2018

JOINT GLOBAL
NEUROFIBROMATOSIS
CONFERENCE

AND WITH THE PARTICIPATION OF:

NOVEMBER 2-6, 2018
MAISON DE LA CHIMIE
PARIS
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EACCME
European Accreditation Council for Continuing Medical Education

2018 Joint Global Neurofibromatosis Conference
Paris, France, 02/11/2018–06/11/2018

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Each medical specialist should claim only those credits that he/she actually spent in the educational activity.

The EACCME® is an institution of the European Union of Medical Specialists (UEMS), www.uems.net. Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME® credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME® credits to AMA credits can be found at www.ama-assn.org/education/earn-credit-participation-international-activities.

Live educational activities occurring outside of Canada, recognised by the UEMS-EACCME® for ECMEC® credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of the Royal College of Physicians and Surgeons of Canada.
Guidelines for Speakers

The Organizing Committee of the JOINT GLOBAL NEUROFIBROMATOSIS Conference will do its utmost to help authors for their presentations and to facilitate their arrival and stay at the conference.

Please take a few minutes to read the following guidelines regarding the on-site organisation of the meeting for the smooth running of the sessions.

A speakers’ preview room will be installed in room 103 on the 1st floor of the Maison de la Chimie, Paris (follow signs on site). Its role will be to manage the following:

- Coordinate and ensure the overall smooth running of the conference sessions,
- Follow the general schedule of the sessions,
- Assist speakers for any requests they may have onsite.

PLATFORM PRESENTATIONS

In order to match with the most recent technology, the conference meeting room will be equipped with a video-projector and a laptop at the lectern.

All speakers are requested to use the PREVIEW ROOM (follow signs on site), which can be accessed once you have picked up your badge at the Welcome desk.

Each speaker should also check in the final Programme that the time of his/her session has not been modified.

We kindly ask you to keep the time limit in mind and remember to save time for discussion!

Qualified personnel will act as liaison between speakers and projectionists: speakers will not have access to the projection rooms; therefore speakers must go to the PREVIEW ROOM to hand in their computer assisted presentations that will be transferred to the conference room on time.

Speakers are entirely responsible for the order, the loading and the pre-projection of their computer assisted presentation, using the equipment made available by the organisers.

PRESENTATION (POWER-POINT STYLE)

To avoid delays caused by switching on computers on the platform, booting up computers and potential compatibility problems, the Organising Committee has made available to speakers the standard A/V system used in the convention sector. There will be a master computer in the meeting room and to ensure smooth transition between speakers and appropriate technical support, the Organisers request that speakers do not connect their own laptop. Every speaker has to go to the Preview room beforehand to provide his/her PowerPoint presentation.

OFFICIAL LANGUAGE

The official language of the Conference is English, which means that all presentations and questions must be delivered in ENGLISH.

FORMAT – PRESENTATION

Only Presentations for PC’s (Windows latest versions) and PC’s compatible (to avoid problems of compatibility between PC’s and MAC, please use fonts compatible with both PC’s and MAC) will be accepted, (no UNIX).

Your presentation should be saved in the .pdf or .pptx format only. Other formats will not be accepted.

- If you have pictures, they must be under the following format: .jpg, .png, or .gif, format (.pict prohibited).
- If you have video files attached to your power point presentation, they must be in the following format: .mpg, .mpeg, .avi, .wmv, mp4 or .mov and must be saved in the same folder as the presentation.

When saving your final presentation on a USB stick, make sure to include your video files and all links to these multimedia files.
Guidelines for Speakers

DEPOSITING OF FILE

- Your computer file must be handed over to the staff of the PREVIEW ROOM, either on a memory stick or a hard drive, as far in advance as possible and ONE AND A HALF hour BEFORE the beginning of each session AT THE LATEST. The presentation for an early morning session should be handed over the evening before.
- In the PREVIEW ROOM, you will be assisted by a technician, who will help you to transfer your presentation to the internal network. You will also be able to review your presentation and to verify that it has been transferred correctly to the network.
- The opening hours of the PREVIEW ROOM during the Conference will be:

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<tr>
<th>Date</th>
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<tr>
<td>Friday, 2 November</td>
<td>from 09:00 to 19:00</td>
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<tr>
<td>Saturday, 3 November</td>
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<td>Sunday, 4 November</td>
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<td>Monday, 5 November</td>
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<td>Tuesday, 6 November</td>
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- All presentations will be considered as confidential by our staff and removed from the system at the end of the conference.

IN THE MEETING ROOM

- Your presentation will be sent directly to the meeting room through the internal computer network of the venue. The PC on the lectern is programmed in 16/9 and is linked to a video-projector.
- Once the presentation is launched, you, the speaker, will control the program. By clicking on the mouse, your computer assisted slides will go on as usual.
- Please, do NOT come at the last minute with your own laptop to the meeting room: you will NOT be able to connect it. Go to the PREVIEW ROOM in time beforehand.
Guidelines for Poster Presentations

The Organizing Committee of the JOINT GLOBAL NEUROFIBROMATOSIS Conference will do its utmost to help authors for their presentations and to facilitate their arrival and stay at the conference.

The Poster exhibition will be accessible from Friday to Monday in the exhibition area of the Maison de la Chimie, Paris.

SET-UP/REMOVAL INSTRUCTIONS

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POSTER SESSIONS

The posters will be discussed in front of the poster boards during the dedicated sessions on Saturday and Sunday.

In the dedicated session slot, the presenting author must be available next to his/her dedicated board to present and discuss the poster.

| BASIC RESEARCH POSTERS | Saturday, 3 November 2018 | From 18:00 to 19:30 | Room 101 – level 1 |
| CLINICAL RESEARCH POSTERS | Sunday, 4 November 2018  | From 17:25 to 18:55 | Room 8 – ground floor |

POSTER DIMENSION AND FORMAT

The poster must not exceed 120 cm in height and 90 cm in width, PORTRAIT ONLY.

LANGUAGE

Your poster must be written in ENGLISH.

POSTER CONTENTS

Each poster should contain, in addition to the scientific elements, the title of the selected abstract as submitted, and the full names and affiliation of contributing authors.

Text, tables and drawings for figures should be large enough to be seen from a 2 meters distance. Illustrations should be used to convey important points; diagrams, graphs, bar charts, scatter grams, pie charts and photographs will enhance your presentation.

Make short statements, avoid long explanatory sentences.

MATERIALS

Your poster should not exceed 120 cm high and 90 cm wide (Portrait only) in order to fit the poster board. Prepare your material beforehand so that it will fit neatly into the space available and can be easily attached to the board. Suitable fixing materials will be provided by the Conference organizers. There will be a poster helpdesk close to the poster area, where staff will be happy to assist you.

Thin cardboard is more suitable than paper. Use a computer or enlarge a typed text by photographic methods. If you can have your poster produced by your institution, the final effect is more professional.
A Review of the Complex and Unique Testing Approaches Available for the Neurofibromatoses

Friday, 2 November, 13:30 – 13:55

Alicia Gomes, MS, CGC, University of Alabama at Birmingham Genomics Laboratory

This talk will review the complexities and unique testing approaches that may be needed to confirm a molecular diagnosis of neurofibromatosis type 1, Legius syndrome, and schwannomatosis. A review of the benefits and limitations of both germline and somatic testing for each condition will be provided as well as specific case reports to highlight unique testing approaches that can be utilized in several clinical scenarios.

The RASopathies: Distinct Syndromes with Shared Phenotypic Findings

Friday, 2 November, 13:55 – 14:20

Karen Gripp, MD, Nemours/Alfred DuPont Hospital for Children, US

The RASopathies are syndromic disorders caused by germline derived mutations in genes affecting the RAS/MAPK signaling pathway. The individual conditions were delineated as distinctive syndromes based on their characteristic findings and include von Recklinghausen disease (now known as neurofibromatosis type 1), Noonan syndrome, cardio-facio-cutaneous syndrome, Costello syndrome and others. These syndromes share central nervous system, cardiac and skin abnormalities, relative macrocephaly and short stature. Tumor predisposition is present in most RASopathies. It can be difficult to differentiate between these conditions, particularly in young patients, because the presentation of each syndrome is variable and may be age dependent. The notable overlap in phenotypes and presenting findings is best understood in the context of their shared underlying biology of dysregulation of the RAS/MAPK signaling pathway.
Don’t Forget Constitutional Mismatch Repair Deficiency (CMMRD)

Friday, 2 November, 14:20 – 14:45

Katharina Wimmer, PhD, Division of Human Genetics of the Medical University Innsbruck, Austria

Constitutional mismatch repair (MMR) deficiency (CMMRD) is a rare, autosomal recessively inherited childhood cancer susceptibility syndrome resulting from biallelic germline loss-of-function mutations in one of the MMR genes. Individuals with CMMRD have a high risk to develop a broad spectrum of malignancies and frequently display features reminiscent of neurofibromatosis type 1 (NF1), mainly in the form of multiple café-au-lait macules (CALMs), but also other NF1 signs. The implications of the phenotypic overlap of CMMRD and NF1 are manifold.

(i) To avoid misdiagnosing paediatric cancer patients with CMMRD as having NF1, which impedes adequate management of the patients and their families, it is important to increase awareness that a clinical phenotype reminiscent of NF1 in addition to any malignancy other than an MPNST, JMML or pilocytic astrocytoma, well known to be associated with NF1, in a child should raise a high level of suspicion of CMMRD syndrome.

(ii) The spectrum of CMMRD-associated childhood malignancies includes high-grade glioma, acute myeloid leukaemia and rhabdomyosarcoma. Reported associations between NF1 and these malignancies are to a large extent based on studies that neither proved the presence of an NF1 germline mutation nor ruled-out CMMRD in the affected. Hence, these associations are challenged by our current knowledge of the phenotypic overlap between NF1 and CMMRD and should be re-evaluated in future studies.

(iii) Awareness that CALMs and occasionally other NF1 signs may be present in a child with CMMRD prior to tumour onset leads to the conclusion that CMMRD is a legitimate differential diagnosis in a healthy child with signs reminiscent of NF1/Legius syndrome without a detectable underlying NF1/SPRED1 germline mutation. In this situation, a diagnosis of CMMRD may provide an opportunity for cancer surveillance of a highly penetrant childhood cancer syndrome prior to onset of the first malignancy. It will also allow predictive genetic testing and may impact family planning. Therefore, physicians and geneticists have begun to discuss if and when to counsel and test for CMMRD in suspected NF1 patients. Potential harm associated with CMMRD counselling and testing in this setting needs to be considered in this discussion. It should be outweighed by expected benefits for both the index patient and at-risk relatives. Appropriate counselling and testing strategies that serve to minimize the potential harm of testing should be available.

Overview of Prenatal Testing and Preimplantation Genetic Diagnosis – NF1/NF2 and Schwannomatosis

Friday, 2 November, 14:45 – 15:20

Myra Wick, MD, PhD, Mayo Clinic, Rochester Minnesota, Departments of Medical Genomics and OBGYN
Kristi Jones MBBS, PhD, Clinical Geneticist, Sydney Children’s Hospitals Network, Genea Fertility, Sydney Australia

Background: Prenatal testing and screening has evolved rapidly over the last decade.

Cell free fetal DNA (cfDNA), which was first described more than 2 decades ago is now used routinely in Europe for determination of fetal RhD status in Rh negative mothers. Cell free fetal DNA for aneuploidy screening is utilized worldwide. Several other potential applications for cfDNA have been reported, including analysis for single gene disorders. Prenatal testing is also evolving rapidly, with routine use of chromosome microarray and whole exome sequencing (WES).

Preimplantation genetic diagnosis (PGD) is a technology utilized by individuals affected by or carrying a monogenic disorder, to prevent transmission of the disorder to future offspring.

Case reviews: All of these technologies are applicable to family planning in NF1/NF2 and schwannomatosis – and these conditions present some specific challenges.

Mosaicism or segmental presentation of NF1 and NF2, including germline mosaicism, are well documented in the literature. A patient with segmental NF1 who desires preimplantation diagnosis is presented.

The primary underlying genetic cause may remain elusive in familial schwannomatosis, and testing may rely on indirect linkage methods. We present the experience of PGD in one such family.

Conclusions: Preimplantation and prenatal screening and testing options are changing rapidly. It is important for obstetric providers and medical geneticists to have a working knowledge of these options and their limitations, as well as societal guidelines regarding these test options.
Less Well Known Dermatological Manifestations in NF1

**Friday, 2 November, 15:20 – 15:45**

**Sirkku Peltonen, MD, PhD, University of Turku and Turku University Hospital, Turku, Finland**

The most common diagnostic signs of NF1 are on skin: café au lait macules, smaller pigmented spots on skinfolds, generally but misleadingly called as freckles, and cutaneous neurofibromas. In addition to these, NF1 is associated with other less well known skin manifestations.

Melanocytes are cells which synthesize melanin pigment and thus contribute to the skin color. Mutations of the NF1 gene lead to up-regulated melanin production, as seen in café au lait macules and freckles where melanocytes harbor second hit mutations in the NF1 gene. In addition to skinfolds, small pigment spots often cover large skin areas especially in trunk. The number and area of spots increase by age. In addition to localized pigmentation, patients with NF1 may have a slightly darker general skin color than their family members without NF1. Single hypopigmented spots may also be seen.

Pale spots in skin may be part of nevus anemicus, which is a congenital vascular anomaly of the skin. Some anemic nevi are visible only after elicitation of vasodilatation in surrounding skin by gentle friction, while some are detectable without friction. Nevus anemicus is most commonly located on upper chest or back.

Juvenile xanthogranulomas are orange papules commonly seen children with NF1. They may be solitary or multiple, and the number varies from single to 20-30. Head and neck are the most common sites. Juvenile xanthogranulomas are predominant in infancy: 40% to 70% appear during the first year of life and they also disappear during childhood. Case reports and molecular studies have connected juvenile xanthogranulomas, NF1 and juvenile myelomonocytic leukemia (JMML). However, JMML is a very rare type of childhood leukemia, and it has been estimated that only 2-3 % of all childhood leukemias are of this type. Recent large cohort studies did not demonstrate any leukemia cases in children with NF1. Since juvenile xanthogranulomas are common in children with NF1, but leukemia is very rare, finding juvenile xanthogranulomas should not lead to active follow-up of blood tests.

Glomus tumours are benign but painful neoplasms of the glomus body of fingers and toes. Glomus bodies are thermoregulatory shunts which regulate capillary blood flow according to temperature. Glomus tumors in the digits are located under nails or in the pulp, usually invisible to eye, but cause localised tenderness, paroxysmal pain, and sensitivity to cold. Glomus tumor can be imaged by MRI and operated by hand surgeon.

EDUCATIONAL SYMPOSIUM (PART 2)

**Chairs:** Dusica Babovic-Vuksanovic, MD; Mimi Berman, MD; Karin Cunha, PhD

A Holistic Model to Discuss the Impact of Muscular Phenotype on Lifestyle of Individuals with NF1

**Friday, 2 November, 16:10 – 16:35**

**Juliana Souza MD, PhD, Neurofibromatosis Outpatient Reference Center, Hospital das Clínicas da Universidade Federal de Minas Gerais, Brazil**

Over the last decades it has been observed that individuals with NF1 can present with significant physical fitness impairment perceived as reduced muscle mass, low muscle tone, global muscular weakness and decreased exercise capacity. These features have been attributed to central nervous system dysplasia and cognitive deficits (due to intrinsic neurofibromin deficiency), clinically observed as poorer motor proficiency and coordination. Nevertheless, recent pre clinical studies have shown a primary peripheral role for neurofibromin in muscle growth and metabolism, suggesting that a lipid storage disease phenotype may underlie muscle weakness in NF1.

Although we still lack a precise understanding of the whole mechanisms involved in the pathophysiology of NF1 muscular phenotype, we can observe its influence on lifestyle and also foresee the potential impact on NF1 shorter life expectancy, poorer quality of life and more frequent and earlier cardiovascular involvement, when compared to the general population. It looks like enough material to propose a holistic model that can propel the NF community towards discussing, studying, proposing and implementing interventions looking forward to approaching such a relevant and potentially modifiable matter.
Lessons Learnt from Breast Cancer Screening in High Risk Women—Screening in Women with NF1, When and How?

Friday, 2 November, 16:35 – 17:00
Eva Trevisson, MD, PhD, University of Padova, Italy

Neurofibromatosis type 1 (NF1) (MIM#162200) is one of the most common autosomal dominant tumor predisposition syndromes, affecting one in 3500 live births. Clinical diagnosis of this condition relies on specific diagnostic criteria (Ach Neu 1988). The disease is caused by germline loss-offunction mutations in the NF1 gene encoding neurofibromin, which acts on the RAS pathway by negatively regulating the GTPase activity of p21RAS. Given its role as a tumor suppressor, somatic mutations of NF1 are found in a number of sporadic tumors (Ratner 2015). NF1 is a multisystem disease displaying various clinical manifestations, including an increased risk of tumor development. The most frequent malignancies are those originating in the central nervous system (particularly optic pathway glioma OPG) and malignant peripheral nerve sheath tumors (MPNST) that usually arise on a pre-existing plexiform neurofibroma. Other neoplasms, such as gastrointestinal stromal tumors (GIST) and pheochromocytomas, have been reported more frequently in NF1 individuals.

Breast cancer (BC) is the most frequent tumor in women and previous studies have reported an higher risk among NF1 patients (Madanikia 2012, Wang 2012, Seminog 2015, Uusitalo 2016), particularly at younger age (< 50 yrs). While standard care for NF1 patients is well established (consisting of periodical detailed clinical evaluations, ophthalmologic examinations and blood pressure measurement), the utility of a specific screening program for BC in NF1 patients has not been investigated so far and is still debated (Sharif 2007, Evans 2012, Howell 2017).

Different BC screening programs have been developed in different populations: standard screening in Italy consists of mammography every two years starting from 50 years of age. BRCA1/2 mutated patients or other high risk women undergo clinical evaluations every 6 months since 25 years and annual mammography/breast MRI since 35 years.

In this presentation I will show the results of our study, focusing on the incidence of BC in a cohort of NF1 Italian women, on possible associated risk factors and on the effects of an earlier BC screening. Our preliminary data suggest that a precocious “high-risk” screening in NF1 women is effective and should be offered to all patients starting at 40 years. Multicenter studies are warranted to evaluate BC risk in NF1 women and the need for a specific screening program.

Oral Manifestations of Neurofibromatosis Type 1

Friday, 2 November, 17:00 – 17:25
Karin Cunha, PhD, Fluminense Federal University, Brazil

A variety of oral manifestations may occur in individuals with neurofibromatosis 1. They are very common and their prevalence is estimated to be around 70-90%. Oral manifestations in neurofibromatosis 1 include alterations in soft tissues, jaws, teeth, and salivary glands, such as neurofibromas in oral mucosa, intraosseous neurofibromas, enlargement of the fungiform papillae of the tongue, enlargement of the mandibular canal, mandibular foramen and mental foramen, alterations in craniofacial morphology, hyposalivation, beyond other alterations. Therefore, it is important that physicians and dentists are aware of oral manifestations in patients with neurofibromatosis 1 so that preventive measures can be introduced, and the proper diagnosis and management of these alterations and their consequences can be performed. The aim of this talk is to present our current knowledge of oral manifestations in neurofibromatosis 1, including their prevalence, diagnosis, and management in the clinical setting.
One of the most devastating consequences of NF1 is the psychosocial impact of the cutaneous lesions. Removal of these tumors can be of immeasurable benefit to the patient. Approximately half of NF1 patients have cutaneous tumors that number well into the hundreds or thousands. Lesions that occur around the face, neck and scalp account for roughly 48% of the total and are highly visible and may be distressing to the affected individuals. Those on the remainder of the body are most common on the trunk (64%), followed by the upper (54%) and lower (31%) extremities. Although more easily concealed, these lesions can also cause these patients to feel awkward and embarrassed in social situations. As a result, these individuals can become socially withdrawn. Their cutaneous neurofibromas serve as a constant visible reminder of their perceived disease.

