ADHD symptoms and daily life functioning. This systematic review aimed to comprehensively summarize findings on biopsychosocial factors related to ADHD symptoms or diagnosis among children and adolescents with NF1 and identify limitations and gaps in existing literature.

Methods: Systematic literature searches were conducted in Scopus, PsycINFO, Web of Science, PubMed, and ProQuest Dissertations and Theses Global in March 2024. The searches identified 2,345 unique articles. Two reviewers independently screened articles and extracted data with discrepancies resolved through consensus meetings with a third reviewer. We included 68 articles that met these criteria: (1) examined the associations between ADHD symptoms (reported by self, parents, caregivers, or teachers) and other variables in individuals with NF1, or conducted between-group comparisons to evaluate differences in other variables between individuals with NF1 who did and did not have an ADHD diagnosis; (2) focused on individuals with NF1 aged 18 years or younger; (3) were published in a peer-reviewed academic journal or as an unpublished thesis or dissertation. We synthesized results across studies in a narrative format, following the Guidance on the Conduct of Narrative Synthesis in Systematic Reviews.

Results: Based on the biopsychosocial model of mental health, we categorized the study findings on factors related to ADHD in children and adolescents with NF1 into three broad groups: biological (n = 33), psychological (n = 47), and social factors (n = 14), which were further divided into subcategories (Figure 1). Findings varied across biopsychosocial factors, ADHD variables (inattention, hyperactivity, impulsivity symptoms, or ADHD diagnosis), measurement methods, study designs, and statistical approaches. Psychological factors were most commonly studied. Findings revealed consistent evidence linking informant-reported executive functioning problems with ADHD, whereas performance-based cognitive measures generally showed no significant associations. Most studies are cross-sectional and included small and nonrepresentative samples.

Conclusion: Our review highlights significant gaps in the current literature regarding the study of biopsychosocial factors related to ADHD in children and adolescents with NF1. Methodological limitations, including small samples, low representativeness, and predominance of correlational designs, hinder the ability to make definitive conclusions and establish causal relationships. Future research should address these issues by utilizing larger, more representative samples, and employing experimental and intervention-based designs. Future research should also explore a broader range of biological and environmental predictors of ADHD. Particularly, there is a critical need for studies examining genetic and peripheral biomarkers, social factors, as well as gene-environment interplay, which could significantly advance our understanding of ADHD in NF1. Finally, leveraging technological advancements, such as ecological momentary assessment, may offer new insights into the dynamic nature of ADHD symptoms and their management, ultimately informing more personalized, real-time interventions to improve daily functioning of children and adolescents with NF1.

Figure 1: Biopsychosocial Factors Associated with ADHD Symptoms/Diagnosis in Children and Adolescents with NF1

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Cognitive Profiles in Pediatric Patients with Neurofibromatosis Type 1: A Retrospective Review Study of 55 Pediatric Patients

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Purpose: Neurofibromatosis type 1 (NF1) is a genetic disorder frequently associated with cognitive impairments[1]. This study characterizes the cognitive profiles of pediatric patients with NF1 using standardized neuropsychological assessments.

Methods: We retrospectively reviewed charts of 55 patients with NF1 who underwent neuropsychological testing before age 18 (between August 1, 1988, and August 1, 2023). Inclusion criteria were a confirmed NF1 diagnosis (molecular or clinical) and standardized developmental or neuropsychological testing by a developmental-behavioral pediatrician or neuropsychologist. Measures included the Wechsler Intelligence Scales: Wechsler Intelligence Scale for Children (WISC-IV/V), Wechsler Adult Intelligence Scale (WAIS-III/IV), and Differential Ability Scales (DAS-II). Standardized testing subtests assessing similar skills were grouped into indexes according to domain. This yielded composites of verbal and nonverbal reasoning, visual-spatial reasoning, working memory, processing speed, and overall IQ. Additionally, data on common comorbidities, including depression, anxiety, attention-deficit/hyperactivity disorder (ADHD), and autism spectrum disorder (ASD) were collected.

Results: Overall IQ in this sample of pediatric patients with NF1 was shifted downward to the left with a mean of 87 ± 16. Verbal reasoning skills were assessed in 49% of patients and emerged as a relative strength, with a mean of 93 ± 15. Working memory was the most notable weakness in this cohort, with the mean score (sample mean=84, SD=15) of 26 patients (47% of sample) falling more than one standard deviation below the standardization sample mean (standardization mean=100, SD=15). Nonverbal reasoning and visual-spatial reasoning were assessed in a smaller portion of the sample (33% and 25%, respectively) with mean scores falling low average (90 ± 17 and 87 ± 18). Processing speed in this sample was low average compared to the general population (M=90, SD=19).

Regarding comorbid conditions, ADHD was diagnosed in 39 of the 55 patients (71%), anxiety in 24 (44%), depression in 14 (25%), and autism in 7 (13%).

Conclusion: Pediatric patients with NF1 exhibit cognitive variability, with weaknesses in working memory that may impact academic performance. Our study is limited by its retrospective design, small sample size and selection bias. Only patients with NF1 whose physicians suspected neurocognitive or developmental issues were referred for developmental or neuropsychological testing, potentially skewing the sample to be more impaired. However, it is also possible that patients with true impairments were overlooked and not referred. High rates of ADHD, anxiety, depression, and autism emphasize the need for targeted interventions and further research on cognitive trajectories and tailored educational strategies.

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The Role of Executive Functioning, Childhood Opportunity, and Social Cognition in the Social Function of Children with Neurofibromatosis Type 1

Matthew C. Hocking, PhD, *The Children's Hospital of Philadelphia; Perelman School of Medicine at The University of Pennsylvania*

Introduction: Youth with Neurofibromatosis Type 1 (NF1) experience social impairments which impact their overall quality of life. Prior research indicates that social impairments are related to executive functioning difficulties. Theoretical models suggest that other social-cognitive (e.g., theory of mind) and socio-emotional (e.g., face processing) skills, along with social determinants of health, shape social outcomes, but minimal research has evaluated these additional factors in NF1. We investigated associations between social functioning and executive functioning, childhood opportunity, theory of mind, and face processing skills in children with NF1.

Methods: Youth with NF1 ($n = 37$) ages 8-14 ($M = 11.8$ years) completed the Theory of Mind (ToM) Comic Strip task (Brunet), the Victoria/Yale Face Processing Battery (VYFPB), and the Patient-Reported Outcomes Measurement Information System (PROMIS) Peer Relationships questionnaire. Caregivers completed the Behavior Rating Inventory of Executive Function (BRIEF) and Social Responsiveness Scale (SRS-2). The Childhood Opportunity Index (COI), a census-tract based measure of social determinants of health, assessed neighborhood opportunity across education, health and environment, and socioeconomic domains. Pearson and Spearman correlations and linear regression analysis assessed associations among executive, social cognitive and socio-emotional domains, neighborhood opportunity, and social functioning.

Results: Executive functioning (BRIEF GEC), overall childhood opportunity, and facial identity recognition (FIR) were significantly correlated with social impairments (SRS-2 Social Communication and Interaction (SCI)), while theory of mind and facial emotion recognition (FER) were not. BRIEF GEC ($\beta = 0.73$, $p < .01$) and overall childhood opportunity ($\beta = -0.18$, $p < .01$), but not FIR, were significantly associated with SRS-SCI scores in regression analysis (overall model: $R^2 = 0.67$, $p < .01$).

Executive functioning (BRIEF GEC) and FER were the only factors significantly correlated with peer relationship quality (PROMIS). While BRIEF GEC ($\beta = -0.22$, $p > .05$), and FER ($\beta = 0.16$, $p > .05$), were not independently significant in regression analysis, the overall model was significant (overall model: $R^2 = 0.23$, $p < .05$).

Conclusion: Findings underscore the importance of multi-domain assessment of both internal, cognitive factors and external, socio-ecological factors when understanding the social functioning of youth with NF1. Notably, the association between neighborhood opportunity and social function is novel. Longitudinal research with larger samples is needed to assess these cognitive processes and community-level factors to inform interventions that enhance the social function in children with NF1.

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Quantifying Self-Care Among Adults with Neurofibromatosis 1: Validation of a New Disease-Specific Measure

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Purpose: Neurofibromatosis 1 (NF1) requires complex self-care, including symptom management, identifying healthcare, and addressing psychological health. The Self-Care of Chronic Illness Inventory (SC-CII) is a widely-used "generic" measure of self-care among people with chronic illness that quantifies monitoring, management, and maintenance of chronic illness, and self-care confidence (Riegel et al., 2012). Riegel and colleagues have subsequently validated several disease-specific versions of the SC-CII for self-care behaviors relating to specific health conditions (e.g., hypertension, diabetes, and heart disease). There exists no self-care measure specific to NF1. The research objective was to create a disease-specific patient-reported outcome measure of self-care in people with NF1.

Methods: Participants were 317 adults with NF 1 recruited from the Neurofibromatosis Registry (age 18-80, 71.3% female, 86.4% Caucasian, 33.5% college-educated, 54.6% with Worst Pain > 4 on a 1-10 scale) who completed measures online. An earlier study (Leif & Zelman, 2024) conducted interviews with 22 individuals with NF1 to establish a compendium of self-care behaviors, and based on these findings, self-care theory and the generic SCI-II, sixty-eight candidate items for the new self-care measure were generated. Each item described a form of self-care scored on a 1-5 Likert frequency scale ranging from Never/Rarely to Very Often. Participants also completed the 20-item generic SC-CII (Riegel et al., 2018) and measures of pain, sleep, quality of medical care and quality of life.

Results: In several iterations of Confirmatory Factor Analysis (CFA), items were dropped at each step to improve fit statistics to establish a set of domains corresponding to important self-care constructs. The final 22-item solution comprised five subscales (Medical Monitoring, Self-Monitoring, Engaging Social Support, Stress Management, and Self-Maintenance) and two four-item pain self-care subscales for those with pain (Personal Pain Care and Pain Medication/ Professional Interventions). The new disease-specific NF1 measure better met criteria for CFA relative to the generic SCI-II, and showed stronger relationships with key outcomes such as anxiety, depression, sleep, pain, autonomy and relatedness with the healthcare team.

Conclusion: This research was the first study to quantify disease self-care in NF1 and is one of few studies to investigate pain management as an aspect of self-care in NF1 (e.g., Buono et al.,2018). This is a promising measure for quantifying self-care that can also stimulate dialogues in healthcare settings about care needs. Subsequent research will continue to establish the suitability of this measure for patients and clinicians.

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Age-Varying Associations Between ADHD Symptoms and Internalizing/Externalizing Behaviors in Children with Neurofibromatosis Type 1: Integrative Analyses of Data from Six Institutions

Dan Liu, PhD, *Florida State University*

Purpose: Children with neurofibromatosis type 1 (NF1) experience higher rates of Attention-Deficit/Hyperactivity Disorder (ADHD) and internalizing and externalizing behaviors compared to their typically developing peers. The severity of these symptoms flucatuates across different ages; however, the age-varying associations between ADHD symptoms and internalizing or externalizing behaviors in children with NF1 remain poorly understood. This study aims to investigate these associations across various ages to inform the development of age-appropriate interventions.

Methods: We integrated individual-level data from 685 children with NF1 (ages 3–18 years) across six institutions in the United States and Australia, using integrative data analysis procedures. ADHD symptoms (inattention, hyperactivity/impulsivity, and combined ADHD) were assessed through parent-reported Conners Rating Scales and ADHD Rating Scales. Internalizing and externalizing symptoms were measured using the Child Behavior Checklist and the Behavior Assessment System for Children. Time-Varying Effect Modeling (TVEM), a nonparametric method, was used to examine the associations between ADHD symptoms and internalizing and externalizing symptoms across ages, while controlling for sex. TVEM estimates these associations as continuous functions of age, providing curvilinear intercept and slope estimates with 95% confidence intervals (CIs). Significant associations are indicated by 95% CIs that do not include zero, and significant age-related differences by non-overlapping 95% CIs at specific age points.

Results: Elevated inattention, hyperactivity/impulsivity, and combined ADHD scores were consistently correlated with elevated internalizing and externalizing symptoms from ages 3 to 17 (see **Figure 1** for an example). The strength of these correlations remained stable across ages, with one exception. Specifically, the correlation between hyperactivity/impulsivity and externalizing symptoms was most pronounced during early childhood (ages 5–8), diminished through late childhood, reached its lowest point during early adolescence (ages 13–14), and then stabilized in middle adolescence **(Figure 2)**.

Conclusion: Findings are consistent with existing research on the associations between ADHD symptoms and internalizing/externalizing behaviors in the general population. The results indicate that children with NF1 who exhibit elevated internalizing and externalizing symptoms should be continuously monitored for ADHD symptoms from early childhood through middle adolescence. Interventions targeting these symptoms may be more effective if they concurrently address ADHD symptoms, with particular attention to developmental stages. For example, early childhood interventions focusing on hyperactivity/impulsivity may be particularly beneficial for mitigating externalizing symptoms, in contrast to interventions in early adolescence.

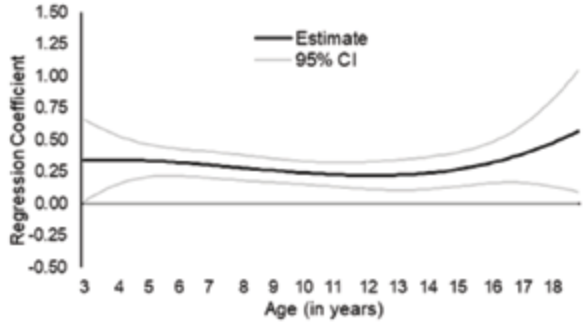

Figure 1. Age-varying associations between inattention and internalizing symptoms. Significant associations are indicated by 95% confidence intervals (CIs) that do not include zero. Significant age differences are indicated by non-overlapping 95% CIs between specific age points.

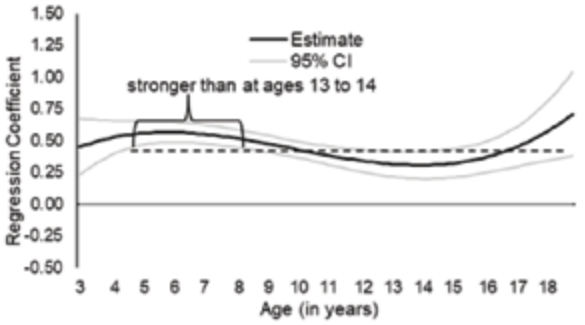

Figure 2. Age-varying associations between hyperactivity/impulsivity and externalizing symptoms. Significant associations are indicated by 95% confidence intervals (CIs) that do not include zero. Significant age differences are indicated by non-overlapping 95% CIs between specific age points.

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Age-Varying Associations Between Executive Function and Internalizing/Externalizing Behaviors in Children with Neurofibromatosis Type 1: Integrative Analyses of Data from Nine Institutions

Dan Liu, PhD, *Florida State University*

Purpose: Children with neurofibromatosis type 1 (NF1) frequently encounter cognitive and behavioral difficulties, such as challenges with executive function and both internalizing and externalizing behaviors. The severity of these issues can change over time. While executive function is known to relate to internalizing and externalizing behaviors differently at different ages in typically developing children, the pattern of these associations in children with NF1 is unclear. This study aims to explore these associations across ages to deepen our understanding and inform the development of age-appropriate interventions.

Methods: Individual-level data from 1,049 children with NF1, ages 3–18 years, across nine institutions in the U.S. and Australia were combined using integrative data analysis. Executive function, including inhibitory control, flexibility, emotional control, working memory, and planning/organization, was assessed with parent-rated Behavior Rating Inventory of Executive Function (BRIEF). Internalizing and externalizing symptoms were measured using the Child Behavior Checklist and the Behavior Assessment System for Children. Time-varying effect modeling (TVEM), a nonparametric method, was used to examine the associations between executive function and internalizing and externalizing symptoms across different ages, while controlling for sex. TVEM estimates these associations as continuous functions of age, providing curvilinear intercept and slope estimates with 95% confidence intervals (CIs). Significant associations are indicated by 95% CIs that do not include zero, and significant age-related differences by non-overlapping 95% CIs at specific age points.

Results: Elevated BRIEF scores, indicating greater impairment in all five domains, were consistently associated with greater internalizing and externalizing symptoms from ages 3 to 18 years. The magnitude of some associations varied significantly across different ages. Notably, the association between emotional control and internalizing symptoms **(Figure 1)** increased over time, becoming significantly stronger in middle to late adolescence (ages 16-18) compared to early childhood (ages 4-7). In contrast, the association between inhibitory control and externalizing symptoms **(Figure 2)** was strongest in early childhood (ages 5-8) and significantly decreased during early and middle adolescence (ages 12-16).

Conclusion: The findings indicate that impaired executive function may contribute to persistent internalizing and externalizing problems among children with NF1 from early childhood through late adolescence. Interventions targeting different executive function domains could help mitigate these emotional and behavioral issues, with their effectiveness potentially varying by developmental stage. For example, early childhood interventions that focus on improving inhibitory control may be particularly effective in reducing externalizing symptoms, while interventions aimed at enhancing emotional control may be especially beneficial for addressing internalizing problems during adolescence.

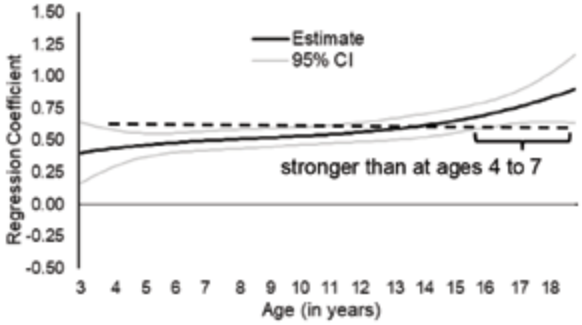

Figure 1. Age-varying associations between emotional control and internalizing symptoms.

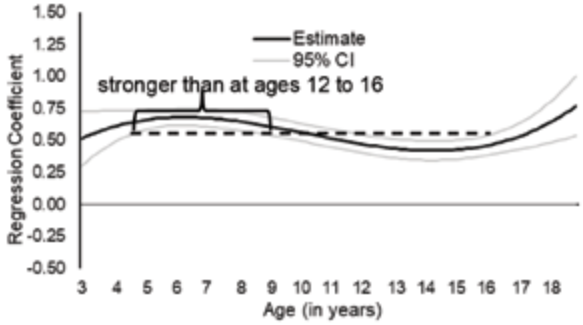

Figure 2. Age-varying associations between inhibitory control and externalizing symptoms.

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Examining the Effect of Non-Invasive Brain Stimulation on Working Memory in NF1

Anna Wild, PhD, *Division of Psychology & Mental Health, The University of Manchester*

Introduction: Neurofibromatosis type 1 (NF1) is a neurodevelopmental condition often associated with learning difficulties, thought to stem from excitation/inhibition (E/I) imbalances. While animal models indicate excess GABAergic activity contributes to learning difficulties, human studies, using magnetic resonance spectroscopy (MRS), suggest a more complex relationship. Here we examine the effect of two different types of brain stimulation – transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) on E/I measured by using functional MRS (fMRS) to obtain dynamic measures of the E/I balance. Our primary aim is to determine whether tDCS and tACS modulate E/I and can improve working memory function in NF1.

Methods: Thirty adolescents with NF1, aged 11-17 years, participated in three visits, receiving a control sham, tACS or tDCS stimulation simultaneously whilst performing a 2-back working memory task. We measured GABAergic activity pre and post stimulation using MRS and measured glutamatergic activity during stimulation using fMRS. In addition, working memory, attention and inhibition tasks were administered pre and post stimulation outside the MR scanner. We used mixed-effects models to compare glutamate, GABA changes and performance changes due to stimulation. Additionally, regression and correlation analyses explored the association of E/I modulation with performance changes, as well as baseline GABA and glutamate's association with baseline performance.

Results: Preliminary results (n=15) suggest tACS significantly improved 2-back working memory relative to sham, but tDCS had no effect. There was no improvement on task performance post stimulation, although tDCS was associated with worse stroop task performance relative to tACS and sham. Application of both tDCS and tACS was associated with increase in excitatory glutamatergic activity relative to sham. No association was found between glutamate and GABA and task performance changes, although baseline GABA was positively associated with greater baseline visual-spatial N-back performance.

Conclusion: Preliminary results suggest that both tACS and tDCS have a modulatory effect on glutamatergic activity. tACS may be superior to tDCS in improving working memory function. Randomised clinical trials are needed to confirm the therapeutic potential of tACS in clinical populations. However, further analysis with the full dataset will clarify GABA's role and assess the overall E/I balance in NF1.

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The Intersection Between Mental Health and Gender Identity in NF1: Results of the IMAGiN Survey Study

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Purpose: Neurofibromatosis Type 1 (NF1) is an autosomal dominant neurogenetic disorder with an incidence of approximately 1 in 2,700 resulting from a germline mutation in the NF1 gene leading to neurofibromin dysfunction and a wide range of clinical manifestations. In addition to conferring a high risk of neural crest lineage-derived tumors such as cutaneous neurofibromas (CNs) and plexiform neurofibromas (PNs), NF1 is associated with cognitive and psychologic challenges such as learning disabilities, autism, mood disorders, and lower IQ. CNs are amongst the most distressing aspects of NF1 due their physical and social impact. While previous research has explored the incidence of mental health disparities in NF1, limited data exist on how race, ethnicity, sexual orientation, and gender influence mental health outcomes and healthcare experiences in individuals with NF1. This survey study assessed correlations between mental health metrics and racial/gender identity in adult participants with NF1.

Methods: A 27-question online survey was developed to assess mental health and life experiences in individuals with NF1. Distribution channels included CTF.org (Children's Tumor Foundation) and private Facebook support groups for NF1. Data cleaning ensured participant validity and response quality. Statistical analysis used chi-square tests and logistic regression analysis.

Results: Of 12,764 completed surveys, data cleaning to eliminate malignant and bot responses resulted in 1549 valid responses (109 transgender/gender diverse (TGD), 1474 cisgender). TGD and cisgender respondents did not significantly differ in the self-reported im-pact of NF1 on mental health. However, TGD respondents reported significantly more poor mental health days over the preceding month. Controlling for race and disease severity, TGD respondents were 1.4x more likely to experience $\geq$10 poor mental health days in a month. TGD respondents also had higher rates of schizophrenia, bipolar disorder, and post-traumatic stress disorder compared to cisgender. Gender identity had a significant correlation with the frequency and prevalence of mental health disorders ($P<.05$). Participants reported diagnostic criteria of NF1 which were categorized as less visible (mutation, parent affected, inguinal/axillary freckling), more visible (cutaneous neurofibromas, café-au-lait macules), or more painful/functionally impactful (optic pathway glioma, plexiform neurofibroma). Participants with more visible features had 2.4 times increased risk of poor mental health in the preceding four weeks compared to those with only less visible symptoms ($P<.05$). Those with more visible and symptomatic symptoms had 1.7 times higher odds of prolonged poor mental health than those reporting only less visible symptoms, significant at a 90% confidence level. Women were 1.2 times more likely than men to avoid mental health treatment due to hesitancy (fear of asking or receiving treatment) and cost ($P<.05$).

Discussion/Conclusion: Our findings suggest that gender identity plays a significant role in the duration and prevalence of poor mental health. Participants with more severe or visible symptoms were at higher risk of experiencing prolonged mental health challenges, underscoring the impact of the physical stigmata associated with NF1. Furthermore, women were more likely than men to avoid seeking mental health treatment due to hesitancy and financial barriers.

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Differentiating Youth with NF1 and Unaffected Youth with Neurocognitive and Cerebellum Neuroimaging Metrics Using Support Vector Modeling

Sara Katharine Pardej, PhD, *Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine*

Purpose: The cerebellum is implicated in several domains of functioning that are known areas of weakness for youth with neurofibromatosis type 1 (NF1), including executive, motor, and language skills. The goal of the present study is to use machine learning (i.e., support vector model) to examine whether cerebellar structural components (in addition to neurocognition) aid in the differentiation of youth with NF1 and unaffected youth. Components that are strong differentiators may be useful clinical trials targets in future interventions.

Methods: The sample included 30 youth (5-16 years) with NF1 (M_{age}=9.05 years SD_{age}=2.25) and 40 unaffected youth (M_{age}= 9.76 years SD_{age}=3.07). The variables included in the support vector model were neurocognitive variables with domains relevant to cerebellar processes, including executive function, motor skills, reading, language, and IQ. Neuroimaging metrics included cerebellar regional volumes, and DTI metrics (Fractional Anisotropy, Mean Diffusivity (MD), Neurite Density Index (NDI), and Orientation Dispersion Index). Three models were run to evaluate the contributions of neurocognitive and neuroimaging data to NF1 versus unaffected classification. Thus, these models included (1) all data; (2) only neuroimaging metrics; and (3) only neurocognitive metrics. To validate the primary model of interest (model 1), permutation testing (n=1000) was conducted, and the resultant sensitivity and specificity values were compared to model 1.

Results: Positive weights indicate that higher values are more associated with an NF1 classification, while negative weights indicate that higher values are more associated with an unaffected classification. Model 1, including neurocognitive data, regional volume, and DTI metrics correctly classified 90.20% participants as being in the NF1 or unaffected group (AUC: 0.99) with high sensitivity (93.10%) and moderate specificity (86.36%). The top five weights were white matter volume (positive weight), PROMIS Mobility ratings (negative weight), mean NDI (negative weight), Vermis IX volume (positive weight), and mean MD (positive weight). By including only neuroimaging metrics in Model 2, the model's predictive accuracy was decreased, with 86.27% of participants being correctly classified (AUC: .91). While sensitivity (93.10%) remained high, the specificity decreased (77.27%). Model 3, including only neurocognitive data, correctly predicted 80.39% of participants as being in the NF1 or unaffected group (AUC: .92), with moderate sensitivity (82.76%) and low specificity (77.27%).

Conclusion: Of the three models run, Model 1 (neuroimaging and neurocognitive data) had the highest rate of accuracy, sensitivity, and specificity. Neuroimaging metrics are important for both identifying "true positives" and "true negatives" as it pertains to classification of NF1 versus unaffected youth. Top neuroimaging weights (in order of absolute strength) in Model 1 were cerebellar white matter volume, NDI, Vermis IX volume, MD, and Vermis VII volume. This lends credibility to the importance of cerebellar white matter, Vermis IX and VII volume, as well as white matter microalterations being important distinguishing features between youth with NF1 and unaffected youth and should be the focus of future neuroimaging research as potential biomarkers of neurocognitive features present in NF1.

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Effects of Musical Practice on Auditory and Executive Functions in Individuals with Neurofibromatosis Type 1

Bruno Cezar Lage Cota, PhD, *Neurofibromatosis Reference Center, Hospital das Clínicas, Federal University of Minas Gerais, Brazil*

Background: Neurofibromatosis type 1 (NF1) is a multisystemic genetic disorder often associated with impairments in executive functions and auditory processing, including musical perception.

Objective: To examine the relationship between auditory and executive function deficits in individuals with NF1 and to assess the potential therapeutic effects of musical practice on these domains.

Methods: Twenty-five individuals with NF1, aged 13 to 25 years, underwent a six-month musical training program and were evaluated pre- and post-intervention using a comprehensive battery of tests. Auditory processing was assessed with the Gaps in Noise (GIN) test, Pitch Pattern Sequence (PPS), and long-latency auditory evoked potentials (P1-N1-P2 and Mismatch Negativity – MMN). Musical perception was measured using the Montreal Battery of Evaluation of Amusia (MBEA). Executive functions were evaluated with the Switch Verbal Fluency Test (SVFT), Digit Span, Corsi Block-Tapping Task, Five Digits Test (FDT), Five-Point Test (T5P), and Attention Network Test (ANT).

Results: Significant correlations were observed between musical, auditory, and executive function measures, including: MBEA and T5P ($p = 0.013$, $r = 0.509$), MBEA and MMN latency ($p = 0.009$, $r = -0.669$), GIN and Corsi ($p = 0.017$, $r = -0.515$), PPS and ANT ($p = 0.006$, $r = -0.563$), and PPS and Digit Span ($p = 0.019$, $r = 0.506$). Musical training led to improvements in executive functions, particularly cognitive flexibility (FDT: $p = 0.015$, $\eta^2 = 0.124$), task switching (FDT: $p = 0.030$, $\eta^2 = 0.155$), and verbal fluency (SVFT: $p = 0.011$, $\eta^2 = 0.158$). Auditory processing also improved, as evidenced by increased GIN scores ($p = 0.017$, $\eta^2 = 0.042$) and reduced MMN latency ($p = 0.001$, $\eta^2 = 0.365$).

Conclusion: The findings suggest a strong relationship between auditory and executive functions in individuals with NF1 and indicate that musical practice may have beneficial effects on these cognitive systems, supporting its use as a complementary therapeutic strategy.

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Longitudinal, Objective Measurement and Analysis of Sleep-Wake Patterns in NF1 Patients

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Purpose: Sleep disturbances and poor sleep quality are frequently reported but underexplored in people with neurofibromatosis type 1 (NF1). Inadequate sleep can have adverse effects on daytime functioning by impacting metabolism, mood regulation, cognition and immunological function. Our study aimed to characterize sleep and circadian phenotypes associated with NF1 and test their dynamic relationship with pain and cognitive function.

Methods: We performed a clinical research study of sleep, circadian rhythms, cognition and quality of life in 100 adult patients with a clinical or molecular diagnosis of NF1 and 100 controls matched for age, sex, race, and ethnicity. Objective metrics of sleep quantity, quality, timing and regularity as well as circadian rest-activity rhythms were obtained through wrist actigraphy and participants completed subjective online questionnaires on sleep and daily sleep logs over a 2-week period. Participants also completed a series of cognitive assessments through the TestMyBrain website every morning including tests on Verbal Paired Associates Memory, Digital Symbol Matching, Trail Making, and Continuous Performance. We tested associations between summary sleep metrics and NF1 status using linear or logistic regression adjusted for matching factors.

Results: The study population was ~85% white, had a mean age of ~41 years and was 75% female. NF1 patients reported shorter time in bed (-34.8 (10.2) min per night; $p<10^{-3}$) and longer time to fall asleep (~31.4 (9.1) min; $p<10^{-3}$) than controls based on daily sleep logs. Insomnia Severity Index (ISI) scores indicated more severe insomnia symptoms in NF1 patients ($p<10^{-3}$), particularly among those on medication, and poorer sleep quality as measured by Pittsburgh Sleep Quality index ($p<10^{-6}$). Epworth Sleepiness Scale (ESS) scores were comparable across groups, suggesting similar daytime sleepiness levels, however lower functional outcomes of sleep were self-reported in patients (FOSQ; $p<10^{-2}$). Analysis of 14-day actigraphy, pain and cognitive assessments is ongoing, and preliminary results suggest milder disruptions in objective sleep metrics.

Conclusion: Our study confirmed significant self-reported sleep disturbances, particularly with sleep duration and quality, in patients with NF1 as compared to an age, race and sex matched control population. Interventions that improve sleep in the general population may thus be beneficial in this population. Our study will further inform on whether sleep disturbances extend to objective sleep measures and impact pain and cognition in a dynamic manner.

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Brain Volume Deviations as Early Predictors of Neuropsychological Risk in Children with Neurofibromatosis Type 1 (NF1)

Prabhumallikarjun Patil, MD, *Emory University School of Medicine; Children's Healthcare of Atlanta*

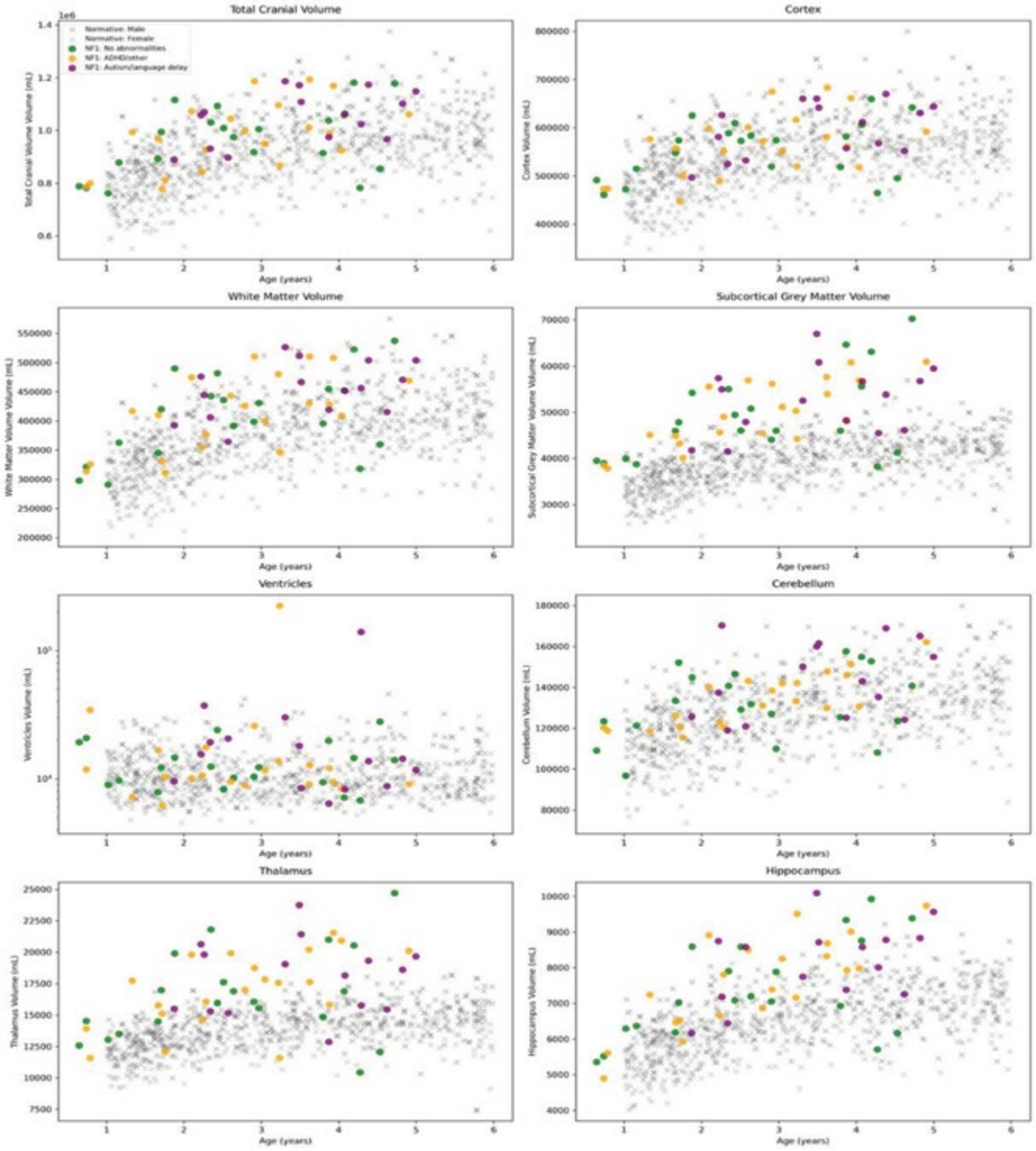
Purpose: Children with NF1 show a range of neurodevelopmental phenotypes with increased risk of neuropsychologic disorders, including autism and attention deficit/hyperactivity disorder (ADHD). The factors that determine which patients with NF1 will develop these problems are not yet known. Here we tested the hypothesis that increased brain volume in specific brain regions may be associated with neuropsychological disorders in NF1 patients and reflect the cerebro-cerebellar circuit involvement as already described in Autism and related neuropsychological disorders.

Methods: We retrospectively analyzed brain MRI from 66 children under age 6 years with NF1. These were compared to an age and sex-matched normative cohort of 772 children drawn from a larger reference dataset of 2,800+ MRI. All images were preprocessed and quality-controlled using the open-source tool MRQy. Brain segmentation was performed using SynthSeg. To account for scanner and site variability, regional brain volumes were harmonized using the "neuroCombat" method. Deviation scores were computed by comparing each NF1 subject's regional brain volumes to age and sex specific normative expectations derived from generalized additive models (GAMLSS) fit to the normative cohort. Neuropsychological risk in the NF1 group was categorized into three subgroups: 1. Autism and language deficiencies, 2. ADHD, and 3. None, based on neuropsychological testing and clinical assessments by NF1 specialists.

Results: Children with NF1 showed significant deviations in brain volume relative to normative expectations, particularly in the thalamus, subcortical gray matter, and cerebellum. NF1 children with neurodevelopmental disorders exhibited more pronounced deviations than those without such diagnoses.

Conclusions: This study shows increased volume in midline regions in preschool-aged children with NF1 compared to a normative pediatric cohort. Greater

deviations in brain volumes of midline structures may be associated with increased neuropsychological risk, though larger datasets are needed to confirm this. This also highlights the role of midline structures and cerebro-cerebellar circuitry in early neuropsychological development in this age group. Our findings support the potential fesibility of AI-based MRI volumetric analysis to gain new information from neuroimaging studies in children with NF1.



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GLIOMA

Longitudinal Changes in Magnetic Resonance Imaging Features of the Anterior Visual Pathway in Children with NF1-OPG

Zhifan Jiang, PhD, *Children's National Hospital, Washington, DC*

Purpose: Neurofibromatosis type 1-associated optic pathway gliomas (NF1-OPG) develop along the anterior visual pathway (optic nerve, chiasm, tract; AVP). Previous research demonstrated that children undergoing treatment for NF1-OPG exhibit significantly larger AVP volumes compared to those who remain under observation without intervention. Our team developed a fully automated deep-learning framework designed to automatically segment the AVP, optic nerves (ONs) and chiasm and to calculate location-specific MRI radiomic features. In this study, we investigate how radiomic features of NF1-OPG cases enrolled in the natural history study change over time.

Methods: Children with newly diagnosed NF1-OPG were included if their MRI contained three MRI sequences (volumetric T1-weighted, T2-weighted and T2-FLAIR) and they had three consecutive MRI studies (baseline, 3 months, and 6 months). Normal or abnormal visual acuity (VA) was assessed at each visit through a quantitative ophthalmic exam. Automatic AVP volumetric segmentation was performed using a knowledge-transferred Swin transformer network. The ONs and chiasm were split through template-based registration for the radiomic measurement at specific locations. A total of 1,172 radiomic features were extracted after image normalization including histogram matching followed by Z-score normalization. We used a linear mixed-effects model to assess longitudinal changes in radiomic features and treatment status.

Results: Four radiomic features exhibited significant differences ($p < 0.05$) between the observation (OBS, N=44) and treatment (TX, N=9) groups, as well as across time points. In T1, texture complexity of the AVP and ONs showed significant variation, while in T2, the prominence of small hyperintensity regions in the AVP and the asymmetry of intensity distribution in the chiasm demonstrated similar trends. At baseline, the OBS group exhibited higher values in all the four radiomic features than the TX group. Over time, the TX group showed a greater increase in T1 texture complexity with AVP increasing by 7.1% at 3 months and 9.8% at 6 months, while ON increased by 7.5% at 3 months and 9.4% at 6 months. In contrast, the OBS group showed smaller changes, with AVP increasing by 1.3% at 3 months and 1.6% at 6 months, and ON increasing by 2.1% at 3 months and 2.4% at 6 months. These findings suggest that these newly identified radiomic features may reflect underlying biological differences or treatment effects.

Conclusion: Deep learning-based analysis reveals an association between new location-specific MRI radiomic features in the ONs and chiasm and treatment decisions. This automated framework has the potential to stratify NF1-OPG patients based on risk of VA loss and aid in their treatment planning.

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Identifying Molecular Drivers in Aggressive Germline vs Somatic NF Mutant Glioma

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Purpose: Germline Neurofibromatosis type 1 *(NF1)* alterations are associated with an increased incidence of non-optic pathway gliomas (non-OPG) and often have worse than expected outcomes based on conventional histologic grading. Patients with sporadic gliomas and intra-tumoral *NF1* mutations also have worse outcomes compared to other sporadic gliomas. Leveraging the comprehensive SPORE sponsored Glioma DHART board (a national, patient driven registry including full clinical, pathological and imaging annotation for people with NF1 and non-OPGs) and the MSKCC biorepository, we evaluated the similarities and differences in molecular profile between *NF1* null (germline) and heterozygous *NF1* (somatic) gliomas.

Methods: We conducted a multi-institution retrospective study, analyzing over 40 patients with either *NF1* sporadic or *NF1* germline driven glioblastoma (GBM) who underwent next-generation sequencing (NGS). We then evaluated non-OPG samples from adults with NF1 provided via the Glioma DHART Board. We compared the molecular profiles and overall survival (OS) between germline and somatic non-OPG of both high and low grade glioma with *NF1* pathogenic variants.

Results: In the somatic *NF1* GBM group compared to the germline GBM group **(Fig 1)** we observed similar molecular expression profiles in several well-established poor prognostic markers such as TERT, CDKN2A/B, TP53, PTPN11 and CDK4 alterations. However, these two groups lacked similarities across other alterations; with *NF1* germline gliomas overall having fewer alterations compared to the somatic group. We observed a similar pattern in *NF1* associated low grade glioma patients with fewer alterations in the germline group when compared to the somatic **(Fig 2)**. CDKN2A/B and TP53 alterations were absent in the low grade glioma cohort, but present in the higher grade cohort.

Conclusions: Germline and sporadic *NF1* associated low grade and high grade non-OPG share some genetic alteration patterns on clinical NGS. Although the profiles were similar between the two groups, further analysis of additional downstream mutations and molecular alterations paired with cellular and clinical phenotype analyses are ongoing.

Fig 1. Oncoplot GBM with Somatic NF1(green) vs Germline NF1 (purple)

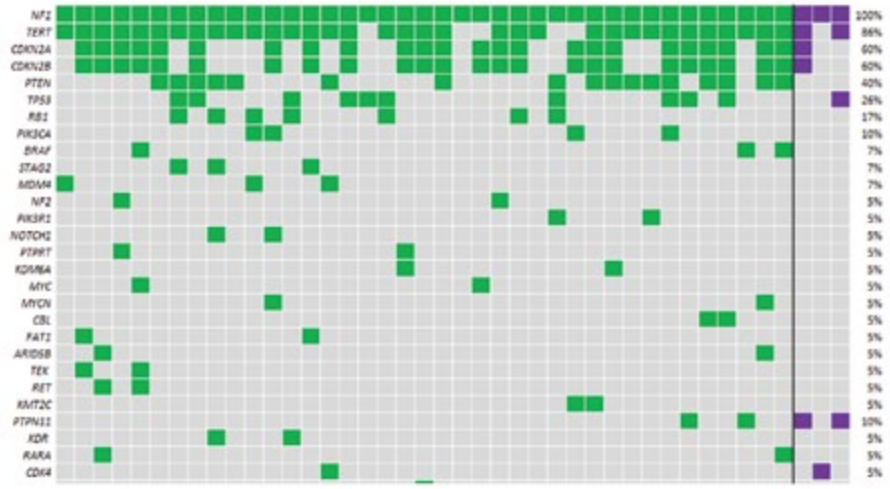

Fig 2. Oncoplot Low grade glioma with Somatic NF1(blue) vs Germline NF1 (purple)

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Part of the Puzzle: Caregiver Considerations for Participating in Novel Clinical Trials to Restore NF1-OPG Related Vision Loss

Matthew C. Hocking, PhD, *The Children's Hospital of Philadelphia; Perelman School of Medicine at The University of Pennsylvania*

Introduction: Vision loss from Neurofibromatosis Type 1 (NF1) related Optical Pathway Gliomas (OPGs) results in lifelong disability and lower quality of life. Current efforts to restore vision are enticing but may be high risk. Understanding family perspectives on engagement with clinical trials for experimental treatment modalities is critical for informing treatment development. This study investigated considerations caregivers would have when deciding to participate in novel visual restoration clinical trials for NF1-OPG related vision loss.

Methods: Participants were identified through NF1-OPG registries and recruited via email and phone. Caregivers were eligible if they had a child with an OPG secondary to NF1, were actively involved in their NF1 care, and lived with the child (ages 1-17) for at least 50% of the time. Twelve caregivers (11 Female, 1 Male, 8.3% racial/ethnic minoritized) participated in structured focus group sessions discussing treatment and resource related factors that may impact treatment decision-making. Focus group sessions were facilitated by psychologists and coded for themes using inductive content analysis.

Results: Several themes emerged: 1) *Part of puzzle* – caregivers viewed vision concerns as one aspect of their child's broader NF1 profile, considering them amongst other physical, psychosocial, and cognitive concerns. 2) *Risk/benefit ratio* – caregivers emphasized the risk/benefit ratio of both participating and not participating in treatment, weighing the potential for side effects and exacerbation of other NF1 concerns versus loss of vision. Caregivers of children with correctable vision impairments were less open to engage with new treatment due to potential adverse effects and family resource burden. 3) *Vision loss severity/probability* – caregivers expressed increased openness to learning about emerging treatment modalities with increased vision loss severity, noting concerns over independence and quality of life. 4) *Trust* – caregivers emphasized the importance of trust in their child's NF1 team and highlighted the importance of proper patient education practices and mutual decision-making when considering various treatment options.

Conclusion: Increased understanding of the factors that families weigh when making decisions about visual restoration therapies is essential to investigators developing clinical trials for NF1-OPG-related vision loss and to clinicians presenting such opportunities to families. Findings underscore the multiple considerations families have when making decisions for their child's care and highlight the need for family-centered approaches in NF1-OPG vision-related treatment that ensure open communication and shared decision making. Future work in NF1-OPG treatment development should disseminate information to the NF1 community in an accessible format to promote family engagement.

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CUTANEOUS AND SUBCUTANEOUS NEUROFIBROMA

Measurement of the Severity Related to Cutaneous Neurofibromas in Neurofibromatosis Type 1: Development and Validation of the Nef-ASI

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No standardized and validated clinician-reported outcome measure exists to assess cutaneous neurofibromas (cNF) in neurofibromatosis type 1 (NF1). An international initiative recently established a core outcome set for NF1-related cNF trials, including number, size/volume, and visibility.

The aim: To develop and validate the Nef-ASI tool designed to assess the overall severity of cNF, integrating these three outcomes.

This CERENEF study at Henri Mondor Hospital, Créteil, France followed six steps. Steps 1-2 involved surveys and working groups (N=25 healthcare professionals, HCP) to integrate cNF size, number/density, and visibility into a single score. Steps 3-6 included scoring sessions with local team members (N=9HCP, grouped by experience) and MDs (N=15) from the French rare skin disease network. Standardized 2D photographs (100cm^2 skin area) were used. Reliability was assessed via intraclass correlation coefficient (ICC >0.8 acceptable), assessor agreement by Bland-Altman plot, and validity by correlation with cNF severity questions.

Development Phase: Steps 1-2 defined cNF size categories (small <5mm, medium 5-9mm, large ≥10mm) with visibility-weighting factors (1, 1.5, 3) and density ranges (% skin surface: 0%, 1%, 5%, 10%, 25%, 50%, ≥75%), which were into three 21-image palettes for different skin phototypes **(Fig 1)**.
Validation Phase: Scoring sessions evaluated reliability, agreement, and feasibility:

Step 3: Inter-rater reliability (9 assessors, 50 photos) showed ICCs >0.8 for MDs and non-MD experts **(Table 1)**. Bland-Altman plots indicated increased data spread in high-score cases **(Fig 2)**.
Step 4: Intra-rater reliability (25 rescored photos) remained high (ICCs >0.9).
Step 5: Fifteen MDs scored 50 photos, with ICCs >0.8. NeF-ASI correlated strongly (0.81-0.88) with cNF severity questions.
Step 6: Feasibility: 87% found NeF-ASI easy to use, with a median evaluation time of 27 seconds per image.

The NeF-ASI demonstrated strong inter- and intra-rater reliability, comparable to other dermatological CROMs like PASI. Its strong correlation with clinical scales confirms its validity. It assesses: i) topical treatments by comparing two 100cm^2 photos (treated vs. placebo) per patient *(Target NeF-ASI)* and ii) systemic treatments using three 100cm^2 photos (back, abdomen, thigh, as defined by Cunha *et al.*) to calculate a *Global NeF-ASI* (average of three scores) across treatment groups. Although developed in France, international experts contributed, ensuring broader applicability.

Thus, NeF-ASI offers a cost-effective, standardized and reliable cNF severity assessment for therapeutic trials, integrating their size, number, and visibility. Its validated 2D-photograph approach enables remote assessments, supporting decentralized trial designs.

Figure 1: Cutaneous neurofibroma palettes constituting the Nef-ASI, corresponding to the 3 phototype groups based on the Fitzpatrick classification (1-2, 3-4, and 5-6).

Figure 2: Bland and Altman plots measuring the level of agreement between 2 assessors randomly selected in each group of 3: medical doctor (MD) experts, non-MD experts and non-experts.

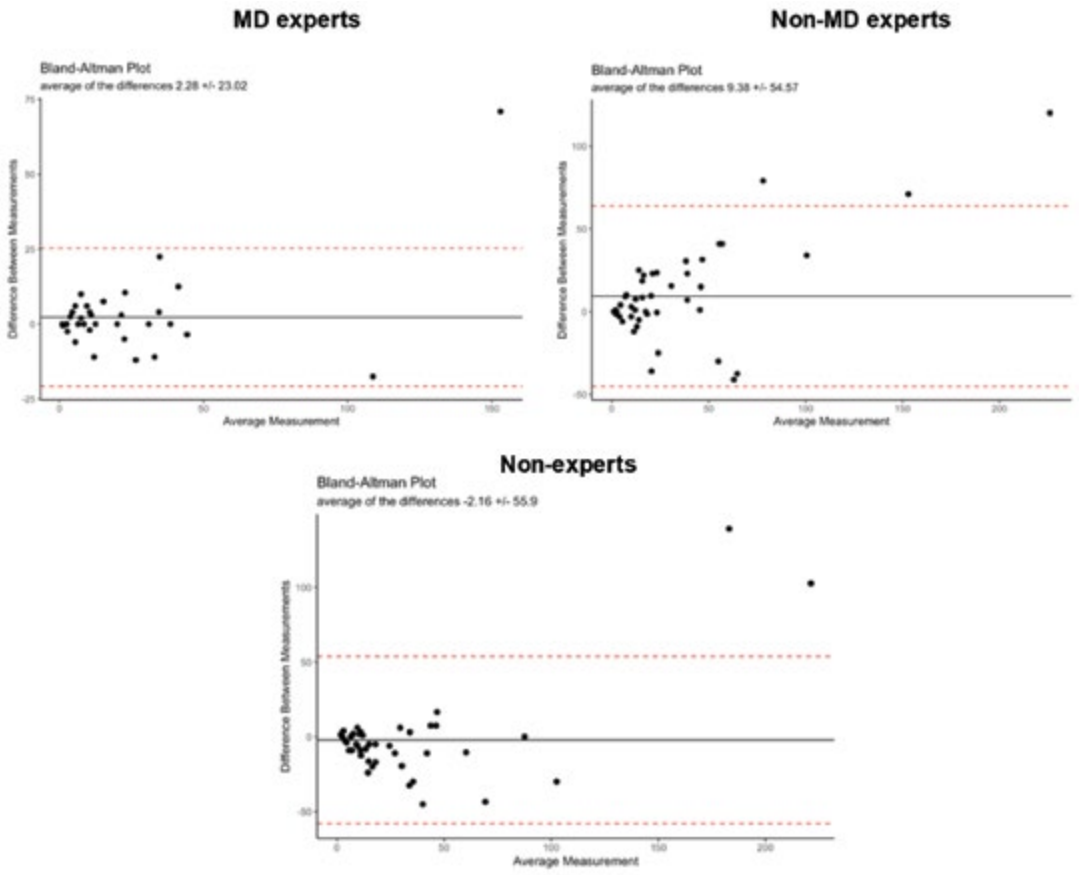

Table 1: Inter and intra-rater reliability assessment of the Nef-ASI using the Intraclass Correlation Coefficient (ICC), and their 95% confidence interval (95%CI). Session 1 corresponded to the results from Step 3, and Session 2 to those from Step 4, highlighting an improvement in the tool's reproducibility with the assessors' training. ICCs range from 0 to 1: <0.5 poor, 0.5–0.74 moderate, 0.75–0.89 good and 0.9–1.0 considered excellent reliability (Koo TK, *et al. J Chiropr Med.* 2016). * indicates the medical doctor (MD) experts, † the non-MD experts, and ‡ the non-experts.

Group	Assessors	Inter-rater Session 1 ICC	Inter-rater Session 1 95%CI	Inter-rater Session 2 ICC	Inter-rater Session 2 95%CI	Intra-rater ICC	Intra-rater 95%CI	Overall inter-rater ICC (95%CI)		
National assessors	N=15	0.825	0.760-0.882					0.776 (0.702-0.846)	0.795 (0.724-0.86)	0.829 (0.767-0.884)
Local assessors	1*	0.871	0.804-0.920	0.952	0.910-0.977	0.905	0.799-0.957			
	2*					0.969	0.933-0.986			
	3*					0.964	0.894-0.986			
	4†	0.828	0.739-0.892	0.822	0.684-0.911	0.878	0.746-0.944			
	5†					0.892	0.760-0.952			
	6†					0.881	0.748-0.946			
	7‡	0.602	0.423-0.743	0.698	0.509-0.839	0.715	0.456-0.863			
	8‡					0.764	0.536-0.889			
	9‡					0.903	0.794-0.956			

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Emergence and Development of Nascent Cutaneous Neurofibromas in Pediatric NF1 Patients

Wangcun Jia, PhD, *Beckman Laser Institute, University of California, Irvine*

Purpose: The aim of our research is to detect the emergence of nascent cutaneous neurofibromas (cNF) in pediatric patients with neurofibromatosis type 1 (NF1) and monitor their development over time using non-invasive imaging techniques. Additionally, we evaluated the feasibility of coagulating cNF of various sizes with lasers and assessed potential side effects through numerical simulations.

Methods: We conducted annual imaging of ten pediatric NF1 patients using two non-invasive instruments: spatial frequency domain imaging (SFDI) and high-frequency ultrasound (HFUS) devices, starting in 2023. SFDI, based on diffuse optical spectroscopy, provides spatially resolved maps of tissue optical properties over a large field of view (15 cm x 20 cm). Once suspected nascent cNF are identified using SFDI, HFUS is used to examine skin structures in the area. We obtained the geometry and volume of the cNF during monitoring, which were used in numerical simulations to evaluate the feasibility of laser coagulation and assess potential side effects. The numerical models incorporate a Monte Carlo light transport component to simulate photon propagation and absorption within the tissue and a finite element heat diffusion component to model the thermal response. Tissue denaturation was analyzed using the Arrhenius integral equation, quantifying thermal damage based on temperature and exposure time.

Results: Emerging cNF, absent in previous imaging, were detected in two patients. One example is shown in **Figure 1**. Initial imaging showed no signs of cNF **(Figure 1a,c)**. However, one year later, SFDI scattering coefficient (μ_s') maps at 851 nm revealed areas with abnormally low values, indicating new development of cNF **(Figure 1d)**. HFUS images in **Figure 1e,f** further corroborate these findings by showing hypoechoic regions within dermis, indicative of cNF[1]. Interestingly, the hypoechoic region in **Figure 1e** appears less defined compared to that in **Figure 1f**, suggesting variability in tissue structure among nascent cNFs and presenting an opportunity for longitudinal study. We observed that existing cNF increased in volume over the year between the two imaging sessions.

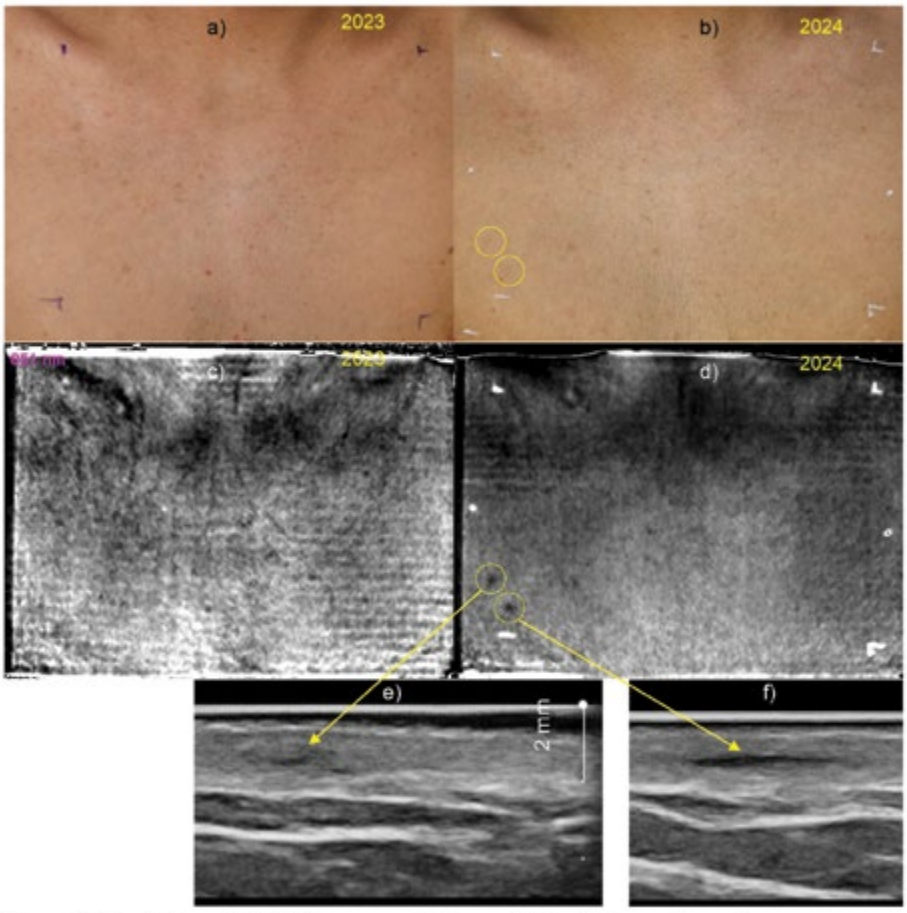

Figure 1: SFDI scattering coefficient (μ_s') maps reveal that nascent cNFs, which were not present one year ago, are detected in a 16-year-old male subject. a-b) color photos; c-d) μ_s' maps at 851 nm of the chest; e,f) HFUS images of the nascent cNF. Yellow circles highlight areas of abnormally low μ_s' in d) while skin appears normal in b).

Numerical simulations indicate that nascent cNF, characterized by their thin, pancake-like morphology **(Figure 1e)**, require minimal amount of energy for photocoagulation, reducing the risk of undesirable thermal damage to the surrounding tissue. In contrast, larger cNF require more energy, increasing the potential for collateral damage and scarring.

Conclusions: Our results suggest that emerging cNF can be effectively detected by SFDI during annual screening. Early detection allows for timely intervention, minimizing risk of undesirable thermal damage to surrounding tissues.

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1. I. Oteiza-Rius et al., "Sonographic Characterization of Red-Blue Neurofibromas in Patients With Neurofibromatosis Type 1," *J Ultras Med* (2024). https://doi.org/10.1002/jum.16632.

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Results of a Phase 1 Trial of Topical Immunotherapy Diphencyprone for Cutaneous Neurofibromas in Neurofibromatosis Type 1

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Purpose: Neurofibromatosis type 1 (NF1) is a tumor predisposition syndrome that leads to the growth of cutaneous neurofibromas (CNs), heterogenous skin tumors with brisk inflammatory infiltrate that cause pruritus, pain, and disfigurement. Diphencyprone (DPCP) is a topical hapten-based immunotherapy that initiates a delayed-type hypersensitivity reaction in the skin and is known to treat cutaneous melanoma metastases. Here we present the results of a single-center, phase 1 open label trial (NCT05438290) of DPCP for the treatment of CNs.

Methods: DPCP 0.04% ointment was administered topically weekly for 10 weeks to up to 20 CNs for 12 adult subjects with NF1. Endpoints included subjective tolerability and measures of pain, stinging, burning, and pruritus. Tri-axial tumor measurements were obtained with digital calipers pre-treatment and 30 days post-treatment. Immunohistochemistry (IHC), bulk RNA sequencing (RNA-seq) and single cell (sc)RNA-seq were performed on CNs resected on days 0 (baseline), 17 (first treatment response), and 107 (post 4-week washout). Spatial transcriptomics (ST) was performed on untreated CNs.

Results: DPCP was well-tolerated; one participant developed a grade 2 inflammatory response which required treatment interruption. There was no significant differences in pruritus, burning, stinging, or pain during the treatment period. The summed target lesion volume of four paricipants decreased as a result of treatment, but increased in eight participants. Both IHC (CD68 and CD11c) and deconvoluted RNA-seq revealed a significant increase in macrophages and dendritic cells. Infiltration and intensity of staining of CD3+ T cells and CD20+ B cells increased on IHC. There were no differences in the number of endothelial cells, keratinocytes, lymphocytes, neuronal/glial cells, or fibroblasts. ST revealed Schwann cell clusters in the core with upregulated neurogenesis and gliogenesis and lymphocytes in the periphery with upregulated defense responses.

Conclusion: DPCP was safe and tolerable in adults with NF1, while efficacy was equivocal. IHC and transcriptional data demonstrated a superficial inflammatory response suggesting inadequate tumor penetration. Future research could evaluate alternative delivery methods of DPCP for improved tumor penetration.

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Using Durometers to Quantify Stiffness as an Outcome Measure for Cutaneous Neurofibroma in NF1

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Individuals with Neurofibromatosis Type 1 (NF1) develop multiple cutaneous neurofibroma (cNF) that no effective drug therapy exists and mainstay treatment remains physical removal. However, clinical trials testing new drugs are currently ongoing, and quantitative techniques based on cNF biology are needed to measure changes in cNF following treatment. The cNF tumor bulk is composed of extracellular matrix and inflammatory cells that dictates their stiffness. No studies have reported measuring the stiffness change in neurofibromas, which would indicate shrinking of the tumor bulk. The goal of this study was to evaluate two different instruments: the Rex Gauge durometer (REX) and the Delfin SkinFibroMeter® (DELFIN), in reproducibly measuring cNF stiffness. 97 neurofibromas from different skin areas of 11 NF1 patients were measured at each of two visits about two weeks apart. The DELFIN had moderate within-tumor agreement (ICC = 0.607, 95% CI:0.512-0.691) and moderate within-visit agreement (ICC = 0.732, 95% CI:0.665-0.786), and the REX had moderate within-tumor agreement (ICC = 0.740, 95% CI:0.631-0.816) and excellent within-visit agreement (ICC = 0.937, 95% CI:0.913-0.953), while accounting for measurements clustered within a visit within a tumor within a patient. We found that both the Delfin SkinFibroMeter® and the Rex Gauge Durometer are easy to use and reliable, providing consistent, objective quantification of cNF stiffness.

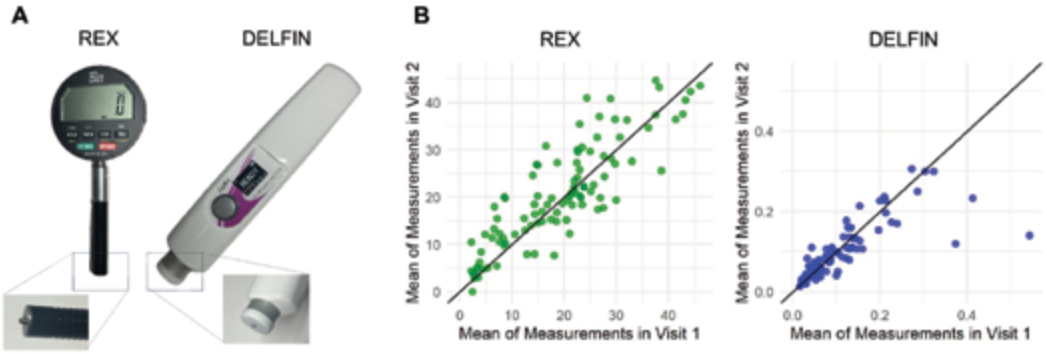

Figure 1. Two instruments to measure stiffness in cNF. A. Pictures of the Rex Gauge Durometer (REX) and the Delfin SkinFibroMeter® (DELFIN). **B.** Scatter plots of the average of the three repeated measurements obtained from Visit 1 and Visit 2 for both instruments. The black diagonal line indicates equal values between visits. Points closer to the diagonal line suggests strong agreement between measurements.

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Integrated GWAS with a Functional Genomics Knowledge Graph (KGWAS) Identifies Six Novel Loci Associated with Cutaneous Neurofibroma Development

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Introduction: Cutaneous neurofibromas (cNFs) are benign skin tumors that develop in the majority of individuals with Neurofibromatosis Type 1 (NF1), often leading to significant physical and psychological morbidity. Despite their prevalence and impact, there is considerable variability in the number of cNFs that develop across affected individuals, posing a major challenge to understanding and treating these burdensome lesions. Although over 3,000 pathogenic variants in the *NF1* gene have been identified, few have been linked to cNF number, suggesting that genetic modifiers outside the *NF1* locus likely play a critical role in influencing individual susceptibility to tumor development.

Purpose: This study aims to identify genetic modifiers of cNF development by leveraging a large, crowd-sourced dataset that integrates genetic, photographic, and clinical information from individuals with NF1.

Methods: A cohort of 607 participants with NF1 aged ≥ 40 contributed photographs covering seven body regions, completed clinical surveys, and provided saliva samples for whole-genome sequencing. Participants were stratified into four groups based on cNF number as measured by the investigators from submitted photographs: 1-10, 11–100, 101–500, and more than 500 tumors. We applied **Knowledge Graph GWAS (KGWAS)**, a novel geometric deep learning framework that integrates genome-wide association summary statistics with a large-scale functional genomics knowledge graph. This method enhances detection power in small-cohort GWAS by assessing the association strength of genetic variants through aggregated biological evidence from interacting molecular elements within the graph.

Results: KGWAS identified six suggestive loci associated with increasing cNF number at a significance threshold of $P < 5 \times 10^{-7}$. These novel SNPs occur within nerve and brain tissue-specific regulatory elements and implicate loci involved in neuronal cell survival, epithelial-mesenchymal transition, and axonal guidance and polarity. These loci may represent novel genetic modifiers influencing cNF formation in individuals with NF1.

Conclusions: Potential genetic modifiers of cNF number in adults with NF1 have been identified, offering new insights into the biological pathways driving tumor development and potential targets for future therapeutic intervention.

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Disclosures: Kavita Sarin is a scientific advisor for NFlection Therapeutics. Carlos Romo is a consultant for Alexion Pharmaceuticals and SpringWorks Therapeutics. Jaishri Blakeley is a consultant for Alexion Pharmaceuticals Therapeutics and SpringWorks Therapeutics.

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Dosimetry for Treatment of Cutaneous Neurofibromas (cNFs) by Surfactant Injection

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Purpose: We previously found that a single injection of two FDA-approved surfactants (deoxycholate and polidocanol) is effective in reducing cutaneous neurofibromas (cNFs) both in-vivo and ex-vivo[1,2]. The purpose of this clinical study was to examine dose-response of these agents.

Methods: 20 adults (ages 26-74; average age 48±16; 5M/15F) with small (2-8mm) cNFs were recruited in an Institutional Board Review-approved study. Tumors amenable to treatment were selected, then randomized into either 1) deoxycholate or 2) polidocanol groups. The individual lesions within these groups were then randomized to treatment or control (minimum of 6 cNFs per condition, with no upper limit for treated cNF). Deoxycholate-treated tumors received intratumoral injection of 10mg/mL deoxycholate (Kybella®, Allergan-AbbVie); polidocanol-treated tumors received intratumoral injection of 1% polidocanol (Asclera®, Merz). Control lesions received no treatment and were observed only for comparison. Each treatment had 6 escalating doses, determined by a fixed ratio of tumor volume to injected surfactant volume. Tumor diameter was used to approximate tumor volume. The 6 doses used were: 0.50x, 0.75x, 1.00x, 1.25x, 1.50x, or 1.75x the volume of the corresponding tumor. Topical anesthetic (5% lidocaine/prilocaine) was applied for 40 minutes before treatment. The following outcome measures were assessed at baseline, 3 months, 6 months, and 12 months post-treatment: 1) tumor height and volume (via 3D Cherry Imaging®); 2) pain score (0-10); 3) clinician and patient assessment of tumor clearance; 4) and side effects (by CTCAE v5 scale), including inflammation, pigmentation, and ulceration. Biopsies were obtained from 13 participants 3 months post-treatment. 18 of 20 subjects completed all assessments, and 396 cNFs total (107 deoxycholate-treated; 102 polidocanol-treated; 187 untreated controls) were measured over all timepoints.