There are several established techniques for removal of the lesions in NF1: surgical excision and/or laser ablation. Surgical excision is a time-tested method that yields a fairly predictable scar, but removal of hundreds of lesions is impractical due to time constraints. This reality forces patients to choose a small subset of their lesions to be removed at one time, producing suboptimal patient satisfaction. Lasers may be excellent for small surface lesions, but the treatment can be time consuming and requires specialized equipment.

I have pioneered a method for the treatment of multiple cutaneous neurofibromas that has numerous benefits. Electrosurgical destruction of cutaneous neurofibromas (often called electrodessication) with needle-point tip mono-polar cautery has proven to be a rapid, effective method of treating large numbers of cutaneous neurofibromas in an efficacious manner. The instrumentation is readily available in all outpatient and in-patient surgical centers. Using a low current provides instant haemostasis with minimal thermal damage to surrounding tissue. The technique is able to treat 500-1000 lesions at any one sitting, in part due to the possibility of a surgeon and an assistant using two or more cautery devices simultaneously. Cosmesis is excellent with only a few instances of scar hypertrophy. Results are quite gratifying to patients and surgeon alike.

A variety of model systems for studying benign and malignant tumor formation in Neurofibromatosis Type 1 (NF1) syndrome. These include models based on genetically engineered mice, human cells and most recently Ossabaw breed swine. Using transcription activator-like effector nucleases (TALENs) and homology-dependent repair (HDR) constructs, we generated NF1+/- (NF1) minipigs harboring a premature termination codon found recurrently in human patients. We have shown that these NF1+/- minipigs spontaneously develop café-au-lait macules (CALMs) and dermal neurofibromas (NFs) with spontaneous loss of heterozygosity (LOH), and retention of the mutant NF1 allele, in melanocytes and Schwann cells respectively. One NF1+/- animal developed an optic pathway glioma-like lesion. We’ve successfully administered 3 different MEK inhibitors to these pigs, achieving pharmacologically relevant levels. Our results suggest that NF1 minipigs, and the cell lines generated from their tissues, will be useful in answering prevailing questions in the field of NF1 and may facilitate development of new therapies for NF1-associated nervous system tumors in humans. Potential uses of the NF1 minipig model will be described in the context of ongoing work in the lab using other model systems.
Platform Talk: SPRINT: Phase II Study of the MEK 1/2 Inhibitor Selumetinib (AZD6244, ARRY-142886) in Children with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PN)  

Saturday, 3 November, 8:40 – 9:00

Andrea M. Gross, MD, National Cancer Institute, Pediatric Oncology Branch, National Institutes of Health, Bethesda

**Background:** PN in NF1 can cause substantial morbidity, and there are no approved medical therapies. In a phase I trial of selumetinib, 17/24 (71%) patients (pts) had a partial response (PR)1. This open-label phase II study (NCT01362803) determines the PR rate of PN treated with selumetinib and changes in PN related morbidities.

**Methods:** Patients 2-18 years old with NF1, inoperable PN and ≥ 1 PN related morbidity received selumetinib at the recommended phase II dose (25 mg/m² PO BID) with continuous dosing (1 cycle = 28 days). Response was evaluated with volumetric MRI analysis (PR = target PN volume decrease ≥20%) and PN related morbidities (pain, disfigurement, functional morbidities) assessed with standardized evaluations after every 4 cycles. Wilcoxon matched-pairs signed rank tests were used to evaluate for change between baseline and pre-cycle13 morbidity assessments.

**Results:** Fifty children (30 male, median age 10.2 years, range 3.5, 17.4) enrolled. Disfigurement (n=44), motor dysfunction (n=33) and pain (n=28) were the most frequent PN related morbidities. As of November 5, 2017: median cycle number 19.5 (range 0, 29) with 38 pts remaining on treatment; median change in PN volume from baseline -27.7% (range -50.6%, 2.2%); best response PR (36 pts, 72%), stable disease (12 pts, 24%); 2 subjects (4%) had no re-staging. Of the 36 total PR, 32 were confirmed on ≥ two consecutive restaging scans and 22 continue to have a PR ≥ 1 year after it was first achieved. Between baseline and end of year 1 evaluations, parent and child-reported pain intensity and pain interference scores significantly improved (p <0.01), as did strength (0-5 scale) and range of motion (degrees) of affected muscle groups/joints (p < 0.01). The most frequent toxicities were nausea/vomiting, diarrhea, asymptomatic creatine kinase increase, acneiform rash and paronychia. Selumetinib dose was reduced in 12 pts, of which 4 were removed from treatment.

**Conclusion:** The response rate from this study (72%) confirms our previously observed response rate (71%). Most responses were sustained ≥6 months. Improvements in PN related pain and motor impairment demonstrate that selumetinib can provide clinical benefit. Data validation and further analyses are ongoing.


Full List of Authors: Andrea M. Gross*,1, Pamela Wolters1, Andrea Baldwin1, Eva Dombi1, Michael J Fisher2, Brian Weiss3, Aerang Kim4, Jaishri Blakely5, Patricia Whitcomb6, Marielle Holmblad7, Staci Martin8, Marie Claire Rederick1, Scott M Paul9, Janet Thermann1, Kara Heisey1, Laurence Doyle1, Malcolm Smith1, John Glod1, Seth Steinberg8, Brigitte C Widemann1

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KEYNOTE LECTURE: Epigenetics: One Genome, Multiple Phenotypes

Saturday, 3 November, 9:00 – 10:00

Danny Reinberg, PhD, Howard Hughes Medical Institute, NYU Langone School of Medicine at Smilow Research Center, Department of Biochemistry and Molecular Pharmacology, New York, USA

Epigenetics encompasses changes in gene expression profiles that occur without alterations in the genomic DNA sequence of a cell. This arises from the dynamic processes that structure regions of chromosomal DNA through a range of compaction in eukaryotes. The altered pattern of gene expression is pivotal to cellular differentiation and development and is inherited by daughter cells thereby maintaining the integrity, specifications, and functions for a given cell type. Aberrancies in this epigenetic process give rise to perturbations that are also inherited and disruptive to normal cellular properties. The histone proteins that package DNA into chromatin are subject to post-translational modifications generating different chromatin structures. The polycomb repressive complexes play pivotal functions in maintaining cellular identity through alteration of chromatin domains. Functions of these complexes will be discussed.
PLENARY SESSION: EPIGENETICS

Chairs: Eric Pasmant, PhD, Université Paris-Descartes, Hôpital Cochin, France; Thomas De Raedt, PhD, Children’s Hospital of Philadelphia, US; Karlyne Reilly, PhD, National Cancer Institute, US

Exploiting Epigenetic Vulnerabilities to Improve Immunotherapy

Saturday, 3 November, 10:30 – 10:55

Thomas De Raedt, PhD, Genetics, Harvard Medical School, Boston; Pediatrics, CHOP/UPenn, Philadelphia

We have shown that MPNSTs are hyper sensitive to treatment of a MEK and BRD4 inhibitor combination. Interestingly, upon treatment we observed a very rapid change in tumor immune micro-environment with a dramatic increase in cytotoxic T-cells. We hypothesized that we could activate the infiltrating T-cells with a PD1-checkpoint inhibitor. Indeed, the combination of MEK and BRD4 inhibitors with PD1-checkpoint blockade caused dramatic tumor shrinkage (on average 80%). Importantly, we show that the cytotoxic T-cells are crucial for the observed tumor shrinkage. Additionally, we show that the observed increase in T-cells is caused by a loss of M2 macrophages. M2 macrophages secrete factors that prevent T-cell infiltration. Interestingly, treatment with the MEK/BRD4 inhibitor combination dramatically reduces the expression of CCL2, a cytokine that attracts monocytes and macrophages, in the tumor. Finally, we show that we are able to shrink tumors in a genetically engineered mouse model for MPNSTs by direct inhibition of the macrophages.

Full List of Authors: Thomas De Raedt*1, 2, Vikram Juneja3, Alessandra Gavin1, Chloe Emmerson1, Arlene Sharpe3, Karen Cichowski1
1Genetics, Harvard Medical School, Boston, 2Pediatrics, CHOP/UPenn, Philadelphia, 3Harvard Medical School, Boston, United States

Learning from Rare Tumors: Tumor Immunogenicity Beyond Somatic Mutation Burden

Saturday, 3 November, 10:55 – 11:20

Josh Waterfall, PhD, Institut Curie, Paris, France

Rare cancers are common, collectively accounting for roughly one quarter of all cancer diagnoses and deaths. Because such homogeneous clinical phenotypes often result from shared molecular mechanisms, the study of rare cancers has been a rich source of discoveries of fundamental oncogenic pathways. While high somatic mutation burden can drive immune recognition of certain tumors, mechanisms of immunogenicity in lowly-mutated tumors is relatively uncharacterized. We will describe how investigating the immune response to malignant rhabdoid tumor, combining patients and animal models as well as bulk and single-cell genomics, has revealed mechanisms of immune recognition in this context as well as opportunities for combination immunotherapy development.
Platform Talk: The Genomic Landscape of Schwannomatosis Schwannomas

Saturday, 3 November, 11:20 – 11:40

Sheila Mansouri, Princess Margaret Cancer Center, University Health Network, Toronto, Canada

**Background:** Schwannomas are characteristic manifestations of NF2 and schwannomatosis syndromes. However, the majority of schwannomas are solitary and sporadic. It is unclear whether and to what extent sporadic and syndrome-associated schwannomas or their histologic subtypes represent distinct biological groups. Clinically, although schwannomatosis schwannomas are considered benign, the majority of patients experience unmanageable pain; however, the underlying mechanism of this pain is not well understood. There is increasing evidence for DNA methylation profiling being able to distinguish biologically relevant tumor subgroups, even within the same cellular lineage and histopathologically similar tumors.

**Methods:** In this study, we used Illumina Methylation EPIC arrays for methylome-based characterization of 88 schwannomatosis schwannomas, in comparison to 90 sporadic schwannomas and 14 NF-2 schwannomas. We performed unsupervised hierarchical clustering selecting 30,000 probes that showed the highest median absolute deviation (MAD) across all beta values.

**Results:** Three different clustering sets were utilized to obtain the most refined differentiation. Schwannomatosis schwannomas formed 3 distinct methylome-based subgroups, which were fully distinct from sporadic schwannomas and NF-2 schwannomas. Additionally, we performed copy number analysis using the DNA methylation data to infer gross chromosomal deletions or gains among the sporadic and syndromic schwannomas, in addition to schwannomas with hybrid features. Methylation subgroups were further correlated with clinical parameters including age, gender, anatomic location, tumor size, germline mutation status (LZTR1/SMARCB1), and 22q LOH, in addition to the histopathologic features associated with each tumor and pain. Furthermore, RNA sequencing was performed to examine gene expression profiles associated with the 3 methylome subgroups and the data was integrated with DNA methylation profiles to establish the biological relevance of hypo- and hyper-methylation of the top varying CpG sites.

**Conclusions:** Our findings suggest that schwannomatosis schwannomas are epigenetically distinct from sporadic schwannomas. Furthermore, schwannomatosis schwannomas form 3 distinct epigenetic subgroups that are significantly associated with tumor anatomic location.

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Platform Talk: Cutaneous and Plexiform Neurofibromas Maintain Distinct Epigenetic Profiles with Predicted Impact on Tumor Biology

Saturday, 3 November, 11:40 – 12:00

Matthew Steensma, MD, Cancer and Cell Biology, Van Andel Research Institute, Byron Center, Grand Rapids, United States

Background: Although prior work confirms a shared cell of origin, there are biological differences between cutaneous (cNFs) and plexiform neurofibromas (pNFs) that are not fully explained by genomic sequence or structural alterations. We hypothesized that epigenetic alterations correlate with established clinical features. Prior work questions whether the epigenetic alterations in cNF and pNF tumors are distinct.

Methods: We performed a clinical trial (n=54 subjects, 85 samples) to establish a collection of fully annotated cNF and pNF specimens from NF1 subjects. Samples were profiled for site-specific methylation differences (EPIC array; Infinium) examining >850,000 loci among cNF’s and pNF’s of varied sizes. Data were analyzed using a modified workflow similar to the ChAMP EPIC array analysis procedure. Differentially methylated region (DMR) analysis was carried out using the DMRcate package in "R."

Results: In this stringent, unbiased analysis we identified ~100,000 significant DMPs (q < 0.05) and >3000 significant DMRs between cutaneous and plexiform neurofibromas, as well as significant methylation changes in cutaneous and plexiform neurofibromas relative to their respective normal tissues. The majority of the differentially methylated regions overlap promoter space (~86%). Additionally, there were approximately 18% of DMRs that overlapped known CpG islands. QA analysis demonstrated distinct methylation profiles between cNFs and pNFs with a high degree of concordance between cNF tumors of varying size. Unsupervised hierarchical cluster analysis of global methylation status revealed strong associations based on histopathologic diagnosis (cNF vs pNF). These differences were not attributable to gender. Chromatin states in hyper- versus hypo-methylated regions differed significantly and the distribution of differently methylated regions (DMRs) relative to transcription start sites also differed. Pathway enrichment analysis (Top 25 GO Terms; Top 10 KEGG Pathways) revealed several strong associations between epigenetic alterations and key signaling pathways. Notable findings include a link to inflammation, pain receptor pathways, ras signaling, and cytoskeleton regulation.

Conclusions: Our study conclusion is that epigenetic differences between cNFs and pNFs do exist. The biological significance of these profiling differences is unclear, but the predicted effects strongly implicate differential methylation events in cNF and pNF progression.

KEYNOTE LECTURE: Schwann Cell Biology

Saturday, 3 November, 13:00 – 14:00

Kristjan R. Jessen, PhD, University College London

The Schwann cell lineage is characterized by a striking phenotypic plasticity. This is seen in the retention of surprisingly broad development options even late in development, and in the persistent instability of the Schwann cell phenotype in adult nerves. Schwann cell plasticity may play a role in the development of Schwann cell related tumours and, in particular, dispose to demyelinating diseases. It does, however, provide a striking advantage in one important situation, namely peripheral nerve injury. In this case, Schwann cells, which in uninjured nerves function to accelerate electrical transmission and maintain nerve homeostasis, are reprogrammed to cells specialized to deal with injury and promote regeneration, repair Schwann cells. This is a key reason for the strong regenerative potential of peripheral nerves. Developmentally, myelin and non-myelin (Remak) Schwann cells originate from the neural crest in three main transitions. The first of these is the generation of Schwann cell precursors (SCP). Unlike crest cells, SCPS are tightly embedded among the axons of embryonic nerves, are acutely dependent on axonal neuregulin1 for survival, and express Schwann cell-related genes. While SCPS therefore unambiguously have a glial phenotype, SCPS also retain one notable feature of the neural crest, namely a broad developmental potential. Thus, during development SCPS give rise to melanocytes, endoneurial fibroblasts and neurons, in addition to Schwann cells. The second transition in the Schwann cell lineage is the generation of immature Schwann cells, while the third transition is the formation of the myelin and Remak cells found in the adult. In injured nerves, the generation of repair cells from myelin and Remak Schwann cells can be considered the fourth main transition in the lineage. This shows many similarities with injury responses of other tissues, including the process of adaptive cellular reprogramming and activation of epithelial mesenchymal transitions/stemness genes. Repair cells activate a sequence of supportive functions that engineer myelin clearance, prevent neuronal death, and help axon growth and guidance. Among the signals that drive Schwann cell development are, Sox10, Zeb2, Krox20 (Egr2), Oct6, Gpr126, and ERK1/2, while the generation of repair cells is subject to major regulation by c-Jun and Merlin, but involves also other signals including Notch, Gpr126, and ERK1/2. A more detailed knowledge of these pathways will lead to a more constructive understanding of Schwann cell pathology and allow the manipulation of these signals to enhance the repair supportive functions of Schwann cells in injured nerves.

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PLENARY SESSION: SCHWANN CELLS AND NEUROFIBROMAS

Chairs: Lu Le, MD, PhD, University of Texas, Southwestern, US; Juha Peltonen, MD, PhD, University of Turku, Finland; Eduard Serra, PhD, ICTP, Spain

**Developmental Origin and Spatiotemporal NF1 Loss of Heterozygosity Leads to Different Types of Cutaneous Neurofibroma**

*Saturday, 3 November, 14:00 – 14:25*

Lu Le, MD, PhD, Dermatology, University of Texas Southwestern Medical Center, Dallas; Neurofibromatosis Clinic; Simmons Comprehensive Cancer Center; Hamon Center for Regenerative Science and Medicine, University of Texas Southwestern Medical Center, Dallas, United States

The neurocutaneous tumor predisposition disorder Neurofibromatosis Type I (NF1) has a wide spectrum of clinical presentations, including developmental, pigment and neoplastic aberrations of the skin, nervous system, bones, endocrine organs, blood vessels and the eyes. Dermal or cutaneous neurofibromas (cNF), a Schwann cell tumor in the skin, affect most adults with NF1 and are a major source of emotional, physical and social distress as NF1 patients can have thousands of these tumors covering most of their skin. Thus, patients with NF1 often identify these tumors as their greatest burden. To date, there is no available medical treatment for cNF, no known way to prevent them from developing. The major barriers that impede progress in this field are the lack of accurate models of these common cNF tumors for drug evaluation and a limited understanding of their pathogenesis as well as the identity of specific cell of origin that directly gives rise to cNF. Here, we uncovered that a Homeobox B transcription factor serve as the lineage marker to trace the developmental origin of cNF neoplastic cells that completely recapitulates human neurofibromatosis, generating a novel mouse model that spontaneously develops both cutaneous and plexiform neurofibroma. In addition to providing insights into the developmental origin of cNF, this novel model also demonstrates genetic modifier for tumorigenesis, yielding vital clues to neurofibroma pathogenesis and now opens the doors for deciphering the evolution of neurofibromagenesis to identify and testing therapeutic targets.

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Funding: This work was supported by funding from Children’s Tumor Foundation, the National Cancer Institute, the US Department of Defense, the Giorgio Foundation, the Neurofibromatosis Therapeutic Acceleration Program and the NF1 Research Consortium Fund.

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**Tumour Progression and Pathogenic Mechanisms of Cutaneous Neurofibromas Revealed by Mice Carrying NF1 Loss in Prss56-Expressing Boundary Cap Cells**

*Saturday, 3 November, 14:25 – 14:50*

Piotr Topilko, PhD, INSERM, France

**Background:** Patients carrying an inactive NF1 allele develop nerve sheath tumours of Schwann cell origin called neurofibromas (NFs). Genetically engineered mouse (GEM) models have significantly enriched our understanding of plexiform forms of NFs (pNFs). However, this has not been the case for cutaneous neurofibromas (cNFs), observed in all NF1 patients, as no previous model recapitulates their development.

**Methods:** Here, we present a novel GEM model carrying conditional NF1 inactivation in neural crest derived, Prss56-positive boundary cap cells and their derivatives.

**Results:** Targeted NF1 loss leads to bona fide paraspinal and subcutaneous plexiform NFs and cutaneous diffuse NFs. Their analysis allowed us to identify sub-epidermal glia as a likely candidate for the cell type at the origin of the cNFs and provides insights on disease mechanisms, revealing a long, multistep pathological process involved in the formation of these slowly-evolving tumors. Moreover, we show that development of cNFs: (i) is associated with abnormal skin innervation and (ii) can be dramatically accelerated by skin injury.

**Conclusions:** This new mouse model is an important asset for future clinical and therapeutic investigations of NF1-associated neurofibromas.

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Platform Talk: Tonic ATP-Mediated Growth Suppression in Peripheral Nerve Glia Requires Arrestin-PP2 and is Evaded in NF1

Saturday, 3 November, 14:50 – 15:10

Robert Coover, PhD, Experimental Hematology and Cancer Biology, Cincinnati Children’s Hospital, Cincinnati, United States

Background: Mutations in RAS-MAPK pathway genes cause Rasopathies including Neurofibromatosis Type 1 (NF1), which results from inactivating mutations in the NF1 gene, and hyperactive RAS-GTP. While normal Schwann cells (SCs) are largely quiescent in adult nerves, NF1 mutant SCs proliferate and form benign SC tumors (neurofibromas).

Methods: We investigated nerve activity–dependent quiescence signaling in vivo pharmacologically with nerve activity inhibiting agents, as well as apyrase (an enzyme that rapidly degrades ATP). We also used a genetically engineered mouse model of NF1 to evaluate possible therapeutic strategies. In vitro analyses were conducted with wt and NF1 deficient SCs, both immortalized patient derived, and primary murine models. Combinations of shRNA, pharmacological intervention, and biochemistry techniques to measure extracellular ATP induced phenotypes were used.

Results: We show that nerve activity–dependent ATP maintains normal SC quiescence. ATP activates the G-protein coupled receptor (GPCR) P2Y2. Downstream of P2Y2, beta-arrestin-mediated signaling results in PP2-mediated de-phosphorylation of AKT, and PP2 activity is required for growth suppression. Elevating ATP levels in vivo reduces neurofibroma cell proliferation. NF1 deficient SCs are resistant to the effects of beta-arrestin-mediated signaling and PP2-mediated de-phosphorylation of AKT, and show reduced growth suppression by ATP.

Conclusions: We conclude that beta-arrested-mediated signaling is a critical component of nerve quiescence signaling to SCs. Also that when NF1 is lost, SCs have aberrant beta-arresting signalling (and by extension GPCR signaling) which results in their ability to evade growth suppressive signals. Our results identify a potential route that SCs utilize to evade normal growth suppression.

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Platform Talk: Trametinib in Pediatric Patients with Neurofibromatosis Type 1 (NF-1)–Associated Plexiform Neurofibroma: A Phase I/IIa Study

Saturday, 3 November, 15:10 – 15:30

Christopher L. Moertel, MD, Pediatrics, University of Minnesota, Minneapolis, United States

Background: NF-1 loss-of-function alterations are associated with development of plexiform neurofibromas (PNs). NF-1–associated PNs can arise early in life in different locations, with variable and significant morbidity. Many patients (pts) progress following surgery, and currently there are no approved systemic therapies. The MEK inhibitor trametinib is being evaluated in pediatric pts across a spectrum of tumor types in a dose-escalation cohort of a phase I/IIa study (NCT02124772) to determine a recommended dose; disease-expansion cohorts include pts with NF-1 PN. Here we present an interim analysis of safety and clinical benefit of trametinib in pediatric pts with NF-1–associated PN.

Methods: Pts aged 1 mo to < 18 y with medically significant, unresectable NF-1–associated PN were treated with trametinib 0.025 to 0.040 mg/kg/d. Presented here are results from the primary objective was safety, and secondary objectives included tumor response assessed by independent review (IR) using published MRI volumetric criteria.

Results: Twenty-six pts received trametinib (0.025 mg/kg/d, n = 21; 0.032 mg/kg/d, n = 1; 0.040 mg/kg/d, n = 4). Presented here are results from the disease-expansion cohort (n = 10). Median duration of exposure was 408 d (range, 360-429 d), and 8 pts (80%) had treatment ongoing at the data cutoff (September 2017). Median age was 5.5 y (range, 1-16 y), and prior therapies included surgery (n = 5), biologics (n = 1), and targeted therapy (n = 1). Treatment-related AEs (TRAES) were reported in 9 of 10 pts (90%), and 1 pt discontinued due to a TRAE. The most frequent TRAEs were paronychia (50%) and rash (40%). No deaths occurred on treatment. Analysis of the full NF-1 PN cohort (n = 26) is ongoing; across this cohort, 12 of 26 pts (46%) had a PR (≥ 20% volume reduction) by IR, and 10 of the 12 responses (83%) were ongoing.

Conclusions: Trametinib demonstrated a manageable safety profile in pediatric pts with NF-1–associated PN. Using volumetric criteria for response determination, the objective responses observed with trametinib support continued investigation in this pt population.

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PLENARY SESSION: PSYCHOSOCIAL IMPACT OF NEUROFIBROMATOSIS

Chairs: Marie-Laure Armand, PhD, Hopital Henri-Mondor, France; Staci Martin, PhD, National Institutes of Health, US; Hilda Crawford, PhD, University of Sydney, Australia

Supporting the Psychosocial Needs of Individuals with Neurofibromatoses: International Perspectives

Belinda Barton, PhD, The Children’s Hospital at Westmead and University of Sydney, Australia

The neurofibromatoses (NF1, NF2 and schwannomatosis) are the most common neurological suppressor syndromes characterised by nerve sheath tumours such as neurofibromas and schwannomas. While these tumours are generally benign, they can grow anywhere in the body and are often associated with significant morbidity including disfiguring cutaneous and plexiform neurofibromas (NF1), unilateral/bilateral deafness and poor balance (NF2), cognitive impairments (NF1) and chronic pain. These complications can have a significant impact on the quality of life (QoL) of individuals with the disorder with research generally indicating poorer QoL in all aspects (i.e. physical function, pain, mental health, social functioning) when compared to the general population, as well as higher rates of depression and anxiety. In addition, adults are often concerned about transmitting the disorder to their children and uncertain disease progression. Despite the potential impact of neurofibromatoses on the wellbeing on individuals, little is known about how to support the psychosocial needs of children and adults with the disorder. This presentation will overview the most common psychosocial concerns reported by children and adults with neurofibromatoses and summarize the clinical practices implemented in different counties that aim to address these concerns. Recommendations on how we can improve on the psychosocial care provided will also be made.

Acceptance and Commitment Therapy (ACT) for Individuals with NF1, Plexiform Neurofibroma Tumors (PNs), and Chronic Pain: Results from a Randomized Controlled Trial

Staci Martin, PhD, National Cancer Institute

Background: Plexiform neurofibroma (PN) tumors are associated with mild to severe pain. A randomized-controlled trial was conducted to evaluate the effects of an ACT intervention among patients 16 to 59 years of age with NF1, PNs, and chronic pain. It was hypothesized that pain interference would decrease from pre- to post-intervention. Exploratory analyses also examined the relationship between engagement in ACT-based skills and pain outcomes.

Methods: Individuals were recruited from CTF’s NF registry and from NF1 clinics around the country. Participants were randomized to receive the ACT intervention immediately (ACT group) or after 8 weeks (Wait List group). The intervention was delivered through two, 2-hour in-person sessions followed by weekly email assignments and biweekly video chats over the course of 8 weeks. Questionnaires assessing pain, pain-related coping, and disease-specific quality of life (QoL) were administered before and after the 8-week intervention. At follow-up, participants reported how often they practiced ACT skills at home.

Results: Forty-six participants completed the intervention (M age = 28.5 years, 46% female). Pain acceptance increased more in the ACT group (n=24) compared to the Wait List group (n=22). Mean pain interference (t = 3.1, p < .01) and pain intensity (t = 3.0, p < .01) significantly decreased from pre- to post-intervention. Finally, engagement in home-based ACT practice had a significant indirect effect on pain interference changes at follow-up, which was mediated by adaptive changes in pain inflexibility post-intervention (F(2,38)=5.60, p<.01).

Conclusions: Results indicate that ACT reduces pain interference and improves physical and social-emotional wellbeing. Future ACT interventions delivered remotely may make this intervention accessible to more patients with NF1.
Platform Talk: Improving Quality of Life (QoL) in Internationally Diverse Patients with NF via Secure Live Video: Results of 3 Randomized Controlled Trials (RCTs) in Adults, Adolescents and Patients Who Are Deaf

Saturday, 3 November, 16:50 – 17:10

Ana-Maria Vranceanu, PhD, Psychiatry/ Integrated Brain Health Clinical and Research Program, Harvard Medical School/Massachusetts General Hospital, Boston, United States

Background: Patients with NF are at high risk for poor QoL relative to the general population and to cancer patients. Despite biomedical advances that have improved outcomes, there remains an enormous need for interventions to reduce the emotional toll of an NF diagnosis. Our team has adapted a mind body program – The Relaxation Response Resiliency Program (3RP) – that teaches mindfulness, coping and positive psychology skills, for the specific needs of: 1) adults with NF1, NF2 and schwannomatosis (3RP-NF); 2) adolescents with NF1 and NF2 (a3RP-NF); 3) adults with NF2 who are deaf (3RP-NF-CART). The individually tailored programs were developed iteratively via focus groups and pilot work with each of the 3 patient populations. The 3 programs teach the same core skills but differ in how the skills are presented, the language used, and the type of stressors addressed. We tested, in 3 individual RCTs, the feasibility, acceptability and preliminary effect of each version of the program versus an attention placebo control on physical and psychological QoL (co-primary outcomes) and anxiety, depression, pain and pain interference (secondary outcomes).

Methods: Patients (63 adults, 51 adolescents, 45 patients who are deaf) were recruited through an international NF registry. Screening and consent occurred via live video, with CART for deaf patients. Data was collected electronically. The intervention programs and control (8 sessions; 90 minutes for adults, 45 for adolescents, and 60 for deaf adults) were delivered by a psychologist. Participants in the intervention received a patient manual and age/symptom tailored meditation recordings for home practice.

Results: Most patients who started the program finished (100% adults, 74.5% adolescents, and 90% deaf patients) and reported high satisfaction. Participants in the 3RP-NF, a3RP-NF and 3RP-NF-CART improved significantly more than control on psychological (p=.04, .03, .045) and physical QoL (p=.05, .03, .05). A similar pattern was observed for anxiety, depression and pain (p<.05). Only adolescents improved in pain interference (p = .038). All improvements were clinically meaningful and maintained over time.

Conclusions: A live video, mind body program tailored for the unique needs of adults, adolescents and deaf patients is feasible, accepted and associated with more improvement in outcomes compared to control. Details on program adaptations, results comparison by patient populations, future directions and implications for NF care will be discussed.

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Disclosure of Interest: A.-M. Vranceanu has a conflict with: Department of Defense grant W81XWH-17-1-0121 to Ana-Maria Vranceanu; Childrens’ Tumor Foundation, 3 clinical awards to Ana-Maria Vranceanu, E. Ricklin: None Declared, V. Merker: None Declared, E. Macklin: None Declared, J. Jordan: None Declared, S. Plotkin: None Declared
Platform Talk: Prospective Patient-Reported Outcomes (PROs) Document Clinical Benefit in Children with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PNs) on SPRINT: a Phase II Trial of the MEK 1/2 Inhibitor Selumetinib (AZD6244,ARRY-142886)

Saturday, 3 November, 17:10 – 17:30

Pamela L. Wolters, PhD, Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD

Background: PNs may cause substantial morbidity, including pain, functional impairment, and disfigurement, which can negatively impact quality of life (QOL). The FDA emphasizes the importance of documenting clinical benefit in addition to PN shrinkage. We prospectively assessed clinical benefit in children with NF1 and PNs using PROs in a phase II trial of selumetinib (NCT01362803).

Methods: Children 2-18 years old with NF1, inoperable PNs, and ≥1 PN-related morbidity were enrolled. Children (>8) and parents (of children >5) prospectively completed PROs assessing tumor pain intensity (Numeric Rating Scale-11), pain interference in daily life (Pain Interference Index), QOL (PedsQL), and global impression of change (GIC) every 2-4 cycles (cy); 1 cy=28 days. Wilcoxon Signed Rank tests evaluated changes in PRO scores from baseline to cy 12, and inductive qualitative analysis identified themes in reported cy 12 changes. Volumetric MRI analysis assessed PN response.

Results: Fifty children (60% male, median age=10.2 years, range=3.5-17.4) enrolled at four sites. As of November 2017, 36 children (72%) had a partial response (PR; >20% decrease in target PN volume). At baseline, 70% rated having tumor pain (31% mild; 24% moderate; 15% severe). From baseline to cy 12, child ratings of worst pain in the past week for their physician-selected target tumor and parent/child mean ratings of pain interference improved significantly (each \(p<0.01\)); 74% had clinically significant decreases of >2 points in their target tumor pain of which 79% had a PR; the other 3 had stable disease. Parent total QOL scores, and physical, emotional, and social (but not school) domain scores improved significantly (each \(p<0.01\)) while child physical domain scores improved significantly (\(p<0.05\)). Parent/child median cy 12 GIC ratings both indicated “much improved” change in tumor pain and other tumor-related problems. Cy 12 qualitative responses described predominantly positive changes (parent=91%; child=78%); most frequent themes noted by parents and children were improved appearance, better function, and decreased pain; less frequently reported negative changes (parent=7%; child=11%) mainly described adverse events.

Conclusions: This is the first trial to document significant improvements in PN-related pain and QOL by prospective standardized PRO evaluations, indicating that selumetinib provides clear clinical benefits in the setting of PN volume reduction in children with NF1 and PNs.

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NF Clinical Trials Consortium

Saturday, 3 November, 17:30 – 17:50

Michael Fisher, MD, Children’s Hospital of Philadelphia

The Neurofibromatosis Clinical Trials Consortium (NFCTC, http://www.uab.edu/nfconsortium) was established by the Department of Defense Neurofibromatosis Research Program (NFRP) to develop and perform clinical trials for the treatment of neurofibromatosis (NF) complications in children and adults. The Consortium is composed of fifteen clinical sites, nine collaborating affiliate sites, and an Operations Center at the University of Alabama at Birmingham. The purpose of the Operations Center is to provide administrative, data management, and statistical support to the NFCTC. Each of the clinical and collaborating sites has expertise in the treatment and management of NF and an established patient population available for clinical trials. The objectives of the Consortium are to develop innovative biologically-based therapies for both children and adults with NF, and to implement trials for multiple clinical manifestations of NF in order to rapidly improve the outcome for children and adults with NF.
PLENARY SESSION: MUTATIONS IN OTHER CANCERS

Chairs: Meena Upadhyaya, PhD, University of Cardiff, Wales, UK; Ophelia Maertens, PhD, Brigham and Women’s Hospital/Harvard University, US

The Expanding Role of NF1 in Sporadic Cancer and Resistance to Targeted Therapies

Sunday, 4 November, 10:00 – 10:25

Ophelia Maertens, PhD, Brigham and Women’s Hospital, Harvard Medical School

RAS GTPase Activating Proteins (RAS GAPs) have emerged as an expanding class of tumor suppressors that, when inactivated, provide an alternative mechanism of activating RAS. The most extensively studied RAS GAP is neurofibromin, the protein product of the NF1 gene. Despite the fact that germline mutations in NF1 are known to underlie the cancer predisposition syndrome Neurofibromatosis Type 1, until recently a role for NF1 in sporadic cancers had not been appreciated. Through genomic, cellular, and mouse modeling studies it has now become evident that the NF1 gene/protein plays a much broader role in cancer than previously recognized. Moreover, accumulating evidence suggests that NF1 plays an important role in mediating resistance to targeted therapies. For example, NF1 loss has been shown to drive resistance to kinase inhibitors in a number of clinical settings, including BRAF- driven melanoma and EGFR- driven lung cancer. My presentation will highlight mechanistic insights into how NF1 cooperates with other genetic events in sporadic cancer and how this can be exploited to develop effective therapies.

The Landscape of Molecular Alterations and Mechanisms of Progression NF1 Gliomas

Sunday, 4 November, 10:25 – 10:50

Antonio Iaravone, MD, Columbia University

Neurofibromatosis type 1 (NF1) is a common tumor predisposition syndrome in which glioma is one of the most prevalent tumor. Gliomagenesis in NF1 results in heterogeneous spectrum of low to high-grade tumors occurring during the entire patients lifespan. The pattern of genetic and epigenetic alterations characterizing glioma that develops in NF1 patients and the similarities with sporadic glioma remain unknown. Here, we present the molecular landscape of low- and high-grade glioma in patients affected by NF1 (NF1-glioma). We found that the predisposing germline mutation of the NF1 gene was frequently converted to homozygosity and the somatic mutational load of NF1-glioma was influenced by age and grade. High-grade tumors harbored genetic alterations of TP53 and CDKN2A, frequent mutations of ATRX associated with Alternative Lengthening of Telomere and were enriched in genetic alterations of transcription/chromatin regulation and PI3 kinase pathways. Low-grade tumors exhibited fewer mutations over-represented in genes in the MAP kinase pathway. Approximately 50% of low-grade NF1-glioma displayed an immune signature, T lymphocyte infiltrates and increased neo-antigen load. DNA methylation assigned NF1-glioma to LGm6, a poorly defined IDH wild type subgroup enriched with ATRX mutations. Thus, the profiling of NF1-glioma defined a distinct landscape that recapitulates a subset of sporadic tumors.
Platform Talk: PEDF Dysregulation Promotes Proliferation and Invasion in Malignant Melanoma with NF1 Driver Mutation

Sunday, 4 November, 11:20 – 11:40

Girish K. Patel, PhD, European Cancer Stem Cell Research Institute

Background: Neurofibromatosis Type 1 (NF1) and Legius syndrome are both inherited conditions, caused by germline inactivating mutations in the NF1 and SPRED1 genes respectively, with similar clinical phenotypes that exhibit pigmentary abnormalities including intertriginous freckling and flat localized hyperpigmented regions of skin termed café-au-lait macules (CALMs). Consistent with activation of the Ras/Raf/ERK signalling in CALM, we hypothesised that the mechanism for melanocyte activation would be similar in melanoma with NF1 driver mutations.

Methods: CALMs and unaffected skin biopsies from patients with classical NF (n=2), 3-bp deletion NF (n=3) and Legius syndrome (n=1) were obtained after regulatory approval by NHS and Ethics committees. The tissue was dissociated and melanocytes selected in culture prior to DNA and RNA extraction for somatic mutation screening and gene expression profiling respectively. Subsequent gene expression was validated by RT-PCR on normal and NF melanocytes, as well as metastatic melanoma cell lines. Pigment epithelium derived factor (PEDF) dysregulation was evaluated by cell viability, proliferation, apoptosis and invasion assays.

Results: All CALMs demonstrated loss of heterozygosity and as expected activation of the RAS-MAPK pathway. Gene enrichment and pathway analysis of the differentially expressed genes identified a number of significantly enriched gene clusters and pathways including 13 pathways, 37 Biological Process and 23 Molecular Function of Gene Ontology functional annotations. 859 genes were significantly differentially expressed (≥1.5 fold & corrected p<0.05) of which 61 genes which were consistently differentially expressed and were validated by qPCR. Intriguingly SerpinF1, which encodes PEDF, was downregulated in all samples and discordant with RAS dependent activation of MITF (the melanocyte maturation factor). PEDF loss was observed in melanoma and only in NF1 mutant melanoma cell lines was responsible for increased proliferation, migration and invasiveness.

Conclusions: CALM melanocytes through dysregulation of PEDF acquire a proliferative potential, which is kept in check by cellular senescence. NF1 mutations are common in malignant melanoma, where loss of senescence combined with dysregulation of PEDF is associated with a more aggressive phenotype.