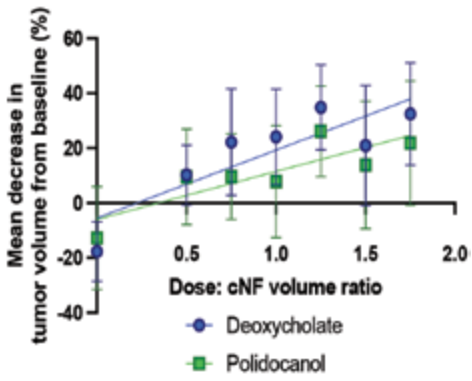
Results: No adverse events > grade 2 occurred. Treatment with both surfactants showed a significant ($p<0.05$) decrease in tumor volume. Tumor reduction increased with the volume injected, for both agents **(Figure 2)**.

Conclusion: A single injection of either polidocanol or deoxycholate into NF1-associated cNFs causes significant, dose-dependent reduction of tumor volume and height, as shown. Injected volumes up to 1.75x the tumor volume are well-tolerated. Injection causes temporary inflammation and mild pain (deoxycholate: 3.2±1.6, polidocanol: 3.3±1.8). Local pigmentation changes occurred in a minority of subjects, which persisted in only one. These local-injection treatments for cNF are well-tolerated and promising.

Figure 1. Mean change in tumor volume from baseline, seperated by timepoint.

	Mean Change in Tumor Volume from Baseline (%)					
	3 Months Post-Treatment		6 Months Post-Treatment		12 Months Post-Treatment	
Dose (escalating)	Deoxycholate (n = 114 treated, 103 control)	Polidocanol (n = 111 treated, 108 control)	Deoxycholate (n = 113 treated, 97 control)	Polidocanol (n = 108 treated, 102 control)	Deoxycholate (n = 107 treated, 97 control)	Polidocanol (n = 108 treated, 103 control)
Control (average)	20.8±25.9	10.8±36.1	18.8±33.3	12.7±44.2	18.8±29.5	11.1±37.8
0.5x	-12.8±25.2	-9.6±30.7	-10.2±26.1	-19.9±27.9	-10.2±23.3	3.0±63.8
0.75x	-19.4±45.6	-6.9±37.4	-26.0±42.5	-9.7±57.6	-25.1±37.7	-7.8±30.6
1.00x	-20.5±37.5%	-18.6±42.5	-17.6±44.8	-18.5±37.4	-24.2±48.7	-0.2±57.4
1.25x	-40.2±27.5%	-26.4±37.7	-38.0±28.6	-30.8±34.8	-30.9±45.5	-25.7±32.2
1.50x	-19.9±44.4%	-24.0±25.9	-20.4±48.1	-18.2±32.6	-14.5±52.9	-18.2±32.6
1.75x	-36.8±62.3%	-13.9±62.3	-34.5±45.1	-32.8±37.6	-32.0±41.2	-27.4±41.0

Figure 2. Dose response, average over 12 months.



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2. Richey P, Funk M, Sakamoto F, *et al.* Noninvasive treatment of cutaneous neurofibromas (cNFs): Results of a randomized prospective, direct comparison of four methods. *J Am Acad Dermatol.* Published online December 10, 2023. doi:10.1016/j.jaad.2023.11.058

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Delayed Diagnosis and Severe Cutaneous Manifestations in Chinese Neurofibromatosis Type 1 Patients

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Objective: While neurofibromatosis type 1 (NF1) phenotypes have been well-characterized in Western populations, comprehensive data from Asian cohorts are notably lacking. To address this critical gap, we aimed to: (1) delineate the clinical spectrum of NF1 in a large Chinese population, (2) identify distinct phenotypic subgroups through unsupervised clustering, and (3) perform direct comparison with U.S. cohort to elucidate potential ethnic variations.

Methods: In this multicenter cross-sectional study, we evaluated 1,313 Chinese NF1 patients (57.3% female; median age 19 years) using physician assessments (52.1%) and structured patient surveys (47.9%). Standardized data collection included tumor burden (cutaneous/plexiform/spinal neurofibromas), cutaneous features (café-au-lait macules, freckling), skeletal abnormalities, and malignant progression. We employed k-means clustering for phenotypic subtyping and conducted comparative analyses against 800 U.S. NF1 patients with Bonferroni-corrected χ^2/t-tests.

Results: Chinese patients demonstrated later diagnosis (86.1% diagnosed after age 20 vs. 23.3% in U.S., $p<0.001$) and more severe cutaneous neurofibromas (27.5% had >500 cNFs vs. 19.9%). Malignant peripheral nerve sheath tumors were significantly more prevalent in the Chinese cohort (8.2% vs 4.4%, $p=0.002$), as were skeletal abnormalities including scoliosis (17.1% vs 41.1%) and fractures (6.4% vs 49.9%) (both $p<0.001$). However, spinal neurofibromas (8.0% vs 27.2%), Lisch nodules (11.2% vs 54.1%) and optic gliomas (2.4% vs 12.7%) were less common (all $p<0.001$). Clustering analysis revealed four distinct subtypes: severe neural (high pNF/MPNST prevalence), cutaneous (abundant cNFs), hereditary (familial cases with intermediate severity), and mild (late-onset, fewer manifestations). Notably, chronic symptoms like itching (13.5% vs 55.7%) and pain (18.3% vs 64.5%) were reported significantly less frequently in Chinese patients ($p<0.001$).

Conclusion: This study establishes key ethnic divergences in NF1 presentation, with Chinese patients exhibiting unique tumor profiles, skeletal morbidity patterns, and delayed diagnosis compared to Western counterparts. Our findings advocate for population-specific surveillance protocols and validate the utility of phenotypic clustering in precision management.

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BONE ABNORMALITIES

Convexity Coronal Imbalance in Dystrophic Scoliosis Secondary to Type 1 Neurofibromatosis: Classification and its Importance for Surgical Decision-Making

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Purpose: Trunk shift causing coronal imbalance (CI) is frequently encountered in unstable dystrophic scoliosis (DS) secondary to Type I Neurofibromatosis (NF-1). Surgical rebalance of coronal malalignment is challenging with high occurrence of iatrogenic aggravation. To develop a comprehensive classification of convex coronal spinal malalignment and propose a treatment algorithm for this specific condition.

Methods: A review of NF-1CI cases where different types of convex CI, rebalancing strategies, as well as the outcomes were identified and analyzed.

Results: Two main convex CI patterns were defined: thoracic CI (type 1) and thoracolumbar/lumbar CI (type 2), and were further subtyped by the compensatory behavior of the upper hemi-curve (straight or curved morphology). The incidence of post-op persistent CI ≥3cm was 0.0% and 63.6% for Type1 and Type 2 groups, respectively. Quantitative analysis revealed that the over correction of the compensatory upper hemi-curve and insufficient correction of the lower hemi-curve playing the role of imbalance driver were more significant in the imbalanced group (△Upper Arc Translation/|△Lower Arc Translation|: 31.8±34.4% vs. 109.6±60.0%, p=0.008; △Upper Arc Inclination/|△Lower Arc Inclination|: 33.5±37.3% vs. 89.8±36.6%, p=0.012). A surgical rebalancing algorithm was proposed to treat each subtype.

Conclusion: This new classification of convex CI emphasized that coronal rebalance should be obtained with cooperative correction that the lower hemi-curve should be aggressively corrected while the morphology of pre-op upper hemi-curve determined whether it played the role of fine-tuning or radical adjustment for coronal realignment. This is particularly true for type 2 NF-1CI patients with poor distal screw purchase and utmost avoidance of pelvic fixation.

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Tumor Resection is Not Necessary for Correction Surgery for NF-1 Patients with Spinal Deformity and Concomitant Intraspinal Tumor

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Purpose: To investigate outcomes and safety of correction surgery without resection of intraspinal tumor for NF-1 patients with spinal deformity and concomitant intraspinal tumor.

Methods: A retrospective analysis was conducted to identify a consecutive series of NF1 patients with intraspinal tumors who underwent correction surgery at our center between January 2000 and January 2022. Radiographic parameters were evaluated for coronal and sagittal spinal alignment combined with pelvic parameter: (1) curve angles of major thoracolumbar/lumbar curves and minor thoracic curves; (2) sagittal parameters included T5–T12 angle, T10-L2 angle, L1-S1 angle, and SVA; (3) pelvic parameters included pelvic tilt, sacral slope, and pelvic incidence; (4) correction ratio: preoperative Cobb angle–postoperative Cobb angle/preoperative Cobb angle.Whole spine MRs were performed for all the patients. The following parameters were determined: (1) level of tumor; (2) single or multiple tumors; (3) range of tumors; (4) maximum stenosis index: rate of maximum tumor area to spinal canal area at cross-section.Preoperative, postoperative and last follow-up neurologic function were analyzed using An American Spinal Injury Association (ASIA) neurologic examination.

Results: A total of 73 patients were included in the study, including 32 males and 41 females. Average age: 11.4 ± 3.7 years, postoperative AISA score increased by 1.7 grades while VAS score increased by 4.5 points. The tumor invasion rate was 63% before surgery and 36% after surgery. Coronal curve angle decreased from 68.3 ° to 19.4 °. Kyphosis decreased from 35.2 ° to 12.2 ° after surgery.

Conclusion: Correction surgery without resection of intraspinal tumor could achieve satisfactory spine deformity correction, and improvement of neurological function.

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Developmental Abnormality of the Sacroiliac (SI) Joint is an Underreported Complication of Neurofibromatosis Type 1 (NF1)

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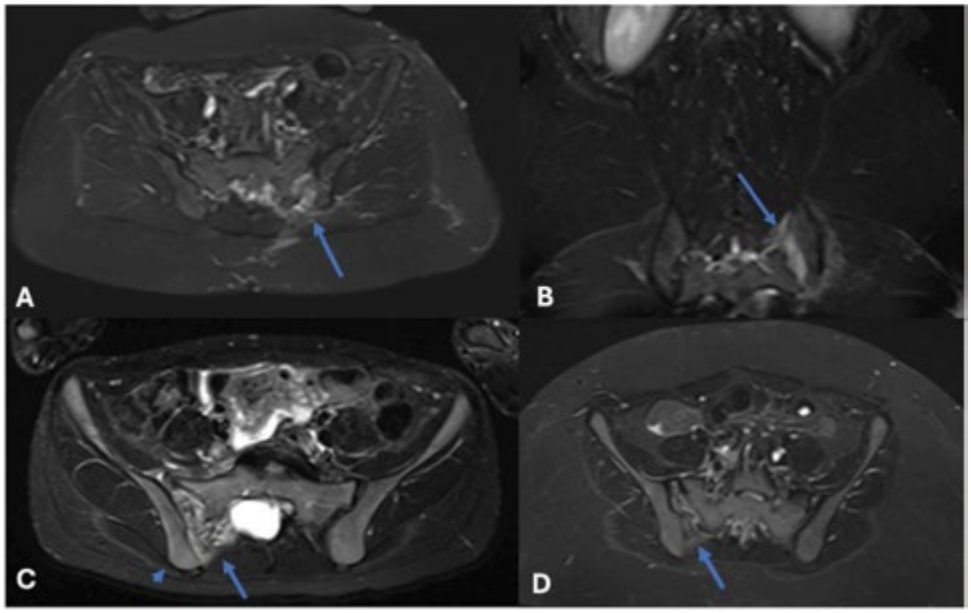
Purpose: To present a possible new NF1-related developmental abnormality in children and young adults with NF1 involving the SI joint.

Methods: We report three participants out of 112 enrolled, with SI joint involvement noted on whole-body magnetic resonance imaging (WB-MRI). These individuals are part of a cohort of 112 participants in a longitudinal NF1 study (NCT05238909) at Ann & Robert H. Lurie Children's Hospital of Chicago in collaboration with the National Institutes of Health. A detailed review of clinical history and imaging was conducted.

Results: Participants 1-3, all female, were 12, 17, and 32 years, respectively, at time of WB-MRI. For each, there was an underlying internal plexiform neurofibroma (PN) adjacent to the SI joint. All three were asymptomatic, without history of joint dislocations or pain. **Participant 1:** The first WB-MRI for this individual showed osseous dysplasia of the right SI joint, including widening of the fibrofatty portion. Follow-up WB-MRI a year later showed osseous dysplasia of the right SI joint with an intervening linear soft tissue abnormality consistent with infiltrative intra-articular PN. The tumor volume was too small to perform volumetrics. **Participant 2:** The first WB-MRI revealed dysplasia of the right posterior iliac crest and right hemisacrum with abnormal widening of the right S1 neural foramen. T2 hyperintense signal was noted in the right SI joint region, extending into the neural foramen, consistent with a focal diffuse PN. The tumor volume was too small to perform volumetrics. **Participant 3:** The first WB-MRI revealed a widened and dysplastic appearance of the left SI joint in the superior aspect. T2 hyperintense signal within the fibrofatty portion of the joint was identified, extending into the subcutaneous tissues. A year later, her second WB-MRI showed a PN with reticular tissue extending from the left SI joint into the surrounding posterior soft tissues. The tumor volume measured 61.7 mL.

Conclusion: Our findings show an underreported developmental abnormality of the SI joint in NF1. MRI characteristics include diffuse, non-mass-like PN tissue infiltrating and widening the SI joint. There is an associated osseous dysplasia of the bones of the SI joint. PN was identified in three asymptomatic individuals, demonstrating the value of WB-MRI.

Figure 1. (A, B) Axial and coronal STIR images show an infiltrative linear plexiform neurofibroma (blue arrows) in the left sacroiliac joint. There is widening of the joint and thinned dysplastic appearance of the posterior iliac crest. (C) Axial STIR image of the pelvis demonstrates a plexiform neurofibroma infiltrating the right sacroiliac joint characterized by linear T2 hyperintense signal that widens the joint (long blue arrow). The adjacent right posterior iliac crest is thinner than the unaffected left side consistent with dysplasia of the bone (short arrow). (D) Axial STIR image of the pelvis demonstrates a linear plexiform neurofibroma infiltrating and widening the right sacroiliac joint (blue arrow).



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Surgical Treatment of Dystrophic Kyphoscoliosis Secondary to Neurofibromatosis Type 1: Is Three-Column Osteotomy Necessary?

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Purpose: To evaluate whether the patients with thoracic kyphosis secondary to neurofibromatosis type 1 (DKS-NF1) must undergo three-column osteotomy during deformity correction surgery.

Methods: 84 patients with DKS-NF1 were retrospectively analyzed, and the average age was 17.7±6.9 years. There were 50 cases with single curve, 18 cases with double curves, and 16 cases with triple curves; kyphosis was found in 42 cases in the thoracic area, 31 cases in the thoracolumbar area, and 11 cases in the lumbar area. According to whether the patient has undergone three column osteotomy, they were divided into two groups: None three column osteotomy group (N-3CO) and three column osteotomy group (3CO). The preoperative, postoperative and follow-up imaging parameters of the two groups were measured and analyzed to evaluate the surgical effect.

Results: 74 patients were divided into the N-3CO group and 3CO consisted of 10 patients. The age of patients in the N-3CO group was significantly younger than that in the 3CO group (15.8 ± 4.8 years vs. 29.4 ± 10.2 years, P<0.001), and the proportion of preoperative traction in this group was significantly higher than that in the 3CO group (26/74 vs. 0, P=0.027). The patients with kyphosis in the 3CO group was mainly located in the thoracolumbar and lumbar area, significantly higher than that in the N-3CO group (10/10 vs. 32/74, P=0.003). The magnitude of kyphosis in the two groups were 73.8±20.9° and 63.1±21.4° before surgery, respectively (P=0.136). After surgery, they were corrected to 43.1±20.9° and 21.1±22.8°, respectively (P=0.003), with correction rates of 43.7±19.6% and 84.1±78.7%, respectively (P<0.001). At the last follow-up, they were maintained at 46.5±20.9° and 24.6±25.5°, respectively (P=0.003). The Cobb angle of the main curve was corrected from preoperative 83.0±29.0° and 66.3±17.7° (P=0.081) to postoperative 50.6±20.8° and 40.8±15.6° (P=0.155), with correction rates of 38.3±16.6% and 39.3±12.7% (P<0.849), respectively. At the last follow-up, they were maintained at 52.3±20.5° and 43.1±18.2°, respectively (P=0.185). The proportion of multi-rod system application and screw density in 3CO group were significantly higher than that in N-3CO group (8/10 vs. 20/74, P=0.002; 72.0±11.3% vs. 61.4±14.6%, P=0.033). The incidence of complications in the two groups was 12.2% (N-3CO group, 9/74) and 20% (3CO group, 2/10), respectively, with no statistically significant difference (P=0.613).

Conclusion: Three column osteotomy is mainly used to treat adult kyphosis in DKS-NF1 patients. While the none-three-column-osteotomy methods were mainly applied in young patients. Posterior approach with posterior column osteotomy was the mostly used plan, while anterior supplementary fusion was also an important option. For severe cases, preoperative Halo gravity traction can be used as a routine strategy. This can basically meet the treatment needs of young patients with DKS-NF1, and has good feasibility and safety as well as ideal deformity correction.

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The Incidence, Mechanism, and Clinical Outcomes of Postoperative Coronal Imbalance in Dystrophic Lumbar Scoliosis Secondary to Neurofibromatosis Type 1

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Purpose: To investigate the incidence, mechanism, and clinical outcomes of postoperative coronal imbalance in dystrophic lumbar scoliosis secondary to Neurofibromatosis Type 1.

Methods: A retrospective analysis was conducted on the data of 33 patients with dystrophic lumbar scoliosis secondary to Neurofibromatosis Type 1 (DLS-NF1) (with the apex located at L1 or below) who underwent surgical correction in our hospital from January 2007 to January 2018. There were 21 males and 12 females, with an average age of 14.5 ± 3.2 years and an average follow-up time of 34.0 ± 26.3 months. 11 cases were fused with distal internal fixation to L4 (i.e., the number of compensatory intervertebral discs at the distal end was 2), and 22 cases were fused to L5 (15 cases) or below (5 cases) (i.e., the number of compensatory intervertebral discs at the distal end was 1 or none). According to coronal balance (CB), patients with scoliosis are classified into three types: Type A (balanced state: CB<30mm); Type B (concave imbalance: CB≥30mm and C7PL located on the concave side of the main curve); Type C (convex imbalance: CB≥30mm and C7PL located on the convex side of the main curve).

Results: Satisfactory correction of lumbar scoliosis and kyphosis were obtained after surgery. CB increased from an average of 22.4±17.5mm before surgery to 27.6±10.9mm after surgery. There were 18 Type A patients, 0 Type B patients, and 15 Type C patients before surgery. After surgery, there were 17 Type A patients, 0 Type B patients, and 16 Type C patients. 16 cases (48.5%, 16/33) had coronal imbalance after surgery, of which 5 cases (27.8%, 5/18) had preoperative Type A conversion to postoperative Type C, and 11 cases (73.3%, 11/15) had preoperative Type C conversion to postoperative Type C (5/18 vs. 11/15, p=0.015). In addition, the proportion of postoperative Type C patients with a distal compensatory disc count of 1 or none was significantly higher than that of Type A patients (17/20, vs. 5/13, p=0.009). All patients with postoperative coronal imbalance showed improvement in decompensation 3-6 months after surgery.

Conclusion: The incidence of postoperative coronal imbalance is higher in DLS-NF1 patients (48.5%), which may be mainly due to preoperative convex coronal imbalance and fewer postoperative distal compensatory discs. However, all postoperative coronal imbalance patients can improve during follow-up.

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OTHER

Coexistence of Neurofibromatosis Type 1 and BMD in the Same Pediatric Patient

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Background: Neurofibromatosis Type 1 (NF1) is an autosomal dominant hereditary disease caused by NF1 gene mutations. The coexistence of NF1 and other genetic disorders in the same person are rare.

Case Presentation: We present a case of NF1 with Becker muscular dystrophy (BMD) in an 8-year-old boy. He had history of multiple café au lait spots since birth and a painless lump at the left chest for over 1 year. The child's past growth and development were similar to those of children of the same age. The patient's brother and parents have normal phenotypes. On physical examination, we found six café au lait spots with a diameter greater than 1.5cm and auxillary freckling. The maximum diameter of the café au lait spot is about 12cm, located on the left chest wall. Hair growth can be seen on the local skin, and a granular mass can be palpated below the skin.

Peripheral blood samples were collected from the child and his family members. Genomic DNA was extracted and whole exome sequencing was performed using high-throughput sequencing, followed by Sanger sequencing validation. A mutation was found in the NF1 gene and DMD gene of the patient, with NF1 c.6855C>A, which was a de novo mutation **(Figure 1)**; DMD EX51-EX52 Del, which originated from the mother **(Figure 2)**. Both of the above variants had been identified as pathogenic variants.

Since the child has no abnormal neurological manifestations such as delayed major motor development or muscle weakness, and no family history of Duchenne muscular dystrophy (DMD), peripheral blood myocardial enzyme spectrum testing was performed, and the serum creatine kinase (CK) concentration was found to be 572IU/L (reference value 22-200IU/L), and creatine kinase MB (CK-MB) was 29IU/L (reference value 0-20IU/L). Combining clinical manifestations and genetic testing results, Becker muscular dystrophy (BMD) can be diagnosed.

Conclusion: The association of NF1 and Becker phenotype MD is quite rare. Although both conditions may lead to neurological damage, no significant neurological impairment was observed in this patient, suggesting that the two genes may function through different pathways.

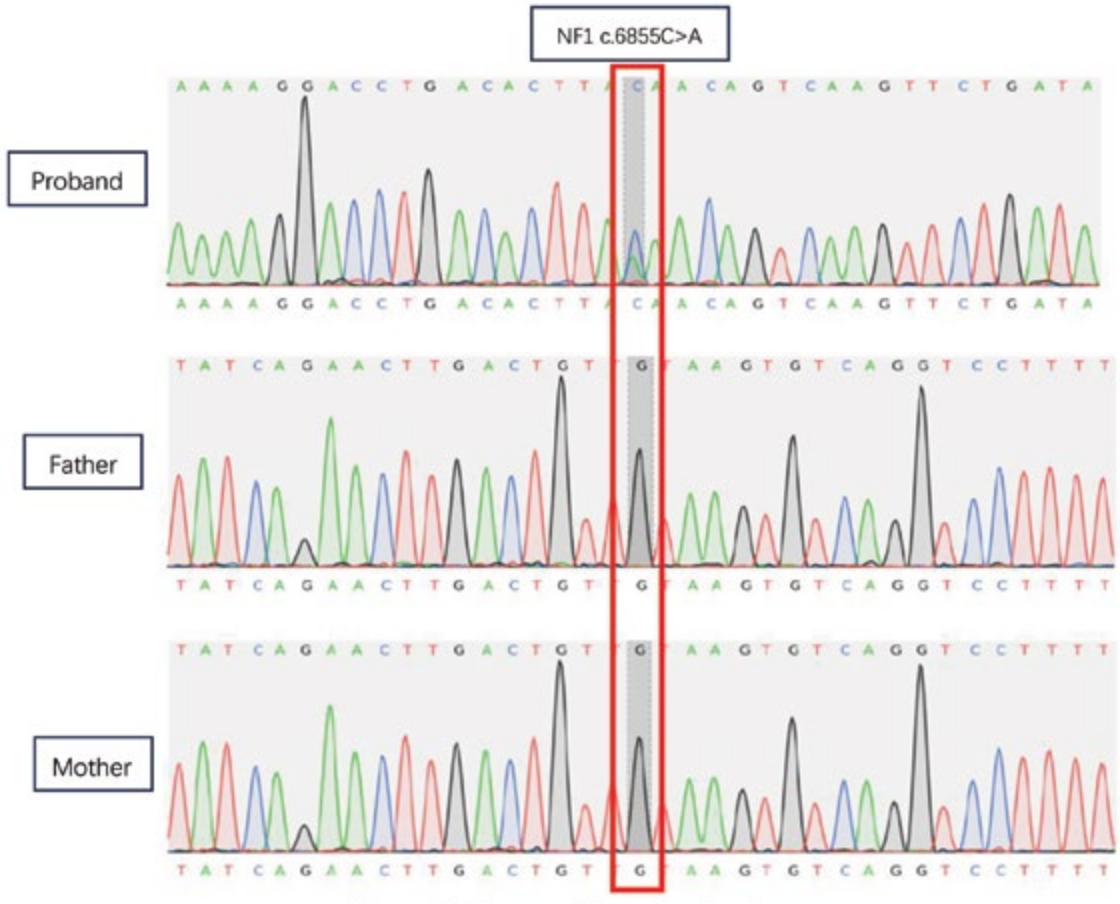

Figure 1 NF1 gene of the proband and parents

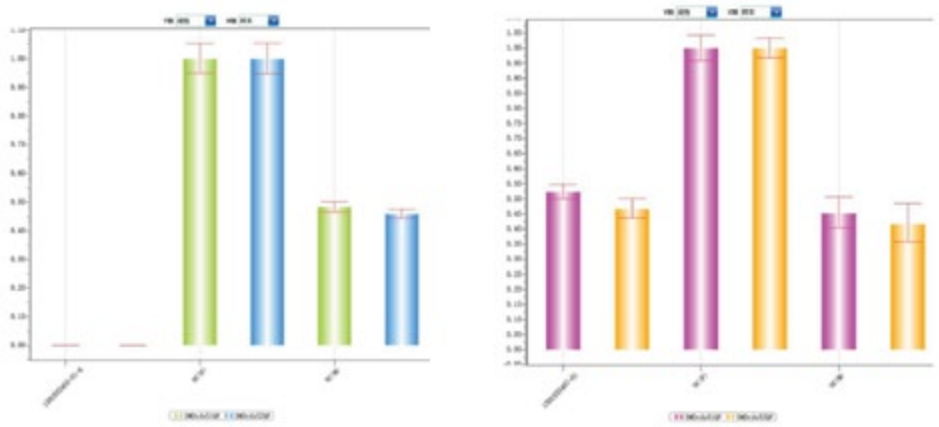

Figure 2 DMD gene mutations of the proband (left) and his mother (right)

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Experience in Treating Plexiform Neurofibromas in the Neck of Children

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Objective: The aim of this study is to observe the efficacy of surgical resection and targeted drug therapy in treating plexiform neurofibromas in the neck of children.

Methods: A 3-year-old child with a progressively enlarging plexiform neurofibroma in the occipitocervical region underwent surgical resection, achieving a resection extent of over 95%. Regular imaging follow-ups were conducted postoperatively, with the follow-up period exceeding five years. Additionally, two other children with plexiform neurofibromas in the neck were treated with the targeted drug selumetinib. After starting the medication, they underwent neck MRI scans approximately every four months for follow-up.

Results: In the child who underwent surgical treatment, significant recurrence of the plexiform neurofibroma in the neck was observed at the first follow-up examination four months after surgery. Imaging studies conducted at different time points postoperatively revealed progressive enlargement of the plexiform neurofibroma in the neck. Among the two children treated with medication, the tumor in one child progressively shrank after taking the drug, while the tumor in the other child remained largely unchanged following treatment.

Conclusion: For plexiform neurofibromas in the neck, both surgical resection and targeted drug therapy have demonstrated efficacy. However, considering the rapid growth of tumors during childhood and the high likelihood of recurrence after surgery, targeted drug therapy or a combination of surgery and targeted drugs appears to be a more rational treatment approach.

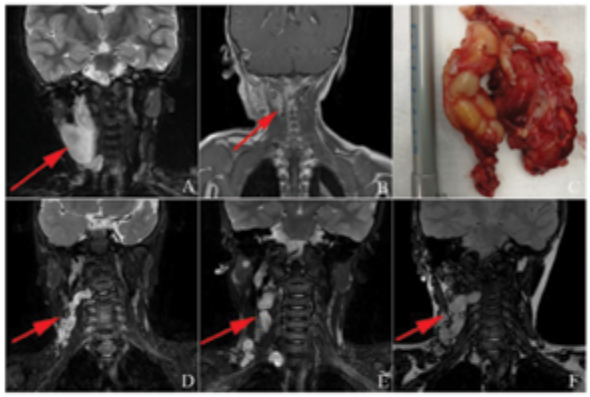

Fig.1 Imaging data before and after surgery for the child who underwent surgical treatment.A: Preoperative MRI showed a large tumor in the neck.B: The MRI performed three days after surgery showed that the tumor had been largely resected.C: The tumor specimen resected during surgery.D: The MRI performed four months after surgery showed tumor recurrence.E: The MRI performed 28 months after surgery showed progressive enlargement of the tumor.F: The MRI performed 50 months after surgery showed continued enlargement of the tumor.

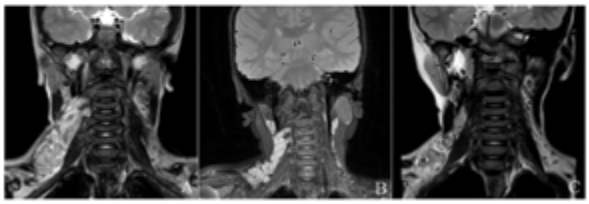

Fig.2 Imaging Data Before and After Targeted Drug Therapy for one Child.A: The MRI performed before the administration of the targeted drug showed a large tumor in the neck. B: The MRI performed four months after the initiation of the targeted drug therapy showed a significant reduction in the size of the neck tumor.C: The MRI performed eight months after the initiation of the targeted drug therapy showed continued shrinkage of the tumor.

Patient with High Tumor Burden, Noonan-like Facies, and Germline *NF1* p.Arg1809 Variant: A Case Report

Alise Blake, MS, CGC, *St. Jude Children's Research Hospital, Oncology*

Background/Purpose: Neurofibromatosis Type 1 is a common genetic disorder characterized by over 2800 pathogenic variants in *NF1,* most with variable phenotypic presentations. However, one of the few described genotype-phenotype correlations occurs at *NF1* p.Arg1809. Pinna et al. reported 14 patients with p.Arg1809 missense variants who had café au lait macules (CALMs) and Noonan-like facies, but lacked cutaneous, subcutaneous, or plexiform neurofibromas (PNs; Eur J Human Genet, 2015). Rojnueangnit et al. identified patients with p.Arg1809 missense variants are significantly more likely to have short stature or pulmonic stenosis ($p<0.0001$), and less likely to have cutaneous neurofibromas, optic pathway glioma (OPG), scoliosis or PNs compared to the NF1 population ($p<0.05$; Hum Mutat, 2015). This case report describes a patient with NF1 p.Arg1809 frameshift variant and high NF1-associated tumor burden.

Case Report: We present a 21-year-old male with maternally inherited *NF1* c.5425delC; p.Arg1809AlafsTer33. Similar to the literature, he has short stature, CALMs, and Noonan-like facies including palpebral ptosis, wide neck, low set ears, and retinal pigment epithelial hypertrophy. Conversely his phenotype has been more severe with a ventricular septal defect and symptomatic OPG in infancy, scoliosis associated with multiple spinal PNs requiring surgical intervention in early childhood, then numerous PNs in late adolescence and early adulthood. At age 17, a rapidly growing thigh PN was resected revealing atypical neurofibromatous neoplasm of uncertain biologic potential (ANNUBP) with loss of CDKN2A and CDKN2B. At age 19 he developed spinal cord compression prompting a large partial resection of a C3-C4 PN followed by treatment with a MEK-inhibitor. However, treatment was discontinued after 15 cycles due to mild cardiotoxicity in the setting of a suboptimal response. Most recently a growing left forearm lesion was resected and histology confirmed ANNUBP with loss of CDKN2A. The patient continues to be followed closely due to numerous remaining nodular PNs. Family history is notable for individuals with spinal neurofibromas and Noonan-like facies, but there is no history of malignant peripheral nerve sheath tumors.

Conclusion: This individual's p.Arg1809 variant correlates well with the reported phenotypic overlap with Noonan syndrome. However, this frameshift variant predicts an alternate stop codon and loss of function due to premature protein truncation or nonsense-mediated mRNA decay which may explain why he does not fit the low tumor profile reported with p.Arg1809. More research is needed to understand *NF1* variant impact on malignant transformation as well as response to, and toxicity from, systemic therapy with MEK inhibition.

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Evaluating Healthcare Transition in Patients with Neurofibromatosis Type 1: A Retrospective Study

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Purpose: Healthcare transition (HCT) is a complex intervention that implies moving from a pediatric health care model to an adult-centered system. Young adulthood is a critical period for patients with neurofibromatosis type 1 (NF1) as many medical complications occur during this timeframe. Youth with NF1 may lack knowledge about their disease, prognosis, complications and the importance of an adequate follow-up through life. They may also face neurodevelopmental and psychological difficulties complicating their self-management skills. The primary objective of our study was to characterize the effectiveness of our transition program from the pediatric center at CHU Sainte-Justine (CHUSJ) to the adult reference center at the Centre Hospitalier de l'Université de Montréal (CHUM) by evaluating the number of transitioned patients. The secondary objective was to feature factors preventing transition to the adult health center to understand the reasons for discontinuation of medical care.

Method: We conducted a retrospective study of NF1 patients aged 17 years and older, followed at the CHUSJ between 2021 and 2024 and who had undergone a transition process to the CHUM. Patients' files were analyzed for demographic data. Patients who had not been seen at the CHUM were called by phone to answer a questionnaire.

Results: Sixty-four patients aged 17 years or older, were included in the study with a sex ratio (H/F) of 1,6. Forty-eight patients (72%) had sporadic NF1 and eighteen patients (28%) had a familial history of NF1. Forty patients (62,5%) attended their consultation at the adult center. Average age for transition was 18 years and 2 months with an average delay of 10 months. Eight patients (12,5%) continued to be followed at the CHUSJ even though they were over 18 years old. Sixteen patients (25%) were not seen at the adult center. Among them, three patients (18,7%) were lost to follow-up before transition, seven patients (43,7%) did not receive an appointment from the adult center, three patients (18,7%) are followed by another caregiver and three patients (18,7%) did not answer the questionnaire.

Conclusion: Healthcare transition is a critical period for patients with NF1 as many patients are lost to follow-up. Our study attempts to assess the effectiveness of our transition program and to highlights limiting factors. A structured transition program reduces the loss of follow-up of patients, tends to prevent medical complications and enhance patient's involvement in the management of their disease.