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Platform Talk: Molecular and Functional Consequences of Somatic NF1 Mutations in Non-Small Cell Lung Cancers

Sunday, 4 November, 11:40 – 12:00

Eric Pasmant, PhD, Service de Génétique et Biologie Moléculaires, Hôpital Cochin, HUPC, AP-HP; EA7331, Université Paris Descartes, Faculté de Pharmacie de Paris

Background: Driver molecular alterations of RAS-MAPK pathway are found in >40% of non-small cell lung cancers (NSCLC). NF1 is a tumor suppressor gene that encodes neurofibromin, an inhibitor of the RAS-MAPK pathway. According to The Cancer Genome Atlas data, NF1 somatic mutations are found in ~15% of NSCLC. However, NF1 mutations are not extensively explored in NSCLC to date.

Methods: We performed NF1 analysis using next generation sequencing in NSCLC surgical specimens. We evaluated the molecular and clinical specificities of NF1 mutated NSCLC. Then, we established NF1-mutated cellular models with different NF1 wild-type (WT) cell lines. We chose two NSCLC (A549 and NCI H-1703, ATCC) and one non-tumorigenic human bronchial epithelial cell lines (HBE4-E6/E7-C1, ATCC). Mono- and bi-allelic NF1 mutations models were established using CRISPR-Cas9 and nickase technologies. In vitro functional tests were performed using these isogenic cell models. In vivo pharmacological tests were performed in patient derived xenografts (PDX) mice models.

Results: In our series of 138 lung adenocarcinoma specimens, 18% showed NF1 mutations and 8% showed NF1 deletions. Most of patients with NF1 alterations were males (72%) and smokers (75%). Overall survival and disease-free survival were statistically better in patients with NF1 alterations patients (N=35) than in KRAS mutated patients (N=30). Then, we established cellular models of NF1-mutated NSCLC. Loss of NF1 expression was confirmed by western blot (WB): partial and total loss-of-expression of neurofibromin was found in mono-allelic and bi-allelic NF1 mutated cell lines respectively. Bi-allelic NF1 mutations increase proliferation and migration capacity in our cell models. Using WB, we showed that pERK/ERK ratio was higher in NF1-mutated cell lines versus WT cell lines, confirming that NF1 loss-of-function triggered RAS-MAPK pathway activation. Transcriptome analysis confirmed this activation. Pharmacological screen (including MEK inhibitors) in our cell and PDX mice models is ongoing.

Conclusions: Our results confirm that NF1 is frequently mutated and represents a distinct molecular and clinical subtype of NSCLCs. Homozygous NF1 mutated cells seem more aggressive compared to heterozygous and WT mutated cells. Moreover, NF1 loss-of-function triggered RAS-MAPK pathway activation in our NF1 mutated cells. A better comprehension of functional consequences of NF1 mutations may open new avenues for NSCLC therapy.

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PARALLEL SESSION: NF2 AND SCHWANNOMATOSIS STATE OF THE ART

Chairs: Michel Kalamarides, MD, PhD; Marco Giovannini, MD, PhD, UCLA, US

An Update on Sensitivity of Pathogenic Variant Testing and Mosaicism in NF2 Using Next Generation Sequencing

Sunday, 4 November, 13:30 – 14:00

D Gareth Evans, MD, Genomic Medicine, University of Manchester, Manchester, United Kingdom

Background: Since the identification of the NF2 gene in 1993, over 4,000 genetic tests have been carried out on unrelated NF2 affected kindreds worldwide (1108 in Manchester). The pathogenic variant detection rate in leukocyte DNA depends on which generation is tested in a family.

Methods: We used sequence analysis latterly with NGS and a test for large rearrangements (MLPA) to identify pathogenic variants in DNA from leukocytes and tumour if available from individuals meeting NF2 criteria.

Results: When testing a member of the second generation of families with NF2 we identified a pathogenic variant in 143/154 (93%), 31/154 (20%) had deletions or duplications detectable on MLPA. In the 11 without a variant on standard testing three have been identified with inversions affecting the 5’ end (one family) and 3’ end (two families) that required additional genomic analysis (both were missed on standard RNA testing) a further two had chromosome translocations identified on cytogenetic analysis and a final case had a 5’ UTR variant (c.-66 -65insT) that causes an early initiation codon. Thus additional testing identifies a pathogenic variant in 149/154 (97%) of NF2 second generation families. In simplex cases (i.e., a single occurrence in a family) the mutation detection rate is around 50-60% (516/951). About 30% of mutations are not detected due to mosaicism. We have currently identified 217 mosaic patients, 40% by analysis of tumour. When the mutation is not detectable in blood, we can now detect below 1% with NGS particularly when a variant is identified on tumour analysis. However, variants may still not be detected after targeted analysis. NGS detected the pathogenic variant at 0.8-9% allele frequency in 26/54 (48%) of leukocyte DNA samples where Sanger had previously failed to confirm this.

Conclusions: A combined approach with NGS sequencing and MLPA alongside additional tests to assess the 5’ and 3’ ends of the NF2 gene is a highly sensitive technique and implies that there is no other gene that causes classical NF2 in multiple family members. NGS is also a major advantage in identifying mosaic NF2 and can provide estimates for offspring transmission.

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Disclosure of Interest: D. G. Evans has a conflict with: Astrazeneca travel paid, M. Smith: None Declared, A. Wallace: None Declared, G. Burghel: None Declared
The Management of Vestibular Schwannomas in Neurofibromatosis Type 2

Sunday, 4 November, 15:00 – 15:20

Michael J Link, MD, Mayo Clinic

Introduction: The treatment of vestibular schwannomas (VS) in patients with Neurofibromatosis Type 2 (NF2) is challenging and in most cases, controversial. Most patients with this disorder will eventually become functionally deaf. The goal of treatment is to provide tumor control, and maintain or restore cranial nerve function.

Methods: We reviewed our experience with surgery and radiosurgery for the treatment of NF2 associated VS.

Results: Between Sept, 1999 and Sept, 2018 I have operated 711 VS, of which 33 (5%) were in patients with NF2. Tumor size ranged from intracanalicular (IC) to > 5.0 cm, (mean: 2.65 cm, median 2.8 cm). Mean clinical and radiographic follow up was 76 months (median: 64 months, range: 14-195 months). Two patients had severe facial weakness at presentation, one of which underwent facial reanimation surgery. Two patients were House Brackman (HB) grade 3 at presentation; one remained HB gr 3 and one was HB gr 4 at last f/u. The remaining 29 patients were HB 1 or 2 at presentation. Sixteen patients (55%) had good (HB gr 1-2) facial nerve function at last f/u, 8 (28%) had moderate (HB gr 3) weakness and the remaining 5 (17%) had severe (HB gr 4-6) facial weakness. These results are significantly worse compared to size and age matched non-NF2 patients. Ten ears had AAO-HNS Class A or B hearing preoperatively. Postoperatively, 3 (30%) maintained useful hearing. Seven patients underwent placement of a cochlear implant and 15 Auditory Brainstem Implants were inserted, one of which had to be explanted for infection.

Between 1990 and 2010, 26 patients with 32 NF2-related VSs underwent SRS using the Gamma Unit (Elekta, Stockholm, Sweden). Median marginal dose and tumor volume were 14 Gy and 2.7 cm, respectively. Twenty-seven tumors (84%) showed no growth (median follow-up, 7.6 years). Kaplan-Meier estimates for 5- and 10-year progression-free survival were 85% and 80%, respectively. Cox proportional hazards demonstrated a significant inverse association between higher marginal doses and tumor progression (hazard ratio, 0.49; 95% confidence interval, 0.17-0.92; P = .02). Audiometric data were available in 30 ears, with 12 having class A/B hearing before SRS. Only 3 maintained serviceable hearing at the last follow-up. Four underwent cochlear implantation. Initially, 3 achieved open-set speech recognition, although only 1 experienced long-term benefit. Facial nerve function remained stable in 50% of cases.

Conclusions: The management of NF2 associated VS is very challenging. There are many strategies available and the treatment strategy in our practice is extremely nuanced and individualized. The primary goals remain tumor control to avoid life-threatening complications from progressive mass effect, and the maintenance or restoration of cranial nerve function.

NF2 Management in China

Sunday, 4 November, 16:40 – 17:00

Hao Wu, MD, PhD, Shanghai Ninth People’s Hospital Affiliated Shanghai Jiao Tong University School of Medicine; Ear Institute Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Translational Medicine on Ear and Nose Diseases, Shanghai, China

NF2 (neurofibromatosis type 2, NF2) is a heritable syndrome that leads to the development of multiple intracranial tumors, including schwannomas, meningiomas and gliomas. Bilateral VSs are identified in 90–95% of NF2 patients. There is about 35000 NF2 patients in China. The treatment options for NF2-associated VSs mainly include observation, stereotactic radiosurgery, microsurgery and drug therapy. Previously in China, NF2 is treated by single department and the primary treatment strategy is microsurgery. Total resection of bilateral VSs usually results in bilateral hearing loss and facial paralysis, which can seriously affect the quality of life. Nowadays in China, multidisciplinary management of NF2 is conducted in few centers. Observation is the most common treatment strategy, which is suitable for patients who are poor candidates for hearing preservation surgery. The goal of treating NF2-associated VSs is to maximize the duration of useful hearing, while minimizing morbidity to the brainstem and facial nerve. However, nowadays the guideline for appropriate management of NF2-associated VSs is still controversial in China. The treatment for NF2 should be individualized according to clinical symptoms and general condition. Nowadays, in China, there is few studies about factors influencing growth rate of VSs, drug therapy, Gamma knife surgery, ABI, and so on. Further studies are needed in order to find out the best treatment for NF2 patients with VSs in China. In our center, MDT is routinely conducted in treating NF2 patients and several studies, involving 500 NF2 patients, about drug therapy and treatment strategy of NF2 are carried out.
New Insights into the Molecular and Cellular Pathogenesis of Optic Glioma

**Sunday, 4 November, 13:00 – 13:25**

**David Gutmann, MD, PhD, Washington University, St. Louis**

Children and adults with neurofibromatosis type 1 (NF1) are prone to the development of benign and malignant tumors of the brain. During the first decade of life, the most common brain tumor is a low-grade glioma involving the optic pathway (optic pathway glioma; OPGs). Since OPGs are rarely surgically removed, the Gutmann laboratory has generated a large series of *Nf1* mutant mouse strains that develop optic gliomas. Using these informative and authenticated genetically engineered mouse (GEM) lines, his team has defined the cells and signals important for gliomagenesis, tumor growth, and glioma-induced vision loss. One of these non-cancerous cell types is a brain macrophage-like cell (microglia), which helps mediate glioma formation, maintenance, and vision loss. In his talk, Dr. Gutmann will present new data on the immunological circuitry that orchestrates glioma development and progression.

Platform Talk: Synodos for NF1 Glioma: In-Depth Genomic Characterization of Pediatric NF1-Associated Gliomas Reveals Recurrent Cooperating Mutations and an Aggressive Tumor Subset

**Sunday, 4 November, 13:25 – 13:45**

**David T. W. Jones, PhD, German Cancer Research Center (DKFZ); Hopp Children’s Cancer Center at the NCT Heidelberg (KITZ), Heidelberg, Germany**

**Background:** Low-grade gliomas arise in 15-25% of children with Neurofibromatosis Type 1 (NF1); however, most of these tumors are treated without a prior tissue diagnosis. As such, very few molecular studies have been performed previously, and little is known about cooperating genetic alterations or other molecular features that may correlate with their heterogeneous clinical behaviour. We therefore initiated a comprehensive, in-depth profiling of a large series of pediatric NF1-associated gliomas.

**Methods:** Tumor and matched germline DNA from 35 pediatric NF1-associated gliomas were subjected to whole-genome sequencing at an average of >70x coverage, to give sufficient power to detect subclonal alterations in these tumors (which can display low tumor cell purity). Transcriptome profiling by RNAseq was also performed. DNA methylation microarrays were used for molecular classification of NF1 tumors relative to sporadic gliomas. An independent expansion cohort of 40 additional tumors was profiled by DNA methylation and targeted panel sequencing.

**Results:** Next-generation sequencing identified the germline *NF1* alteration in all cases, confirming the power of this technique. Truncating alterations were far more frequent than missense mutations. Copy-neutral loss of heterozygosity (CN-LOH) was a frequent mechanism of inactivating the second *NF1* allele, occurring in approximately half of all tumors examined. Recurrently mutated genes in addition to *NF1* included the *FGFR1* and *PIK3CA* genes. While most tumors showed similarities to subsets of sporadic low-grade gliomas based on DNA methylation, about 10% harbored additional cell cycle gene alterations (*CDKN2A/B* loss, *TP53/PPM1D* mutation) and more closely resembled a recently described subset of IDH-wildtype anaplastic astrocytoma (Reinhardt et al., Acta Neuropathol. 2018).

**Conclusions:** This first large-scale genomics study of pediatric NF1 glioma revealed several novel insights, including the secondary mechanisms of *NF1* loss, the presence of additional cooperating oncopgenes, and the existence of a subset of tumors with more aggressive molecular features. Our findings have important clinical relevance in terms of optimizing diagnosis, prognosis and treatment for children with NF1-related glioma.

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Platform Talk: Larger Tumor Volume is Associated with Visual Acuity Loss and Axonal Degeneration in Children with Optic Pathway Gliomas Secondary to Neurofibromatosis Type 1

Sunday, 4 November, 13:45 – 14:05

Robert Avery, DO, Children’s Hospital of Philadelphia, Philadelphia

**Background:** Approximately 50% of optic pathway gliomas secondary to Neurofibromatosis type 1 (NF1-OPG) cause vision loss. It has been proposed that larger NF1-OPGs are more likely to be associated with visual acuity (VA) loss and axonal loss, measured using optical coherence tomography (OCT). In this cross-sectional study, we determined whether MRI measures of tumor dimension (diameter and width) and volume can identify children who experienced VA loss and axonal loss from their NF1-OPG.

**Methods:** Children with NF1-OPGs enrolled in an ongoing prospective study of VA were eligible if they had undergone 3-Tesla MRI that included a T1-weighted volumetric sequence and concurrent OCT measures of the circumpapillary retinal nerve fiber layer (cpRNFL) thickness, a biomarker of axon integrity. The maximum diameter of the optic nerves and optic tracts along with the width and height of the optic chiasm were measured from the T1 sequence using our semi-automated algorithm. The individual and combined volume (ml) of these components comprising the proximal anterior visual pathway (AVP) were also measured. VA and cpRNFL thickness were reported on a per-eye basis.

**Results:** Fifty-two study eyes (26 children) with NF1-OPGs met inclusion criteria, of which 40% (N=21) had abnormal VA and 60% (N = 31) had normal VA. Total AVP volume > 1.75 ml detected VA loss with 86% sensitivity and 92% specificity. Linear regression demonstrated that for every 1 ml increase in optic chiasm volume or AVP volume, the cpRNFL declined by 6 or 10 microns, respectively. In children with OPGs involving the optic chiasm with or without optic tract involvement, an optic chiasm width greater than 17mm demonstrated a 89% and 86% positive predictive value for VA loss and abnormal cpRNFL thickness (<80 microns), respectively.

**Conclusions:** Volumetric measures of NF1-OPGs accurately identified children with VA loss and axonal degeneration. Larger tumor volumes and dimensions were associated with more severe axonal injury and greater VA loss. Both tumor volume and cpRNFL thickness may be helpful in making treatment decisions in children with NF1-OPGs that present with abnormal VA or are uncooperative with VA testing.

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NF1 Optic Pathway Glioma: Treat or Observe? A Proposal for a Randomised Selection of Treatment Versus Observation

Sunday, 4 November, 14:05 – 14:30

Amedeo Azizi, MD, PhD, Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria

**Background:** While 15-20% of children with Neurofibromatosis type 1 (NF1) develop optic pathway glioma (OPG), only half of these patients are symptomatic and only about 20% receive therapy for their OPG. Different risk factors for visual deterioration have been characterised such as age, involvement of the posterior optic tracts, optic disc pallor and female sex. Yet, data on the natural history of OPG and information on the actual impact of therapy on vision remains scarce and data are almost exclusively of retrospective nature. The selection criteria for treatment in previous trials of NF1 OPG were visual deterioration, tumour growth or an ill-defined “threat to vision”.

**Present challenges:** While novel agents such as MEK inhibitors push forward in upcoming trials other questions still remain to be answered: Do we select the right patients for treatment? Do treatment strategies result in visual improvement? When is the best time point to treat? Do we know who will profit from treatment? Treating patients that present with visual deterioration or optic disk pallor may be at high risk for further visual loss, but might already have arrived at a stage of disease that may not be salvaged. Treating children early in the course of disease might perhaps increase the chances of visual improvement, but would risk to treat patients that might not show further progression.

A recent survey among international experts (Walker et al., unpublished data) underlined the ongoing dissent on whom or when to treat. Future trials should therefore not only compare different treatment arms but also investigate patient selection criteria. Whereas some patient groups, such as young patients with bilateral vision loss would immediately receive treatment, others, such as young patients with extensive OPG involving the posterior tracts but with minor or no visual loss might be considered for randomisation between treatment and observation.

**Conclusion:** Identifying the correct selection criteria for treatment initiation in children with NF1 associated OPG may help to in improve visual outcome and should be considered in the design of future trials.
PARALLEL SESSION: FUNCTIONAL GENETICS AND INTERPRETATION/CLASSIFICATION OF
VARIANTS

Chairs: Akihiko Yoshimura, PhD, Keio University, Japan; Eric Legius, MD, PhD, University of Leuven, Belgium; Frans Verheijen, PhD, Erasmus MC, Netherlands

Splice Effect Assessment: The Important First Step of NF1 Variant Interpretation

Sunday, 4 November, 13:00 – 13:25

Katharina Wimmer, PhD, Division of Human Genetics of the Medical University Innsbruck, Innsbruck, Austria

The NF1 gene is one of the largest human genes spanning 280kb of genomic DNA and a coding sequence of ~8.4 kb in 57 constitutively and 3 alternatively spliced exons. Already in the early days of NF1 mutation analysis it was suggested that an RNA-based assay may overcome diagnostic challenges resulting from the complexity of this large gene lacking mutational hotspots. A seminal paper published in 2000 showed that indeed mutation analysis strategies including RNA-based mutation analysis protocols as core assays reach the highest mutation detection rates. The two main obstacles of effective mutation detection at transcript level, i.e. illegitimate splicing and non-sense mediated decay, are effectively circumvented in the currently applied direct cDNA sequencing assay by using RNA extracted from puromycin treated short-term lymphocyte cultures.

The systematic application of this assay for nearly 20 years reveals that ~30% of NF1 patients have mutations affecting mRNA splicing and two thirds of these mutations either completely elude genomic DNA (gDNA)-based mutation analysis protocols or they defy ready classification as (likely) pathogenic mutations without knowledge of splice effect. This explains to a large extend the success of RNA-based mutation analysis in the NF1 gene.

Evaluation of the splice effect in patients’ tissues plays in several ways an important role in studies aimed at assessing genotype-phenotype correlations for NF1 mutations.

The large number of non-canonical NF1 mutations fully characterized at mRNA level represent a highly suited data set to study molecular mechanisms of splice site definition and disruption and by this to improve tools to predict splicing effects of genomic variants also in other genes.

Although the effect on mRNA splicing will be described for a very large number of NF1 mutations in near future, it can reasonably be expected that private mutations (i.e. not reported in any other patient) are still identified in a significant percentage of patients. Hence, direct cDNA sequencing to assess a potential splice effect directly in patients’ tissues will remain an important tool in NF1 diagnostics and research even when massive parallel sequencing becomes the core assay of the comprehensive mutation analysis protocols. Individual examples show that replacement of these methods by ex-vivo splicing assays using mini-gene constructs should be critically evaluated as they may not (fully) reflect the natural situation.

Functional Assessment of NF1 Missense Variants Identified in Individuals Suspected of Neurofibromatosis Type 1

Sunday, 4 November, 13:25 – 13:50

Rick van Minkelen, PhD, Department of Clinical Genetics, Erasmus MC, Wytemaweg 80, 3015CN Rotterdam, The Netherlands

Neurofibromatosis type 1 (NF1) is caused by inactivating mutations in the NF1 tumour suppressor gene. The broad phenotypic spectrum and age-dependent symptoms associated with NF1 makes clinical diagnosis challenging, particularly in young individuals. Mutation detection is therefore an useful part of the clinical work-up. However, the large size and high mutation rate at the NF1 locus makes not only mutation detection, but also variant interpretation, challenging. NF1 encodes neurofibromin (NF1), a GTPase activating protein (GAP) for RAS. To obtain insight into the pathogenicity of NF1 variants identified in individuals suspected of NF1, we have applied assays that interrogate NF1 function. We have investigated the effects of 26 NF1 variants on NF1 RAS GAP activity and on the interaction between NF1 and the SPRED1 gene product. In 9 cases (35%), we obtained evidence for an effect of the variant on NF1 function.

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Effects of Mutations in SPRED1 on Legius Syndrome and Relationship to NF1

Sunday, 4 November, 13:50 – 14:15

Akihiko Yoshimura, PhD, Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo, Japan

SPRED (Sprouty-related protein with an EVH1 domain) family proteins, initially discovered as c-kit- and c-fms-binding proteins, have been shown to suppress the Ras-ERK pathway. SPREDS form a subfamily of the Sprouty/Spred family, which is characterized by the Sprouty-related C-terminal cysteine-rich (SPR) domain. SPREDS also contain N-terminal EVH1 domain and central c-kit binding domain (KBD). Constitutional heterozygous loss-of-function mutations in the SPRED1 gene cause a phenotype known as Legius syndrome, which consists of symptoms of multiple café-au-lait macules, axillary freckling, learning disabilities and macrocephaly. It has been demonstrated that SPRED1 functions as a negative regulator of the RAS-ERK pathway and interacts with neurofibromin, the NF1 gene product. We found that the SPRED1 EVH1 domain interacts with the N-terminal 16 amino acids (aa) and the C-terminal 20 aa of the GTPase-Activating Protein (GAP)-related domain (GRD) of neurofibromin, which form two crossing $\alpha$-helix coils outside the GAPdomain. These regions have been shown to be dispensable for GAP activity and are not present in p120GAP. Several mutations in these N- and C-terminal regions of the GRD in NF1 patients and pathogenic missense mutations in the EVH1 domain of SPRED1 in Legius syndrome reduced the binding affinity between the EVH1 domain and the GRD. EVH1 domain mutations with reduced binding to the GRD also disrupted the ERK suppression activity of SPRED1. These data clearly demonstrate that SPRED1 inhibits the Ras-ERK pathway by recruiting neurofibromin to Ras through the EVH1-GRD interaction, and this study also provides molecular basis for the pathogenic mutations of NF1 and Legius syndrome.

We also characterized SPR domain of SPRED1. Mutations in the SPR (V408E, P415A, P425A/L, C416R, C418R, P422R) disrupt the suppressive function of SPRED1 against the EGF-mediated ERK activation. SPR is rich in cysteine residues and has been shown to be palmitoylated through the palmitoyl acyltransferase. We found that SPRED1 with these mutations in the SPR lacked palmitoylation in HEK293 cells, and localized as inclusion bodies but not in the cell surface membrane. Substitution of cysteine residues suggest that multiple cysteine residues are palmitoylated, but palmitoylation at C426 seems to be most important for membrane localization. Molecular characterization of mutations found in Legius syndrome will facilitate the understanding of SPRED1 function and symptoms of the diseases.

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Platform Talk: Neurofibromin a New SUMO Target Involved in Neurofibromatosis Type 1 Disease

Sunday, 4 November, 14:15 – 14:35

Mohammed Bergoug, PhD, CNRS, Centre de biophysique moléculaire, Orléans, France

Background: Neurofibromin (Nf1), the protein responsible for neurofibromatosis type 1 disease, has been identified as regulated by post-translational modifications (PTM) such as phosphorylation and ubiquitination. In our team we are studying its regulation by another PTM called SUMOylation. In a previous work, our team showed that Nf1 colocalizes with PML (promyelocytic leukemia) nuclear bodies (PML-NBs) in the nucleus. PML-NBs are dynamic proteic structures, and PML protein is their key organizer which localizes at their surface and recruits an ever growing number of proteins whose common known features are SUMOylation or presence of SUMO interaction motifs. Furthermore, Nf1 carries 15 SUMOylation consensus sites. These observations suggest that Nf1 might be modified by SUMOylation.

Our aims in studying Nf1 SUMOylation are:
Define whether Nf1 is SUMOylated
Identify on which amino acids the SUMOylation occurs
And how it regulates Nf1 functions

Methods: To detect the SUMOylated forms of Nf1 and its GRD (GAP related domain) and SecPH domains we used Immunoprecipitation, 6His Pulldown, and Western blot.

We also used site directed mutagenesis to identify on which amino acids the Nf1 SUMOylation occurs.

And we are currently performing Ras-GTP and Phospho-ERK quantification to evaluate the Ras-GAP activity of an Nf1 mutant affected in SUMOylation.

Results: This work led us to demonstrate that endogenous Nf1 is SUMOylated. Its GRD and SecPH domains are modified by SUMO2 and they display different forms of SUMOylation. One amino acid was identified as a SUMO acceptor. Our work is now focused on the functional role of SUMOylation at this site and at the level of the entire Nf1 protein. We would also like to know if SUMOylation might play a role in Nf1 disease, and for this purpose we will test if patient’s mutations in Nf1 affect the protein SUMOylation profile.

Conclusions: Results already obtained open wide perspectives for studying Nf1 functions regulation by SUMOylation. Our work will focus on the role of SUMOylation in RAS-GAP activity, Nf1 interaction with its partners, its subcellular localization and its stability.

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PARALLEL SESSION: LEARNING DISABILITIES

Chairs: Thijs van der Vaart, MD, PhD, Erasmus University Medical Center, Netherlands; Jonathan Payne, PsyD, Murdoch Children’s Hospital, Australia; Nicole Ullrich, MD, PhD, Boston Children’s Hospital, Harvard University, US

Multi Parametric Imaging and Behaviour in an Early-Phase Mechanism Trial of Simvastatin for NF1-Autism

**Sunday, 4 November, 15:00 – 15:50**

Stavros Stivaros, PhD, Shruti Garg, PhD, University of Manchester, UK

Autism Spectrum Disorder is a strongly occurring comorbidity in NF1. Statins rescue the social and cognitive phenotype in animal knockout models, but translational trials, with NF1 subjects >8yrs on general cognition and behaviour outcomes, have shown mixed results. This single-site triple-blind RCT of simvastatin vs. placebo breaks new ground by studying for the first time: i) younger children (n=30, mean age 8.1yrs, SD1.8); ii) children with NF1 plus comorbid autism; iii) multi-parametric imaging outcomes. Assessment (baseline and 12 week endpoint) included: peripheral MAPK assay; awake magnetic resonance imaging spectroscopy (MRS; GABA and glutamate+glutamine (Glx)); arterial spin labelling (ASL); apparent diffusion coefficient (ADC); resting-state functional MRI; and autism behavioural outcomes (Aberrant Behaviour Checklist and Clinical Global Impression). Simvastatin was well tolerated. Imaging was fully acquired in 24/30 subjects (placebo N=15/16; simvastatin, N=11/14). Evidence for simvastatin treatment effect was seen in: i) increased frontal white matter MRS GABA (t(12) = -2.12, p=.055); GABA/Glx ratio (t(12) = -2.78, p=.016), and reduced deep grey nuclei Glx (t(18) = -3.08, p=.006); ii) increased ASL perfusion in ventral diencephalon (Mann-Whitney-p<0.01); iii) decreased ADC in cingulate gyrus (Mann-Whitney p<0.01). Machine-learning classification including all imaging outcomes showed 79% (p<.05) accuracy in endpoint group allocation (placebo vs. simvastatin), compared to chance level at baseline. Autism symptom response was seen in 3/12 (25%) simvastatin cases compared to none in placebo. Multiparametric imaging suggests evidence of a simvastatin effect towards normalising function in brain areas previously associated with NF1 pathology and the social brain network. We show feasibility of peripheral MAPK assay and measurement of autism symptom change.


Social Behavior Deficits in a Spred1 Knockout Mouse Model of Legius Syndrome

**Sunday, 4 November, 15:50 – 16:15**

Hilde Brems, PhD, Department of Human Genetics, UZ/KU Leuven, Leuven, Belgium

Loss-of function mutations in the *SPRED1* gene lead to a RASopathy disorder termed Legius syndrome. This syndrome presents with a clinical phenotype similar to Neurofibromatosis type 1, however milder. The features include café-au-lait macules, axillary freckling, macrocephaly, along with frequent incidence of mild learning disabilities and autism spectrum disorder (ASD). The *SPRED1* protein is member of the SPROUTY/SPRED protein family, which acts as a negative regulator of RAS-MAPK signaling. A mouse model for Legius syndrome, the *Spred1* knockout mouse, recapitulates learning deficits seen in this syndrome. Here, we investigated whether *Spred1* knockout mice can model social behavior deficits analogous to the ASD symptoms seen in Legius syndrome. ASD compromises of deficits in social and communicative behaviors, and restricted and repetitive patterns of behavior. *Spred1* knockout mice exhibited deficits across a range of social behavior tests compared with their wildtype littermate controls, including impairments in social dominance and social communication. *Spred1* knockout mice also exhibit abnormal response to novelty in several behavioral tasks. Treatment of *Spred1* knockout mice in adulthood with the specific MEK inhibitor PD0325901 could rescue selected social behavior deficits. These results suggest that social behavior deficits relevant to RASopathy disorders can be reliably modelled in *Spred1* knockout mice, and that RAS-MAPK pathway over-activation plays a key role in mediating these social deficits.

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Platform Talk: Genotype and Behavioral Phenotypes in Children and Adolescents with Neurofibromatosis Type 1

Sunday, 4 November, 16:15 – 16:35

Andre Rietman, PhD, Child and adolescent psychiatry/psychology, Erasmus Medical Centre, Rotterdam, Netherlands

Background: Neurofibromatosis type 1 (NF1) presents a variable phenotype and genetic factors are important determinants of this variation. The NF1 phenotype can be determined by specific germ-line mutations and also by the timing and location of the somatic second-hit events that underlie many of the hallmark somatic features. The cognitive deficits associated with NF1 are generally presumed to be highly variable, but studies addressing this variability and the underlying factors are lacking. In this study, we describe the association between the NF1 genotype and cognitive and behavioral characteristics of a cohort of children with NF1 and of monozygotic twin pairs with NF1.

Methods: In this prospective cohort study, the records of all pediatric NF1 patients born 1990-2013 and seen at our NF1 outpatient clinic were reviewed. Data of neuropsychological assessment included intelligence (IQ) and parent-rated behavioral problems (global behavioral/emotional problems, ASD traits and ADHD symptoms). Pathogenic NF1 variants were classified as likely to result in the absence of neurofibromin (group X) or as likely to result in the expression of abnormal neurofibromin (group P). To obtain intra-class correlations (ICC) of IQ within monozygotic twin pairs, a linear mixed model was used. The effect of mutation type on IQ was determined using Mann-Whitney U tests, one-way ANOVA, and linear regression analyses. All parts of the study were approved or waived by the medical ethical committee.

Results: The variability in IQ (87.6±15.4) in a cohort of children with NF1 (n=359) resembles the variability in the general population. Monozygotic twin pairs with NF1 (n=11) show the same high ICC as unaffected monozygotic twins (ICC=0.90). Individuals with group X mutations did not differ from individuals with group P mutations in IQ or behavioral/emotional problems (p=0.34). However, the P group did show more ADHD symptoms (p=0.048) and more severe ASD traits (p=0.02).

Conclusions: The variability of cognitive function in our cohort of individuals with NF1 was not significantly different from the reference population indicating that the contribution of bi-allelic inactivation or specific genetic modifiers to IQ in NF1 is probably minimal. Notably, carriers of a group P mutation showed more severe ADHD and ASD symptoms compared to those with a group X mutation. Our study adds to the limited knowledge regarding the causes of variability in the behavioral NF1 phenotype.

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**Platform Talk: Risk of Psychosocial, Cognitive and Health Impairments in Adult Survivors of Childhood Glioma with Neurofibromatosis Type 1**

**Sunday, 4 November, 16:35 – 16:55**

**Peter de Blank, MD, Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH**

**Background:** Neurofibromatosis type 1 (NF1) is associated with an increased risk of tumors as well as non-malignant health conditions. The effect of NF1 on incidence and severity of late outcomes in adult survivors of childhood glioma is poorly understood.

**Methods:** 147 >5yr survivors of childhood glioma from the Childhood Cancer Survivor Study (CCSS) were compared to 2,629 non-NF1 glioma survivors and 5,051 siblings for neurocognitive impairment (CCSS neurocognitive questionnaire), emotional (Brief Symptom Inventory-18), socioeconomic outcomes, chronic health conditions (CTCAE v4.0) and late mortality (occurring >5yrs from diagnosis) using logistic and Poisson regression adjusted for age, sex, race, and treatment exposures (central nervous system radiation, surgery and chemotherapy). Specific chronic medical conditions commonly associated with NF1 (including epilepsy, vision loss, hypertension and headache) were also compared between groups.

**Results:** Compared to siblings, NF1 survivors (age at diagnosis [mean, range]: 6.8yr, 0.3-20.9yr; follow up: 14.4yr, 2.1-24.9yr) were more likely to report anxiety (RR[95%CI]: 2.2[1.1-4.4]), difficulty with task completion (1.5[1.1-2.0]), not being married (1.8[1.1-2.9]), and not having attended college (1.8[1.2-2.2]). NF1 glioma survivors reported more severe/life-threatening chronic health conditions (42.2% vs. 28.1%) than non-NF1 survivors at baseline, but the risk of developing new chronic health conditions or conditions associated with NF1 >5yrs from diagnosis was not different between NF1 and non-NF1 survivors (RR 0.85[0.53-1.38]). However, NF1 survivors had significantly worse late mortality (41.1% 30yrs from diagnosis) compared to non-NF1 survivors (17.5% 30yrs from diagnosis, p<0.001) and siblings (0.9% 30yrs from entry, p<0.001). Among specific causes of late mortality, death due to second neoplasm was more likely in NF1 survivors than in non-NF1 survivors (15.7% vs 4.4% 30 years from diagnosis).

**Conclusions:** NF1 glioma survivors experience worse psychosocial and neurocognitive outcomes than non-NF1 glioma survivors but were not at increased risk for developing late chronic health conditions. Nevertheless, the risk of late mortality is significantly higher in NF1 survivors compared to non-NF1 survivors. Risk of late mortality due to second malignant neoplasm is an important consideration when choosing upfront treatment options for children with NF1-associated gliomas.

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Platform Talk: Understanding the NF1 Experience and the Priorities of NF1-Affected Individuals and Families For Cognitive and Social-Emotional Research

Sunday, 4 November, 16:55 – 17:15

Tess Kennedy, Children’s National Health System, Washington, United States

Background: Patient engagement has become increasingly recognized as a valuable, essential aspect of NF research. Through active patient engagement, clinicians and researchers can discover the primary concerns and research interests of individuals with NF and their families. Providing patients and families the opportunity to share their concerns and interests is expected to enhance the development, methodology, and feasibility of clinical trials in NF.

Methods: Data has been collected through an IRB- and CTF Registry-approved REDCap survey developed to ascertain information on the respondents’ NF-related morbidities (neurologic, dermatologic, cognitive, social-emotional), priorities and interests regarding cognitive and social-emotional research, and willingness to participate in such research. The survey was dispensed to 4,565 individuals consented to the CTF Registry including children with NF1 ages 10-17 years, adults age >18 years with NF1, and parents/caregivers of individuals with NF1 (with or without NF1 themselves). The email was opened by 35% (N=1615).

Results: To date, 658 individuals have participated in the survey, with 76% being completed in full. Less than 10% of respondents have participated in cognitive research, while upwards of 50% indicated that they have sought out opportunities for cognitive research. Over 80% of respondents believe that cognitive research is very/extremely important. The top two areas that respondents indicated should be funded were learning/academics and emotional functioning. ADHD was ranked last for funding. The respondents willingness to participate in certain areas of research mimicked their ranking of funding (learning/academic and emotional), with some differences between respondents.

Conclusions: Initial analysis of this rich data highlights that most of the survey respondents believe cognitive and social-emotional research is very important in NF, but a relatively small number have actually participated and this may be related to limited dissemination of information and opportunities to the broader NF community. These results also highlight that the respondents consider academically based problems and emotional challenges to be research priorities, which may or may not align with research foci in the scientific community. Continuing to engage patients and families with NF is expected to enhance the value and engagement in cognitive research.

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PARALLEL SESSION: GENOTYPE/PHENOTYPE OF NF1

Chairs: Ludwine Messiaen, PhD, University of Alabama at Birmingham, US; Hildegard Kehrer-Sawatzki, PhD, University of Ulm, Germany; Douglas Stewart, MD, National Cancer Institute, US

Genotype-Phenotype Correlations in NF1: The Current State of Knowledge

Sunday, 4 November, 15:00 – 15:25

Ludwine Messiaen, PhD, genetics UAB, University of Alabama at Birmingham, Birmingham AL, United States

Introduction: As neurofibromatosis type 1 (NF1) is characterized by a highly variable and age-dependent clinical presentation and given the wide NF1 allelic heterogeneity, genotype-phenotype correlations are exceptional in NF1. Although each specific genotype-phenotype correlation may affect only a small percentage of individuals, together they impact counseling and management of a significant number of NF1 individuals, thus increased efforts towards identification of additional clinically relevant genotype-phenotype associations are needed.

Methods: Our research currently focuses on the evaluation of the clinical presentation of individuals heterozygous for recurrent NF1 missense or one-amino-acid pathogenic variants.

Six recurrent missense or one-amino-acid deletion pathogenic variants occur with a prevalence of minimum 0.5% each in the UAB cohort comprising ~8,100 unrelated NF1 individuals, and these were included in this study.

We compare the aggregated phenotypes of the studied groups with cohorts of individuals with NF1 missense / one-amino-acid deletion pathogenic variants affecting codons 1809, 844-848, 992 as well as with large-scale previously reported cohorts of individuals with “classic” NF1. We apply two-tailed Fisher's exact test, adjusting P values for multiple comparisons using Benjamini-Hochberg procedure with false discovery rates at 0.05 and 0.01.

Results and relevance: The prevalence of the following features shows statistically significant results between cohorts carrying one of six different pathogenic variants analyzed: Noonan-like features, pulmonic stenosis, externally visible plexiform neurofibromas, cutaneous neurofibromas, symptomatic spinal neurofibromas and optic pathway gliomas. Our new and previously reported data demonstrate genotype-phenotype correlations involving these codons that may be valuable for the management and genetic counseling of a significant number of NF1-affected individuals.

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Acknowledgements: This work was supported by the Children's Tumor Foundation by the Isaac and Sadie Fuchs Genotype-Phenotype Study and by internal funds from the Medical Genomics Laboratory UAB.