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Increased Myelin Concentrations in Children with Neurofibromatosis Type-1 Shown by Quantitative T1 Magnetic Resonance Imaging

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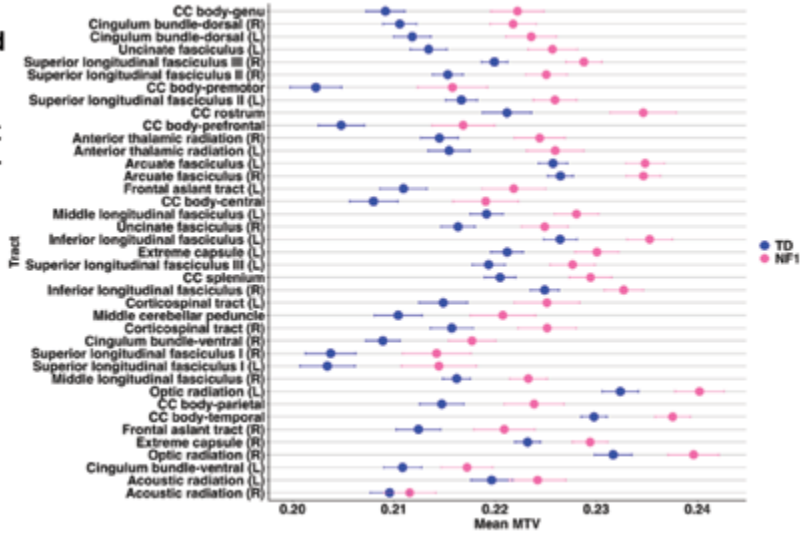
Purpose: Preclinical models of neurofibromatosis type-1 (NF1) show aberrations in myelin content, however, there is a need for *in vivo* techniques that can detect myelin alterations in clinical populations[1,2]. We investigated quantitative T1 mapping (QT1) as a clinically feasible tool for measuring myelin content in children with NF1.

Methods: Fifteen children with NF1 (M_{age}=9.59, SD_{age}=2.24; 8 male) and 23 typical developing (TD; M_{age}=10.2, SD_{age}=3.48; 11 male) peers participated in this prospective study. We collected structural, multi-shell diffusion-weighted, and QT1 MRI data on a GE Premier 3T scanner. QT1 maps were produced using open-source Python code. The macromolecular tissue volume (MTV), a proxy for myelin concentration, was extracted from 39 white matter tracts using TRActs Constrained by UnderLying Anatomy within FreeSurfer[3]. We extracted R1 values from 360 brain regions per subject based on the Human Connectome Project multi-model parcellation[4]. Previous work shows the R1 (1/T1) and MTV measurements generated by QT1 are reliable measures of myelin content[5,6]. We compared MTV in the 39 white matter tracts and cortical R1 in 360 regions between-groups using analyses of covariance, including age and sex as covariates in R 4.4.1 (R Core Team, 2024).

Results: Thirty-six of 39 tracts showed significantly increased MTV in NF1 relative to TD (p_{FDR} <.05), indicating elevated white matter myelin in NF1 **(Figure 1)**. The largest effect sizes were found in the genu of the corpus callosum (p_{FDR}<.001, Cohen's d=1.40), the right (p_{FDR}<.001, d=1.40) and left cingulum bundle-dorsal (p_{FDR}<.001, d=1.37), and the left uncinate fasciculus (p_{FDR}<.001, d=1.37). We did not find any significant differences in cortical R1 between NF1 and TD, suggesting similar levels of cortical myelin content in both groups.

Conclusion: Our findings suggest increased white matter myelin content in NF1 relative to TD. Mouse models of NF1 shows proliferation of oligodendrocyte precursor cells[7]. We expect that the affected oligodendrocyte lineage may contribute to the elevated myelin observed in this work, however, further studies are needed to uncover the exact mechanism. The significance of these findings warrants further investigation with a larger sample size and greater statistical power. Myelin is often linked to cognitive functioning, thus future studies should investigate relationships between myelin content and cognition in NF1[8]. Our results and the short scan time required (approximately 3 minutes for a QT1 acquisition) suggest QT1 may be a promising technique for clinical translation.

Figure 1. Average tract MTV and standard errors in NF1 and TD groups, ordered from largest effect size at the top to smallest effect size at the bottom. Thirty-six of 39 tracts show significantly elevated MTV in NF1 relative to TD (p_{FDR}<.05). *CC=corpus callosum; L=left; MTV=macromolecular tissue volume; NF1=neurofibromatosis type 1; R=right; TD=typical developing.*



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A Retrospective Study on Epidemiological and Disease Characteristics of Chinese Neurofibromatosis Type 1 Patients in Real World: Promise Study

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Background: Neurofibromatosis type I (NF1) affects about 1 in every 3000 people worldwide. 30-50% NF1 patients develop plexiform neurofibromatosis (PN) which grow rapidly in early childhood and can cause severe complications. This systemic disease imposes a heavy psychosomatic and financial burden on patients and their caregivers.

Methods: We performed a retrospective analysis of the NF1 patients visited 5 hospitals between Jan 2019 and May 2024. The prevalence, disease characteristics, progression free survival (PFS) and treatment pattern of NF1-PN were assessed.

Results: Totally 2277 NF1 patients were enrolled.

51.08% (1163/2277) of NF1 patients developed PN. The median age of patients at PN diagnosis was 14 years old. Head & neck (51.5%) was the most common locations of PN. 55.03% (640/1163) of patients with PN had PN related complications and 26.41% (169/640) of them had more than 1 complication. The most common complications are disfigurement (63.44%), functional abnormality (31.88%) and pain (30.57%).

Table 1. Prevalence of NF1 manifestations

NF1 manifestations	Rate
Café-au-lait	87.09% (1983/2277)
Cutaneous neurofibroma	39.35% (896/2277)
Axillary and inguinal freckle	62.98% (1434/2277)
Lisch nodule	7.58% (115/1517)
Optic pathway glioma	0.70% (16/2277)
Bone dysplasia	22.68% (367/1618)

22.79% (265/1163) patients received surgery. The mean surgery times was 1.14. 27.69% (72/260) patients had PN complete resected, 28.46% (74/260) patients had PN subtotal resected and 43.85% (114/260) had PN partial resected. 5.67% (66/1163) patients had drug therapy and selumetinib was the mostly used drug(93.9%, 62/66). 56.66% (659/1163) patients had follow-up and 32.56% (380/1163) patients only had follow-up without any treatment. The median PFS of 327 patients with PN evaluated by doctor was 23.36 months (95%CI 22.11～25.36). 173 patients were evaluated as progression, 47.40% of progressions were not realized by patients. 107 patients who received surgery were evaluated by doctors after surgery. The median after-surgery PFS was 23.33 months (95%CI 16.03～27.50). Patients who received complete resection had significant longer PFS than patients who received partial resection (47.44 months vs 23.36 months, HR 0.50, 95%CI 0.30-0.82, P=0.006).

Conclusion: The retrospective analysis shows PN can be found in 51.08% NF1 patients and can cause severe disease burden. PN is a progressive disease. Early diagnosis, early treatment and regular follow-up are important to NF1-PN. Only a small proportion of PN can be fully resected and partially surgery can't prolong the PFS of PN. With drug treatment approved for PN, inoperable PN needs new treatment plan.

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Outcomes of Paediatric Patients with Neurofibromatosis 1 (NF1) Treated with MEK Inhibitors in the North of England

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Purpose: In 2009, National Health Services (NHS) England established two Highly Specialised Services (HSS) for Complex NF1 in Manchester and London. In May 2022, NICE approved Selumetinib for the treatment of symptomatic inoperable plexiform Neurofibromas in children from 3-18 years. A national pathway and shared care agreement with paediatric oncologists was published in 2023. We analysed the outcomes of patients on MEK inhibitors over 8 years in northern England.

Methods: Retrospective notes from eligible children discussed in the national MEK inhibitor MDT as per National pathway for England (2017-2024) were assessed for consideration of treatment with MEK inhibitors. The main indicators for treatment were pain, disfigurement, and threat to function.

Results: Out of 65 eligible children discussed in MDT, 26 did not meet the full approval requirements. 8 children were enrolled in trial and 3 were on a waiting list for a trail. Their data is not included here. Since 2017, 28 children (17 males, 11 females) aged 1 to 17.1 years (average 8.5 years) have been treated with MEK inhibitors. Main indicators included disfigurement (5), pain (10), threat to function (13). All children approved for disfigurement had craniofacial plexiform. Threat to function included airway compromise (3) and swallowing and visual disturbances (1 each). 8 children had symptomatic compression-Spinal cord with 3 requiring additional decompression surgery. Side effects were reported in 21 patients: skin rashes and paronychia (17), loose stools and vomiting (2), and fatigue (1). Adjustments made for side effects included drug breaks and dose reductions, but treatments were not stopped for any of these patients.

Conclusion: None of the children stopped MEK inhibitors because of lack of efficacy or tolerability. Pain is a significant indicator and many of these children experienced pain relief even at lower doses of MEK inhibitors which meant that they had less side effects. Threat to function remains the main indicator for treatment with symptomatic cervical cord compression accounting for 53 % of cases. Children requiring decompression in infancy have a very difficult spinal stability course with morbidity. This group of children will need better exploration of their pathway of care.

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Disclosures: Dr. Grace Vassallo is a medical advisor for Alexion.

Multiomic Analyses at Single-Cell and Spatial Resolution Reveal Distinct Evolution Patterns and Immune Composition in PRC2-Loss Versus PRC2-Retained MPNST

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Purpose: Neurofibromatosis Type 1 (NF1) is a common neurogenetic and tumor predisposition syndrome, leading to the development of benign plexiform neurofibroma (PN) that can progress to malignant peripheral nerve sheath tumors (MPNST). Loss of function of polycomb repressive complex 2 (PRC2) which methylates histone H3 on lysine 27 (H3K27me3), is a common feature of MPNST and is associated with poorer prognosis in MPNST patients. We previously demonstrated MPNST with PRC2 loss exhibit a more immune suppressive environment. To deeply understand the role of PRC2 loss in MPNST pathogenesis to inform novel treatment options for NF1-MPNST, we performed multiomic analyses to explore how PRC2 loss impacts tumor evolution and immune composition.

Methods: Paired PN and MPNST samples from 12 patients (PRC2 loss: N = 7; PRC2 retained: N = 5) were collected to investigate the molecular events and microenvironment during tumor progression. At the genomic level, we defined clonal evolution from PN to MPNST with whole exome sequencing (WES) data by a novel pipeline developed in our laboratory: Precise DNA Variant Calling (PDVC). At the transcriptomic level, we integrated bulk and single-cell RNAseq data to determine how PRC2 loss influences meta-signatures and immune cell signaling pathways through deconvolution analyses. At the epigenetic level, we performed parallel proteomic and histone analyses to determine how PRC2 loss affects histone post-translational modifications and immune responses. Finally, at the spatial proteomic level, we utilized multiplexed Hyperion Imaging Mass Cytometry (IMC) staining to construct niche-similarity networks and validate the different tumor-stroma-immune composition in PRC2-loss vs -retained MPNST as well as their precursor lesions.

Results: Diverse clonal evolution patterns including distinct types of NF1, CDKN2A, and MTAP structural variants were identified in PRC2-loss vs -retained MPNST through PDVC-WEX pipeline. Global proteomics and transcriptomics analyses indicated MPNST with PRC2 loss have poorer antitumor immune infiltration and responses compared to those with PRC2 retained. The trial study of IMC data provided a differential landscape of morphological features, spatial cellular interactions, and niche-similarity networks in PRC2-loss vs -retained MPNST. All IMC results and supplementary single-cell RNAseq data from NCI will be available before the Material Review Process of 2025 NF Conference by CTF.

Conclusion: Our findings demonstrated that MPNST with PRC2 loss are “cold tumors” with poorer antitumor immune responses and a more malignant progression path from PN, compared to those MPNST with PRC2 retained, which can potentially offer clues to develop treatments targeting the immune system for NF1-MPNST.

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The Spectrum of Skin Toxicities in Children with NF1 Treated with MEK Inhibitors

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Purpose: Skin side effects are among the most common toxicities described in patients receiving treatment with MEK inhibitors (MEKi); impacting quality of life and treatment interruption. Recognition of the wide spectrum of their clinical presentation is an essential first step in directing appropriate management.

Methods: Records of all children and young adults who were treated with MEKi for symptomatic inoperable plexiform neurofibromas by the National Health Service England, Highly Specialised Service for Complex NF1 in Manchester, since 2017 were identified. This service has dedicated joint dermatology, paediatric neurology, paediatric oncology and specialist nurse clinics for all children and young adults treated with MEKi.

Results: Thirty-six children and young adults were treated with MEKi (2017-2025). Eight of these were part of a clinical trial and their data will not be presented here. Twenty-eight patients are currently being treated with MEKi for symptomatic inoperable plexiform neurofibromas (17 male and 11 female; median age 8.5-years; age range at the start of treatment 1-year to 17-years and 1-month). Out of 28 patients, 21 experienced side effects from their MEKi. The most common side effects were skin related changes in 85% of patients (n=18). A broad spectrum of skin toxicity was observed and the clinical severity of these was highly variable, ranging from mild xerosis of the skin and depigmentation of hair colour to widespread (near total skin involvement) eczema-like and acneiform eruptions.

We also observed a high frequency of paronychia, again of varying severities. Drug breaks were sometimes necessary, and two children required nail bed surgery before MEKi could be restarted. Necrotic skin breakdown around a PortaCath occurred in one child, necessitating its removal. Hitherto unreported skin changes, including spider naevi and an inflammatory collarette of scale surrounding an established café-au-lait patch, have also been noted. We have also observed significant psychosocial distress and additional symptom burden.

Conclusion: The spectrum of skin changes in children with NF1 receiving treated with MEKi is widening as their use and clinical expertise increases. Recognition of these is essential, supporting successful use of MEKi and helping to inform managements guidelines. Further studies to explore the predisposing and protective factors for the development of skin toxicities with MEKi therapy are indicated and these may provide the key for improved prophylactic/early intervention measures.

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Call for Neurofibromatosis Specialty Care Clinics in South Carolina

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Purpose: Grounded in patient-centered care, this study assesses the perceived need for a dedicated multidisciplinary clinic for individuals and families with neurofibromatosis type 1 (NF1) in South Carolina, USA.

Methods: A 62-question online cross-sectional survey, available in English and Spanish, was distributed to South Carolina residents over age 18 years who were either adults who do not have NF1 but have children with NF1, adults with NF1 who have children with NF1, and adults with NF1 who do not have children or may have children without NF1, to capture a wide range of experiences. Survey responses were analyzed using descriptive statistics to summarize key findings and chi-squared and Fisher's exact tests for categorical comparisons. Free-response data were examined by content analysis and evaluated by a second researcher.

Results: Of a total of 52 survey responses analyzed in the study, 90.4% indicated agreement that a specialty clinic should exist in South Carolina. More than 70% of participants reported adherence to medical advice for NF1 and saw a doctor at least once per year, with children and adults seeing several relevant specialists. Analysis of participant free text responses identified 4 clinical care gaps and 4 educational gaps in current care-seeking behaviors.

Conclusion: Establishing a dedicated, multidisciplinary care center for individuals with NF1 in South Carolina can address 3 out of the 4 clinical care gaps and 1 out of the 4 education gaps identified by the content analysis and is highly supported by participant preference.

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The Development of Nurse Led Transition Clinic and Teenage Peer Support as Part of the Transition Pathway for Children and Young People with Neurofibromatosis Type 1 (NF1)

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Purpose: The nationally commissioned Neurofibromatosis Type 1 (NF1) complex service at Guys Hospital recognised the role of structured transition in improving health outcomes for young people with NF1[1]. Historically, transition to adult service was facilitated by the young person's clinician. Evaluation in 2022 identified the need for specialist nurse involvement for effective transition.

Method: The development of the transition service constituted 3 elements; the introduction of the 'NF1 hospital passport', initiation of a nurse-led transition clinic, and the reinstatement of 'Teenage Days'. Transition is co-ordinated by a multi-disciplinary team (MDT) approach.

The transition clinic, launched in March 2025, is led by two paediatric NF clinical nurse specialists. It runs monthly, aiming to prepare young people for independent management of healthcare and ensure a smooth handover to adult services. This is consolidated through the completion of the 'NF1 hospital passport' during their appointment[2]. Supervision is provided by consultant paediatric neurologists.

'Teenage Days' began in 2014 but were disrupted by the COVID-19 pandemic. They were reintroduced as annual events. Attendees' experiences were evaluated through patient and parent surveys. Frequency was increased to twice a year to improve accessibility and awareness of peer support.

An MDT approach added an NF1 social worker and physiotherapist (PT) to assist young people in areas affecting their independence, such as PT support for fatigue in patients on MEK inhibitors, and education around financial support from the NF1 social worker.

Results: Evaluation of the 'NF1 Hospital Passport' showed young people found it helpful in saving time and promoting independence by reducing the need to explain their condition. Feedback from NF1 youth advisory groups is needed for broader insights[3].

Attendance at 'Teenage Days' showed progress: 16 children expressed interest, and 5 attended. Non-attendance was due to holidays, sickness, or travel. Attendance increased from zero when offered virtually the previous year. Feedback indicated smaller groups (4-5 attendees) reduced social anxiety, and desired topics were sleep, diet, pain management, anxiety, and bullying.

Parents reported that attending the events increased their child's confidence and understanding of their condition. Attendees wished for more time with other children with NF1.

Conclusion: Nurse-led transition in NF1 is crucial to ensure young people experience smooth, coordinated, and holistic care. This should include education, psychosocial support, and safety netting with MDT support. Providers for children with NF1 should offer opportunities for socialisation with other children their age who share their condition.

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Proposed Role of Physiotherapy in the Management of Fatigue for Paediatric Patients with Neurofibromatosis Type 1 Undergoing Selumetinib Treatment

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Background: Neurofibromatosis Type 1 (NF1) is a neurological genetic condition characterised by the growth of benign tumours called plexiform neurofibromas. Selumetinib is an oral MEK-Inhibitor licensed by the NHS for treating symptomatic and inoperable plexiform neurofibromas in children aged 3-18. Research shows the drug improves pain and function in paediatric patients; though side effects, including skin irritation and fatigue, can occur[1]. Physiotherapy interventions have been well documented in managing chemotherapy-induced fatigue (CIF) and could be a solution for Selumetinib-related fatigue[2].

Method: Fatigue is a homeostatic dysfunction caused by an increase in energy production demand by an external stimulus. For patients, it manifests as an increase in physical exertion required to complete a task they could previously complete with ease. Fatigue can also be cognitively driven, causing a reduction in cognitive performance. In both instances, fatigue is a multidimensional construct that can also impact mental health, pain, and sleep quality[3].

During Selumetinib trials, 44% of patients reported fatigue. It hasn't been established whether patients experienced physical and/or cognitive fatigue[1,4]. Physiotherapy interventions are effective for similar CIF cases and this poster will explore these interventions.

Results: Physical activity (PA) focuses on increasing muscle strength and aerobic capacity, via hypertrophic change, increased mitochondria, and blood flow[5], which relieves fatigue and improves quality of life in patients. It also improves psychological health and sleep quality, which in turn positively impacts fatigue[2].

Energy Conservation Techniques (ECT) involve changing an activity or environment to decrease the energy required to complete tasks. Techniques include identifying activities that drain and recharge the child's battery; and adapting lifestyle changes to optimise energy while avoiding the 'boom and bust' phenomenon; over-exertion followed by extreme fatigue. Timetabling high-energy activities and monitoring fatigue changes is also important[6].

Collaborative goal setting involves setting achievable goals with the child's support network. Goals must be self-identified by the patient, rather than set by therapists and parents, to help drive behaviour change and motivation. Goal setting enhances positive behaviour change around PA and ECT[7].

Supporting families during chemotherapy aids emotional wellbeing, helping both parents and children. Providing education about the above strategies and empowering them to progress without supervision enables a cohesive treatment programme[8].

Conclusion: Physiotherapy has been shown as an effective tool in CIF in paediatric patients and should be considered as a therapeutic adjunct in NF1 paediatric patients undergoing Selumetinib treatment.

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Neurorradiological Findings in 125 Patients with NF1 at the Neurofibromatosis Unit of the Italian Hospital in Buenos Aires, Argentina

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Purpose: This paper presents the neurorradiological findings of 125 patients with NF1 treated at the Neurofibromatosis Unit of the Italian Hospital in Buenos Aires.

Methods: the Neurofibromatosis Unit at the Italian Hospital of Buenos Aires has been engaged in the diagnosis, monitoring, and treatment of patients with neurocutaneous disorders since 2022.

We present the neuroradiological findings of 125 patients with NF1 evaluated at our multidisciplinary team in the time period between 2023 and 2024. We focused on documenting neuro-oncological pathology, the presence and localization of focal areas of signal intensity (FASI), vascular anomalies, plexiform tumors of the head and neck, sphenoidal dysplasia and its association with adjacent lesions, as well as reporting the NF1 neurorradiological findings at the brain in patients with specific phenotypes of neurofibromatosis, such as spinal variants of this condition and mosaicism.

Results: the average age of the patients was 33 years. The youngest patient was one and a half years old, while the oldest was 86 years old. Neuro-oncological pathology was detected in 30 patients (37.5%). The most common neoplasm was glioma, accounting for 73.3% of cases (22/30). Optic nerve glioma was observed in 17 patients. Among the remaining 5 patients, one presented with glioblastoma and two had pilocytic astrocytomas at the level of the brain parenchyma. Two patients received a presumptive diagnosis of low-grade glial neoplasm and have not yet been biopsied, as these were identified as incidental findings. Two patients with systemic NF1 presented with craniopharyngiomas and required surgical intervention. Approximately 13.3% of the patients had meningiomas, and one patient was diagnosed with a hemangiopericytoma confirmed through pathological anatomy, which was radiologically interpreted as a meningioma. Neurovascular findings were present in 10% of the patients, including the detection of Moyamoya syndrome in one patient. A left vertebral artery dissection was identified in a young 39-year-old patient, while another young patient experienced a stroke in the context of a diagnosis of malignant peripheral nerve sheath tumor (MPNST).

Other notable findings included Arnold-Chiari malformation type 1 in 3 patients, cortical dysplasia in 1 patient, stenosis of the Sylvian aqueduct in 2 patients. The presence of FASI was detected in 47% of our patients, with the most frequent locations being the globus pallidus (24%) and the cerebellum (23.2%). Other common sites included the cerebral peduncles (13.6%), thalamus (12.8%), and brainstem at 10.4%, predominantly in the midbrain. Of the 125 patients, 8 were diagnosed with mosaicism and three with spinal neurofibromatosis. None of them exhibited any brain abnormalities related to NF1. One patient with mosaicism presented retinal vascular anomalies in the right eye, which, when combined with focal skin involvement on the same side (an atypical large café-au-lait spot situated on the neck), led to the diagnosis.

Approximately 10% of the evaluated patients presented with head and neck plexiform neurofibromas. Sphenoidal dysplasia was a positive finding in 8 patients (5.6%). Two of them presented this alteration in isolation. Another two associated it with gliomas of the optic nerve on the same side, while 4 presented giant plexiform neurofibromas surrounding the orbit.

Conclusion: The neuroradiological findings associated with NF1 are extensive, highlighting the complexity and variability of this condition, which should be well understood by specialists in the field of NF1.

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NF1 and Additional Genetic Alterations in Other Genes

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Purpose: The purpose of this study is to emphasize the importance of considering further genetic workups, even for patients already diagnosed with neurofibromatosis type 1 (NF1). By reviewing the clinical phenotypes of five patients with NF1 who exhibited additional features warranting further genetic evaluation, we discovered additional genetic alterations in other genes. This highlights the need for comprehensive genetic assessments to better understand and personalize patient care and treatment.

Methods: Present detailed clinical phenotypes of five patients with NF1 and additional genetic alterations in other genes.

Results: Table 1: Clinical Characteristics and Genetic Alterations in Five Patients

Patient	1	2	3	4	5
Clinical features prompting additional genetic testing	DD, ASD, myoclonic epilepsy	High arched feet, hammertoes, muscle atrophy of extremities, decreased sensation and tingling of feet, walking difficulty	DD, ASD, micropenis and buried penis, corpus callosum abnormality and colpocephaly	Bilateral renal cysts, complex postaxial polydactyly, pigmentary retinal dystrophy, & poor bilateral vision	Hemiplegic migraines, mild asymmetry in the internal carotid arteries with tortuous vertebral and basilar arteries.
***NF1* variant**	*NF1*:c.4369A>C, (p.Lys1457Gln)	*NF1*:c.278G>A, p.(Cys93Tyr)	*NF1* deletion	*NF1*:c.3113+1G>A	*NF1*:c.4625T>G, p.(Leu1542Arg)
Additional genetic variants in other genes	*SLC6A1*: c.1228G>T p.(Asp410Tyr) associated with myoclonic-atonic epilepsy	arr[GRCh37] Xp22.33(298124_504691)x1, 17p12(14110120_15419589)x3: The Xp22.33 deletion includes the upstream enhancer region of the *SHOX* gene, and the 17p12 duplication involves the *PMP22* gene consistent with Charcot-Marie-Tooth disease type 1A.	A heterozygous 1.4 Mb microdeletion encompassing *NF1, SUZ12P, CRLF3, ATAD5, ADAP2, RNF135, UTP6, SUZ12,* and *LRRC37B, C17orf42, RAB11FIP4* and *C17orf79.*	7.5% heteroplasmy of *MT:TL1*:m.3243A>G linked to Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes (MELAS). *BBS1*:c.1169T>G, p.(Met390Arg) & *BBS1*: c.1285C>T, p.(Arg429*) consistent with Bardet-Biedl syndrome Prenatally found 47,XXY (Klinefelter syndrome)	*COL4A2*:c.883G>T, p.(Glu295Ter) associated with brain small vessel disease 2

Abbreviations: DD: delayed development; ASD: Autism spectrum disorder

Conclusions: Many individuals likely carry pathogenic variants in multiple genes. When a patient diagnosed with NF1 exhibits additional atypical features, further genetic evaluation is recommended. This approach can enhance our understanding of the patient's phenotype and enable more personalized medical care.

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Feasibility, Acceptability, and Preliminary Efficacy of an Online Platform to Promote Evidence-Based Care for Underserved Patients with Neurofibromatosis 1 (NF1)

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Purpose: The majority of U.S. NF1 patients do not regularly attend specialized NF clinics; this population is less likely to receive NF1 care recommended by the American Academy of Pediatrics (AAP) and American College of Medical Genetics and Genomics (ACMG). To address this gap, we tested whether an educational intervention for patients/parents and primary care providers (PCPs) could be used to increase receipt of recommended care at annual well visits.

Methods: We conducted an open pilot study of *My NF Guide* with 10 adults and 10 parents of children with NF1 from across the U.S. who don't attend a specialized NF clinic. Adults/parents completed an online form that generated two letters with personalized NF1 care recommendations based on AAP/ACMG guidance: one for themselves and one for their/their child's PCP. Adults/parents completed pre-visit surveys, post-visit surveys, and interviews to assess the intervention's *feasibility* [patient/parent-reported use of intervention components], *acceptability* [Telehealth Usability Questionnaire (TUQ); Client Satisfaction Questionnaire (CSQ-8)], and *preliminary efficacy* [patient/parent-reported receipt of recommended care; Consumer Assessment of Healthcare Providers and Systems (CAHPS) Visit Rating; Patient Activation Measure (PAM-13)].

Results: All 20 eligible participants (n=10 English-speaking, n=10 Spanish-speaking) enrolled in the study, completed the *My NF Guide* online intake form, and read their personalized care recommendations. 18 participants (90%) gave their PCP this information; 2 Spanish-speaking adults did not due to not attending the PCP visit or not understanding what materials to share. Participants were highly satisfied with *My NF Guide* (median CSQ-8 = 28/32) and found it easy to use (median TUQ = 6.4/7). The median proportion of recommended NF1 care completed was 89% for children and 60% for adults (Figure 1). Participants' satisfaction with their/their child's PCP visit was high (median CAHPS rating = 8.5/10); all participants gave ≥7/10 ratings except one Spanish-speaking adult whose PCP refused to use intervention materials. Participants' confidence in managing their/their child's care improved by 7 points after using the intervention (median PAM-13 = 65.5/100 pre-intervention, 72.5/100 post-intervention), exceeding the minimal clinically important difference for this measure.

Conclusion: *My NF Guide* is a feasible and acceptable tool to improve NF1 patient/parents' knowledge of and primary care providers' implementation of evidence-based NF1 care, although Spanish-speaking adults may need additional support to communicate with PCPs. The efficacy of this intervention is currently being assessed in a nationwide, decentralized randomized clinical trial enrolling children and adults with NF1 who do not attend specialized NF clinics (NCT06262113).

Figure 1. Completion of AAP- and ACMG-recommended health screenings and education

A. Children with NF1

Recommendation	ENP1	SPP13	ENP2	ENGP3	ENP8	SPP12	ENGP7	SPP11	SPP10	SPP9
Age	1.5	2	3	4	6	6	14	15	15	16
Weight/Height										
Blood pressure										
Skin exam										
Neuro exam										
Scoliosis										
Tibial bowing										
Developmental										
Early puberty										
Eye exam										
MPNST										
Reproductive										

Completed at PCP visit	Completed by other provider	Not completed	N/A	Unknown/did not wish to say

B. Adults with NF1

Recommendation	ENA3	SPA15	ENA9	ENA10	SPA7	SPA13	SPA12	ENA8	ENA1
Age	36	40	50	54	54	61	63	67	76
Blood Pressure									
Skin Exam									
Neuro Exam									
Scoliosis									
Neuropathy									
Chronic Pain									
Depression									
Breast Cancer									
MPNST									
Reproductive									

Legend: Each column represents one open pilot participant (n=19 individuals who attended their PCP visit), with participant ID labeled as EN for English-speakers or SP for Spanish-speakers.

Each row represents AAP- or ACMG-recommended NF1 care, including measuring weight/height and blood pressure; performing skin and neurological exams; visually assessing for signs of scoliosis, tibial bowing, and early puberty; reviewing developmental milestones and school progress; screening for symptoms of neuropathy, chronic pain, and depression; ordering/ recommending eye exams and breast cancer screening (mammography or breast MRI); and educating participants on MPNST warning signs and reproductive options/offspring recurrence risk.

Green indicates care completed by the PCP or by another healthcare provider within the AAP-/ACMG-recommended timeframe. Red indicates care was not completed as recommended. Gray indicates screening/education was not needed based on the participant's age, sex, or reported health history. White indicates that a participant did not report whether or not care was performed.

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Cardiac Surveillance for NF1 Children: What Have We Learned So Far?

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Purpose: Timely diagnosis and management of NF1 and cardiac abnormalities in patients would provide a benefit for this population with these issues.

Introduction: Neurofibromatosis (NF1) is known to be one of the most common genetic disorders, with an incidence of 1 in 3000 individuals[1]. NF1 is a multisystemic disorder, in which cardiovascular disease burden in children with NF1 includes hypertension, congenital heart disease, hypertrophic cardiomyopathy amongst other manifestations. We presented a previous study by the authors in 2021 that reported a high incidence (~9%) of children with NF1 having cardiac abnormalities.

Methods: A retrospective chart review of all patients <21 years of age identified with NF1 (2021-2024) at a single referral center. Clinical information was obtained from their charts and clinic database. All patients met NIH clinical criteria for NF1 or have confirmation of NF1 with genetic testing. As part of our routine assessment, all patients confirmed with NF1 were seen by a pediatric neurologist and referred to a pediatric cardiologist for full cardiac evaluation. Data collected: demographic data, age at time of EKG and echocardiography findings and their implications.

Results: We are conducting the study analysis comparing both studies. We have preliminary data showing that between 9-10% of NF1 patients have cardiac abnormalities.

Discussion and Conclusion: Abnormal cardiac findings in patients with NF1 are underestimated due to lack of a proper investigation and management protocol. Cardiovascular manifestations are known to be part of the NF1 vasculopathy spectrum[2,3]. However, there is scarcity of echocardiography/electrocardiography findings in NF1 patients. We still need to unveil the effect of *NF1* mutations on the cardiovascular system. Some studies that found a possible role of neurofibromin in the myocardium and its development, reported that potential pathogenic mechanisms are impaired apoptosis and aberrant proliferation during embryonic stages[4,5]. A recent study also showed a role in congenital heart disease, suggesting a role in epithelial-mesenchymal transition regulation[6]. In this study, patients with NF1 had surveillance, including EKG and echocardiogram. We will compare our results from the two different centers (Cleveland Clinic and UT Pediatric Neurosciences at Dell Children's). This is a preliminary study evaluating the prevalence of cardiac abnormalities in children with NF1. Results emanatory from these preliminary studies help us in recommending a complete cardiac evaluation in all newly diagnosed patients with NF1, so that timely treatment and intervention with an early detection can be instituted.

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Cardiological Manifestations in Pediatric Patients with Neurofibromatosis Type 1

Agustin Lombardi

Introduction: Neurofibromatosis type 1 (NF1) is a genetic disorder that affects multiple organ systems, with cutaneous and neurological manifestations being the most commonly recognized. However, the cardiological complications associated with NF1 often remain underdiagnosed, despite their potential to significantly impact long-term health. It is estimated that between 15% and 30% of patients with NF1 experience cardiovascular abnormalities, ranging from structural issues to functional heart dysfunctions. These manifestations, if not detected early, can substantially affect the patient's quality of life. Given the complexity of these cases, cardiological surveillance in NF1 patients is not only a medical necessity but also a clinical challenge requiring a high level of awareness, particularly among pediatric cardiologists. Furthermore, with the increasing use of novel treatments for plexiform neurofibromas, there is a growing need to recognize the potential cardiotoxic effects of these therapies. Early detection of cardiological complications can make a significant difference in preventing irreversible cardiovascular damage.

Objectives: To assess the prevalence and types of cardiological manifestations in pediatric patients with NF1 at a high-complexity pediatric hospital.

Materials and Methods: We conducted a retrospective review of clinical records from pediatric patients diagnosed with NF1 (ages 0-24) followed at our institution. A total of 117 patient records were reviewed, including their electrocardiograms (ECGs) and echocardiograms.

Results: The cohort included 117 patients with a mean age of 12.26 years. Of these, 36.7% (43 patients) had no prior cardiological evaluation. Among the 74 patients who had undergone cardiological assessments, 16.2% (12 patients) presented with cardiovascular abnormalities. Six patients had abnormal echocardiograms: 3 with mitral valve insufficiency due to prolapsed valves, 1 with multiple ventricular fibromas, 1 with a ventricular septal defect (VSD), and 1 with a combination of an atrial septal defect (ASD), VSD, and patent ductus arteriosus (PDA). Five patients showed ECG abnormalities: 2 with short PR intervals, 2 with supraventricular tachycardia, and 1 with first-degree atrioventricular (AV) block. One patient was diagnosed with hypertension.

Conclusion: Cardiological monitoring is crucial for all pediatric patients with NF1, as a significant proportion (15-30%) exhibit cardiovascular manifestations that can be identified and managed if detected early. Pediatric cardiologists should be well-versed in the potential cardiovascular issues associated with NF1 to enable timely diagnosis and intervention. Additionally, with the rise of novel therapies for plexiform neurofibromas, it is essential to be aware of the potential cardiotoxic effects of these treatments. Early identification of these complications can help mitigate the need to discontinue critical therapies, ensuring better outcomes and quality of life for NF1 patients.

Myopia and Neurofibromatosis

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Introduction: Neurofibromatosis (NF) is a genetic disorder that affects the development of neural crest derived tissues, including ocular structures in the eye, such as the sclera, choroid, ciliary body and part of the cornea and predispose to refractive defects such as myopia.

There are publications about the prevalence of myopia in patients with NF1, which is higher than the prevalence of myopia in the general population. Myopia has an overall prevalence of approximately 30%.

The aim of this work is to describe the prevalence of myopia and astigmatism in a series of patients with NF1 and compare it with the general population.

Materials and Methods: A retrospective and cross-sectional study of patients with NF1, treated at the Clinicas Hospital, the Italian Hospital, and Garraham Hospital was performed. The clinical and demographic characteristics of patients seen between January 1, 2024 and January 31, 2025 were collected from medical records. The sample was described with measures of central tendency and dispersion for continuous numerical variables and percentage for categorical variables. To compare the prevalence of myopia in the sample studied and the estimated prevalence in the general population, a one-way test of proportions was performed. A p value of less than 0.05 was considered statistically significant. R software was used for the statistical analysis.

Results: The study included a total of 123 participants, with a mean age of 31.2 years (SD = 16.9). Of the total sample, 56.9% (n = 70) were women and 43.1% (n = 53) were men. Of the participants, 98.4% (n = 121) were of Argentine nationality, while 1.6% (n = 2) were foreigners. Participants were recruited from three medical centers: 32.5% (n = 40) from Garraham, 30.9% (n = 38) from HCJSM, and 36.6% (n = 45) from Hospital Italiano. The prevalence of myopia was 49.6% (n = 61), with a right eye involvement of 41.5% (n = 51), and 47.2% (n = 58) in the left eye. When comparing with the general population, a statistically significant difference was observed X-squared = 21.563, p-value = <0.001. Regarding astigmatism, 52.8% (n = 65) presented astigmatism.

Discussion: In this study, a significantly higher prevalence of myopia and astigmatism was observed in patients with neurofibromatosis type 1 (NF1) compared to the general population, further research is needed to clarify this relationship.

Conclusion: These results suggest that refractive defects are more common in NF1, could be an additional feature?

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Cancer Risk in Patients with Neurofibromatosis Type 1: Hereditary Tumor Cohort Study in Japan

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Purpose: Previous studies reported that patients with neurofibromatosis type 1 (NF1) develop neoplasms at a significantly higher rate than the general population (Uusitalo E et al., 2016, Landry JP et al., 2021). However, few studies have evaluated the risk of cancer in Japanese NF1 patients. This study retrospectively investigated the risk of cancer development in patients with *NF1* pathogenic variant (PV) confirmed by genetic testing.

Methods: We reviewed the history of malignant tumors in 52 participants from our Mid-West Japan Hereditary Tumor Cohort Study (https://cgm.hsc.okayama-u.ac.jp/en/cohort/) who were genetically confirmed to harbor *NF1* PV. In addition, we analyzed whether the risk of malignant tumors was associated with the type or location of *NF1* PVs.

Results: The mean age at the initial visit was 36 ± 21.4 years (range: 2–76 years), with 55.8% of the subjects being female. A history of malignancy was identified in 34.6% (18/52) of the patients. Among adults (over 18 years), 43.6% (17/39) had a history of malignancy, including malignant peripheral nerve sheath tumors (MPNST) in 7.7% (3/39) and breast cancer in 20% (5/25) of women. The mean age at breast cancer diagnosis among adult women (54.8 years). Notably, a trend toward a higher risk of malignant tumors was observed in patients harboring missense variants.

Conclusions: The mean age at diagnosis of breast cancer in carriers of the *NF1* PV was higher than the commonly reported age of onset of 30-50 years for breast cancer in this patient population. This finding suggests that breast cancer surveillance should be extended beyond the age of 50 years in this population. Our study suggests that Japanese patients with NF1 have a high risk of developing malignant tumors. Further large prospective studies are needed to validate these findings and to explore additional risk factors for malignancy in NF1.

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Neurofibroma of the Vocal Cord – A Rare Case and Review of the Literature

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Purpose: Nerve sheath tumors of the vocal cord are extremely rare. We describe the case of a Neurofibromatosis 1 (NF1) presenting with hoarseness and underlying vocal cord neurofibroma.

Methods and Results: A 41-year-old patient with NF1 presented with one year of hoarse voice. He described a sudden change in his voice that was noticed during the day while speaking. The impairment remained constant since onset and was without pain, but speech required more effort. No dysphagia was noted. He had no history of smoking. Videostroboscopy was performed in laryngology clinic, demonstrating fullness of the right true fold with relatively normal appearing epithelium and reduction in vibratory capacity. These findings were suggestive of a subepithelial cyst. The patient underwent direct microlaryngoscopy with microflap excision. Rather than a cyst, a subepithelial fusiform mass of right true vocal fold was found, with dense anterior and posterior attachments was visualized. It was dissected free while preserving the vocal ligament and much of the superficial lamina propria and epithelium as possible. Pathology showed coexpression of S100 protein and CD34 in the neoplastic cell pop ulation with approximately equal frequency supporting the diagnosis of neurofibroma. Following surgery, the patient reported a significant improvement in vocal quality and decreased strain producing sound. His Voice Handicap Index-10 Score improved from 32 to 20 (< 11 considered normal). Follow up video stroboscopy showed mild erythema, stiffness and a whitish patch of the right vocal cord, consistent with early stage of healing. In contrast to the preoperative findings, the vocal fold closure was complete and not impaired by the mass. Literature review shows that this is a rare presentation and will be described in detail.

Conclusion: Neurofibroma of the vocal cord is a rare presentation in patients with NF1. Patients presenting with voice changes should be referred for laryngeal evaluation.

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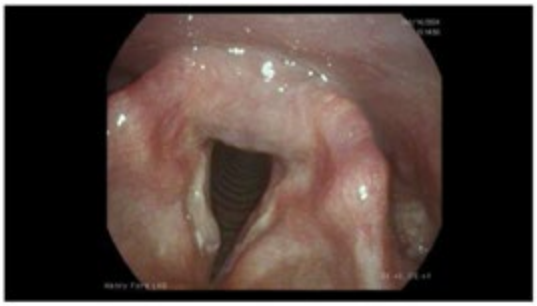

Fig 1: Preoperative image from videostroboscopy showing fullness of the midportion of the right true vocal fold

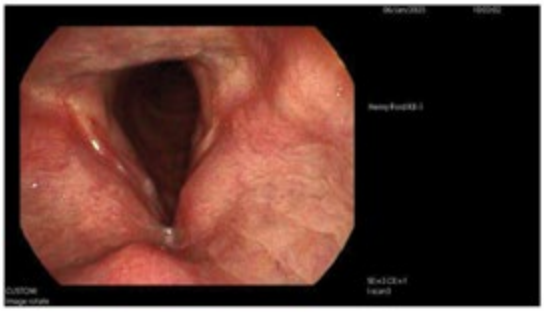

Fig 2: View of right true vocal fold with decrease in the fullness, but expected inflammation for this point postoperatively

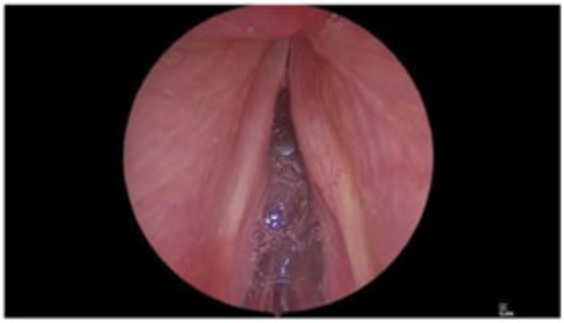

Figure 3: Intraoperative photo showing fusiform submucosal mass lesion involving much of the membranous vocal fold on the right

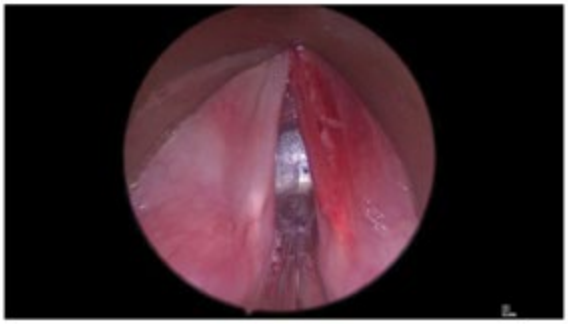

Figure 4: intraoperative view at conclusion of procedure showing a smooth vocal fold edge, preserved epithelium other than the incision itself, but greater erythema than is typical for these procedures in the non-neoplastic setting.

Validation of Novel NF1-Specific Patient-Reported Outcome Measures to Assess Pain Related to Plexiform Neurofibromas for Clinical Trials: Preliminary Data Analysis

Arjun Yogaratnam, BS, *Johns Hopkins University*

Purpose: Patient-reported outcome (PRO) measures are used to evaluate the effects of treatments on symptoms and function. To date, there are no validated PRO measures for pain related to plexiform neurofibromas (pNF), which are needed for clinical trials and critical for drug approval. We previously developed PRO measures assessing pNF-related pain intensity and interference in daily life from extensive qualitative research. The current study examines the psychometric properties of these two novel surveys in individuals with neurofibromatosis type 1 (NF1) and pNFs to support validation.

Methods: Individuals with NF1-pNF, ages ≥ 8 years, with pNF-related pain, and a consistent analgesic regimen (none or stable ≥ 3 months) or MEK therapy (none or ≥ 12 months), are eligible for either in-person or remote enrollment at 3 NF centers (target N=120). At enrollment, participants complete a demographic form and PROMIS Anxiety and Depression measures. During a practice session, they download the Catalyst Metricwire mobile app, and they review and complete the two new PRO measures: 1) PAin INtensity Scale for pNF (PAINS-pNF) assessing spike and chronic tumor pain intensity of a target pNF (0=no tumor pain to 10=worst tumor pain) and 2) the Pain Interference Index for pNF (PII-pNF) assessing tumor pain interference (0=none to 6=completely) as well as validated measures of pain intensity (eCAS; 0-100) and pain interference (PROMIS-PI; T-scores, Mean=50, SD=10). During the study, participants complete the PAINS-pNF and eCAS nightly for two consecutive weeks and the PROMIS-PI and additional measures at days 7 and 14. Data analyses were conducted using non-parametric statistics.

Results: To date, 55 participants enrolled with 53 completing the study (2 withdrew after consent); mean age=33 years; range=8-69; 47% male, 62% White, and 91% not Hispanic. For the new measures, internal consistency was excellent (Cronbach's alpha: PAINS-pNF=0.91, PII-pNF=0.96) with all item-total correlations >0.7. Spearman correlation coefficients indicated excellent construct validity for the two PAINS-pNF items of spike and chronic tumor pain intensity with the eCAS tumor pain item (r_s=0.90, r_s=0.80, respectively, both $p<.001$) and for the PII-pNF total score with the PROMIS-PI (r_s=0.75, $p<.001$). Known groups validity was demonstrated by significantly different median PII-pNF scores between NF1 symptom severity groups (p=0.011; mild=0.33 vs. moderate-severe=1.29) and higher median PAINS-pNF spike pain intensity in participants taking analgesics compared to those who do not (p=0.004; pain meds=5.0 vs. no pain meds=1.0). Median ratings of spike and chronic pain also were significantly different between descriptive pain categories (e.g., none, mild, moderate, severe; $p<.001$). Higher PROMIS Anxiety and Depression T-scores were correlated with greater PII-pNF total scores (r_s=0.33, p=.017; r_s=0.30, p=.029, respectively) suggesting a relationship between social-emotional function and pain interference in daily life, but not with PAINS-pNF spike or chronic tumor pain intensity.

Conclusion: The new NF1-specific PRO measures assessing pNF-related spike and chronic pain intensity and pain interference in daily life show excellent reliability and validity based on preliminary data. These measures are being used as secondary endpoints in a phase III MEK inhibitor trial that will determine their sensitivity to assess change with treatment. Recruitment for individuals with NF1-pNF ≤ 21 years and ≥ 40 years for the current study is ongoing to achieve target enrollment and complete the validation process.

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"Flipping a coin to whether or not they had the condition and then rolling a dice to decide how severe it was going to be": Pregnancy Experiences of Expectant Parents with Neurofibromatosis Type 1 (NF1)

Gamze Kaplan, *The University of Manchester, Division of Psychology and Mental Health*

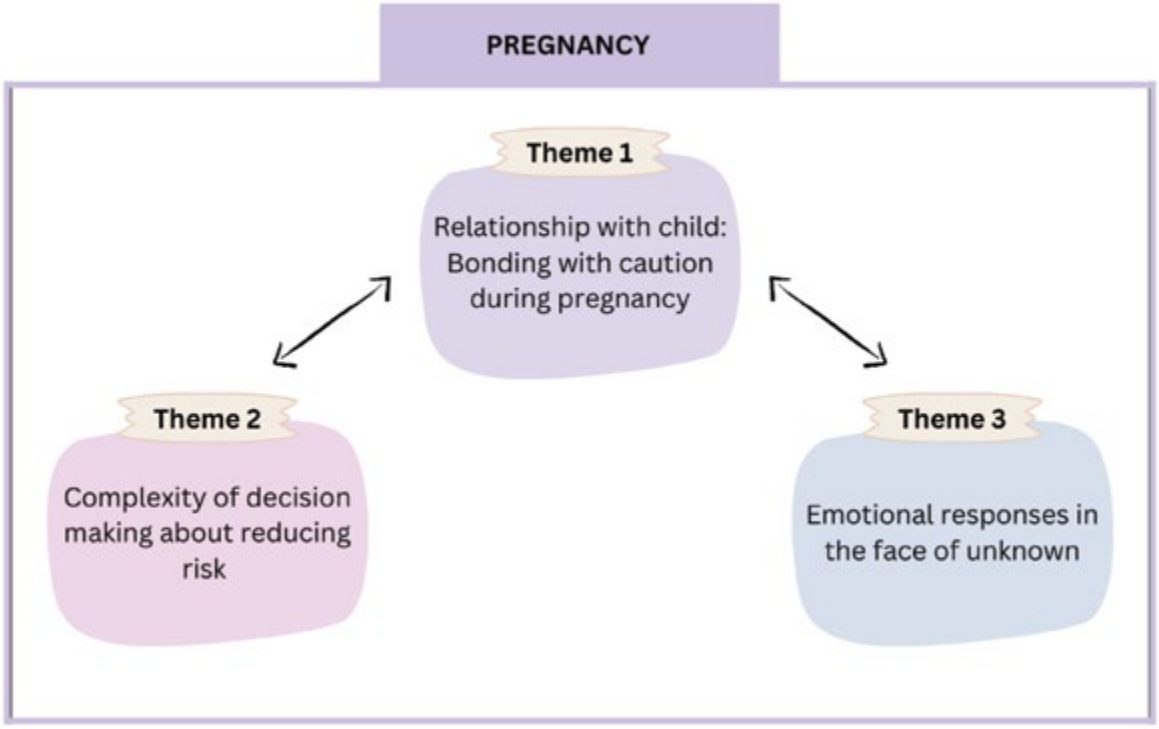
Purpose: The aim of this study was to explore the pregnancy experiences of expectant parents with NF1, examining their emotional connection with unborn children and their access to healthcare services.

Method: A qualitative research design was employed to investigate the experiences of expectant parents. Semi-structured interviews were conducted individually with fourteen expectant parents with NF1 and/or their partners. The transcribed data was analysed using a reflexive thematic analysis approach (Braun and Clark, 2022), facilitated through NVivo software.

Results: Three themes were generated based on the thematic analysis (a) *Relationship with child: bonding with caution during pregnancy,* (b) *Complexity of decision-making about reducing risk,* and (c) *Emotional struggles in the face of unknown* (illustrated in **Figure 1**). Expectant parents maintained emotional caution while bonding with their unborn baby, often delaying preparations as a safeguard against potential loss or the inheritance of NF1. Decisions regarding conception methods or genetic testing were influenced by prior medical and personal experiences but were primarily shaped by their perceptions of the condition's severity and its potential impact on their child. Uncertainty and unpredictability—surrounding pregnancy, personal health, and the child's future—were central to their emotional journey, contributing to anxiety, guilt, and deliberate emotional restraint. To cope, they relied on both internal strategies (e.g., self-reassurance) and external support from partners and family.

Conclusion: The influence of uncertainty and perceived severity of NF1 on expectant parents' experiences highlights the need for more empathetic healthcare support, enhanced psychological resources, and stronger external networks to address distress and support informed decision-making.

Figure 1. Three themes were reached through reflexive thematic analysis.



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A Tertiary Care Center Experience on Prevalence, Characteristics, and Outcomes of Moyamoya Syndrome in Neurofibromatosis Type 1 (NF1)

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Background: Neurofibromatosis Type 1 (NF1) is an autosomal dominant disorder with significant cerebrovascular complications, including Moyamoya syndrome (MMS) (Miller et al., 2019). MMS is characterized by progressive stenosis of the internal carotid arteries and fragile collateral vessel formation, leading to an increased risk of stroke (Brosius et al., 2022). Despite its known association with NF1, systematic screening guidelines for early detection remain absent (Duat-Rodríguez et al., 2013). Our institution's protocol of routine MRI/MRA screening provides a unique opportunity to assess MMS prevalence, imaging characteristics, and clinical outcomes in NF1 patients.

Purpose: This study aims to:

1. Determine the prevalence of MMS and its association with other cerebrovascular anomalies in NF1 patients.
2. Characterize clinical presentations, including stroke, transient ischemic attacks, seizures, headaches, and motor impairments in individuals with MMS.
3. Assess the natural history of MMS
4. Evaluate the efficacy of surgical revascularization (e.g., encephaloduroarteriosynangiosis [EDAS]) in MMS patients.
5. Explore genotype-phenotype correlations in NF1 patients with MMS.

Methods: This retrospective cohort study uses medical records and imaging data from individuals with NF1 treated at McGill University Health Centre (MUHC) from 1989 to 2024. Data includes demographics, MRI/MRA findings, clinical symptoms, treatment interventions, and long-term neurological outcomes. Descriptive statistics summarize clinical and imaging findings, while logistic regression and Kaplan-Meier analysis assess disease severity predictors and recurrence-free survival rates, respectively.

Results & Anticipated Conclusion: The study is expected to provide novel insights into the prevalence and natural history of NF1-associated MMS, and guide screening recommendations and clinical decision-making for NF1 patients. Our goal will be to contribute to evidence-based guidelines for early MMS detection and intervention in NF1 populations.

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Decision-Making Around Pre-Implantation Genetic Testing by Individuals with Neurofibromatosis Type 1: A Qualitative Study

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Purpose: Preimplantation genetic testing for monogenic conditions (PGT-M) with in vitro fertilization (IVF) is a reproductive technology available to reduce the chance of passing known genetic variants to the next generation. While prior research has explored reproductive decision-making for individuals with NF1, few studies describe the factors that influence their choices to pursue or decline IVF with PGT-M.

Methods: Adults with NF1 in the United States who had received reproductive counseling and considered IVF with PGT-M were recruited from the Children's Tumor Foundation (CTF) NF Registry. We conducted 20 semi-structured, virtual qualitative interviews that assessed participants' reproductive choices and lived experiences in the context of NF1. Interview transcripts were de-identified, coded, and thematically analyzed using the Framework Method. Coding matrices were created to synthesize data and generate themes illuminating the factors that influence decision-making around IVF and PGT-M for individuals with NF1.

Results: We interviewed 20 individuals with NF1: 11 who pursued and 9 who declined IVF with PGT-M **(Table 1)**. Four key themes emerged around reproductive decision-making: (1) Personal values and preferences, (2) Practical considerations, (3) Input from others, and (4) Retrospective feelings and future considerations. Personal values and preferences included opinions about passing NF1 on to future generations, perceptions of personal and/or family members' severity of NF1, feelings around the unpredictable clinical variability of NF1, preferences for biological children and family size, and personal spiritual or philosophical beliefs. Practical considerations, which also served as barriers to pursuing IVF with PGT-M, included financial burden, medical risk to self posed by IVF and pregnancy, and IVF and PGT-M success rates. Input from others included advice from medical providers and opinions/preferences of reproductive partners. Participants reflected on retrospective feelings about their reproductive journeys; many expressed content with their decisions, some voiced that they did not anticipate the emotional burden and lengthy duration of the IVF process, and a few struggled with decisions around the fate of embryos affected with NF1.

Conclusion: The choice to pursue or decline IVF with PGT-M for NF1 is a personal and nuanced decision. Providers can consider highlighting the clinical variability of NF1, emotional, financial, and time commitments, and success rates of IVF with PGT-M when facilitating reproductive decision-making. Many participants reported worsening NF1 symptoms during IVF or pregnancy and/or expressed concerns about carrying future pregnancies due to this perceived medical risk. Future research could address these concerns to improve reproductive counseling for individuals with NF1.

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Figure 1: Flowchart of study participants: Abbreviations: IVF (in vitro fertilization), PGT (pre-implantation genetic testing)

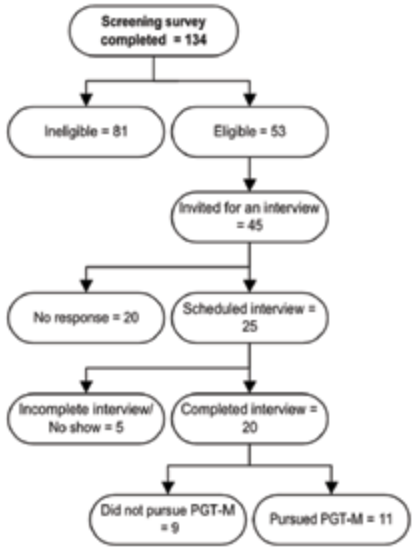


Table 1: Participant Characteristics (n = 20)

Participant Characteristics	n=20	%
Age	Median = 41 (Range: 23-54)	
Sex assigned at birth		
Female	15	75
Male	5	25
Race/Ethnicity (participants can select more than one option)		
African American/Black	2	9
Latine/Hispanic	4	18
East/Southeast Asian	1	5
West Indian	1	5
White	14	63
Highest Level of Education		
Less than a high school diploma	2	10
High school diploma or equivalent	2	10
Undergraduate degree	6	30
Graduate or professional degree	10	50
Household Income		
Less than $30,000	2	10
$30,000 - $59,999	2	10
$60,000 - $89,999	3	15
$90,000 - $149,999	7	35
$150,000 or more	5	25
Declined to answer	1	5
Health Insurance status at time of IVF/PGT counseling		
Insurance through a current or former employer or union	16	80
Medicaid	1	5
Other type of health insurance coverage	1	5
Not sure	1	5
Declined to answer	1	5
Age of Dx of NF1		
0-10 years old	15	75
11-17 years old	3	15
18-24 years old	2	10
25-34 years old	1	5
Perceived Severity of NF1		
Mild	6	30
Moderate	12	60
Severe	2	10
Family History of NF1		
Family History of NF1	10	50
Sporadic	10	50

Expanding Inclusivity in NF1 Research: Assessing the Potential Impact of Recruiting Outside NF Clinics

Evan Koch, BA, *Massachusetts General Hospital, Boston, MA*

Purpose: The majority of neurofibromatosis type 1 (NF1) patients in the United States do not receive care at specialized NF1 clinics, yet most NF1 clinical trials predominantly recruit from these clinics. This study examines whether recruiting from outside of the NF Clinic Network (NFCN) could help engage rural populations and those facing financial barriers to promote broader access and inclusion in NF research.

Methods: A retrospective comparative analysis was conducted on two adult NF1 cohorts from across the U.S. We identified a cohort of individuals who attend NFCN clinics from a survey distributed through the NF Registry in May 2022. We identified a cohort of individuals who do not attend NFCN clinics by combining data from the same NF Registry survey with the data from all the adults enrolled in the decentralized My NF Guide study as of 2/1/2025 (NCT06262113). We assessed for socioeconomic differences in age, gender, race/ethnicity, region, living area type, medical insurance coverage, financial barriers to care, household income, and education level using chi-squared tests.

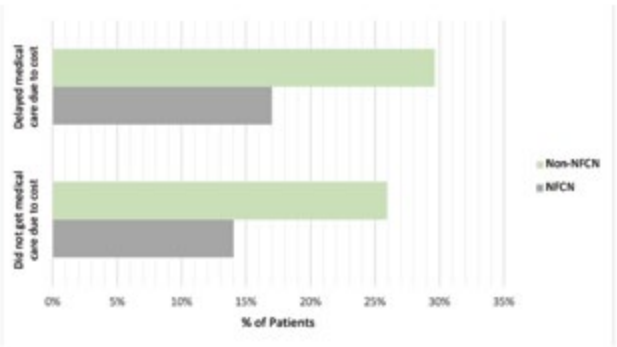
Results: We compared 58 adults with NF1 who attend an NCFN clinic to 136 adults with NF1 who do not attend an NFCN clinic (n=89 from the NF Registry and n=47 from the My NF Guide study) **(Table 1)**. Individuals who did not attend NFCN clinics were more likely to live in rural areas (7% vs 21%, p=0.051) and certain regions of the U.S. (e.g. the Far West and Southeast) than individuals who attend NFCN clinics (p=0.045). There was also a trend towards individuals who did not attend NFCN clinics reporting that they were more likely to delay (p=0.105) or forgo (p=0.095) medical care due to cost compared to those who attend an NFCN clinic **(Figure 1)**. Across the two groups, there were no significant differences in age, gender, race/ethnicity, medical insurance, household income, and education.

Conclusion: Our findings suggest that recruiting adults with NF1 who do not attend NFCN clinics could be effective in engaging rural populations and those facing financial barriers to care. Pursuing decentralized strategies for NF clinical trials may increase the inclusion of these underrepresented individuals in NF research by addressing participation barriers and promoting wider access to trials. However, additional strategies may be needed to ensure broader diversity in NF trial participation, particularly across education and income levels.

Table 1. Participant Demographics

	NFCN attendees from NF Registry (n=58)	Non-NFCN patients from NF Registry and My NF Guide Study (n=136)
Age (median, range)	43 (18-70 years)	48 (18-78 years)
Female Gender (n, %)	42, 84%	94, 82%
Race/Ethnicity (n, %)		
White	44, 76%	111, 82%
Native American	2, 3%	3, 2%
Black	4, 7%	8, 6%
Latino or Hispanic	4, 7%	3, 2%
Asian	2, 3%	4, 3%
Multirace	0, 0%	5, 4%
Other	2, 3%	2, 1%
Income Quintile (n, %)		
Lowest 20%	13, 22%	32, 24%
20-40%	12, 21%	34, 25%
40-60%	11, 19%	26, 19%
60-80%	11, 19%	18, 13%
Top 20%	8, 14%	15, 11%
Prefer not to say	3, 5%	11, 8%
Education (n, %)		
Some high school	0, 0%	5, 3%
High School	22, 38%	43, 29%
Associate's Degree	6, 10%	16, 13%
Bachelor's Degree	19, 33%	41, 32%
Graduate Degree	11, 19%	31, 23%
Living Area Type (n, %)		
Urban	19, 33%	32, 25%
Suburban	33, 57%	70, 51%
Rural	4, 7%	29, 21%
Not Sure/Other	2, 3%	5, 3%
Region (n, %)		
Far West	3 5%	23, 15%
Rocky Mountain	3, 5%	9, 7%
Southwest	3, 5%	10, 8%
Plains	3, 5%	5, 3%
Great Lakes	9, 16%	16, 13%
Southeast	11, 19%	41, 32%
Mid-Atlantic	15, 26%	19, 13%
New England	11, 19%	10, 9%
Other	0, 0%	1, 1%

Figure 1. Patient-reported financial barriers to care in the past 12 months



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Outcomes of NF1 Patients Diagnosed with Gastrointestinal Stromal Tumors

David Bonilla, ScB, *Massachusetts General Hospital, Boston, MA*

Purpose: Individuals with neurofibromatosis type 1 (NF1) are at an increased risk of developing gastrointestinal stromal tumors (GIST) with a reported prevalence of 3.7% in this population. Despite a breadth of research on sporadic GIST, outcomes for the NF1 population remain understudied. Here we describe the clinical outcomes for a cohort of patients with NF1-associated GIST towards understanding differences between the sporadic form and informing optimal clinical management.

Methods: An institutional review board approved search of electronic medical records at Mass General Brigham (MGB) between 11/1999 and 11/2024 using the ICD-10 codes for "neurofibromatosis" and "gastrointestinal stromal tumor" identified 63 patients. Four patients were excluded because they did not have a diagnosis of NF1; two additional NF1 patient were excluded for lack of GIST. We then retrospectively reviewed the charts of these 57 patients for data on demographics, diagnosis method, surgical records, and vital status.

Results: The median age was 51.0 years (range 26.1-78.5) and included 26 men (46%). A diagnosis of GIST was confirmed pathologically in 50 patients (88%) through endoscopy (14 patients) or surgery (36 patients). A presumed diagnosis was established radiographically in 7 patients. The location of GISTs were stomach (4, 7%), duodenum (18, 32%), jejunum (22, 39%), ileum (4, 7%), and unspecified (11, 19%). 16 (28%) GIST were multifocal upon initial diagnosis. The median follow-up time for GIST was 5.1 years (range 0.4 to 18.1). Forty-five patients were alive at last contact and 12 patients died including 5 who died from complications of GIST, 4 who died from MPNST, and 3 of non-NF1-related causes. The 5-, 10-, and 15-year GIST-specific survival was 94%, 90%, and 80%. For comparison, the 5-, 10-, and 15-year NF1-specific survival was 92%, 85%, and 76%, with a median of 17.2 years with 95% CI (13.9, NA). Six patients had, at any point, a diagnosis of MPNST (11%), six had pheochromocytoma (11%), and four had other GI tumors (7%, two neuroendocrine tumors, one desmoid tumor, and one low-grade serous carcinoma of the omentum).

Conclusion: To our knowledge, this is the largest single institution report on the outcomes of GIST in patients with NF1. Our findings confirm the distinct presentation of NF1-related GIST compared to sporadic GIST and provide novel outcome data on GIST-specific survival after diagnosis. Our team continues to analyze data on tumor pathology, recurrence, and treatment as important additions to the natural history of GIST in this unique population.

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Clinical and Humanistic Burden Among Adults with Neurofibromatosis Type 1 and Plexiform Neurofibroma in the United States

Xiaoqin Yang, PhD, *Merck & Co., Inc., Rahway, NJ*

Purpose: Adults with neurofibromatosis type 1 (NF1) and plexiform neurofibroma (PN) experience tumor growth, debilitating pain, disfigurement, and functional limitations. However, the impact of symptoms on patients' health-related quality of life (HRQoL) remains understudied. This study characterized patient-reported clinical burden and its impact on HRQoL among adults with NF1 PN.

Methods: Adults ≥18 years with NF1 PN in the United States (US) completed an online, cross-sectional survey available from August-October 2024. Participants were recruited via a provider network and social media outreach. Respondents were required to be MEKi treatment-naïve or have ≤1 month of use. Patient-reported outcome (PRO) measures were collected, including modified PAINS-pNF (PN-related pain intensity; 0-10 scale, higher=worse), Pain Interference Index (PII)-pNF (PN-related pain interference; 0-6 scale; higher=more interference), Plexiform neurofibromas Quality of Life measure (PlexiQoL) (impact of PN on daily life and well-being; 0-18 scale; higher=worse), Pediatric Quality of Life Inventory (PedsQL) NF module (skin sensations, cognitive functioning; 0-100 scale; lower=worse), Patient-Reported Outcomes Measurement Information System (PROMIS) item banks (Physical Function, Depression, Anxiety, Fatigue), Work Productivity and Activity Impairment Questionnaire plus Classroom Impairment Questions, and EQ-5D-5L (health utility index range -0.573 to 1; lower=worse).

Results: Among 120 respondents (mean age, 41.8; standard deviation [SD], 14.9), 53.3% were male, 49.2% White, and 99.2% MEKi treatment-naïve. Most (61.7%) had ≥3 PN and 73.3% had PN in the head, neck or spine. Most patients (65.0%) experienced disfigurement. More than half of patients reported moderate-to-severe (pain intensity ≥4) chronic (51.7%) and spike (83.3%) tumor pain over the past 7 days (PAINS-pNF: mean chronic pain intensity=3.6 [SD, 2.5]; mean spike pain intensity=6.3 [SD, 3.2]), with considerable interference with daily activities (PII-pNF: mean=3.1 [SD, 1.6]). Mean PlexiQoL score (10.2 [SD, 5.2]) indicated considerable disease-specific impact. Patients experienced substantial skin sensations (tingling, pins and needles, burning) and cognitive issues, with PedsQL mean scores of 23.8 (SD, 20.2) and 36.9 (SD, 23.2), respectively. PROMIS T-scores showed that high percentage of respondents reported moderate-to-severe physical function limitations (65%), fatigue (49.2%), depression (50.0%), and anxiety (48.3%). Only 15 (12.5%) patients were employed, with average work productivity loss of 39.3% among them. Overall HRQoL impact was considerable (mean EQ-5D-5L utility index: 0.38 [SD, 0.39]). Burden was higher among patients with more PN; with PN on head, neck, or spine; or with higher pain scores.

Conclusion: This systematic assessment in adults with NF1 PN in the US highlights the significant impact of NF1 PN on patients' HRQoL across multiple domains, including tumor-related pain, physical limitations, psychological distress, reduced work productivity, and low health utility scores. These findings underscore the need for novel therapeutics and psychosocial interventions in adults with NF1 PN.

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Disclosures:
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Burden Among Caregivers of Adult Patients with Neurofibromatosis Type 1 and Plexiform Neurofibroma in the United States

Xiaoqin Yang, PhD, *Merck & Co., Inc., Rahway, NJ*

Purpose: Adults with neurofibromatosis type 1 (NF1) and plexiform neurofibroma (PN) experience high disease burden, including debilitating pain, motor deficits, and functional impairment. However, the impact of NF1 PN on caregivers' humanistic burden remains understudied. This study characterized quality of life (QoL), work productivity and activity impairment, and psychosocial impacts among caregivers of adults with NF1 PN.