Identification of the Factors Underlying the Cognitive Variance Associated with Neurofibromatosis Type 1

Sunday, 4 November, 15:25 – 15:50

Ype Elgersma, PhD, Erasmus University Medical Center

The common autosomal dominant syndrome Neurofibromatosis type 1 (NF1) (1:3000) presents a highly variable phenotype. This is largely triggered by somatic second-hit mutations, as bi-allelic NF1 inactivation underlies the formation of (plexiform) neurofibromas, café-au-lait spots and malignant peripheral nerves sheath tumors. In addition, NF1 severity is influenced by certain genotypes as the 17q11.2 microdeletion syndrome and a few specific missense mutations. The cognitive deficits associated with NF1 are generally presumed to be highly variable as well, but detailed studies addressing the extent of the variability and the underlying factors are lacking. Here we report that in a large, unselected cohort of children with NF1 (n=359), the variability in cognitive performance resembles variability of the general population. Moreover, monozygotic twin pairs with NF1 (n=11) show the same high interclass correlation as unaffected monozygotic twins, indicating that the variance in IQ is mainly genetically determined. We subsequently investigated if specific genetic determinants affect the cognitive phenotype. Individuals with 17q11.2 microdeletions were found to have lower IQ-scores compared to other types of mutations (p<0.001). IQ of individuals with missense mutations or small in-frame deletions in the NF1 gene, did not differ from individuals carrying mutations that would result in the inability to express full-length Neurofibromin (p=0.34). We conclude that the variability of cognitive function in individuals with NF1 is largely determined by natural variation in genetic background, and that the contribution of bi-allelic inactivation or genetic modifiers of NF1 is minimal. Only 17q11.2 microdeletions were found to worsen the cognitive phenotype of NF1.
Looking Beyond the Lamppost: Exome Sequencing in >92,000 People in a Single US Healthcare System to Investigate Prevalence, Penetration, and Phenotype in NF1 and Legius Syndrome

Sunday, 4 November, 15:50 – 16:15

Jung Kim, PhD, Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

Like many monogenic disorders, the clinical and research-study ascertainment of individuals with NF1 and Legius syndrome is typically triggered by syndrome phenotype. The "phenotype first" approach is a traditional and proven one in clinical cancer genetics, and historically has been very productive in linking phenotype with germline variation. However, risk estimates derived from phenotype-linked ascertainment likely 1) over-estimate syndrome severity and penetration, 2) miss non-penetrant risk-variant carriers, 3) miss rare or unknown manifestations of disease and 4) give an incomplete picture of the phenotypic spectrum, especially at older ages. In this investigation, we used the genotype-first approach to evaluate the prevalence, penetrance, and phenotype of individuals harboring pathogenic NF1 and SPRED1 germline variation in >92,000 exomes from the Geisinger cohort (98% white; 60% female; median age 59 years; age range 2-89 years). Germline DNA from Geisinger MyCode participants underwent exome sequencing as part of the DiscovEHR collaboration of Geisinger (Danville, PA) and the Regeneron Genetics Center (Tarrytown, NY). NF1 and SPRED1 variants with minor allele frequency < 1% were classified as pathogenic (P), likely pathogenic (LP), variant of unknown significance (VUS), likely benign (LB), and benign (B) per ACMG-AMP guidelines. We used ICD9/10 codes to query the electronic health record (EHR) for diagnoses related to malignancy. In individuals with NF1 and SPRED1 P/LP variation, cancer diagnoses were confirmed by the Geisinger Cancer Registry and are exclusive of non-melanoma skin cancers. Since NF1 is a clinical diagnosis and not a genetic one, caution is needed in estimating prevalence and penetrance based on genotype and EHR-derived phenotypes only. Our genotype first approach to ascertain NF1 and SPRED1 P/LP prevalence and penetrance in a large population-based, exome-sequenced cohort is novel. In participants with unusual or severe phenotypes, the identification of P/LP variation in NF1 and non-NF1 genes shows how the genotype first approach can boost efforts to identify modifiers of NF1.

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Platform Talk: Comprehensive Screening of NF1 Missense Mutations for Genotype-Phenotype Correlations Using Drosophila and Mammalian Cell Models

Sunday, 4 November, 16:15 – 16:35

James Walker, PhD, Center for Genomic Medicine, Mass General Hosp, Boston, United States

Background: NF1 encodes a large ~320 kDa protein, termed neurofibromin, the only known function of which is to serve as a Ras-specific GTPase-activating protein (GAP). However, pathogenic missense mutations are found throughout NF1, suggesting other parts of the highly conserved protein are also essential for its function. We hypothesize that amino acid substitutions outside of the GAP domain may disrupt important novel functional domains, interactions with other proteins, or alter the regulation, stability or subcellular localization of neurofibromin – any of which could perturb its activity and potentially contribute to the varied clinical symptoms of NF1.

Methods: We have investigated the molecular and cellular consequences of NF1 missense mutations using both an invertebrate model of NF1 (Drosophila) and human cells. Drosophila was used to rapidly assess the residual function of dNf1 transgenes bearing corresponding mutations from patients. The approach relies on the fact that the majority of amino acids mutated in human neurofibromin are conserved in Drosophila, allowing us to correlate cellular and mammalian phenotypes to specific mutations in different regions of neurofibromin. The most informative NF1 mutations from our Drosophila screen are subsequently modeled in human induced pluripotent stem cells (hiPSCs) using CRISPR/Cas9 gene editing.

Results: We have conducted a functional assessment of the ability of dNf1 transgenes bearing conserved pathogenic missense mutations from NF1 patients to rescue established phenotypes in a fruit fly model of NF1: increased Ras signaling, reduced systemic developmental growth, aberrant circadian rhythms and defective cognitive function. To determine whether mutations in transgenic neurofibromin alter protein interactions, we have used affinity purification and mass spectrometry. Altered subcellular localization of mutant transgenic neurofibromin in fly neurons was assessed using confocal microscopy. Selected mutations of interest were engineered into hiPSCs using CRISPR/Cas9 and used to derive neurons to create isogenic disease models enabling specific NF1 mutations to be correlated with phenotypes.

Conclusions: This study, combining functional testing in vivo in Drosophila, with subsequent validation in human cells, allows us to correlate cellular and molecular phenotypes to specific patient-derived NF1 mutations in different regions of neurofibromin. This knowledge may aid the discovery of new biomarkers and therapeutic targets for NF1.

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1Center for Genomic Medicine, Mass General Hosp, 2University of Massachusetts, Boston, United States
Platform Talk: NF1-Deficiency is Linked to Estrogen Signaling in Breast Cancer with Evidence of Genotype-Phenotype Correlation

Sunday, 4 November, 16:35 – 16:55

Matthew Steensma, MD, Cancer and Cell Biology, Van Andel Research Institute, Byron Center, United States

Background: Breast cancer is an established phenotype of Neurofibromatosis Type 1 (NF1). NF1-related breast cancer survival is diminished regardless of age at presentation, gender or tumor receptor status. The role of neurofibromin-mediated tumor suppression in breast cancer is unclear.

Methods: We developed NF1-deficient rats bearing mutations in the GRD of the NF1 gene orthologue. Germline NF1-haploinsufficiency was induced using CRISPR-Cas9 gene editing. Animal survival, hormone receptor status, kinase signaling (pERK, pMET, pSTAT3, pAKT) and NF1 transcript variation (PCR) were all assessed. We also assessed NF1 loss in sporadic human breast cancer using the METABRIC breast cancer dataset. To identify biological networks impacted by NF1 deficiency, we constructed unbiased gene co-expression networks using weighted gene correlation network analysis (WGCNA).

Results: Germline, NF1 deficient female rats exhibited early, penetrant mammary adenocarcinoma. The observed breast cancer phenotype was more penetrant but not exclusive to the nonsense mutant lines. Nonsense mutations exhibited significantly diminished survival compared to missense mutant lines. ER+/PR+/Her2+ expression was noted in the vast majority of tumors (IHC). Estrogen-dependence was verified by estrogen-ablation in NF1 rats where rapid tumor regression was observed (mean 4.7% size reduction/day (p<0.0001)). Conversely, NF1-deficiency correlated with increased ER phosphorylation in mammary epithelial cells, tissue and mammary adenocarcinomas. We also identified distinct neurofibromin protein isoforms in mammary tissue that were altered during tumorigenesis. Alternative splice variants at mutant NF1 loci correlated with diminished survival among isogenic strains.

METABRIC data analysis revealed that NF1 shallow deletions occur in 25% of breast cancer patients and are associated with a significantly higher tumor grade, stage, and size (p <0.0001). Moreover, NF1 shallow deletions were associated with a 1.4 fold increase in mortality (p = 0.001) relative to diploid NF1 status. Unbiased WGCNA revealed a network connected to ESR1 (estrogen receptor), androgen receptor (AR) and FOXA1. Unsupervised clustering of genes demonstrated a strong association of NF1 shallow deletions with both ER+ and ER- tumor subsets.

Conclusions: These results demonstrate a significant role for NF1 in both NF1-related and sporadic breast cancer, and highlight a potential functional link between neurofibromin and the estrogen receptor.

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1Cancer and Cell Biology, Van Andel Research Institute, Byron Center, United States
Platform Talk: Genetic Basis of Variable Clinical Expression in Neurofibromatosis 1: Multiple Loci Identified in the First Genome-Wide Association Study

Sunday, 4 November, 16:55 – 17:15

Audrey Sabbagh, PhD, UMR 216 IRD MERIT

**Background:** Neurofibromatosis type 1 (NF1) is a Mendelian disease with full penetrance. Absence of genotype-phenotype correlation demonstrated the minor role of the NF1 gene in the great variability of NF1 clinical expression with the rare exceptions of (i) missense mutations in codons 844-848 and Arg1809, one short inframe deletion (p.Met992del), and (ii) the recurrent large deletions of the NF1 locus. Evidence of modifier genes was provided by animal models and intra-familial phenotypic correlations. A large genotype-phenotype database dedicated to NF1 was established in France: a standardized phenotypic description and the NF1 genotype were collected in more than 1,500 patients. We aim to carry out the first NF1 genome-wide association study (GWAS) in collaboration with the National Genotyping Center and the Neurofibromatosis Reference Center.

**Methods:** The GWAS analysis was performed on the discovery cohort (997 patients) and the replication cohort (501 patients) using the Illumina OmniExpressExome chip. The chip includes ~ 700,000 tag SNPs (capturing a large part of the common variation of the human genome) as well as >260,000 functional exon variants. Association tests with 14 clinical traits (5 quantitative and 9 binary traits collected with a standardized case report from) were performed using a mixed-effects model, considering the age, gender, and inherited or de novo nature of the NF1 mutation as fixed effects.

**Results:** NF1 genotyping of the cohort allowed exclusion of patients with mutations associated with a known genotype-phenotype correlation (missense mutations in codons 844-848 and Arg1809, the short inframe deletion p.Met992del, and large NF1 locus deletions). Our analysis confirmed the strong heritability of most of the NF1 clinical traits. Moreover, we identified several loci showing significant genome-wide associations (p<10^-8) with NF1 major phenotypic traits in both discovery and replication cohorts.

**Conclusions:** The results of this study will contribute to a better understanding of the genetic basis of NF1 expression variability and its underlying pathophysiological mechanisms. Our study will be completed by identification of the specific modifier genes and their functional studies in relevant cellular models by genome editing approaches.

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KEYNOTE LECTURE: The Translational Mouse: From Genetic Models to the Mouse Hospital and Co-Clinical Trials, for a Novel Understanding and Treatment of Cancer

Monday, 5 November, 8:30 – 9:30

Pier Paolo Pandolfi, PhD, Director, Cancer Center & Cancer Research Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

The Co-Clinical Trial Project is a cutting-edge new platform for clinical trial optimization, which we have developed at our Cancer Center at Harvard. The platform also rests on a "Mouse Hospital" infrastructure, which is equipped as a human hospital, if not better, to perform experimental clinical trials in mouse models of disease, exactly as they would be run in the human hospital.

In the "Co-Clinical" Approach for Cancer Therapy optimization, mouse models of cancer, which are representative of the diversity of human cancer, are treated with the same drug, and following the very same clinical protocol offered to human patients enrolled in experimental clinical trials in the human hospital. This allows for "mice-to-human-to mouse" stratification and cross-validation of response and resistance to specific treatment modalities, and for the identification of effective therapies that overcome such resistance.

Drugs can be tested on Organoids and "Immune-deficient" Patient Derived Xenograft: (PDX) models that are generated from biopsies from the same very same patients enrolled in the trials. Importantly, however, "Co-Clinical" Trials can also be run by enrolling "immune-competent" genetically engineered mouse models of cancer (GEMMs) bearing genetic mutations associated to human cancer to assess how the various cancer genetic make ups and therapeutic treatments impact and are impacted by the immune microenvironment. Exciting new data from these platforms and on-going analyses will be presented.
PLENARY SESSION: MICROENVIRONMENT

Chairs: Piotr Topilko, PhD, INSERM, France; D. Wade Clapp, MD, Indiana University, US; Alison Lloyd, PhD, University College, London, UK

Cellular and Extracellular Microenvironment of Cutaneous Neurofibromas

Monday, 5 November, 9:30 – 9:55

Juha Peltonen, MD, PhD, Institute of Biomedicine, University of Turku, Turku, Finland

The growth of cutaneous neurofibromas (cNFs) is a result of cell proliferation and deposition of extracellular matrix (ECM) which constitutes a significant proportion of the tumor volume. Tumor growth requires permissive and enhancing, but also restricting factors since the size of the tumors typically does not exceed few centimeters. Ultimately an equilibrium is reached and growth markedly slows or stops. The main framework of the extracellular matrix of cNFs comprises of an abundant but relatively loose network of small diameter collagen fibrils organized in fine bundles. The majority of the neurofibroma cells can adhere to this framework which in turn has the potential to influence their gene expression profiles. The ECM also may direct the migration of blood derived lymphocytes and precursors of mast cells and macrophages. The non-fibrillar ECM effects the movement of soluble small molecular effectors in cNFs, such as growth factors and cytokines. Most of the cNF cells express receptors of sex hormones explaining the apparent association of neurofibroma growth with puberty and pregnancy.

Previous studies by us and others have shown that the Schwann cells with a hit in both NF1 alleles (NF1-/-) are particularly sensitive to sex hormone regulation, and on the other hand NF1-/- Schwann cells actively express stem cell factor (SCF) to which the NF1 +/- mast cells are particularly sensitive. Our recent focus has targeted the tumor immunology of cNFs. Immunity has a central and complex role in tumor growth, which is utilized when developing new treatment modalities for tumors. Transformed cells are normally eradicated by cooperation of innate and adaptive immune cells. Immune surveillance could be expected to recognize neurofibromin neoepitopes resulting from the second hit of the NF1 gene, but this is not taking place in cNFs. Mast cells are increasingly more interesting as druggable targets in tumor biology and neurogenic itch. Thus, tumor immunology of cNFs has been our recent focus. In the first phase of the work, T cell subpopulations of cNFs have been characterized with appropriate CD antibodies, and the two main mast cell populations by their enzyme profiles.

Cxcr3 Expressing Leukocytes Are Necessary for Neurofibroma Formation in Mice

Monday, 5 November, 9:55 – 10:20

Nancy Ratner, PhD, Divisions of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine

Plexiform neurofibroma is a major contributor to morbidity in Neurofibromatosis type I (NF1) patients. Macrophages and mast cells infiltrate neurofibroma, and data from mouse models implicate these leukocytes in neurofibroma development. Anti-inflammatory therapy targeting these cell populations has been suggested as a means to prevent neurofibroma development. Here, we compare gene expression in inflamed nerves from NF1 models which invariably form neurofibroma to those with inflammation driven by EGFR overexpression which rarely progresses to neurofibroma. We find that the chemokine Cxcl10 is uniquely up-regulated in NF1 mice that invariably develop neurofibroma. Global deletion of the Cxcl10 receptor Cxcr3 prevented neurofibroma development in these neurofibroma-prone mice. Cxcr3 expression localized to T cells and dendritic cells (DCs) in both inflamed nerves and neurofibromas. These data support a heretofore unappreciated role for T cells/DCs in neurofibroma initiation.

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This work was supported by 1 R01 NS28840; The Children's Tumor Foundation; The Neurofibromatosis Therapeutic Acceleration Program and DOD W81XWH-11-1-0057 (to NR), and 5 F30 NS096796-02 (to JSF).
Platform Talk: Dermal Fibroblast-Derived Exosomes Enhance Angiogenesis in a Tissue-Engineered Skin Model of Neurofibromatosis Type 1

Monday, 5 November, 10:20 – 10:40

Vincent Roy, Axe médecine régénératrice, Centre du CHU de Québec/LOEX; Surgery, Université Laval, Quebec

Background: Neovascularization is a critical process for tumor formation in neurofibromatosis type 1 (NF1). In neurofibroma development, complete loss of neurofibromin gene (NF1) function in Schwann cells is required. Plexiform neurofibromas are highly vascularized tumors that can progress to malignancy. It is known that NF1 haploinsufficiency in endothelial cells exaggerates angiogenesis. Here we hypothesize that NF1-/+ human dermal fibroblasts secrete small vesicles that communicate a pro-angiogenic signaling and play a crucial role in modifying tumor microenvironment. The purpose of this study is to evaluate the protein content of exosomes derived from dermal fibroblasts and validate their effect on endothelial cells and angiogenesis.

Methods: Tissue-engineered skin (TES) was generated with dermal fibroblasts and keratinocytes, isolated from NF1 patients and control individuals, and microvascular endothelial cells (MVECs) using the auto-assembly method. Micro-capillary networks were analyzed and compared to healthy control. Exosomes produced for 72h from fibroblast sheets were isolated using a commercial kit and exosome concentration and size were evaluated with a nanosizer. Exosomal proteins were also analyzed using an angiogenesis protein array. NF1 exosome’s direct influence on MVECs was evaluated by a tube formation assay on Matrigel®.

Results: MVEC seeded in NF1-TES formed a denser network with increased nodes, junctions and capillary branching frequencies compare to controls. No variation in exosomal size and particles and proteins concentrations were shown between the studied populations. Analyses of NF1-derived exosomes reveals pro-angiogenic profiles that significantly enhanced tube formation on Matrigel® after 24h of co-culture with MVECs. Finally, uptake of small vesicles by MVECs was also confirmed by labelling exosomes with PKH26.

Conclusions: Our study suggests that NF1 haploinsufficiency alters dermal fibroblast function and creates a pro-angiogenic signal that increases capillary formation. Our NF1-TES model could become a unique tool to better characterize the pathogenic mechanisms associated with skin tumor genesis. Ultimately, it could provide better tools to develop new therapies for patients through the development of personalized medicine strategies.

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Platform Talk: Targeting the Hyaluronan-Rich Peripheral Nerve Sheath Tumor Microenvironment to Improve Drug Efficacy and Delivery

Monday, 5 November, 10:40 – 11:00

Bryant J. Keller, University of Minnesota – Twin Cities, Minneapolis

**Background:** Malignant Peripheral Nerve Sheath Tumors are aggressive soft tissue sarcomas that manifest at a high rate in individuals with the genetic cancer predisposition syndrome Neurofibromatosis Type 1. Although many therapeutic avenues have been explored, little improvement has been seen in the poor prognosis. Surgical resection and nonspecific chemotherapeutics remain the only standard of care. Evidence is mounting that a desmoplastic reaction involving high deposition of the glycosaminoglycan Hyaluronic Acid (HA) in the Extra-Cellular Matrix (ECM) can lead to poor drug penetrance and efficacy. Human MRI examples display large regions of poor uptake of contrasting agent, and a human tissue microarray shows 100% positivity of HA deposition through all samples, including plexiform neurofibromas. Breaking down this physical barrier is a promising potential avenue to improve drug penetrance, perfusion, and efficacy.