Methods: Primary caregivers of adults ≥18 years with NF1 PN who were MEK inhibitor-naïve or new users (≤ 1 month) in the United States (US) completed a cross-sectional online survey between August-October 2024. Caregivers were recruited via NF1 PN provider/patient networks and social media engagement. Validated self-report measures included Zarit Burden Interview (ZBI, range 0-88; higher=greater burden), Work Productivity and Activity Impairment questionnaire adapted for caregiving (WPAI:CG), and EQ-5D-5L QoL). Characteristics of caregivers and their adult dependents with NF1 PN were collected. Descriptive statistics summarized burden across physical, emotional, and social domains.

Results: Among 100 caregiver respondents (mean age [standard deviation, SD] 49.0 [15.1]; 67.0% female; 59.0% White), most were married/partnered (76.0%) and had provided care for ≥4 years (61.0%). Their adult dependents with NF1 PN (mean age [SD] 39.6 [16.0], 59.0% male) had lived on average with NF1 for 22.2 years and PN for 9.6 years, with 59.0% having ≥3 PN. Caregivers included parents (25.0%), spouses/partners (19.0%), or children/siblings (28.0%). Despite 37.0% of caregivers being homemakers and 24.0% retired, the need for additional caregiving support remained high (31.0% reported additional support from spouse/partner, 24% from family members, and 28.0% from paid caregivers). Most (69.0%) received >10 hours of additional caregiving support weekly. Based on ZBI scores (mean [SD] score: 36.6 [15.6])., 48% of caregivers reported moderate-to-severe or severe burden (scores >40). Among employed caregivers (18.0%), mean [SD] overall work productivity loss was 47.5% [23.9%], with 15.6% [27.7%] work hours missed and a 41.7% [22.8%] reduction in on-the-job productivity over the past 7 days. Among all caregivers, the ability to perform daily activities outside of work was reduced by 50.2% [20.5%]. While overall health status appeared preserved (EQ-5D-5L utility: 0.84 [0.18]; Visual Analog Scale, VAS: 80.3 [9.5]), 66.0% experienced anxiety or depression, with 11.0% reporting severe or extreme symptoms.

Conclusion: Caregivers of adults with NF1 PN in the US experience substantial burden across multiple domains, including work productivity loss, activity impairment, and psychological challenges. These findings underscore the need for new therapeutic interventions for patients with NF1 PN and comprehensive support programs for their caregivers.

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Stress in Neurofibromatosis Type 1 (NF1): Longitudinal Relationships Between Disease Severity, Caregiver Stress, and Children's Stressful Life Events Among Youth with NF1 and Plexiform Neurofibromas (PN)

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Purpose: Children with neurofibromatosis type 1 and plexiform neurofibromas (NF1-PN) and their families are at risk for heightened stress. Stress can be conceptualized as distinct stressful life events (SLEs) or generalized ratings of overall stress. Different stressors may impact medical and psychological functioning in varying ways. Understanding SLEs in children with NF1-PN and overall caregiver stress is necessary for targeted supportive interventions. This analysis extends previous presentations from the NCI natural history study (NCT00924196) by describing types of stressors and investigating the relationship between stress and disease severity among children with NF1-PN. We hypothesized that (1) medical variables would relate to caregiver stress and child-experienced SLEs, and (2) baseline disease severity would predict stress measures at 3-year follow-up.

Methods: Caregivers completed questionnaires about their child's demographics (e.g., race, age, psychiatric diagnosis), number of SLEs experienced by their child (Life Events Checklist), and their own stress (Rating of Overall Stress Scale [ROSS]; 0=not stressed to 10=very stressed). Medical professionals rated NF1 disease severity and number of NF1 complications. SLEs were categorized as family, school, loss, and financial (Elliot-DeSorbo et al., 2009).

Results: Baseline data from 108 children's caregivers (M_{age}=11.7 years, range=2.6-27.7) were analyzed (70% 3-year follow-up retention). Half (Baseline:50.5%, Follow-up:54%) of caregivers reported high stress (ROSS ≥ 7). At baseline, most (n=81, 75%) reported ≥1 SLE for their child, with 51% (n=55) endorsing ≥2. The most frequent SLEs were financial-related (n=35, 33%) and school-related (n=34, 32%). No demographic factors were related to total number of SLEs. As hypothesized, number of baseline medical complications were positively related to total SLEs (ρ=.234, p=.015) and specifically, family-based SLEs (ρ=.263, p=.006). Children with a parent-reported psychiatric diagnosis had more SLEs (M=2.58) than those without (M=1.53; p=.023). Prediction hypotheses could not be analyzed due to violated assumptions for regression.

Conclusion: Caregivers of children with NF1-PN experience a high degree of overall stress in their daily lives. Parent-reported SLEs about their child were related to the child's medical complications, with the most common types of SLEs being financial or school-related. Furthermore, having a child with a psychiatric diagnosis may put families at increased risk for SLEs. Both clinicians and researchers should be aware of caregiver stress levels and common types of SLEs in youth with NF1-PN to inform best care.

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Epilepsy in a Cohort of Pediatric Patients with Neurofibromatosis Type 1 in a Tertiary Center in Argentina

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Objectives: To describe the electroclinical characteristics of epilepsy in a pediatric series with NF1 and compare them with those reported in the literature. To analyze electroclinical and radiological patterns and therapeutic response.

Materials and Methods: We performed a retrospective descriptive analysis of patients with NF1 evaluated by Neurology at Garrahan Hospital during the last 10 years. We included children from 1 month to 17 years old with positive clinical criteria of Neurofibromatosis type 1 (NF1) according to the latest guidelines of the National Institute of Health of the United States (NIH) and who had associated epilepsy. We defined epilepsy according to the ILAE 2022 criteria. We classified epileptic seizures according to ILAE 2017. Two authors analyzed electroencephalograms (EEGs). We excluded any patient with paroxysmal non-epileptic episodes or symptomatic seizures.

Results: 203 records were reviewed, of which 22 (10.8%) were reported with seizures, 2 had presented acute symptomatic generalized seizures and were therefore excluded, 20 (9.8%) presented epilepsy, 10 (50%) were focal epilepsies, 8 (40%) generalized and 2 (5%) epileptic encephalopathy of difficult control. Of the focal seizures, 5 had pathological electroencephalogram and only presented structural epilepsy due to a high-grade glial lesion.

Of the generalized epilepsies, 8 had EEG without foci or paroxysms, only 2 had lesions, one for a Moyas Moya syndrome and the other for a neuronal migration disorder.

As for treatments, 15 controlled seizures with one drug, 2 with two, 2 with four and 1 with five antiepileptic drugs.

Conclusions: Our series confirms that children with NF1 are more predisposed to epilepsy than the rest of the child population, 10.8%. We also evidenced that we did not register significant percentage differences between focal and generalized epilepsies. And that focal epilepsies one patient had a structural lesion. In turn, we recorded that most of these patients controlled their epileptic seizures with an antiepileptic drug and only two had refractory epileptic encephalopathy.

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Association Between Neurofibromatosis Type 1 and Mixed Pheochromocytoma: A Case Report of a Pediatric Patient

Francina Lombardi, *FLENI, Buenos Aires, Argentina*

Introduction: Neurofibromatosis type 1 (NF1) is a genetic disorder characterized by an increased risk of developing tumors, including pheochromocytomas. These tumors, rare in the general population, have a higher incidence in individuals with NF1. Pheochromocytomas are neuroendocrine tumors that affect the adrenal glands and are responsible for excessive production of catecholamines. In some cases, dopamine-secreting pheochromocytomas are present and are known as mixed pheochromocytomas. This variant refers to pheochromocytomas that present morphological features of both typical pheochromocytomas and neuroblastomas. This variant is particularly rare in pediatric patients with NF1, which poses significant diagnostic and therapeutic challenges. Identification of this type of pheochromocytoma is crucial due to the clinical implications in the management of hypertension and other associated complications.

Objectives: The objective of this study is to describe the clinical, diagnostic, and therapeutic characteristics in a pediatric patient with NF1 who developed a mixed pheochromocytoma, in order to enhance the understanding of this rare association and optimize its management.

Materials and Methods: A retrospective, observational study was conducted on a 13-year-old pediatric patient diagnosed with NF1 and mixed pheochromocytoma. Data were collected regarding clinical presentation, diagnostic studies, surgical interventions, and treatments.

Results: A 12-year-old male patient, diagnosed with NF1, began with recurrent, progressively severe headaches. Upon admission to our institution, records showed elevated blood pressure, prompting a brain and spinal MRI, which revealed a nodular mass in the left adrenal gland, initially presumed to be a neurofibroma. Urinary catecholamine tests were requested, the results were 9.5 mg/24 hours (NV $<$ 5.2 mg).

Scintigraphy showed a left adrenal mass suggestive of neuroblastoma. The patient underwent laparoscopic left adrenalectomy with tumor resection. The biopsy confirmed adrenal neoplasm composed of pheochromocytoma/ganglioneuroblastoma. No further treatment was required, and the patient remained asymptomatic, with no cardiovascular compromise, normalizing blood pressure readings. Follow-up imaging was unremarkable.

Conclusion: Mixed pheochromocytomas in pediatric patients with NF1 are an extremely rare entity, with an estimated incidence in the literature of approximately 0.1% to 5.7% of patients with NF1. These tumors should be considered in the differential diagnosis of children with unexplained hypertension. Early diagnosis and surgical intervention are crucial for the successful management of these patients. Given the risk of recurrence, continuous long-term follow-up is recommended to detect potential recurrences and manage associated complications.

Management of pediatric patients with NF1 and mixed pheochromocytoma requires a multidisciplinary approach to ensure timely diagnosis and appropriate treatment.

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Neurological Manifestations in Neurofibromatosis Type 1: Experience of a Tertiary Center with a Cohort of 203 Argentine Children

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Introduction: NF1 is an autosomal dominant genetic disorder with expression variability, it is due to mutation in the neurofibromin gene. It can be associated with epilepsy, neurodevelopmental disorders and cerebrovascular disease.

Objectives: To describe the most prevalent neurological manifestations in a series of pediatric patients with NF1 seen at Hospital Garrahan in Argentina in the last 10 years.

Population and Methods: We performed a descriptive, retrospective analysis of the medical records of patients with clinical criteria for NF1 with an age range of zero to 15 years, evaluated by neurology at Hospital Garrahan in the last 10 years.

Results: We registered 203 patients, 99 females with a mean age of 3 years. 133 patients reported neurodevelopmental disorders with learning difficulties in 54%, psychopedagogy was the most indicated therapy (85%). 22 (11.3%) presented focal and generalized epilepsy not always associated with structural lesions, 12 of them (54%) with pathological electroencephalogram. The most indicated drug was clobazam. 83 patients 22 (11.3%) presented focal and generalized epilepsy not always associated with structural lesions, 12 of them (54%) with pathological electroencephalogram. The most indicated drug was clobazam.83 patients (50%) had hamartomatous lesions in the cerebellum, 2 patients reported Moya Moya syndrome.

Conclusion: Our study shows that neurodevelopmental disorders are frequent in this population, among them the most diagnosed was learning disorder. Epilepsy is more common than in the general population, and focal seizures are more frequent, requiring prolonged antiepileptic drugs but with good control. In agreement with previous series, bright lesions in cerebellum were frequently observed.

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Outcome of Brain Imaging in Asymptomatic Children with NF1

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Purpose: To analyse the outcome of MR scans of the brain undertaken on children aged 6-18 years as part of research trials.

Methods: In 2009 NHS England commissioned two Highly Specialized Services for complex NF1. The Manchester service has undertaken two neurodevelopmental studies in children aged 6-18 years where neuroimaging was part of the research protocol and in whom they were clinically asymptomatic at enrolment into the study. The first study took place in 2019 to 2020 and the second started in 2024 and is ongoing.

Results: The MR brain images and records of 57 children were available to us. 4 of the records were incomplete and in 8 children the imaging is still being reviewed. We have complete records on 45 children with an age range of 6-18 years and a mean / median age of 14 years. 25 are male and 20 female. Positive findings triggering further intervention were identified in 18 (40%) of children. In 2019 cohort one 14-year-old male had an asymptomatic dysembryoplastic neuroepithelial tumour (DNET). One had an asymptomatic optic pathway glioma (OPG). She has never needed treatment and has had several years of follow up with complete stability of her OPG. One child in whom the scan raised the possibility of need for cerebrospinal fluid (CSF) flow studies was lost to follow up. In 5 children nonspecific changes were seen in which it was impossible to differentiate between myelin vacuolation changes and low-grade gliomas. Their ongoing surveillance imaging continues to require yearly follow up but they remain asymptomatic. In the cohort of children 2024-2025, 3 children have asymptomatic OPGs, 3 children have slightly smaller pituitary glands and will require clinical review for growth and pubertal status and 4 children again have nonspecific changes where low grade gliomas cannot be excluded. These will all enter the clinical complex NF1 paediatric pathway in the service.

Conclusion: The question of asymptomatic annual screening for children with NF1 continues to be raised by families and clinicians alike. Our review of neuroimaging of asymptomatic children who had a brain scan as part of a research study demonstrates that a significant proportion of these scans reveal changes that require further imaging and further clinical reviews. Reassuringly however, none of the positive findings changed the individual's clinical outcome. Our study supports the present recommendation that MR scans should only be undertaken in children with NF1 in whom there are clinical concerns.

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Bridging the Gap: Developing a Transition Plan for Pediatric to Adult Care in Neurofibromatosis Type 1

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Purpose: Neurofibromatosis Type 1 (NF1) is a genetic condition that requires lifelong management. Patients with NF1 face significant challenges in their transition from pediatric to adult care. This may include lack of follow-up with specialists, limited or no knowledge regarding their condition, and difficulty navigating the complex healthcare system. Patients with NF1 often become lost to follow up as they graduate from pediatric care. Our objective was to develop a successful transition program for pediatric patients with NF1 seen through the Seattle Children's NF program to adult NF care through patient education, systematic transition readiness tasks, and direct handoffs to their adult NF1 provider.

Methods: After a review of literature and resources regarding transitioning care for patients with NF1, specific transition tasks and goals were created based on the patient's age, starting from 13 years of age with goal of transitioning by age of 21. A transition database was developed to track patients along their transition journey. Tasks and goals for each patient based on their age was automatically included in the patient's after visit summary (AVS). Each patient was also provided a Portable Medical Summary that would summarize their medical history and ongoing plans, helping them to feel empowered to provide accurate and complete information when seeing new medical providers as they transition into adult care. Quarterly video meetings between Seattle Children's and adult NF1 programs at University of Washington Alvord Brain Tumor Center as well as with Swedish Ivy Center were held to discuss patients that were being referred from pediatric to adult NF1 care, ensuring communication about continuity of care. NF1 program nurse coordinators ensured that each task and goal was completed.

Results: 15 patients were transitioned to adult care from June 2022-Feb 2025. Pediatric NF1 providers more readily referred their patient for adult care by age 21. Patients and families reported feeling more prepared for and willing to transition to adult care. Patients reported feeling satisfied with their adult NF1 medical home.

Conclusion: Seattle Children's NF Program was able to develop a successful transition plan for pediatric to adult care in patients with NF1 and will expand the transition program for patients with NF2 related schwannomatosis and other types of schwannomatosis.

Age	Transition Task
13-15	• Initial discussion with patient and family and provide information about transition. • Provide a transition handout. • First private discussion with patient without parent/guardian in the room. Continue at every visit. • Discuss Electronic Medical Record changes at age 13, encourage patients to activate a personal account.
16	• Provide first transition readiness assessment questionnaire (TRAQ) to patient and family. • Discuss ways in which patient can take the lead in their care (e.g. make appointments, pick up medicine) • Discuss establishment of care with pediatric specialties. • Discuss molecular testing, if not already completed. • Start discussion of reproductive implications.
17-19	• Provide second TRAQ questionnaire to patient and family. • Discuss guardianship or health proxy documents, if needed. • Begin discussion of transitioning to an adult primary care provider. • Begin Portable Medical Summary.
20	• Provide final transition readiness assessment. • Complete/update Portable Medical Summary
20/21	• NF team discusses patient with Swedish Ivy Center or University of Washington Alvord Brain Tumor Center at quarterly meeting including: o Date last seen and when due for next visit, imaging, etc. o Clinical and social history with any special considerations. o Ensure referral placed and received with all supporting documents.

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Dietary Considerations for People with NF1: A Systematic Review of the Literature

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Purpose: Neurofibromatosis type 1 (NF1) is a neurocutaneous genetic condition that can present with multiple manifestations. Limited pre-clinical and clinical data suggest that certain metabolic disturbances, body composition differences, and nutritional deficiencies may be more prevalent in people with NF1. Data on the role of dietary patterns in people with NF1 and their effects on NF1-related manifestations is scarce.

Methods: A systematic literature review was conducted; the MeSH database of PubMed was utilized to identify controlled vocabulary and keywords associated with NF1, diet, and nutrition. These terms were combined using boolean operators to query PubMed, Embase, PLOS One, and Cochrane. The inclusion criteria consisted of articles in English, full text available, human studies, and published between 2000-2024. The results were screened, and key details were extracted.

Results: 394 published articles were identified, out of which 14 met the inclusion criteria. 379 papers were excluded due to duplication or not meeting all inclusion criteria. Articles included 1 case report, 1 case series, 1 literature review, 7 retrospective studies, and 4 prospective trials. Topics included vitamin D and bone metabolism, nutritional supplements, glucose metabolism, and nutritional intake. A prospective study suggested a link between vitamin D deficiency and a greater number of cutaneous neurofibromas. One phase 2a clinical trial with 6 participants suggests that L-carnitine supplementation is safe and feasible and might result in marginal improved function in muscle performance in children with NF1. Another prospective study compared Mediterranean and Western diets with or without curcumin supplementation; however, the number of participants in each group on this project was no more than 3, some did not follow the assigned diet, the endpoints were not clear and follow up only lasted 6 months.

Conclusions: Data regarding the effects of diet or nutritional supplements in people with NF1 is very limited. Available studies suggest that a diet composed of elements, including but not limited to, medium-chain fatty acids, calcium, and vitamin D, aids in bone growth and density, and muscle lipolysis. The results of these studies might only be applicable to a small population of people with NF1 and did not result in conclusive or significant clinical benefit. Further research is needed to better comprehend metabolic abnormalities in people with NF1 and the role that nutrition plays in patients' function and quality of life.

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NF1 Patient Reported Pain Associated with Glycolytic Bias of Peripheral Blood Mononuclear Cells in Pilot Study

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Introduction: Neurofibromatosis 1 (NF1) is a common, yet incurable, genetic disorder inducing various types of nervous system tumors and other deleterious symptoms. The clinical phenotype arises from a wide range of *NF1* mutations, and its hereditary mosaicism of NF1 poses significant challenges for understanding the relationship between genotype and phenotype. Given those limitations, studies on the impact of NF1 genetic lesions on biological functions are essential for understanding, predicting, and treating the debilitating effects on patients. Emerging evidence suggests that neurofibromin mutations dysregulate cellular and organismal metabolism involving mitochondria. NF1 patients exhibit decreased respiratory quotients and varying alterations in their basal metabolic rates. We then hypothesized that mitochondrial dysfunction among NF1 patients contributes to adverse health outcomes.

Methods: As a test, 55 subjects were enrolled in a study: 44 NF1 patients and 11 chaperones without neurofibromatosis or Schwannomatosis (control). Subject blood was collected and fractionated within three hours of collection to isolate peripheral blood mononuclear cells (PBMCs). Cells were subjected to bioenergetic analyses (mitochondrial function) using an Agilent Seahorse XF Pro for correlative studies with clinical phenotypes and symptoms. For patients, fatigue and pain symptoms were measured by the Functional Assessment of Chronic Illness Therapy-Fatigue scale (FACIT-F) and Numerical Pain Rating Scale (NRS-11). Biomedical evaluation of liver function, creatinine kinase, hemogram, and echocardiogram was also carried out.

Results: Seahorse analyses demonstrated a significant (p 0.008) difference in the extracellular acidification rate (ECAR) between NF1 patients and those lacking NF1 defects but not for oxygen consumption rates (OCR). The findings showed a glycolytic bias in metabolism for NF1 patients. When comparing bioenergetic results to clinical symptoms at sampling, pain had a significant, albeit low, correlation with OCR (r (93) 0.31, *p* 0.003, Pearson test) but not for ECAR (r (93) 0.09, *p* 0.4, Pearson test). Regarding fatigue, 68% of patients reported severe fatigue at least once during the study, but there was no correlation with fatigue reports and measured OCR (r (93) -0.05, *p* 0.63, Pearson test) or ECAR values (r (93) 0.03, *p* 0.77, Pearson test).

Conclusion: NF1 patients have dysregulation in cellular respiration towards a less aerobic more glycolytic pathway. While often observed for cancer cells (Warburg effect), this quality for NF1 PBMCs shows a germline defect in which likely all patient cells possess the mitochondrial dysfunction and so impact health outcomes. In fact, correlative studies implicated pain, but not fatigue, was associated with mitochondrial dysfunction for patients.

Can MEK Inhibitor Therapy Be Safely Discontinued in a Responding Plexiform Neurofibroma? A Case Report

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Background: The use of MEK inhibitors (MEKi) has been recommended for treating inoperable, symptomatic plexiform neurofibromas (ISPN) in individuals with Neurofibromatosis type (NF1)[1,2]. Following our clinical care protocol for MEKi therapy in ISPN management[3], we began treatment in a 9-year-old male patient (DBF) with an extensive intraneural plexiform neurofibroma affecting the pelvis and right lower limb **(Figure 1A)**, accompanied by intractable neuropathic pain unresponsive to prior therapy, including imatinib. Pre-treatment PET-CT showed no malignant transformation. Selumetinib access was granted by court order, and oral therapy began at 25 mg/m^2 twice daily in 28-day cycles starting December 2023.

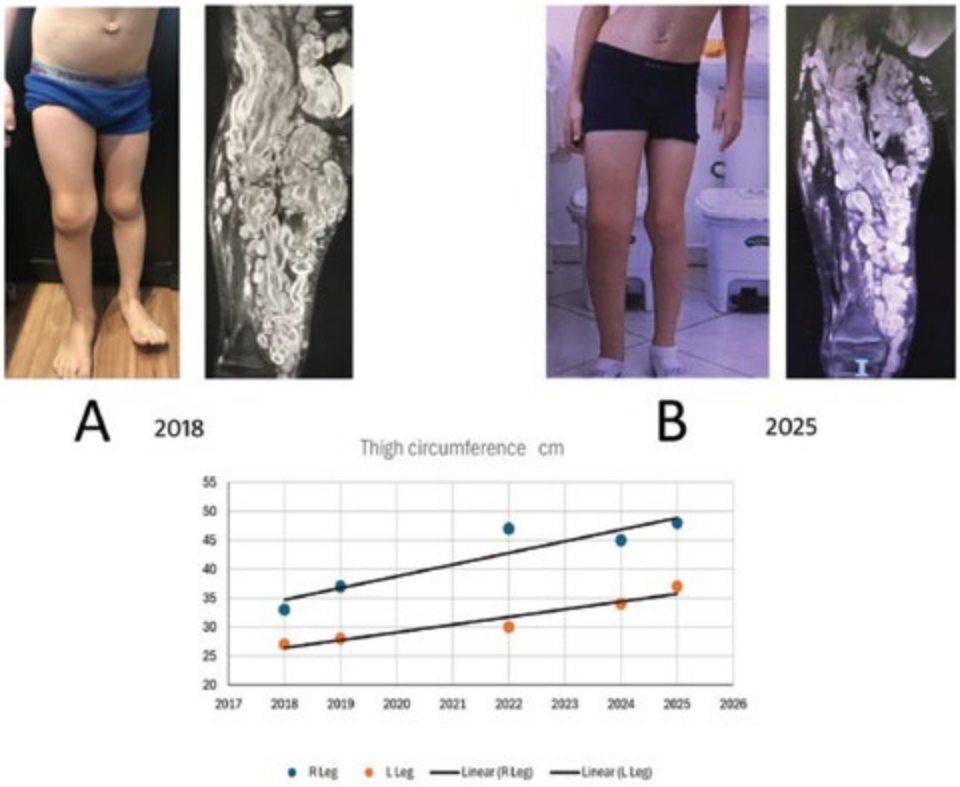
Purpose: To explore clinical considerations regarding the discontinuation of selumetinib therapy, focusing on timing, criteria, and strategies to safely halt treatment without causing tumor regrowth or neuropathic pain recurrence, whether due to patient preference or financial limitations[4].

Methods: MRI scans were conducted every 6 months, as volumetric MRI analysis was unavailable at our institution. Pain levels were assessed using a standardized pain scale, and thigh circumference measurements were taken both prior to and during treatment (December 2023 to April 2025).

Results: After 3 months of treatment, DBF reported a significant improvement in pain management, using non-opioid analgesics intermittently, and enhancement in quality of life, including his return to physical activities at school. Additionally, the patient family reported a subjective perception of slowed ISPN growth, although MRI imaging did not show a reduction in tumor size **(Figure 1B)**. **Graphic 1** illustrates the changes in thigh circumference from 2018 to 2025. By April 2025, clinical assessments indicated sustained improvement in pain, quality of life, and a decrease in the rate of tumor growth **(Figure 1B)**, with minimal adverse events reported.

Conclusion: The most recent clinical assessment led to a critical discussion between the patient's family and the medical team regarding the next steps in treatment. Given that the drug is FDA-approved (and by ANVISA in Brazil) with no prescribed protocol for discontinuation when treatment responses are favorable, the question of when and how to stop therapy remains unclear. We believe it is time for the NF community to engage in a thorough discussion about the outcomes of stopping MEKi therapy for plexiform neurofibromas. This dialogue is particularly important to better support individuals who may not be able to tolerate the side effects, cannot afford the treatment, or prefer to discontinue the medication.

Figure 1 - Images of the right lower limb and MRI showing the extent of the plexiform neurofibroma.



Graphic 1. Thigh Circumference (cm) - There is a 4-degree angle between the two legs growth trends, indicating that the right leg, with plexiform neurofibroma, has been growing faster than the left leg, with a peak acceleration in 2022, which returned to the usual growth pattern after starting treatment with selumetinib (Dec 2023).

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Caregiver-Reported Sleep Disturbances in Children and Adolescents with NF1

Chelsey Ortman, MD, *Department of Pediatric Neuroscience, Division of Pediatric Neurology, Dell Medical School, The University of Texas at Austin*

Purpose: The aim of this study is to identify the prevalence of specific sleep issues within a single-center representative cohort of children with neurofibromatosis type 1 (NF1). Secondary aims include exploring sleep-related behaviors and daytime sleepiness.

Introduction: Children and adolescents with NF1 have a higher risk of developing behavioral and cognitive issues compared to their peers. Sleep issues have been long suspected as contributors to cognitive and behavioral dysfunction, however there are few recent studies describing sleep disturbances in patients with NF1. By understanding the prevalence of sleep abnormalities and types of sleep behaviors in children with NF1, further evaluation of how sleep ultimately affects quality of life, behavior, performance, and other domains in children can be achieved.

Methods: A parent and caregiver survey based on an abbreviated Children's Sleep Habits Questionnaire (CSHQ) assessed sleep issues in patients <18 years old with NF1 in a multidisciplinary NF clinic. The study was approved by the IRB (STUDY00007564). All data was collected via REDCap, where questions were posed via email to parents and caregivers.

Results: A preliminary total of 14 patient caregivers were surveyed, with an average age of 9.8 years, median age 9.5 years, range 2-17 years. To summarize, approximately 29% (n=4) of children fell asleep sometimes during daily activities. Loud snoring was described for 57% (n=8), and teeth grinding for 35% (n=5). Three (3) patients (21%) of patients were counted as waking up more than once nightly. About 71% (n=10) were portrayed as sometimes restless during sleep. Six (6) children (43%) were reported as waking up earlier than necessary at least 20% of the time.

Discussion and Conclusion: We are still collecting information from parent and caregiver surveys provided to our NF population. However, these preliminary results show that sleep issues might be more prevalent in children and adolescents with NF1 than the general population. The preliminary data highlights that a significant number of children with NF1 were described as having sleepiness with daily activities, snoring, teeth grinding, restlessness with sleep, and difficulties staying asleep. We suspect that these sleep issues may be a contributing factor for behavioral and cognitive problems. We will collect more information to continue investigating these underrepresented problems within our population, so timely management and treatment can be provided.

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Clinical - SWN (including *NF2*-SWN)

(The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
			SCHWANNOMA
Zhao	Jared	5423	Facial Nerve Schwannoma Eroding the Horizontal Semicircular Canal: An Uncommon Cause of Conductive Hearing Loss
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DeMarsh	Samantha	5577	Molecular Misdirection? CHEK2 and LZTR1 Mutations Confounding a Schwannomatosis Diagnosis
Navarro	Laura	5857	Single-Center Retrospective Case Series on *LZTR1* Germline Mutations in Patients Without Established Diagnosis of *LZTR1*-Related Schwannomatosis
Ahlawat	Shivani	5884	Impact of Bevacizumab on Peripheral Lesions in Patients with *NF2*-Related Schwannomatosis
Welling	D. Bradley	5907	Hearing Preservation and Restoration Surgery in *NF2*-Related Schwannomatosis
Welling	D. Bradley	5909	Hemorrhage During Excision of Bevacizumab-Treated Vestibular Schwannomas
Elzaafarany	Osama	8272	Clinical and Molecular Characterization of Clinically Diagnosed Schwannomas: A Cohort Study from Moffitt Cancer Center
			MENINGIOMA
Welling	D. Bradley	5914	Natural History of Meningioma Growth in *NF2*-Related Schwannomatosis
Plotkin	Scott	8363	Neratinib for *NF2*-Related Schwannomatosis with Progressive Tumors: Interim Analysis from the INTUITT-NF2 Platform-Basket Trial
			OTHER
Evans	Gareth	5401	Occam's Razor or Hickam's Dictum: Demyelination in Paediatric *NF2*-Related-Schwannomatosis
Teranishi	Yu	5679	Distinct Immune Microenvironment and Epigenetic Signatures in *NF2*-SWN Related vs. Sporadic Grade 3 Meningiomas: Implications for Prognosis and Targeted Therapy
Stultz	Reagan	8323	Nomenclature Revision of Neurofibromatosis Type 2 (NF2) to *NF2*-Related Schwannomatosis (*NF2*-SWN) – Patient and Provider Perspectives

SCHWANNOMA

Facial Nerve Schwannoma Eroding the Horizontal Semicircular Canal: An Uncommon Cause of Conductive Hearing Loss

Jared Zhao, BS, *Johns Hopkins University School of Medicine*

Purpose: This case report aims to present a facial nerve schwannoma manifesting as unilateral hearing loss, discuss the erosion of facial nerve schwannoma into the inner ear, and how this erosion influences the decision-making process regarding surgical intervention.

Methods: This case report was compiled by thoroughly reviewing the patient's medical chart—including clinical notes, imaging studies, and operative reports—and synthesizing these findings with current peer-reviewed literature on facial nerve schwannomas.

Results: A young adult male presented with a 10-month history of intermittent left ear fullness and hearing loss, which began acutely after a presumed episode of acute otitis media. Audiometry indicated left-sided conductive hearing loss, yet all cranial nerves were functioning normally. Both otomicroscopy and CT scans suggested a middle ear mass involving the ossicles, initially raising suspicion for cholesteatoma. However, MRI revealed that the lesion was more likely a facial nerve schwannoma, with CT imaging revealing the tumor extending from the mastoid region to the sinus tympani, eroding into the horizontal semicircular canal, and encompassing the stapes. Surgical decompression of the facial nerve was performed, and a biopsy confirmed a schwannoma. Genetic testing (for NF2, SMARCB1, and LZTR1) was negative. A follow-up spine MRI detected a 1 mm peripheral nerve sheath tumor in the cauda equina, meeting the criteria for schwannomatosis-NOS (not otherwise specified). Postoperatively, the patient developed mild lower facial weakness and tinnitus in the left ear. Despite stable tumor size on imaging, conductive hearing loss persisted due to the involvement of the ossicular chain. There is also a recognized risk of future sensorineural hearing loss should the tumor continue to grow.

Conclusion: This case underscores the importance of thorough imaging and vigilant monitoring to distinguish facial nerve schwannomas from common middle ear pathologies, as bony erosion can result in conductive or sensorineural hearing and vestibular loss. Detecting a small peripheral nerve sheath tumor in the cauda equina highlights broader syndromic considerations and the need for long-term surveillance.

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Nodular Growths of *NF2*-Related Schwannomatosis Vestibular Schwannoma: Clinical and Therapeutical Consequences

Michel Kalamarides, MD, PhD, *Sorbonne Université - Department of Neurosurgery, Groupe Hospitalier Pitié-Salpêtrière, Paris, France*

Background: Neurofibromatosis type 2-related Schwannomatosis (NF2-SWN) is characterized by the development of bilateral vestibular schwannoma (VS) inducing hearing loss. VS size and tumor growth rate are not associated with the corresponding hearing changes, making difficult to plan a therapeutic strategy aimed at preserving hearing. However, VS measurement is very important for the follow-up and treatment monitoring, and should be volumetric and easy to perform.

Objectives: To characterize the growth of VS in NF2 and correlate it with hearing loss.

Methods: Observational, retrospective analysis of NF2-SWN VS followed at a single institution in adults and comparison with a sporadic VS series using an AI auto-segmentation tool for 3D volumetric analysis. This technique makes it possible to superimpose sequential images to determine the precise pattern of VS growth. The tool was trained to automatically identify and delineate schwannomas based on image features and greyscale matching.

Results: A total of 43 tumor in 32 NF2-SWN patients (41% male, mean age 34 years) and 6 sporadic-VS were included. In the NF2-SWN population, during the follow-up (average duration of 6 years), the tumors increased in size by 2.3cm^3, and in 14 cases, tumor growth was associated with a significant decrease in hearing. No significant differences in tumor volume or growth rate were observed between the patients whose hearing remained stable and those whose hearing deteriorated. However, 3D tumors reconstruction revealed distinct growth patterns. NF2-SWN VS exhibit nodular, multinodular, or homogeneous growth in contrast with the sporadic VS, which all exhibited homogeneous growth (42% vs 100%; $p < 0.01$). Regarding NF2-SWN tumors, nodular growth was significantly associated with hearing loss ($p = 0.02$). More specifically, 100% of tumors with nodular growth while hearing loss showed a growing nodule in the posterior-inferior part of the VS, while hearing was preserved in case of nodular growth in the antero-superior part of the VS ($p = 0.001$).