**Methods:** To accurately model high grade Peripheral Nerve Sheath tumors (PNST), we implemented our previously described genetically engineered mouse (GEM)-PNST consisting of Dhh-Cre; Nf1fl/fl; PTEN fl/fl (Keng V., et al., Cancer Research. 2012). This model manifests multi-focal, 100% penetrant peripheral nerve sheath tumors with a median lifespan of 18 days post birth. These tumors also display elevated HA levels in the ECM as well as collapsed and sparse vasculature. This model is amenable for treatment with a human pegylated hyaluronidase (PEGPH20) from Halozyme (San Diego, CA) to deplete HA and improve drug delivery.

**Results:** The GEM-PNST model, when treated with PEGPH20, displayed a dose-dependent decrease in tumor HA levels with no obvious toxicities to the animals. Taking advantage of the natural fluorescence of the broad-spectrum chemotherapeutic doxorubicin, sections cut from animals that had received PEGPH20 treatment before doxorubicin showed a quantifiable increase in perfusion. Furthermore, CD31 staining suggests an increase in blood vessel patency post-PEGPH20 treatment. Dual treatment of PEGPH20 with doxorubicin slightly improved longevity of these animals as compared to the monotherapy. Preliminary results also show an exciting increase of life when PEGPH20 is combined with the targeted MEK inhibitor PD0325901.

**Conclusions:** PEGPH20 shows promising therapeutic benefit in targeting physical barriers in MPNSTs. Improved drug delivery and efficacy will open avenues to further drug combinations in this currently incurable malignancy.
Platform Talk: A Phase 0 Pharmacodynamic and Pharmacokinetic Study of Everolimus in Vestibular Schwannoma (VS) and Meningioma Patients

Monday, 5 November, 9:30 – 9:50

Matthias A. Karajannis, MS, MD, Pediatrics, Memorial Sloan Kettering Cancer Center

Background: Inactivation of NF2 activates the mTORC1 signaling pathway. Inhibition of mTOR signaling has been shown to diminish growth of NF2 deficient tumors in preclinical studies, and clinical data suggest that everolimus, an orally administered mTORC1 inhibitor, may slow tumor progression in a subset of NF2 patients with VS. To date, no data exist regarding penetration of everolimus into tumor tissue or molecular target modulation in human VS or meningioma patients. To assess the pharmacokinetics, pharmacodynamics and potential mechanisms of treatment resistance, we performed a pre-surgical ("phase 0") clinical trial of everolimus in patients undergoing surgery for VS or meningiomas.

Methods: Adult patients with meningioma or VS requiring tumor resection were eligible and received everolimus 10 mg daily for 10 days immediately prior to surgery. Everolimus blood levels were determined immediately prior to and after surgery. Tumor samples were collected intraoperatively. Total everolimus concentrations in tissue were determined using mass spectrometry. Primary molecular endpoint was complete inhibition of phospho-S6 in tumor tissue. Phospho-S6 expression and related molecular markers were compared to control tissues from untreated patients matched by histology and NF2 status.

Results: Ten patients completed protocol therapy, including 5 patients with NF2-related meningioma, 3 patients with sporadic meningioma, and 2 patients with NF2-related VS. Median pre- and post-operative plasma levels of everolimus were found to be in a high therapeutic range (17.4 ng/ml and 9.4 ng/ml, respectively). Median tumor tissue drug concentration was 24.3 ng/g (range 9.2–169.2), and median tumor tissue to post-operative plasma drug concentration ratio was 0.39. We observed only partial inhibition of phospho-S6 in the treated tumors, indicating incomplete target inhibition (p = 0.005). Consistent with prior observations that inhibition of mTORC1 may lead to MAPK pathway activation through a PI3K-dependent feedback loop, we observed a statistically significant increase of phospho-ERK (p < 0.03) versus untreated controls.

Conclusions: In patients with meningioma or VS, treatment with everolimus leads to incomplete inhibition of mTORC1 signaling and upregulation phospho-ERK. These data may explain the limited anti-tumor effect of everolimus observed in clinical studies for NF2 patients, and identify upregulation of phospho-ERK as a likely resistance mechanism that could be exploited with combination therapies.
Platform Talk: A Single Arm Phase 2 Study of the Dual mTORC1/mTORC2 Inhibitor Vistusertib Provided on an Intermittent Schedule for Neurofibromatosis 2 Patients with Progressive or Symptomatic Meningiomas

Monday, 5 November, 9:40 – 9:50
Scott Plotkin, MD, PhD. Neurology, Massachusetts General Hospital, Boston, United States

Background: Meningiomas are the second most common tumor in NF2 patients, with a cumulative prevalence of 80% by age 70 and a high prevalence of multiple meningiomas. Surgery remains standard of care for these tumors, yet outcomes remain suboptimal for many patients with multiple tumors. Loss of NF2 expression is associated with activation of the mTOR pathway, but treatment with the mTORC1 inhibitor everolimus is not associated with tumor shrinkage. We hypothesized that inhibition of both mTORC1 and mTORC2 pathways would result in increased activity against meningiomas.

Methods: This single center, phase II, open label study evaluated adults (≥18 years) with NF2 and progressive meningiomas treated with vistusertib. Subjects received vistusertib 125 mg BID two consecutive days per week. Radiographic response was defined as ≥20% decrease in tumor volume from baseline. The primary endpoint was radiographic response rate in the target meningioma; secondary endpoints included radiographic response in non-target meningiomas and vestibular schwannomas.

Results: We enrolled 18 subjects (5M;13F) with a median age of 40 years (range 18-60 years). Baseline volume of target meningioma was 14.2 ml (range, 3.3-69.2 ml) and median pretreatment growth rate was 33%/year. A radiographic response was seen in 1/18 (6%) target meningiomas and in 2/20 (10%) of non-target meningiomas and in 2/21 (9.5%) of vestibular schwannomas. Three target meningiomas (17%) progressed during treatment. Seven subjects (39%) discontinued treatment by choice due to tolerability and 8 remain on study. Adverse events included fatigue, nausea, vomiting, anorexia, rash, mucositis, and hypophosphatemia.

Conclusions: Vistusertib treatment is associated with radiographic response rates of 5-10% in meningiomas and schwannomas in NF2 patients, with only a minority of meningiomas progressing during treatment. Grade 2 toxicity led to treatment discontinuation in a significant minority of patients. Future analyses will address the relationship between tumor shrinkage and activation of TORC1/2 pathways in archival tumor specimens.

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Platform Talk: Management of NF2-Associated Vestibular Schwannomas in Children and Young Adults: Influence of Surgery on Tumor Volume and Growth Rate

Monday, 5 November, 10:40 – 11:00
Isabel Gugel, Centre of Neurofibromatosis and Rare Diseases; Department of Neurosurgery, University Hospital Tübingen, Tübingen

Background: The occurrence of bilateral vestibular schwannomas (VS) in patients with Neurofibromatosis Type 2 (NF2) is a typical hallmark of the disease and their growth control by microsurgery or chemotherapy is one of the main issues in treatment concept. In the current study, we evaluated tumor volume and growth rate of VS before and after hearing preserving surgery (decompression of the internal auditory canal (IAC) with various degrees of tumor reduction according to acoustic evoked potential (AEP) stability) in NF2 patients under the age of 25.

Methods: In thirty-six NF2 patients (72 tumors) tumor volumetry using contrast T1-weighted MR images with thin slices (< 3 mm) was performed using the BrainLab Software. Growth rate was calculated using the gradient between a minimum of two MRI points. Significance was tested with two independent sample t-test and paired sample t-test by the Statistical Package for Social Services (SPSS Version 24) SPSS software.

Results: We enrolled 18 subjects (5M;13F) with a median age of 40 years (range 18-60 years). Baseline volume of target meningioma was 14.2 ml (range, 3.3-69.2 ml) and median pretreatment growth rate was 33%/year. A radiographic response was seen in 1/18 (6%) target meningiomas and in 2/20 (10%) of non-target meningiomas and in 2/21 (9.5%) of vestibular schwannomas. Three target meningiomas (17%) progressed during treatment. Seven subjects (39%) discontinued treatment by choice due to tolerability and 8 remain on study. Adverse events included fatigue, nausea, vomiting, anorexia, rash, mucositis, and hypophosphatemia.

Conclusions: Decompression of IAC and hearing preservation by partial tumor resection has no stimulatory influence on tumor growth rate but slows tumor growth. Thus, timing of surgery is important and should precede medical treatment with growth inhibitors like bevacizumab. The exact effect of surgery on the extent of hearing preservation and the duration of hearing stability post-surgery is currently evaluated.

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PLENARY SESSION: PAIN AND PRURITIS IN NEUROFIBROMATOSIS

Chairs: Allan Belzberg, MD, Johns Hopkins University, US; Rosalie Ferrer, MD, SGuy’s and St. Thomas’ NHS Foundation Trust and King’s College, UK; Scott Plotkin, MD, PhD, Massachusetts General Hospital, US

Pathophysiology of Itch

*Monday, 5 November, 11:20 – 11:45*

Laurent Misery, MD, *Université de Bretagne Occidentale*

Although poorly known until now, itch is very frequent in patients with NF. It is not restricted to the tumours but frequently associated with tumours. Its pathophysiology is poorly understood in these conditions but there is probably a role of the inhibitory effect of neurofibromin. Peripheral and central sensitizations to itch are also involved.

Therapeutic Management of Neuropathic Pain

*Monday, 5 November, 11:45 – 12:05*

Nadine Attal, MD, PhD, *INSERM U 987 and CETD, Boulogne Billancourt, France*

Neurofibromatosis is often associated with neuropathic pain. Such pain is considered as extremely difficult to treat. Recent guidelines for pharmacological treatment recommend tricyclic antidepressants (particularly amitriptyline), serotonin–norepinephrine reuptake inhibitors (particularly duloxetine), pregabalin and gabapentin as first line, while second line treatments include tramadol, and, for localized peripheral neuropathic pain, lidocaine plasters and capsaicin high concentration patches. Third line treatments include strong opioids (with rigorous monitoring) and botulinum toxin A (for peripheral neuropathic pain in specialist settings). Stimulation techniques are increasingly proposed alone or in combination with pharmacotherapy, because of a generally better side effect profile; they include transcutaneous electrical nerve stimulation and noninvasive brain neurostimulation techniques particularly repetitive transcranial magnetic stimulation. Invasive techniques such as spinal cord stimulation are proposed for refractory cases. Therapeutic perspectives include the development of compounds acting on new targets and the implementation of an individualized therapeutic approach.

Biology of Neuropathic Pain and Potential Links to NF

*Monday, 5 November, 12:05 – 12:30*

Michael Caterina, MD, PhD, *Johns Hopkins Medical Center*

Neuropathic pain is a common pathological condition that results from injury or other insults to the nervous system, such as trauma, diabetes mellitus, viral infection, chemotherapeutic agents, or neoplastic diseases such as peripheral nerve sheath tumors. Neuropathic pain often presents with a characteristic set of symptoms and clinical signs distinct from those associated with purely inflammatory pain. Accordingly, the pathophysiological mechanisms and the therapies that have proven most effective at treating these distinct conditions differ from one another. Pain is a common but poorly understood and inadequately treated symptom of peripheral nerve sheath tumors such as those caused by neurofibromatosis and schwannomatosis. In this presentation, I will discuss some of the basic biological mechanisms underlying neuropathic pain and how these mechanisms might relate to pain in peripheral nerve sheath tumors.
**Platform Talk: Molecular Mechanisms Underlying Schwannomatosis Pain**

*Monday, 5 November, 12:30 – 12:50*

Larry Sherman, PhD, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton

**Background:** Schwannomatosis patients present with intractable pain that can occur in the absence of a detectable mass and which is not always relieved by tumor resection. A recent study suggested that although most schwannomatosis patients experience some degree of pain, patients with SMARCB1 mutations have less severe pain compared to patients with LZTR1 mutations (Medicine (Baltimore). 2018; 97(5):e9717).

**Methods:** We found that inducible conditional disruption of the Smarcb1 gene in Schwann cells does not lead to changes in peripheral nerve morphology or Schwann cell proliferation or cell cycle-related gene expression. However, mice with targeted Smarcb1 disruption in Schwann cells demonstrate increased pain sensitivity.

**Results:** Dorsal root ganglion (DRG) and trigeminal ganglion neurons from mice with Schwann cell-targeted disruption of Smarcb1 express elevated levels of the TRPV1, a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin. We also find that TRPA1, an ion channel that acts as a sensor for environmental irritants, and the calcitonin gene related peptide (CGRP), which has been implicated in pain signaling, are elevated in sensory neurons of mice with Schwann cell-targeted Smarcb1 mutations. Wild type DRG cells grown in Smarcb1-null Schwann cell conditioned media demonstrated elevated cobalt uptake, a marker of TRPV1 activity, compared to cells grown with wild type Schwann cell conditioned media, and DRG cultures treated with Smarcb1-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients expressed elevated levels of TRPV1, TRPA1 and CGRP as indicated by immunocytochemistry. Proteomic, DNA microarray and chromatin immunoprecipitation analyses identified several proteins that are elevated in Smarcb1 mutant Schwann cells and whose transcription is directly regulated by Smarcb1. Several of these proteins directly influence the expression of TRPV1 in sensory neurons.

**Conclusions:** Collectively, these data indicate that loss of Smarcb1 in Schwann cells leads to the secretion of factors that induce the expression of pain mediators in sensory neurons, and suggest a mechanism for schwannomatosis pain in patients bearing SMARCB1 mutations. We are testing if the effects of factors derived from Lztr1 mutant Schwann cells are distinct from those of Schwann cells with Smarcb1 mutations to determine if loss of either gene results in distinct mechanisms of pain signaling.

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**FONDATION ARC POUR LA RECHERCHE SUR LE CANCER KEYNOTE LECTURE:**

**Targeted Therapy in Melanoma**

*Monday, 5 November, 13:30 – 14:30*

Caroline Robert, MD, PhD, Gustave Roussy Cancer Center, Paris

Melanoma therapy was revolutionized by two strategies over the last recent years: targeted therapy for BRAF-mutant melanoma, and immunotherapy relying on checkpoint inhibitors, regardless of BRAF mutation.

Targeted therapy combining anti-BRAF + anti-MEK achieves the highest response rate – around 70% – in patients with metastatic BRAF-mutant melanoma, however, secondary resistances are frequent. After one year of treatment, half of the patients show signs of relapse.

A vast variety of resistance mechanisms have been identified and several strategies of combination or sequential treatments are evaluated in order to delay the occurrence of resistance.

These targeted agents are now evaluated in earlier disease stages in the adjuvant and neoadjuvant settings with very promising results.

They are associated with a large spectrum of adverse events, new to the clinicians, but that are usually tolerable and easy to manage.

The complex controlling the initiation of protein translation, eIF4F, is a nexus of resistance that is involved in most of the resistance mechanisms. It can be targeted by eIF4A inhibitors that are in early clinical development.
Platform Talk: Transposon Mutagenesis and CRISPR/Cas9 Screening Reveal Pathways Driving Malignant Peripheral Nerve Sheath Tumor Development and Maintenance

**Monday, 5 November, 14:30 – 14:50**

**German L. Velez Reyes, PhD, Medical School, University of Minnesota, Minneapolis, United States**

**Background:** Malignant Peripheral Nerve Sheath tumors (MPNSTs) are highly aggressive, soft tissue, Schwann cell sarcomas. Half of these tumors occur sporadically, while the rest occur in the context of Neurofibromatosis Type 1 Syndrome (NF1), usually developing from pre-existing plexiform neurofibromas. NF1 is characterized by loss-of-function mutations in the gene encoding neurofibromin, a negative regulator of the Ras pathway.

**Methods:** To better understand genetic factors that give rise to MPNSTs, we performed a Sleeping Beauty (SB) transposon screen in mice. The results implicated Wnt/b-catenin, PI3K-AKT-mTOR, and other pathways. We next sought to validate SB screen gene hits in a human cellular model using CRISPR/Cas9 technology as a tool to induce loss-of-function mutations in tumor suppressor gene (TSG) and oncogene candidates. A total 103 genes were independently targeted with multiple single guide RNAs (sgRNA) in immortalized human Schwann and MPNST cell lines and effects on transformation assessed. Transformation was assessed by anchorage-independent colony formation in soft agar, transwell migration, and xenograft tumor formation in NRG mice.

**Results:** In these assays, more than 30 genes scored as TSG candidates. Our results revealed a role for the Wnt/b-catenin, Hippo/Yap, RhoA, and growth factor receptor signaling pathways in human neurofibroma and MPNST development and maintenance. Specifically, we found that TAO1 and GDI2 loss of function lead to very potent transformation of immortalized human Schwann cells and tumor formation in a xenograft model. TAO1, deleted in up to 8% of MPNSTs, encodes a Ser/Thr kinase that negatively regulates the Hippo/Yap pathway, which has been implicated in the genesis and progression of MPNST. We have found that expression of TAO1 is also reduced in human neurofibromas, MPNSTs, and MPNST cell lines. On the other hand, GDI2 is a regulator of the RhoA pathway, which has also been found to act as a suppressor of lung cancer metastasis. We have found reduced GDI2 copy number, expression, and promoter hypermethylation in MPNSTs.

**Conclusions:** Our screening methods have found novel candidate cancer genes involved in the development of human MPNSTs. These have generated hypothesis-driven pre-clinical studies that we are pursuing at the moment. We are devising methods to target the pathways implicated by our studies and that are represented in human MPNSTs.

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Disclosure of Interest: G. Velez Reyes: None Declared, N. Koes: None Declared, G. Kaufmann: None Declared, M. Berner: None Declared, D. Largaespada has a conflict with: DL is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., and Genentech, Inc. is funding some of his research. The business of all these companies is unrelated to the contents of this abstract. Others authors have no conflict of interest to disclose.
Platform Talk: Early Development and Autism Risk in Neurofibromatosis Type 1

Monday, 5 November, 14:50 – 15:10

Anna Kolesnik, Centre for Brain and Cognitive Development, Birkbeck University, London

Background: Diagnosis of Neurofibromatosis Type 1 (NF1) occurs based on distinct physical features, although up to 80% children experience cognitive and behavioral difficulties. No studies to date provide descriptions of early development, nor routes to social impairment. Prevalence for NF1 and Autism Spectrum Disorder (ASD) reaches 25% in population-based samples (Garg et al.2013;Garg et al.2013) vs. 1.5% in the general population (Baird et al.2006), which is not explained by learning difficulties. Identifying early electrophysiological and behavioral indices of cognitive function may help predict later ASD symptoms in NF1 and help construct individualized intervention protocols.

Methods: We present the first report from a prospective study of 10-month old infants with NF1 (n=10,(Kolesnik et al.2017)), compared to a cohort of infants with familial risk of ASD (BASIS; separated by outcome at age 3: ASD (n=34), atypical development(n=44), or typical development (n=89)) as well as low-risk controls(n=75). Behavioral domains include observer examinations and parent reports of cognitive and adaptive function. We further discuss eye tracking quality metrics: lost data, accuracy and precision, from the infants with NF1 (5-14m), infants at risk of ASD and infants without family history of neurodevelopmental disorders (5-14m).

Results: At 10 months, infants with NF1 show striking impairments in motor function relative to low-risk infants, a pattern similar to infants with later ASD (Mullen Scales of Early Learning, Fig1). Both NF1 and HR-ASD groups showed communication delays, although social skills were not notably impaired in NF1. Initial assessment of eye-tracking data suggests high retention rate and adequate accuracy and precision over the gaze-point from infants with NF1. Consistently, our provisional Index of Head Motion reveals increased movement of the head in infants with NF1 relative to the typically developing controls throughout the eye-tracking battery.