Conclusion: NF2- SWN VS exhibit growth patterns different from those of sporadic VS, frequently presenting with a nodular appearance, which is consistent with the genetics of these tumors. The growth of a nodule, even of small size, in the posterior-inferior part of the tumor is strongly correlated with hearing loss, probably by displacement/stretching of the cochlear nerve.

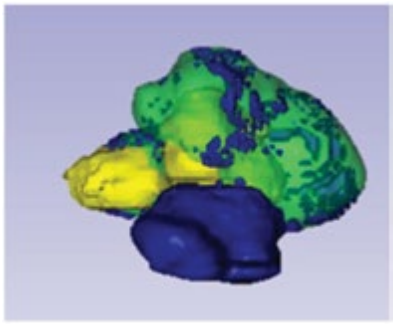

Figure. Example of overlayed tumor. The more recent tumor mask is in blue shade, the opacity of the mask has been changed to visualize the previous tumor mask in yellow. After nine months of follow-up, a new tumor nodule emerged in the postero-inferior surface of the vestibular schwannoma associated with severe hearing deterioration.

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Molecular Misdirection? CHEK2 and LZTR1 Mutations Confounding a Schwannomatosis Diagnosis

Samantha DeMarsh, DO, *Morgan Adams Foundation Pediatric Brain Tumor Research Program, Children's Hospital Colorado, Aurora, Colorado; Center for Cancer and Blood Disorders; Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, Colorado*

Purpose: To present an unusual case of tumor and germline genetics complicating the diagnosis and treatment of schwannomatosis in a young male presenting with acute onset weakness and decreased sensation in extremities.

Methods: An 11 year old male with a history of a repaired aortic coarctation and bicuspid valve presented with progressive weakness and neuropathy . MRI of the brain demonstrated several schwannomas including bilateral vestibular schwannomas and numerous intradural extramedullary masses were visualized throughout the spinal canal with the largest at C5-C6, resulting in severe compression of the spinal cord with cord edema. He underwent osteoplastic laminectomies (C3-C6) for tumor resection with pathology confirming Meningioma, WHO Grade 1. Presumed clinical diagnosis of schwannomatosis associated with NF2 was made, but somatic and germline genetic testing revealed mutations present in CHEK2 and LZTR1.

Results: Somatic testing of the tumor sample confirmed a low tumor mutational burden (24 variants, 4.74 variants/megabase) with no clinically significant (Tier I/II) DNA or RNA variants present by our *Comprehensive Neuro-Oncology Next-Generation Sequencing Panel Analysis.* Two variants of unknown significance (VUS) with large deletion events were captured; one with the chromosome 22 deletion event including the NF2 locus. Two heterozygous sequence variants included c.556A>C p.(Asn186His) identified in exon 4 of the CHEK2 gene (NM_007194.3) and c.22G>T p.(Gly8Trp) of the LZTR1 gene (NM_006767.3). The paired normal peripheral blood specimen was analyzed for both the LZTR1 and CHEK2 variants and both were confirmed as germline. Single gene sequencing on NF2 is being conducted to assess for mutations and copy number variants of the target gene to specifically assess for a "second hit" being the loss of wild type NF2 by deletion. This step would be crucial to the formation of bilateral acoustic schwannomas with the presence of LZTR1 germline and somatic mutations.

Conclusion: The mutations present in LZTR1 and CHEK2 add to the complex heterogeneity of schwannomatosis in this case. Brigatinib has demonstrated broad antitumor activity in patients with NF2 schwannomatosis and would be ideal therapy in this patient given the threat to function cord from multiple, more difficult to resect meningiomas, but there is no literature to date describing its efficacy in LZTR1 schwannomatosis. Additionally, it is unclear if the aggressive presentation in this case is further complicated by the CHEK2 mutations that is currently classified as a VUS.

Figure 1: Somatic Mutation Results

Tier III Variants With Unknown Clinical Significance

Gene	Transcript	Genomic Position	DNA Change	Protein Change	Allele Frequency	Tier
LZTR1	NM_006767.3	chr22:21336682	c.22G>T	p.Gly8Trp	64.1%	Tier 3
CHEK2	NM_007194.3	chr22:29121001	c.556A>C	p.Asn186His	83.2%	Tier 3

Copy Number Variation Events

Genomic Position	Copy Number Change
chr22:26,250,000-51,300,000	Deletion
chr5:65,400,000-180,750,000	Deletion

Figure 2: Germline Analysis Results

Previously-Reported Tier III Variants With Unknown Clinical Significance

Gene	Transcript	Genomic Position	DNA Change	Protein Change	Germline/ Somatic Status	Classification
LZTR1	NM_006767.3	chr22:21336682	c.22G>T	p.Gly8Trp	Germline	Likely Benign
CHEK2	NM_007194.3	chr22:29121001	c.556A>C	p.Asn186His	Germline	VUS

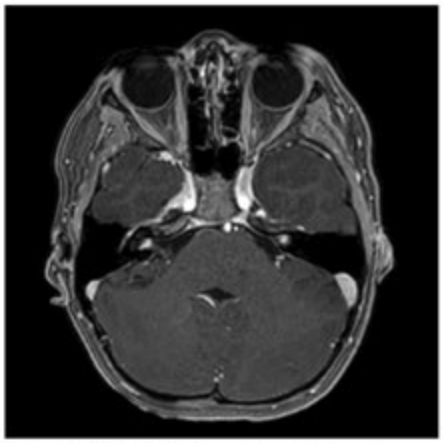

Figure 3: MRI Brain with evidence of bilateral acoustic schwannomas.

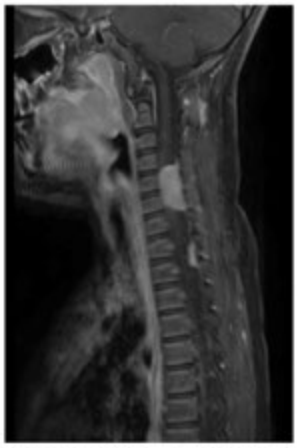

Figure 4: MRI Spine demonstrating compressive extramedullary masses.

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Single-Center Retrospective Case Series on *LZTR1* Germline Mutations in Patients Without Established Diagnosis of *LZTR1*-Related Schwannomatosis

Laura Navarro, BS, *University of Illinois at Chicago*

Introduction: Germline loss-of-function mutations in the leucine zipper-like transcription regulator 1 *(LZTR1)* gene are associated with the development of non-neurofibromatosis type 2 (NF2) related schwannomatosis (non *NF2*-SWN). Diagnostic criteria for *LZTR1*-related schwannomatosis (*LZTR1*-SWN) include the presence of schwannomas on imaging and a confirmed *LZTR1* mutation. Schwannoma typically develop between 20-40 years of age. This case series presents three patients with incidental findings of *LZTR1* germline mutations without confirmed schwannomas and thereby they do not meet diagnostic criteria for *LZTR1*-related schwannomatosis at this time.

Methods: We conducted a single-center retrospective study at the University of Illinois at Chicago, reviewing patients who underwent genetic testing between 2022-2024 and were found to have *LZTR1* mutation without established diagnosis of *LZTR1*-SWN. Data collected included clinical features, imaging results, genetic and oncologic history, family history, age, and gender.

Results: Three patients with confirmed *LZTR1* mutations were included. Patient 1 is a 51-year-old female with a history of invasive ductal carcinoma of the breast who underwent genetic screening due to her cancer history. She presented with bilateral tinnitus and thigh pain, but no focal neurologic deficits. Her family history included renal cell carcinoma and breast cancer. Neuroimaging, including MRI spine and MRI internal auditory canal (IAC) showed no schwannomas. Patient 2 is a 32-year-old female with papillary thyroid carcinoma, screened for genetic mutation due to a family history of ovarian, breast, and cervical cancer. She presented with bilateral tinnitus and balance problems but had no focal neurologic deficits. MRI of the spine and IAC were negative for schwannomas. Patient 3 is a 33-year-old female with a history of breast sarcoma and Cowden syndrome, who underwent genetic screening due to her cancer history. There were no focal neurologic deficits or pertinent positive symptoms at presentation. MRI of the abdomen revealed a T2 hyperintense liver lesion, and a PET scan identified hilar lymphadenopathy, which was biopsied and found to be benign.

Conclusion: This case series presents three patients with *LZTR1* germline mutation, which were incidentally found on a screening test due to personal or family history of cancer. Given their young ages, they may develop schwannomas in the future, potentially meeting diagnostic criteria for *LZTR1*-SWN. Further research is needed to explore the potential association between *LZTR1* germline mutation, *LZTR1*-SWN, extraneural malignancies and other cancer predisposition syndromes.

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Impact of Bevacizumab on Peripheral Lesions in Patients with *NF2*-Related Schwannomatosis

Shivani Ahlawat, MD, *The Russel H. Morgan Department of Radiology and Radiological Science, Johns Hopkins Medical Institutions*

Purpose: Surgical management remains the mainstay of treatment for peripheral schwannomas in patients with *NF2*-related schwannomatosis (*NF2*-SWN) with emerging but limited non-operative therapies most commonly targeting progressive/symptomatic vestibular schwannomas (VS). There is paucity of data on the response of non-VS schwannomas (particularly peripheral schwannomas) to systemic treatment. Here we describe the whole-body magnetic resonance (WB-MRI) features of peripheral schwannomas in patients with *NF2*-SWN treated with bevacizumab during a clinical trial for progressive/symptomatic VS.

Methods: In this multi-institution, open-label phase II clinical trial, WB-MRI at 3.0T was performed at baseline, 25-weeks, and 49-weeks after treatment with bevacizumab (7.5 mg/kg every 3 weeks for 46 weeks) using standardized STIR, DWI/ ADC mapping, pre- and post-contrast T1-weighted imaging. Two readers recorded size and signal characteristics of non-target peripheral lesions > 10 mm (maximum 5 largest lesions/patient, each from a different body region). 2D-area (product of two perpendicular measurements), inter-reader reliability and percent change (baseline- 49 weeks) was calculated. Radiological response was defined as complete response (CR, disappearance), partial response (PR, ≥20% decrease from baseline), progressive disease (PD, ≥20% increase from baseline), and otherwise stable disease (SD).

Results: Baseline WB-MRI detected 119 non-target peripheral lesions consistent with schwannomas with a median of 8 peripheral schwannomas per patient (range: 0-37) across 14 participants, the majority located in the lower extremities. Thirty-three peripheral schwannomas in 11 patients (median age: 30yrs (range:14-79yrs)) were characterized. Median size (mm) at baseline, 25-weeks, and 49-weeks was 26 (range: 10-88), 26 (range: 10-86), and 25 (range: 9-86), respectively. Lesions were measured with high inter-reader reliability (ICC range 0.990-0.997). Based on largest lesion diameter, all non-target lesions were stable at week 49. Based on 2D-area, the majority of the lesions were SD (31/33; 94%) followed by PR (1/33;3%) and PD (1/33; 3%). There was no change in minimum ADC values or other signal characteristics from baseline to 49-week WB-MRI.

Conclusions: In *NF2*-SWN, WB-MRI enables detection and characterization of peripheral schwannomas as small as 10 mm with high-inter reader reliability. Despite histological similarities to central vestibular schwannomas (treated for confirmed progression), non-target peripheral schwannomas predominantly had SD by 1- and 2- dimensional measurements on bevacizumab.

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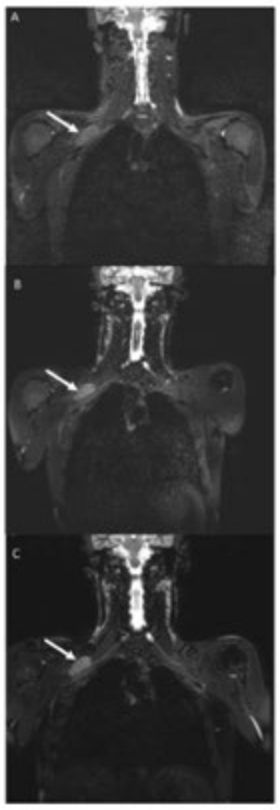
Figure 1: Stable disease in brachial plexus lesion at baseline (a), 25-week (b) and 49 weeks (c).



Hearing Preservation and Restoration Surgery in *NF2*-Related Schwannomatosis

D. Bradley Welling, MD, PhD, *Massachusetts Eye and Ear Infirmary*

Purpose: To determine what factors influence hearing preservation in patients with *NF2*-related schwannomatosis (*NF2*-swn) undergoing surgical resection of vestibular schwannomas (VS).

Methods: Retrospective review of a single institutional patient data base of *NF2*-swn patients undergoing surgical removal of VS with the goal of hearing preservation. To be included subjects must have a preoperative word recognition score (WRS) of greater than 50%. Factors evaluated for influence on hearing preservation included tumor size, tumor location, preoperative pure tone average (PTA), preoperative WDS, onset age of NF2, auditory brainstem function, genetic mutation type, inflammation in the cochlea, and the extent of tumor resected. Contribution of each factor was evaluated with univariate analysis, multivariate analysis. When hearing was not preserved, auditory brainstem implants (ABI) or cochlear implants (CI) results are also included.

Results: From 319 patients with *NF2*-related schwannomatosis, 47 patients with speech discrimination >50% were identified between 1988 to 2024. Postoperatively 19 of the 47 patients had WRS >75%. Univariate analysis identified factors of normal wave V on ABR preoperatively and lack of brainstem contact by the tumor as significant at the 0.05-alpha level. Tumor size and age of onset approached significance. However, multivariant analysis did not identify any factors which reached statistical significance implying relationships between the covariates. When a machine learning (ML) method (SuperLearner) was used, we were able to achieve a 75% prediction accuracy of hearing preservation implying that there is indeed useful information for predicting hearing preservation in this data. The three most influential variables in the ML models were normal wave V on ABR, lack of brainstem compression, and pre-operative WRS.

Conclusions: Factors influencing post operative hearing in *NF2*-swn VS resection when combined with ML may aid in predicting surgical hearing outcome. Further confirmational and multi-institutional studies are needed.

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Hemorrhage During Excision of Bevacizumab-Treated Vestibular Schwannomas

D. Bradley Welling, MD, PhD, *Massachusetts Eye and Ear Infirmary*

Purpose: To determine if the chronic use of bevacizumab, prior to withholding for elective surgery in the preoperative period, increases the risk of hemorrhage during vestibular schwannoma (VS) resections.

Methods: Retrospective review of estimated intraoperative blood loss volume during surgical resection of 50 bevacizumab-treated and 56 bevacizumab-untreated vestibular schwannomas (VS) from patients with *NF2*-related schwannomatosis (*NF2*-SWN). Two index cases are included. Additionally, masked histopathologic examination of tissues from bevacizumab treated and untreated VS were evaluated.

Results: The mean blood loss during VS resection was statistically increased in patients with prior bevacizumab treatment (421ml) compared to untreated patients (285ml) ($p=0.015$). Intraoperative hemostasis using standard techniques was difficult and some cases resulted in early termination of the surgical procedure prior to total resection. Eighteen percent of bevacizumab- treated patients had gross total or near total tumor resection while untreated-tumors had 52% gross total or near total resections (p-value=0.001). The length of time on bevacizumab preoperatively correlated with intraoperative blood loss ($p=0.049$). The median time patients were treated with bevacizumab prior to surgery was 23.5 months and ranged from 2.75-120.75 months. The median time off bevacizumab prior to surgery was 11.5 months and ranged from 0.75-87.25 months. Histopathologic examination revealed no readily apparent difference in blood vessel density, integrity, or growth patterns.

Conclusions: Bevacizumab treatment prior to excision of *NF2*-SWN VS in this retrospective case series is associated with increased intraoperative hemorrhage. Bleeding was difficult to control using standard techniques. Underlying pathologic mechanisms are not yet understood and are under further investigation.

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Clinical and Molecular Characterization of Clinically Diagnosed Schwannomas: A Cohort Study from Moffitt Cancer Center

Osama Elzaafarany, MD, *Moffitt Cancer Center, Tampa, FL*

Background: Schwannomatosis is suspected in patients with two or more schwannomas and is commonly associated with *NF2, LZTR1,* or *SMARCB1* mutations. *NF2*-related schwannomatosis, the most prevalent type, typically presents with bilateral vestibular schwannomas, multiple meningiomas, and ependymomas. *LZTR1*-related schwannomatosis may include unilateral vestibular schwannomas. Despite diagnostic advances, the clinical and genetic heterogeneity of *NF2* remains poorly understood.

Purpose: This study aims to characterize the clinical presentation, pathological features, and molecular landscape of schwannomatosis in patients who underwent schwannoma resections at Moffitt Cancer Center.

Methods: Tissue samples from 18 patients with suspected schwannomatosis were analyzed via pathology and next-generation sequencing (NGS) using the Moffitt STAR panel. Clinical data were retrieved from medical records and correlated with diagnostic criteria per the updated classification system.

Results: The cohort included 18 patients (median age 45; range 24–85), comprising 10 males and 8 females. A prior cancer history was present in 17%, including melanoma (11%) and T-cell lymphoma (6%). Tumor resection sites included paraspinal (33%), peripheral nerve (22%), cauda equina (17%), cervical nerve root (11%), intracranial (11%), and extremity (6%). According to updated NF2 criteria, eight patients (44%) did not meet diagnostic thresholds for schwannomatosis. *LZTR1*-related schwannomatosis was diagnosed in four patients (22%), *NF2*-related schwannomatosis in three (17%), and one (6%) as schwannomatosis not otherwise specified (NOS). One patient (6%) had a meningioma with an *NF2* mutation. NGS identified diverse genetic alterations: isolated *NF2* mutations were most common (28%), followed by co-occurring *NF2, SMARCB1,* and *LZTR1* mutations (11%), and *SOX10* mutations (11%). Additional variants—each in a single case (6%)—included mutations in *LZTR1, TET2, CHEK2, SMARCB1, DNMT3A, EGFR,* and *ERCC4.* One case lacked sufficient material for testing. The molecular findings highlight the heterogeneity of schwannomatosis, with frequent involvement of tumor suppressor and chromatin remodeling genes.

Conclusion: Histopathological and molecular assessments underscore the clinical and genetic diversity of *NF2*-related tumors. These findings support the need for comprehensive imaging, molecular profiling, and multidisciplinary care to optimize diagnosis, surveillance, and treatment strategies in patients with schwannomatosis.

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MENINGIOMA

Natural History of Meningioma Growth in *NF2*-Related Schwannomatosis

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Purpose: To determine the natural growth rate of meningiomas in the setting of *NF2*-related Schwannomatosis (*NF2*-SWN) without surgical intervention for comparison with clinical trials.

Methods: Retrospective chart review of a single institutional research patient data repository for those who have been genetically diagnosed with *NF2*-SWN with observable meningiomas. Inclusion criteria included patients who have had genetic analysis for *NF2*-SWN and radiographic evidence of a suspected meningioma. Meningiomas were followed if they measured at least 5mm in one dimension without prior surgical or radiation therapy intervention with a minimum of two MRIs 6-months apart. Variables considered that may be associated with growth rate of meningiomas were mutation, location, age of onset and age of diagnosis for *NF2*-SWN, sex, and family history.

Results: One hundred-seventy-two patients with clinical diagnosis of *NF2*-SWN were reviewed. Eighty *NF2*-SWN patients were eligible and 180 meningiomas were followed. The mean follow-up was 8.6 years (range 1-23 years). The average growth rate from the baseline was 1.95 mm per year (range –0.4 mm/yr to 17 mm/yr). The mean percent increase was 81.5% from baseline to the latest accessible MRI with an average percent increase of 10.8% per year from baseline. Only 3 of 177 untreated meningiomas decreased spontaneously over the follow up period. ANOVA was performed to determine if constitutional *NF2*-SWN mutation grade severity affected average tumor growth rate from baseline but was not statistically significant (p-value = 0.107). Relative percent change of greatest diameter per year was also examined against genetic severity and did not reach statistical significance (p-value = 0.1193).

Conclusion: In this retrospective chart review, radiographically diagnosed meningiomas in patients genetically diagnosed with *NF2*-SWN were observed to grow 2mm per year unassociated with genetic severity. Baseline growth patterns will be used to investigate possible drug therapies to control meningiomas in *NF2*-SWN patients.

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Disclosures: DBW is a consultant for Salubritas, Inc. and Recursion Pharmaceuticals. JTJ is a consultant for Alexion pharmaceuticals; Children's Tumor Foundation; Recursion Pharmaceuticals; Shepherd Therapeutics; Akeila Bio; Merck & Co; Michigan State University; Navio Theragnostics; Springworks Therapeutic; Elsevier, Inc; The Doctor Lounge; Shepherd Therapeutics; Akeila Bio; and Khora TX. SRP is co-founder of NFlection Therapeutics and NF2 Therapeutics; consults for Akouos; and is on the scientific advisory board of SonALAsense.

Neratinib for *NF2*-Related Schwannomatosis with Progressive Tumors: Interim Analysis from the INTUITT-NF2 Platform-Basket Trial

Scott R. Plotkin, MD, PhD, *INTUITT-NF2 Consortium*

Background: *NF2*-related schwannomatosis (*NF2*-SWN) is a rare hereditary tumor syndrome that predisposes affected individuals to vestibular schwannomas (VS), non-vestibular schwannomas (NVS), meningiomas, and ependymomas. Deregulation of ErbB receptor signaling has been demonstrated in schwannomas, meningiomas, and ependymomas, and previous trials of EGFR or HER2 inhibitors have shown some efficacy against VS. Neratinib is a potent irreversible pan-erbB inhibitor. We report the results of the interim analysis of the neratinib treatment arm of INTUITT-NF2.

Methods: We conducted a multicenter, phase II, open-label basket trial of neratinib for subjects (≥12 years old) with NF2 and progressive tumors (baskets: VS, NVS, meningioma, or ependymoma). In stage 1, 20 participants were accrued (minimum of 2 participants per tumor basket). Tumor response was evaluated by MRI every 3 months in year 1 and every 6 months thereafter. Radiographic response was defined as ≥20% decrease in tumor volume below baseline. Progressive disease was defined as ≥20% increase in tumor volume from baseline. Primary outcome was the radiographic response rate (RR). Pre- and post-treatment growth rates were determined using pre-baseline and study MRI scans.

Results: Twenty subjects (median age=27 years, range, 12-45 years, 3 pediatric, 12 females) were treated with neratinib. Target tumors included 10 VS, 3 NVS, 5 meningiomas, and 2 ependymomas; non-target tumors included 20 VS, 14 NVS, 13 meningiomas, and 2 ependymomas. The radiographic RR for target and all tumors was 10% and 13%, respectively. By tumor basket, the radiographic RR for all tumors was 0% for VS, 35% for non-VS, 17% for meningioma, and 0% for ependymomas. Average annualized tumor growth rates decreased for VS, NVS, meningioma, and ependymoma during neratinib treatment (table). No serious adverse events were reported. Five grade 3 treatment-related adverse events were reported (all diarrhea). Three patients held or delayed dose due to grade 2/3 toxicity (diarrhea, rash, malaise), and two patients discontinued treatment during cycle 1 of treatment (diarrhea, malaise).

Conclusions: Neratinib treatment showed modest efficacy in treatment of tumors associated with *NF2*-SWN. Based on this interim analysis, the team will explore whether an additional 20 participants will be enrolled in the two most promising tumor baskets.

	VS		NVS		Meningioma		Ependymoma	
All tumors, N	30		17		18		4	
Baseline volume	6.7 cc		6.1 cc		4.4 cc		15.4 mm	
Average annualized growth rate (pre/post treatment)	Pre	Post	Pre	Post	Pre	Post	Pre	Post
% per year	46%	25%	237%	-11%	72%	42%	41%	11%
cc per year	0.5	0.4	9.5	0.2	0.9	0.9	0.5	0.1

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OTHER

Occam's Razor or Hickam's Dictum: Demyelination in Paediatric *NF2*-Related-Schwannomatosis

Gareth Evans, MD, FRCP, *Consultant Clinical Geneticist, Highly Specialised Services for NF2, St. Mary's Hospital, Manchester, UK*

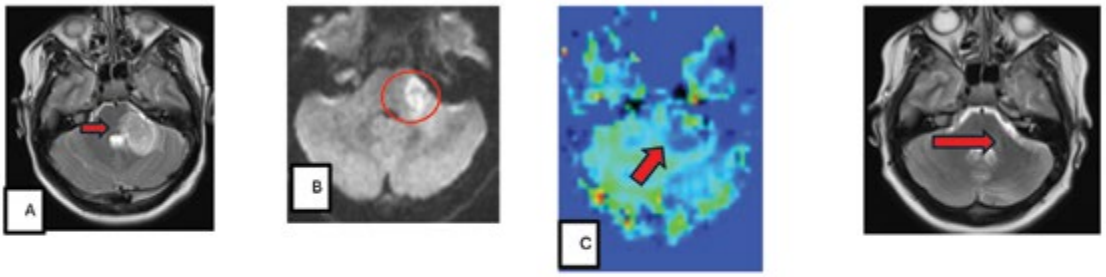
Purpose: Neurofibromatosis type 2 (NF2)-related-schwannomatosis is an autosomal dominant genetic condition affecting 1 in 25,000 individuals. It is characterised by benign vestibular schwannomas and other types of brain and spinal tumours. *NF2*-related-schwannomatosis is much less common than NF1. While demyelination like multiple sclerosis (MS) is well recognised in adult neurofibromatosis type 1 there are only isolated reports of MS in NF2 in adults. We describe a teenage boy presenting with his first demyelinating event and the challenges of management and diagnosis.

Methods: Case report

Results: A 14-year-old boy known to the NHS funded Highly Specialised Service (HSS) NF2 service in Manchester England presented with a five-day history of loss of sensation on the left side of the face with trauma to his left buccal mucosa sustained during eating. His positive neurological signs were loss of sensation in the left trigeminal nerve distribution, reduced left blink reflex, ataxia and right sided upper motor neurone signs. He was afebrile and infections markers were negative.

Neuroimaging demonstrated a lesion in the left middle cerebellar peduncle with features concerning for a high-grade neoplastic lesion. A biopsy of this lesion was carried out and multiple investigations organised. Histology showed no signs of malignancy but showed necrotic cells with features of demyelination. Culture of the lesion for bacteria and Tuberculosis was negative. Extensive basic blood tests, blood cultures and viral PCRs were also negative. Discussions were held in several multi-disciplinary teams (including HSS NF2 Manchester (Adult and Paediatric Forums), Paediatric Neuro-oncology, and Paediatric Neuroradiology), The adult neurologists in the HSS NF2 service recommended treating as a first demyelinating event. Three days of high dose prednisolone weaned over 6 weeks were administered with sustained improvement. A repeat scan in 6 weeks, three months and 6 months and one year showed near complete resolution of the lesion. Anti-MOG and Aquaporin antibodies were negative as were blood and cerebrospinal fluid (CSF) studies for central nervous system (CNS) lymphoma. Chest X-ray and abdominal ultrasounds normal. Six months later his neurology was back to normal, and this is sustained one year later.

Conclusion: There is no recognized association between NF2 and CNS demyelination in children. To our knowledge this is the first described paediatric case with both. Multi-disciplinary team approach is helpful in the management of such rare and challenging cases.



Imaging: Initial MRI (Fig A-B in Feb 2024) demonstrated a lesion in the left middle cerebellar peduncle. This new T2 hyperintense mass lesion (A) extended into the left side of the superior cerebellar vermis and left inferior cerebellar hemisphere. This demonstrated contrast enhancement and diffusion restriction. (B) Imaging features were highly concerning for a high-grade neoplastic lesion, although there were some more equivocal features like the lack of increased perfusion on ASL (C). Resolution post treatment (D)

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Distinct Immune Microenvironment and Epigenetic Signatures in *NF2*-SWN Related vs. Sporadic Grade 3 Meningiomas: Implications for Prognosis and Targeted Therapy

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Purpose: *NF2*-related schwannomatosis (*NF2*-SWN) is an autosomal dominant tumor predisposition syndrome driven by NF2 mutations, leading to multiple CNS tumors that often necessitate repeated surgeries. Among these, meningiomas (MGMs) are the second most common tumor type and a major contributor to mortality. While Brigatinib demonstrates efficacy against benign *NF2*-SWN-associated MGMs (NF2ptMGMs), its impact on aggressive NF2ptMGMs remains limited, underscoring the need for alternative therapeutic strategies, including immunotherapy. Recent our research suggests that epigenetic mechanisms influencing the immune tumor microenvironment (TME) contribute to NF2ptMGM progression, either independently of or in conjunction with NF2 mutations. Notably, the frequency of high-grade NF2ptMGMs does not significantly exceed that of sporadic MGMs, implying that the immune TME may play a critical role in tumor prevalence and progression. Given that recurrence-free survival—a commonly used metric in MGM research—is largely dictated by the extent of surgical resection, we instead analyzed overall survival (OS), along with genetic/epigenetic features and immune TME characteristics, in Grade 3 NF2ptMGMs (Gr3NF2ptMGMs) and Grade 3 sporadic MGMs (Gr3spMGMs).

Methods: A long-term retrospective study (80.9 ± 97.9 months) analyzed 16 Grade 3 MGMs (4 Gr3NF2ptMGMs, 12 Gr3spMGMs). Tumor characteristics were assessed using whole-exome sequencing, DNA methylation analysis, and multiplex immunofluorescence (CD163, FOLR2, SPP1, Ki67, CD4, CD8, GrB, FOXP3, PD1).

Results: Gr3NF2ptMGMs had significantly better OS than Gr3spMGMs (1-year: 100% vs. 66.7%; 3-year: 100% vs. 33.4%; 5-year: 50% vs. 0%, $p < 0.01$). Kaplan-Meier ($p = 0.001$) and Gray's test ($p = 0.002$) confirmed significance. No differences were observed in mitotic count, Ki-67, CNV, hTERT, pTERT methylation, CDKN2AB co-deletion, or H3K27me3 status.

All Gr3NF2ptMGMs belonged to the "Intermediate" epigenetic group, while 91.7% of Gr3spMGMs were "Malignant." $CD163^+$ macrophages were significantly higher in Gr3NF2ptMGMs (22.5% vs. 10.3% in total cell counts (TTC), $p = 5.45e\text{-}10$), while $PD\text{-}1^+CD8^+$ T cells were significantly lower (0.1% vs. 0.9% in TTC, $p = 2.8e\text{-}7$). Tissue-resident macrophages ($CD163^+FOLR2^+$) were enriched in Gr3NF2ptMGMs (11.5% vs. 3.8% in TTC, $p = 0.02$), whereas $SPP1^+$ macrophages were more abundant in Gr3spMGMs (5.7% vs. 0.25% in TTC, $p = 6.6e\text{-}5$).

Conclusion: Gr3NF2ptMGMs have a better prognosis than Gr3spMGMs, likely due to differences in immune TME. These findings highlight the need for immune-targeted therapies in high-grade MGMs in *NF2*-SWN patients.

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Nomenclature Revision of Neurofibromatosis Type 2 (NF2) to *NF2*-Related Schwannomatosis (*NF2*-SWN) – Patient and Provider Perspectives

Reagan Stultz, MS, *Medical College of Wisconsin*

Introduction: In 2022, the nomenclature of neurofibromatosis type 2 (NF2) was revised to *NF2*-related schwannomatosis (*NF2*-SWN)[1]. The new nomenclature incorporated genetic and phenotypic advances with a goal to improve diagnostic accuracy, better reflect the similarities between different schwannomatosis disorders, and reduce confusion between neurofibromatosis type 1 (NF1) and NF2. This study explores knowledge, values, and use of the revised nomenclature among patients with *NF2*-SWN and healthcare providers who serve them.

Methods: Two separate parallel mixed-methods surveys were distributed to patients and providers via patient advocacy groups and professional societies. Survey questions included quantitative responses utilizing multiple choice and Likert scale formats, and qualitative short-answer responses. Three knowledge-based questions were included.

Results: Respondents included 87 patients and 47 providers. A majority of patients (63%) and providers (79%) knew about the nomenclature revision prior to the study. When asked the type of tumors that are associated with *NF2*-SWN, respondents correctly answered schwannomas (93% patients vs.100% providers) but incorrectly answered neurofibromas (53% patients vs. 23% providers).

The majority of patients (54%) liked the former nomenclature, compared to only 23% of providers ($p=<0.001$). The inverse was true regarding feelings about the new nomenclature ($p=<0.001$). Both patients and providers who incorrectly selected neurofibromas as an associated finding of *NF2*-SWN were more likely to like/strongly like the former nomenclature (patients $p=0.004$; providers $p=0.016$). Forty percent of patients agreed/strongly agreed that they were confused by the new nomenclature whereas 85% of providers disagreed with this statement ($p=<0.001$). Forty-one percent of patients and 89% of providers agreed/strongly agreed that they appreciate that the new nomenclature accurately reflects current knowledge in the field ($p=<0.001$). Patients are more likely to use the former nomenclature, while providers more frequently use the new terminology ($p=<0.001$).

Conclusions:

- Continued educational efforts about advances and nomenclature revision in *NF2*-SWN for both patients and healthcare providers are needed.
- Increased sensitivity and recognition of different perspectives when discussing *NF2*-SWN may be useful during patient interactions.
- Consistent use of the updated nomenclature is essential to support clarity and integration of the new terminology.

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1. Updated diagnostic criteria and nomenclature for neurofibromatosis type 2 and schwannomatosis: An international consensus recommendation. *Genet Med.* 2022 Sep;24(9):1967-1977.

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Disease Agnostic (The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Gade	Purva	5140	Secretory Mitophagy: An Adaptive Survival Mechanism in NF2 Tumors Promoting Tumor Progression Through Export of Damaged Mitochondria and Tumor Suppressors
Tsuchiya	Takahiro	5364	Genetic Analysis in Vestibular Schwannomas and Non-Vestibular Intracranial Schwannomas
Faulkner	Jennifer	5540	A Comparison of Two Electronic Data Capture (EDC) Platforms to Create an NF Registry
Rosser	Tena	5604	Neurofibromatosis Type 1 and Schwannomatosis Virtual Case Conference Series
Moore	Marc	5617	Identification of AAV Engineered Capsids that Allow Efficient Nervous System Transduction Through Comparative Biodistribution Studies
Parry	Christina	5639	Clinical Metadata Representation in the NF Data Portal
Wan	Kyle	5673	NF Simplified: Enhancing Access to Cutting-Edge Research for the Neurofibromatosis Community Through AI-Driven Summaries and Expert Validation
Nath	Aditya	5762	Improvements to the NF Data Portal and Synapse Data Platform
Anstett	Kara	5796	Patient and Caregiver Preferences when Considering a Hypothetical Gene Therapy for Patients with Neurofibromatosis Type 1 (NF1) or Schwannomatosis (SWN)
Kelts	Kate	7963	An Analysis of the Children's Tumor Foundation NF Registry, 2012–2025

Disease Agnostic

Secretory Mitophagy: An Adaptive Survival Mechanism in NF2 Tumors Promoting Tumor Progression Through Export of Damaged Mitochondria and Tumor Suppressors

Purva Gade, *George Mason University, Fairfax, VA*

Background and Purpose: Neurofibromatosis Type 2 (NF2) is a hereditary tumor disorder caused by mutations in the NF2 gene, resulting in the loss of the tumor suppressor protein Merlin. NF2 patients develop tumors such as vestibular schwannomas and meningiomas, which cause hearing loss, tinnitus, and balance dysfunction. Current therapies are largely palliative, addressing symptoms rather than targeting tumor growth. Thus, there is a need to uncover novel mechanisms contributing to NF2 tumor progression. Cancer cells undergo metabolic reprogramming to survive oxidative stress by altering mitochondrial dynamics through fission and fusion. In healthy cells, mitophagy removes damaged mitochondria via lysosomal degradation. This process is regulated by mitochondrial proteins, including PINK. Our study identifies a novel alternative pathway secretory mitophagy by which NF2 tumor cells export damaged mitochondria via extracellular vesicles (EVs), while simultaneously exporting tumor suppressor molecules, such as Merlin. This process promotes tumor survival under oxidative stress.

Methods: Meningioma cells with NF2 mutations were treated with oxidative stress inducers combined with lysosomal inhibition to block canonical mitophagy. EVs were isolated from conditioned media and analyzed for Merlin, FIS1, and PINK1 content. Immunoprecipitation (IP) of PINK1-positive EVs was performed to detect co-exported Merlin. To investigate FIS1's role, we conducted siRNA-mediated knockdown followed by oxidative stress treatment, then measured EV secretion, cell survival, and secretory mitophagy levels.

Results: Oxidative stress combined with lysosomal inhibition led to significantly increased EV secretion enriched for damaged mitochondria. These EVs contained Merlin, FIS1, and PINK1, indicating active export of both damaged mitochondria and tumor suppressor proteins. Immunoprecipitation confirmed Merlin co-localized with PINK1+ EVs, demonstrating tumor suppressor export. FIS1 knockdown significantly reduced secretory mitophagy and cell survival, confirming that FIS1 is critical for this adaptive process.

Conclusion: This study identifies secretory mitophagy as a previously unrecognized survival strategy employed by NF2 tumor cells. By exporting damaged mitochondria along with tumor suppressor molecules like Merlin, NF2 tumor cells enhance their survival under oxidative stress and further disrupt tumor suppressor signaling networks, promoting unchecked growth. Targeting secretory mitophagy could offer a novel therapeutic strategy to restore intracellular tumor suppressor levels and impair tumor stress adaptation mechanisms in NF2-associated tumors.

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Genetic Analysis in Vestibular Schwannomas and Non-Vestibular Intracranial Schwannomas

Takahiro Tsuchiya, *Department of Neurosurgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan*

Purpose: Schwannomas are benign neoplasms arising from myelinating Schwann cells of the nerve sheath. The majority of the intracranial schwannomas are vestibular schwannomas (VS). Among non-vestibular intracranial schwannomas (non-VS), trigeminal and jugular foramen schwannomas are predominantly observed. While the loss of *NF2* function plays a significant role in sporadic schwannoma tumorigenesis, a recent, large-scale study revealed the involvement of additional recurrent gene mutations, in addition to the *SH3PXD2A::HTRA1* fusion gene, in sporadic schwannomas. However, the genetic landscape of non-VS remains unclear. In this study, we conducted a comprehensive genetic analysis of VS and non-VS.

Methods: Targeted panel sequencing and microsatellite analysis of 22q were performed in 51 patients with sporadic intracranial schwannomas (21 patients with VS and 30 with non-VS). The proportion of known schwannoma variants was assessed depending on the tumor origin site. Additionally, the *NF2* alterations were examined to determine whether they differed between VS and non-VS.

Results: The median tumor size of non-VS was significantly larger than that of VS (38 mm, 27 mm, $p < 0.01$). In contrast, there were no significant differences between the VS and non-VS in median age at surgery (41.5 years vs 45.2 years, $p = 0.37$), female gender (42.8% vs 50.0%, $p = 0.83$), cystic lesions (71.4%, 73.3%, $p > 0.99$), postoperative stereotactic radiosurgery (14.3% vs 13.3%, $p > 0.999$), or regrowth after surgery (9.5% vs 16.7%, $p = 0.75$). Somatic *NF2* mutations were frequently identified in tumor samples (25 patients, 49.0%); non-VS showed a significantly lower frequency of *NF2* mutations than VS (80.9% vs 26.7%, odds ratio: 11.0 [95% confidence interval: 2.59–59.5], $p < 0.001$). Despite the absence of statistical significance in the frequency of 22q loss of heterozygosity (LOH) between VS and non-VS, any *NF2* alterations (*NF2* mutation or 22q LOH) exhibited a significant difference (95.2% vs 56.7%, $p < 0.01$). Truncating mutations (frameshift, nonsense, and splice-site mutations) were the most common type of mutation (22 mutations, 75.9%). The variant allele frequency of *NF2* was found to be high, indicating its role as a significant contributor to tumorigenesis.

Conclusion: Our study has shown that *NF2* alterations were significantly predominant in VS, and the potential involvement of factors other than *NF2* inactivation in tumorigenesis, especially in non-VS. Further comprehensive molecular analyses are necessary to elucidate the mechanisms underlying these observations.

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A Comparison of Two Electronic Data Capture (EDC) Platforms to Create an NF Registry

Jennifer L. Faulkner, MS, CRS, *University of Arkansas for Medical Sciences*

Purpose: Compare the utility of maintaining a neurofibromatosis registry in both REDCap and OpenClinica platforms.

Methods: A patient registry was created to help manage UAMS' adult neurofibromatosis clinic. REDCap (Vanderbilt University, USA) was the first choice due to its ease of use and ubiquity. After curating data elements, Epic Business Intelligence for UAMS was consulted to examine the feasibility of extracting data. Diagnoses of NF1, NF2, and/or Schwannomatosis were used to determine the registry population. ICD-11 codes were used to identify the presence of complications and/or tests, and treatment history was gathered using pharmacy and service orders. This information plus patient encounters were gathered quarterly. After receiving extracted Epic data, they were transformed for upload into REDCap. The data were edited and expanded via manual entry by the physician accessing patient charts.

After months of using REDCap, it was decided to build a registry that enabled varying numbers of rows, representing encounters, that could be added as needed to a grid. This led to OpenClinica being chosen as an alternative EDC platform. In addition to housing the same data elements as REDCap, OpenClinica also enabled this addition of rows, allowing comparison of different encounters on a single page.

Results: Of the 76 data elements selected, 61 were able to be easily extracted and imported into REDCap, and with somewhat more difficulty, OpenClinica. REDCap can clone projects which proved useful in creating "snapshots" of each project before cloning and adding newly extracted data. Both are user friendly, but in different ways. OpenClinica can add rows to a grid, facilitating following a patient through different services. REDCap has useful status icons and the capability to build graphics.

Conclusion: Both EDC platforms are useful for creating and maintaining patients' registries. REDCap offers a smooth pipeline for data import and export while OpenClinica offers the ability to increase the number of data elements captured on a given page at will.

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Disclosures: Dr Erika Santos Horta is the recipient of the "Adult NF Clinic Program Building Pilot Project." Dr Erika Santos Horta has also worked as a member of advisory board for SpringWorks Therapeutics and Alexion Pharmaceuticals.

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Neurofibromatosis Type 1 and Schwannomatosis Virtual Case Conference Series

Tena Rosser, MD, *Children's Hospital Los Angeles*

Background: The Children's Tumor Foundation (CTF) Virtual Case Conference (VCC) was established in April 2021 as an easily accessible, online platform to educate medical professionals within the NF Clinic Network (NFCN) on topics related to Neurofibromatosis type 1 (NF1) and Schwannomatosis (SWN) patient care. The VCC is organized by a subcommittee of the CTF Clinical Care Advisory Board (CCAB). One hour-long conferences are held monthly, with a case-based discussion by an early career faculty member guided by an NF-experienced mentor and an expert panel. VCC sessions are recorded and subsequently viewable online to members of the NFCN on a private YouTube channel.

Results: Topics in 2024 included MEK inhibitor side effects, NF1-associated headaches, NF1 and NF2-SWN ophthalmologic findings, orthopedic issues in NF1, neuropsychological aspects of NF1, cutaneous neurofibromas in NF1, pain management in SWN, women's health and reproduction and NF1-related vasculopathy. In 2024, there was an average of 188 registrants per session with 379 unique registrants and a corresponding average of 54 attendees per session with 210 unique attendees overall. Attendees included 45% MD or MD/PhDs, 7% PhDs, 9.1% APPs, 10% genetic counselors, 1% trainees and 22.8% others. NFCN clinic directors or co-directors made up 28% of the audience. The most frequently represented specialties included neuro-oncology (24.4%), genetics (24.4%) and neurology (13.1%). Feedback from attendees was very positive with the majority agreeing that the objectives were met, sessions improved knowledge and the virtual format was good for learning.

Conclusions: The CTF's VCC is a highly effective and well-received way to promote case-based learning provided by NF1/SWN experts to medical professionals within the CTF's NFCN. There are still opportunities to expand these educational sessions to reach a broader audience and to incorporate additional junior mentee presenters within the NF1/SWN field.

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Identification of AAV Engineered Capsids that Allow Efficient Nervous System Transduction Through Comparative Biodistribution Studies

Marc Moore, PhD, *Teesside University*

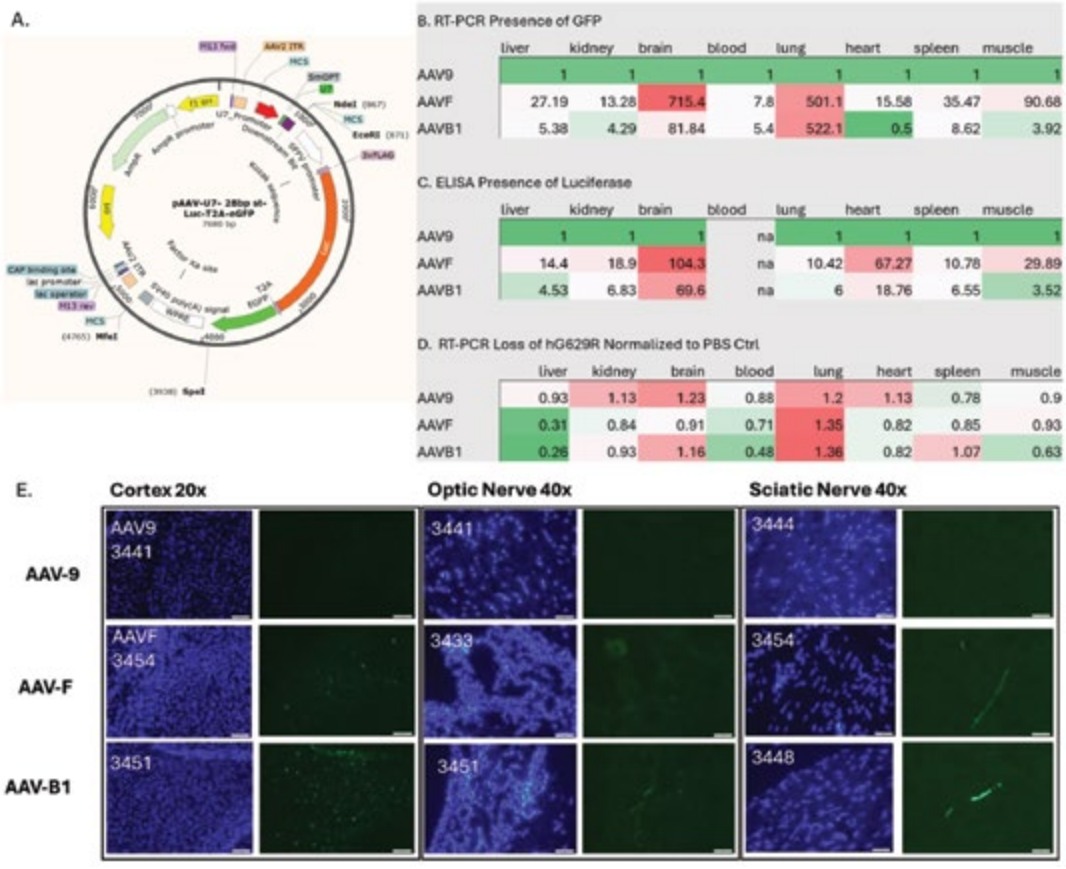
Background: The efficacy of a gene therapy mediated by an adenoviral-associated virus (AAV) vector is partly determined by the tropism of the viral capsid used. For genetic conditions affecting the nervous system there is currently lack of understanding around the ideal AAV capsid to be used.

Aims: To enable the translational development of AAV mediated gene therapies for nervous system related disorders, we examined the efficacy of delivery of various AAV capsids by assessing biodistribution through use of a packaged bioreporter expression cassette.

Methods/Materials: To enable analysis of biodistribution alongside examination of exon skipping efficacy of virally-delivered U7-SnRNA encoding the lead NF1 exon 17 ASO constructs, we have designed, cloned and validated a SFFV-driven biosensor containing T2A-linked Luciferase and eGFP expression cassette. AAV vectors were packaged in AAV-9, AAV-F and AAV-B1 capsids. These were administered through an ICV route into adult heterozygous NF1 humanized G629R mice at 3.7e9 vp. At four weeks post-administration, various body wide tissues were harvested and divided up for RNA harvest for eGFP expression analysis and exon skipping quantification, and for protein for luciferase assay assessment. Tissue was also embedded for subsequent cryo-sectioning for fluorescent histochemical analysis.

Results: On the basis of eGFP expression at the mRNA level (in liver kidney, brain, blood, lung, heart, spleen, muscle), intensity of fluorescence on sectioned tissues (brain cortex, optic nerve, and sciatic nerve) and luciferase enzymatic activity (in liver kidney, brain, blood, lung, heart, spleen, muscle), we establish that AAV-B1 and AAV-F are far superior to AAV-9 for the transduction of and the delivery of cargoes to the CNS by ICV routes of administration. For example, at the brain transcript level AAV-F is 715 fold and AAV-B1 81 fold greater than AAV-9; and at the protein level AAV-F is 104 fold and AAV-B1 is 69 fold greater than AAV-9. AAV-F and AAV-B1 capsids also affect high levels of NF1 exon 17 skipping in liver and blood based on loss of the humanized allele. Consideration of the readouts as a whole suggest that AAV-F is the most efficient of the three capsids for delivery/transduction efficacy.

Conclusions: With the identification of AAV serotypes that allow efficient transduction and delivery of transgenes to the nervous system, we provide validated opportunity for gene therapies for CNS-related genetic disorders to their reach maximal potential. Further, detection of on-target NF1 exon skipping in blood makes for a translatable biomarker for clinical trials.



B. RT-PCR Presence of GFP	liver	kidney	brain	blood	lung	heart	spleen	muscle
AAV9	1	1	1	1	1	1	1	1
AAVF	27.19	13.28	715.4	7.8	501.1	15.58	35.47	90.68
AAVB1	5.38	4.29	81.84	5.4	522.1	0.5	8.62	3.92

C. ELISA Presence of Luciferase	liver	kidney	brain	blood	lung	heart	spleen	muscle
AAV9	1	1	1	na	1	1	1	1
AAVF	14.4	18.9	104.3	na	10.42	67.27	10.78	29.89
AAVB1	4.53	6.83	69.6	na	6	18.76	6.55	3.52

D. RT-PCR Loss of hG629R Normalized to PBS Ctrl	liver	kidney	brain	blood	lung	heart	spleen	muscle
AAV9	0.93	1.13	1.23	0.88	1.2	1.13	0.78	0.9
AAVF	0.31	0.84	0.91	0.71	1.35	0.82	0.85	0.93
AAVB1	0.26	0.93	1.16	0.48	1.36	0.82	1.07	0.63

Fig: ASO Delivery with different AAV capsids. A. Map of pAAV Biosensor with U7-SnRNA Luciferase-T2A-eGFP allowing easy biodistribution analysis. B. q-RT-PCR showing biodistribution of eGFP expression. Both AAVF and AAVB1 are normalized to AAV9 eGFP expression levels. C. ELISA assay showing luciferase expression in protein lysates. Both AAVF and AAVB1 are normalized to AAV9 Luciferase expression levels. D. q-RT-PCR showing exon skipping (loss of humanized allele) normalized to PBS injected animals. E. Fluorescent imaging examples of NF-relevant tissues showing nuclei (DAPI-blue) and GFP expression in cortex, optic nerve, and sciatic nerve for each capsid.

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Clinical Metadata Representation in the NF Data Portal

Christina Parry, PhD, *Sage Bionetworks*

Purpose: The Neurofibromatosis (NF) Data Portal (nf.synapse.org) is a central hub for collaborative NF research, facilitating the sharing of research data. Despite the wealth of available data, clinical datasets with structured patient-centered metadata are scarce on the portal. This scarcity is largely due to the absence of a standardized set of attributes for patient data collection within the NF community, leading to the use of diverse data models and ontologies across clinical projects. Here, we present a collaboration between NF clinician-scientists and Sage Bionetworks' data management team, utilizing Simple [A]'s metadata modeling software to develop a harmonized NF clinical data model.

Methods: Clinical data models from at least 10 NF-related interventional, observational, and retrospective studies will be collected and analyzed to identify common data elements (CDEs) and reusable components for specific phenotypes (e.g., optic pathway gliomas may need additional metadata). The metadata management software, CoreModels, will support the development of the harmonized clinical data model by facilitating the creation of semantic and synonymous mappings. To increase interoperability, existing data standards such as Monarch Disease Ontology (MONDO) and Observational Medical Outcomes Partnership (OMOP) will be integrated into the NF CDEs.

Results: The primary outcome will be a pilot version of an open-source harmonized NF clinical data model, structured as a modular and expandable framework to serve as a recommended template for clinical researchers. This model will enable standardized data collection in future studies, with the flexibility to evolve as our understanding of NF diseases advances and new data elements become relevant. Additionally, faceted search functionality will be prototyped in the NF Data Portal to enable users to identify specific cohorts based on clinical metadata elements, such as treatment outcomes and biomarkers. Feedback and refinement with regard to usability and effectiveness is to be evaluated by the *Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) Biomarker Working Group* and at least five NF clinicians and/or researchers.

Conclusion: As a rare disease, NF inherently has low patient sample sizes, underscoring the need for greater harmonization across studies. This new harmonized clinical metadata model and implementation in the NF Data Portal is the first step towards being able to integrate data from multiple studies, effectively increasing the available sample size. In the future, these high-value harmonized clinical datasets could support machine learning models for drug discovery and other key insights, ultimately leading to better outcomes for patients.

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Disclosures: Cruce Saunders is the founder and an owner of Simple [A]. Modar Al Nachar and Mike Markley are employees of Simple [A], the company that develops Core Models software. The remaining authors declare no disclosures.

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NF Simplified: Enhancing Access to Cutting-Edge Research for the Neurofibromatosis Community Through AI-Driven Summaries and Expert Validation

Kyle Wan, *The Nueva School, San Mateo, CA*

Introduction: Persons with rare genetic diseases such as neurofibromatosis (NF) face significant challenges in accessing accurate and understandable health information, an essential factor for making informed care decisions. While scientific and medical information is regularly published by experts, it is often presented in highly technical language that is difficult for non-specialists to understand.

Purpose: This project aims to bridge the gap between complex scientific research and patient understanding by providing accessible, accurate, and up-to-date health information tailored to the needs of the NF community.

Methods: We developed NF Simplified, an innovative online platform that leverages large language models (LLMs), specifically GPT-4o, to translate complex scientific literature into patient-friendly summaries. To mitigate the risk of LLM inaccuracies or "hallucinations," we implemented a streamlined interface that facilitates crowd-sourced expert verification of summaries before publication. This dual-layer approach ensures the content is both up-to-date and accurate while significantly reducing the manual burden on scientists to curate and simplify research findings. We assessed the readability of published abstracts of NF research articles and their NF Simplified summaries using the Flesch-Kincaid Reading Ease score (range, 0-100, with higher scores indicating easy to read language) and compared readability using a t-test.

Results: Within the first month of launch, NF Simplified published 35 verified articles, collaboratively edited by 13 members of the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) International Collaboration. Readability scores of these summaries improved significantly, with an average increase of 20 points on the Flesch-Kincaid Reading Ease scale (from 21 to 41; $p < 0.0001$, t-test) as compared to the original abstract, demonstrating a substantial enhancement in content accessibility. Expert editors reported being pleased with the quality of the summaries although there were still occasional critical errors found requiring expert correction and two editors commented that the summaries overstated the impact of the work.

Conclusion: NF Simplified significantly improved readability of essential health research, and has the potential to empower NF patients and caregivers by promoting better health literacy and outcomes. However, some summaries remain at a post-high school reading level, highlighting the need for further refinement. Moving forward, we will continue to optimize LLM prompts, and conduct user surveys to assess the clarity, accuracy, and relevance of summaries from the patient and caregiver perspective. This scalable, AI-assisted model presents a promising framework that can be adapted to support other rare disease populations, effectively bridging the gap between cutting-edge scientific research and patient understanding.

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Improvements to the NF Data Portal and Synapse Data Platform

Aditya Nath, MS, *Sage Bionetworks*

Purpose: The Neurofibromatosis (NF) Data Portal provides a space for NF researchers to share, find, and re-use original research data and analysis tools. Sharing data can spark collaborative opportunities and accelerate scientific progress by improving the speed and accuracy of the research pipeline. Yet, following the F.A.I.R principles that data be Findable, Accessible, Interoperable and Re-usable presents several challenges including: developing data model standards for diverse data types, maintaining accurate and organized content, establishing data-sharing governance, protecting patient data privacy, harmonizing data from diverse sources, making data findable through an accessible user interface, and providing documentation for users with diverse expertise.

Due to these challenges, the team at Sage Bionetworks has been trying to improve the ability to interact with data uploaded on the NF portal.

Methods/Results: We have been able to visualize the growth in data/users by using Snowflake Streamlit dashboards created in-house. We've also added metadata to surface more information directly for funders and researchers. This allows better access to information such as embargo date endings and other grant and funding-related questions. We have also been working to make our datasets visible in Google dataset search. Search functionality on the NF portal is also being improved. A chatbot has been developed for use with the synapse platform, and an NF-specific chatbot is also under development. The Synapse chatbot can be used now to find NF specific data, but the NF specific chatbot will have more NF-specific functionality.

Discussion/Conclusion: We have shown increases in user engagement due to these improvements and are continuing to improve the portal. We have plans to add Data Use ontology terms to make data re-use easier in the future. We have also been working to package metadata for our NF datasets using the Croissant standard, which allows ease of use for machine learning purposes. These improvements are ongoing, and we will continue to update the NF community on changes to the portal, and hopefully, the changes will continue to improve how users can interact with the portal.

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Funding: The NF Data Portal and NF Open Science Initiative is funded by The Gilbert Family Foundation, Children's Tumor Foundation, and Neurofibromatosis Therapeutic Acceleration Program.

Patient and Caregiver Preferences when Considering a Hypothetical Gene Therapy for Patients with Neurofibromatosis Type 1 (NF1) or Schwannomatosis (SWN)

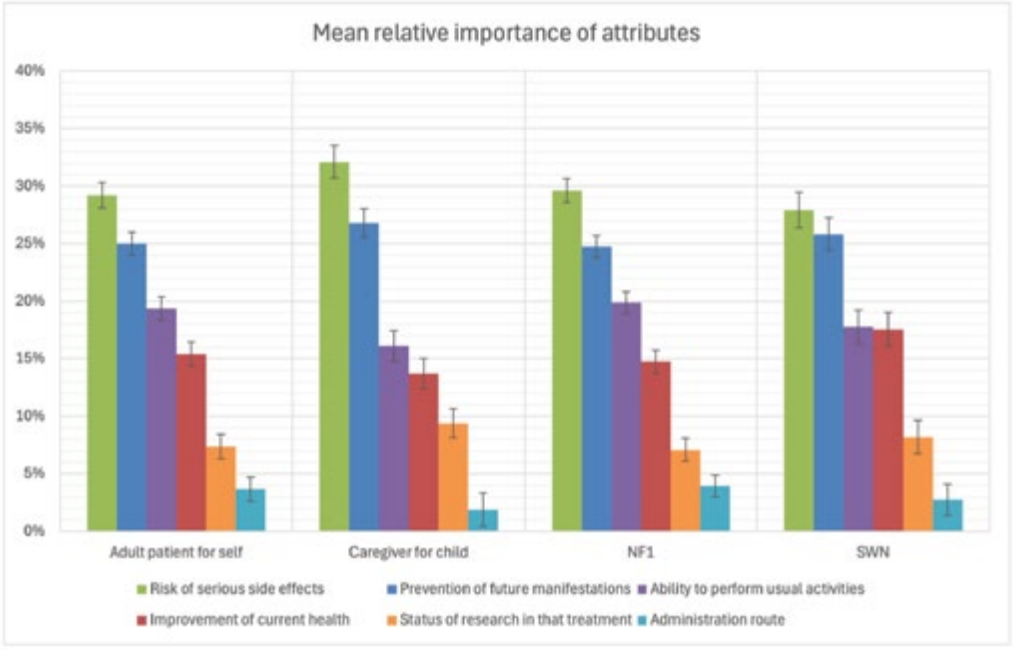
Kara Anstett, MS, CGC, *NYU Grossman School of Medicine, New York, NY*

Purpose: To investigate patient and caregiver preferences for a hypothetical NF1/SWN gene therapy and guide patient-centered clinical trial development.

Methods: A discrete choice experiment (DCE) was conducted among adults and caregivers of children with NF1/SWN in the United States. DCE is a quantitative method used to elicit preferences without directly asking participants to state them. In DCEs, participants are presented with a series of choice questions asking them to choose between two or three hypothetical treatment alternatives. Each alternative is described using a set of treatment characteristics ("attributes"), and each attribute is described using several "levels". For this study, the attributes (levels) are: chance of preventing future disease manifestations (10%, 50%, 90%), improvement in current health (no improvement, some improvement, significant improvement), risk of serious side effects (1%, 15%, 30%), ability to perform usual activities on treatment (unable, some problems, no problems), administration route (oral, IV, direct injection), and evidence of treatment safety/side effects (*in vitro, in vivo,* human studies). Participants were randomized into one of two blocks, each containing 12 choice sets presenting two hypothetical gene therapies and an opt-out alternative. Data were analyzed using a mixed effects conditional logistic regression model accounting for the effects of Patient/Caregiver and NF1/SWN groups, using the mclogit function in R. The estimated preference weights for each attribute were used to assess relative importance and trade-offs involved in decision-making.

Results: 591 of 822 (72%) participants who met screening criteria completed the survey, including 439 adults (296 NF1; 87 *NF2*-SWN; 55 non-*NF2*-SWN) and 211 caregivers (196 NF1;14 *NF2*-SWN), with 59 responding for both self and caregiver. The most important attributes were risk of serious side effects and chance of preventing future disease manifestations, followed by improvement in current health, ability to perform usual activities, evidence of treatment safety/side effects, and lastly, administration route **(Figure 1)**. When assessing trade-offs, participants would tolerate greater risk of serious side effects to prevent future manifestations than to improve current health. Risk of serious side effects carried more weight when considering treatment for a child, and impact on usual activities was more important for adults than children. Improvement of current health and prevention were more important in SWN than NF1.

Figure 1: Mean relative importance of 6 gene therapy treatment attributes when choosing a hypothetical gene therapy option to treat the NF1/SWN of oneself or their child, stratified by adult (all disease types), caregiver of a child (all disease types), NF1 (all ages), and SWN (all ages).



Conclusion: We describe the preferences of patients and caregivers for potential NF1/SWN gene therapies. Further analysis of the data is underway. Understanding patient preferences should inform patient-centered development of the first gene therapy trials for NF1/SWN.

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An Analysis of the Children's Tumor Foundation NF Registry, 2012–2025

Kate Kelts, RN, BSN, *Children's Tumor Foundation*

Purpose: In 2012, the Children's Tumor Foundation (CTF) established the NF Registry, a US-based secure website where individuals with NF or their caregivers can enroll to stay informed about research and provide anonymous information to help researchers understand the burden of NF. Participants complete annual surveys reporting symptoms, treatments, and outcomes. Data analysis focused on participant demographics, clinical manifestations, and user behavior.

Methods: Participants were recruited through CTF's social media, newsletters, events, the NF Clinic Network, and advocacy groups in the US and Europe. Consent was obtained when joining the NF Registry. Statistical analysis was conducted on all participants as of March 18, 2025, using Tableau and Snowflake. All met diagnostic criteria for one of the three NF types.

Results: The NF Registry includes 11,770 consented participants, with 10,969 responding to surveys. Most are diagnosed with NF1 (86.5%), followed by NF2-SWN (10.8%) and Schwannomatosis (2.8%). A majority are white (74%) and female (57%) **(Figure 1)**. To illustrate available data, we highlight one clinical finding per diagnosis. For NF1, 19% reported having an Optic Glioma, with 66% receiving no treatment/ observation only **(Figure 2)**. Similar analyses were conducted for NF2-SWN and Schwannomatosis. Regarding user behavior, 24% of participants completed multiple surveys, and most returning participants completed a maximum of two surveys. Survey participation increased steadily since 2012, with a notable spike each May during NF Awareness Month **(Figure 3)**.

Conclusion: Our findings align with existing NF literature and highlight the need for greater participation from underrepresented communities and international populations. Limited annual survey completion restricts longitudinal analysis, and further efforts are needed to boost engagement. The CTF NF Registry provides robust, multidimensional data and remains a valuable asset for NF research.

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[2]Accenture Federal Services, Virginia, USA

Figure 1. Participant Demographics

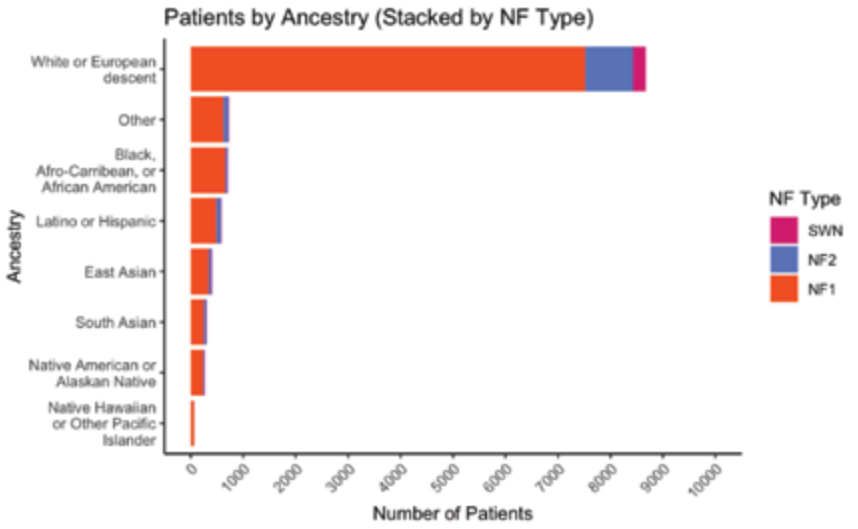


Figure 2. Frequency of Optic Glioma Treatments for NF1 Patients

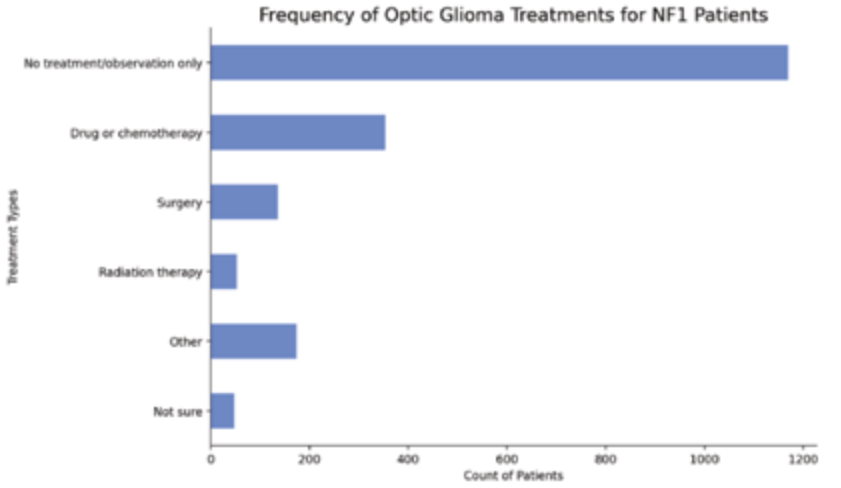
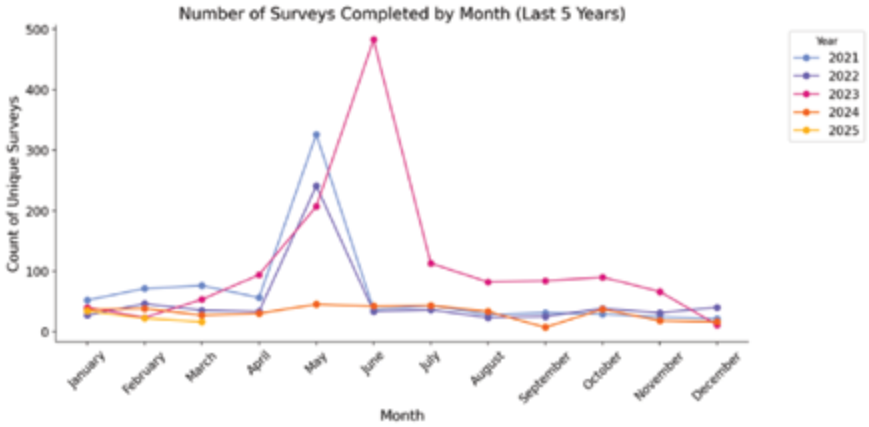


Figure 3. Survey completion over time



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