Conclusions: There are notable developmental difficulties in 10-month-old infants with NF1, which are similar to infants with later ASD diagnosis. These findings highlight the importance of motor functioning delay over social skills for future animal models and early treatment protocols. The next step is assessing whether these delays persist at 14 months, which will be presented and discussed. We will further consider the impact of motor delay on neurocognition, based on quality metrics and head-motion data from our eye-tracking battery.
Platform Talk: Antisense Directed Gene Therapy to Treat Intragenic Neurofibromatosis Type I (NF1) Mutations

Monday, 5 November, 15:10 – 15:30

Michael Daniel, Genetics, University of Alabama at Birmingham, Birmingham, United States

Background: Antisense oligonucleotides offer an approach to skipping an exon in which an NF1 mutation is located. Antisense directed gene therapy for exon skipping has been used successfully to treat various genetic disorders, including Duchenne muscular dystrophy (DMD). In an effort to develop an exon skipping approach to NF1, we first sought to determine which NF1 exons could be skipped and still maintain neurofibromin function.

Methods: We mined and integrated available data of reported patient mutations resulting in individual exon or multi-exon deletions with computational predictions of exon skipping effects on the structural and physicochemical features of the mutant protein. Sources for patient information are the Leiden Open Variation Database, Human Gene Mutation Database, and various online publications. This information was combined with available structural data from the Protein Database. Changes in features were assessed with respect to their potential to impair neurofibromin functionality.

To determine if skipping of an exon still results in a functional NF1 protein, we generated mNF1 cDNAs that have each exon of interest deleted. These are tested in vitro in NF1 null HEK293 cells for restoration of neurofibromin function. Each cDNA is transiently transfected into cells and then evaluated for NF1 and p-ERK protein levels via Western blot as well as levels of GTP-bound Ras, and finally for ELK1 transcriptional activity via a luciferase readout.

Results: We used bioinformatics to prioritize NF1 exons for skipping based on known skips in unaffected individuals, lack of skips reported in NF1 individuals, and exons that are not within critical NF1 functional domains (such as the GRD); prioritized exons include 12, 17, 18/19, 20, 40, 46, and 51. We created representative cDNAs for each exon and evaluated NF1 levels and Ras-activity. Deletion of certain exons (e.g. 18/19) abolishes NF1 activity while other deletions (e.g. exons 40 and 46) retain function in the assay.

Conclusions: Our goal was to assess if antisense directed gene therapy could be used to treat NF1 mutations. To this end, we determined which NF1 exons could be skipped while still maintaining NF1 function.

Feasibility of Gene Replacement Therapy in NF1-Related MPNST

Monday, 5 November, 15:30 – 15:50

Verena Staedtke, MD, PhD, Neurology, Johns Hopkins University, Baltimore, United States

Background: Neurofibromatosis Type 1 (NF1) is a RASopathy that represents a major risk for the development of the malignant peripheral nerve sheath tumor (MPNST), in which biallelic-inactivating NF1 mutations in Schwann cells result in tumor development due to Ras hyperactivation. MPNSTs are very difficult to treat and current therapies have shown little long-term benefit. In the treatment of NF-1 related MPNSTs, gene therapy remains a largely unexplored area despite the known uniform monogenic alteration as underlying cause of tumor formation and the fact that clinical gene therapy has been increasingly successful in the treatment of NF-1 related MPNSTs, in which biallelic-inactivating NF1 mutations in Schwann cells result in tumor development due to Ras hyperactivation. MPNSTs are very difficult to treat and current therapies have shown little long-term benefit. In the treatment of NF-1 related MPNSTs, gene therapy remains a largely unexplored area despite the known uniform monogenic alteration as underlying cause of tumor formation and the fact that clinical gene therapy has been increasingly successful owing to improved gene delivery technologies. Among these, adeno-associated viruses (AAVs)-based delivery vectors have emerged as safe and effective.

Methods: NF1-GRD domain was cloned in viral expression vectors and tested for inhibition of Ras activity and growth in MPNST cells. Thirteen AAV serotypes expressing EGFP were produced and tested for transduction in MPNST and human Schwann cells. NF1-GRD was further optimized for anti-Ras activity by fusing with a membrane-attaching motif.

Results: We first demonstrated the ability of NF1-GRD in suppressing the Ras activity and cell growth in MPNST cells using a lentivirus vector. Following, we systematically assessed 13 AAV subtypes for their capacity of transducing three human NF-1 derived MPNST cell lines and human Schwann cells. Among 13 different strains tested, 5 AAV serotypes appeared particularly promising, with very efficient transduction rates in MPNST and Schwann cells. Finally, to further optimize the therapeutic efficacy of NF1-GRD, we created a novel NF1-GRD construct with cell membrane-targeting resulting in superior and specific inhibition of MPNST cells.

Conclusions: This approach has the potential to add a new dimension to the treatment of NF1-related MPNSTs and sets the foundation of engineering a novel AAV vector with higher transducing efficacy in MPNST and Schwann cells. In the long-term, the scope of such a therapy could ultimately be extended to a preventative application for NF1 haploid individuals who are at high risk for a higher tumor burden/cancer or NF-1 patients in general.

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Platform Talk: Preclinical Assessment of MEK1/2 Inhibitors for Neurofibromatosis Type 2-Associated Schwannomas Reveals Differences in Efficacy and Drug Resistance Development

Monday, 5 November, 15:50 – 16:10
Cristina Fernandez-Valle, PhD, University of Central Florida, Orlando

Background: Neurofibromatosis type 2 (NF2) is a genetic tumor disorder caused by loss of function of the NF2/merlin tumor suppressor gene. A hallmark of NF2 is formation of bilateral vestibular schwannomas (VS) for which no FDA-approved drug is presently available. Because merlin modulates activity of the Ras/Raf/MEK/ERK pathway, we investigated repurposing drugs targeting MEK1/2 to treat NF2-associated schwannomas.

Methods: Mouse and human merlin-deficient Schwann cell (MD-MSC/HSC) lines were screened against six MEK1/2 inhibitors. Efficacious drugs were tested in orthotopic allograft and NF2 transgenic mouse models. Proteome and pathway analyses were conducted. Drug efficacy was examined in primary human VS cells with NF2 mutations and correlated with differential DNA methylation patterns.

Results: Trametinib, PD0325901, and cobimetinib were the most potent in reducing MD-MSC/HSC viability. Each decreased pERK1/2 and cyclin D1, increased p27, and induced caspase 3 cleavage in MD-MSCs. Proteome analysis was consistent with cell cycle arrest and activation of pro-apoptotic pathways in trametinib-treated MD-MSCs. The three inhibitors slowed the growth of MD-MSC allografts compared to controls. However, decreased pERK1/2, cyclin D1, and Ki67 levels were observed in PD0325901 and cobimetinib, but not trametinib treated grafts. Eight weeks of trametinib treatment reduced tumor burden and average tumor size compared to controls in the NF2 transgenic mouse model; tumors did not exhibit reduced pERK1/2 staining compared to control. Both trametinib and PD0325901 modestly reduced viability of several primary human VS cells with NF2 mutations. DNA methylation analysis of PD0325901-resistant versus -susceptible VS identified differentially methylated regions in genes that could contribute to drug-resistance.

Conclusions: This comprehensive pre-clinical study demonstrates efficacy differences and possible emergence of drug resistance among MEK inhibitors in schwannoma models and supports further investigation of MEK inhibitors alone and in combination with other targeted drugs as treatments for NF2-associated schwannomas.

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Platform Talk: Novel Drug Discovery for NF2-Deficient Meningiomas: Brigatinib Causes Tumor Shrinkage in NF2-Deficient Meningiomas

Monday, 5 November, 16:10 – 16:30

Long-Sheng Chang, PhD. Center for Childhood Cancer & Blood Diseases/Department of Pediatrics, The Res Inst at Nationwide Children’s Hosp/The Ohio State Univ College of Medicine, Columbus, OH

Background: Originating from meningothelial cells of the arachnoid layer lining the brain, meningiomas cause significant morbidities by compressing adjacent brain tissues, cranial nerves, and blood vessels. Meningiomas can occur spontaneously or are frequently found in NF2 patients carrying NF2 mutations. As an FDA-approved drug is presently not available for NF2-deficient menigioma, we aimed to identify novel targeted drugs that delay tumor progression or cause tumor shrinkage.

Methods: A panel of isogenic NF2 and NF2+ arachnoidal cells and NF2+ meningioma cells was used to screen ~2000 compounds of diverse mechanisms of action. The anti-tumor activity of the selected drug combination, pharmacokinetic (PK) analysis, PathScan RTK Signaling Antibody Arrays, and Western blots were performed.

Results: From the single agent screen, 45 potent compounds were selected for further combination screening, and 33 drug combinations, including the combination of brigatinib, an inhibitor of multi-RTKs including ALK, and MK2206, an AKT inhibitor, were identified that exhibited a greater synergistic growth inhibition in NF2- cells versus NF2+ cells. As a single agent, brigatinib effectively blocked tumor growth and reduced tumor size in the intracranial NF2-deficient Ben-Men-1-LucB meningioma model, while MK2206 only modestly suppressed tumor growth. Combined treatment with brigatinib and MK2206 further reduced tumor size. Upon cessation of treatment, the tumors treated with brigatinib or the brigatinib/MK2206 combination regrew. Importantly, these regrown tumors were responsive to these drugs when retreated again. PK analysis revealed that both brigatinib and MK2206 crossed the blood-brain barrier and accumulated in tumor-containing brain tissues. Intriguingly, Ben-Men-1 cells did not express ALK but expressed several phospho-RTKs (p-RTKs) with strong expression of p-ErbB2, ErbB3, FGFR1, TrkA, and VEGFR2. Brigatinib treatment reduced the levels of most of these p-RTKs with the most significant reduction in EGFR, ErbB2, ErbB3, VEGFR2, several Eph receptor members, as well as FAK. In addition, brigatinib treatment attenuated the downstream signals of these kinases, including p-AKT and p-ERKs.

Conclusions: The anti-tumor effects of brigatinib in NF2-deficient meningiomas are mediated through inhibition of multiple growth-promoting RTKs but not ALK. Brigatinib and its combination with an AKT inhibitor should be further evaluated in patients with NF2-deficient meningiomas.

Full List of Authors: Long-Sheng Chang*, Sarah S Burns1, Janet L Oblinger1, Marc Ferrer2, Jie Huang1, Ming Poi3, Vijaya Ramesh4, On behalf of the Synodos for NF2 Consortium5 and Full list of Investigators of the Children’s Tumor Foundation (CTF)-supported Synodos for NF2 Consortium: https://www.synapse.org/#!Synapse:syn2343195/wiki/62126

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Support: the CTF and US Department of Defense
Platform Talk: NF105: A Neurofibromatosis Clinical Trials Consortium (NFCTC) Phase II Study of Cabozantinib (XL184) for Neurofibromatosis Type 1 (NF1) Associated Plexiform Neurofibromas

Monday, 5 November, 14:30 – 14:50

Michael Fisher, MD, Children’s Hospital of Philadelphia, Philadelphia, PA, United States

Background: Pre-clinical mouse models of plexiform neurofibroma (PN) demonstrated the efficacy of altering the microenvironment with Cabozantinib, a multi-targeted tyrosine kinase inhibitor of KIT, MET, VEGF, and AXL. Here we report the activity of Cabozantinib in adolescents and adults with NF1-associated PN.

Methods: The NFCTC conducted a multi-institutional phase II study (NCT02101736) of Cabozantinib in subjects ≥16 years with NF1 and clinically significant PN defined as potentially life-threatening, impinging on vital structures or significantly impairing quality of life. The primary study aim was response determined by volumetric MRI analysis. Cabozantinib was administered once daily on a continuous dosing schedule (1 cycle=28 days) for up to 24 cycles. Patients started at an initial 40 mg oral daily dose with a dose escalation to a target dose of 60 mg after 2 cycles. Dose reductions for toxicity were allowed to a minimum dose of 20 mg/day. Success was defined as ≥ 25% of subjects achieving and maintaining a partial response (PR) (≥ 20% decrease in PN volume from baseline) after 12 cycles without significant toxicity.

Results: 23 subjects enrolled (mean age 23 years); 19 were deemed evaluable, defined as completion of at least 1 cycle of therapy and having had at least one follow-up PN imaging. 4 subjects were not evaluable due to failure to meet eligibility (n=1), subject withdrawal (n=1), lost to follow-up during first cycle (n=1) or enrollment error removed prior to receiving study drug (n=1). The median baseline PN volume was 556.6 cm³ (range 56.8-2954.0 cm³). Eight patients (42%) met criteria for PR by the 12th cycle. No subject had PN progression on treatment. The most common toxicities were CTCAEv4 grade 1-2 diarrhea, constipation, fatigue, headaches, palmar-plantar erythrodysesthesia (PPE) and leukopenia. Three subjects experiencing grade 3 serious adverse events (nausea and vomiting, PPE, skin infection) remained on study after dose reductions.

Conclusions: Cabozantinib is only the second class of agents to demonstrate substantial clinical activity for PN. It was well tolerated in patients ≥16 years old. This trial has been expanded to include a cohort of children aged 3 to 15 years.

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Supported by DOD Award W81XWH-12-1-D155 and Exelixis
Platform Talk: Clinical Presentation and 5 Year Survival of 83 Individuals with NF1 Associated Malignant Peripheral Nerve Sheath Tumour in a National Neurofibromatosis Centre

Monday, 5 November, 14:50 – 15:10

Rosalie Ferner, MD, Neurofibromatosis Centre, Department of Neurology, Guy’s and St Thomas’ NHS Foundation Trust

Background: Neurofibromatosis 1 (NF1) associated malignant peripheral nerve sheath tumour (MPNST) is difficult to diagnose and has been reported as having a poor prognosis. There is a 15.8% lifetime risk of developing NF1-MPNST. The aim of this study was to contribute to understanding the natural history of NF1-MPNST by reviewing the clinical manifestations and 5 year survival in a large cohort of patients at a national institution.

Methods: We conducted a retrospective review of patients with NF1 and MPNST under the care of our national neurofibromatosis service from 2009-2018. Demographics, presenting symptoms, site, size and grade of tumour were assessed. Presence of neuropathy, other malignancy and family history of MPNST were documented. Follow-up and 5 year survival was determined.

Results: 83 patients had MPNST out of 1596 (4%) with NF1, 47 males, 36 females. Age range at diagnosis was 10-81 years, median 31. Clinically segmental NF1 was present in 4 patients. 82% had pain; 33% had neurological deficit/breathing problems; 61% had hard texture of palpable tumours and 57% noted rapid growth of a visible tumour. 7 individuals had clinical and neurophysiological length dependent, axonal neuropathy; 10% had a family history of MPNST; 27% had another malignancy. Lower limb (25%), abdomen and pelvis (21%) were the commonest sites for MPNST. Size of tumour range was 2-24 cm, median 9 cm. 69% of MPNST were high grade tumours; 5 patients had a previous history of MPNST and presented with new primary tumours. Follow-up range was 0.16-30.1 years, median 4.1 years. 5 year survival was 59% with no significant difference between males and females. 47% of survivors were asymptomatic at last review.

Conclusions: We have presented a comprehensive review of the phenotype and survival of a large cohort of individuals with NF1-associated MPNST. The majority of MPNST were high grade and the 5 year survival was 59%. Survival has improved since earlier studies and is consistent with 5 year survival rates for NF1 and Cancer reported in a recent Finnish study. This potentially reflects earlier diagnosis and/or improved management in cohesive national centres.

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*Neurofibromatosis Centre, Department of Neurology, Guy's and St Thomas' NHS Foundation Trust, †Histopathology, Royal National Orthopaedic Hospital, ‡Neurofibromatosis Centre, Department of Neurology, Guy’s and St. Thomas’ NHS Foundation Trust, §Sarcoma and Melanoma Unit, Royal Marsden Hospital, §Psychology, University of Westminster, London, United Kingdom
Platform Talk: Risk of Subsequent Neoplasms (SNs) After a Primary Tumor in Patients with NF1

Michael Fisher, MD, Pediatrics, Children's Hospital of Philadelphia, Philadelphia, United States

Background: Concern about SNs has resulted in limited use of radiation (RT) in NF1 patients, despite the fact that RT results in superior tumor control (vs. chemotherapy [CT]) for low grade glioma (LGG) and provides superior nerve-sparing (vs. surgery alone) for malignant peripheral nerve sheath tumors (MPNSTs). Alkylator CT is used sparingly in NF1 patients, due to concerns about increased risk of therapy-related leukemia, limiting treatment options for LGG. Gaps in knowledge exist regarding: A) SN risk in childhood cancer survivors with and without NF1; B) SN risk in NF1 patients with a primary tumor treated with RT or alkylator CT.

Methods: Cumulative incidence of SNs was calculated treating death as competing risk. Proportional subdistribution hazards multivariable regression analysis was used to identify risk factors.

Results: (A) We used the Childhood Cancer Survivor Study cohort of 5+y survivors with NF1 (n=176) and without NF1 (n=24,181). Excluding basal cell carcinoma from the SNs, a significantly higher 20y cumulative incidence of SNs was observed in the NF1 cohort (10% vs. 4.1%, p=0.0003). Multivariable analysis (adjusting for primary tumor type, age at diagnosis of primary tumor, alkylator CT and RT) found the NF1 cohort at a 3.1-fold higher risk of SN (95%CI, 1.8-4.9, p<0.0001) than the non-NF1 cohort. (B) In order to capture events from primary tumor diagnosis to sentinel event (and overcome the limitations of the CCSS 5y survivor cohort), we constructed an independent retrospective cohort of 169 NF1 patients treated at 2 centers with dedicated NF clinics (UB, CHOP). Mean age at diagnosis of primary tumor was 5.0±5.0y; 71% had a primary brain tumor; treatment exposure included: alkylators CT (63.3%), RT (14.2%). Twenty-eight SNs (glioma: n=14; MPNST: n=8; other: n=6) were observed at a median of 8.6y (range 0-18.4) from primary tumor dx. Multivariable analysis (adjusted for age at diagnosis and primary tumor) found that exposure to RT was associated with a 2.3-fold increased risk of SNs (95%CI, 0.9-5.2, p=0.04). In contrast, exposure to alkylator CT was not associated with increased risk of SNs (HR=1.1, 95%CI 0.4-2.9, p=0.9).

Conclusions: NF1 patients with a primary tumor were at a significantly higher risk of developing SNs vs. non-NF1 cancer patients. Among NF1 patients, exposure to RT, but not alkylator CT was associated with increased SN risk. These findings provide evidence for refining management of primary tumors in NF1 patients.

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Platform Talk: Dorsal Root Ganglia Volume Differentiates Schwannomatosis and Neurofibromatosis Type 2

Tim Godel, Heidelberg University Hospital, Heidelberg, Germany

Background: Schwannomatosis and neurofibromatosis type 2 (NF2) are hereditary tumor syndromes that are primarily characterized by the development of peripheral nerve sheath tumors. Although there is considerable pathomorphological overlap, genetic origins and clinical symptoms vary greatly. Dorsal root ganglia (DRG) have been shown to play a key pathophysiological role in the development of peripheral neuropathies and pain syndromes. Since they are also described as a vulnerable site in the NF2 murine model, we here investigated DRG volume of schwannomatosis and NF2 patients in comparison to healthy controls.

Methods: In this prospective multicenter study, 16 schwannomatosis, 14 NF2 patients and 26 healthy controls were examined by MR-Neurography at 3 Tesla. A 3D T2-weighted sequence was used for imaging the lumbosacral plexus, covering DRG L3 to S2. DRG volume for each patient and level was calculated and tested for statistical significance using one-way ANOVA with Bonferroni correction. P-values of < 0.05 were considered significant. ROC curves of DRG volume of NF2 and schwannomatosis patients were used to assess diagnostic accuracy.

Results: Compared to schwannomatosis patients, DRGs of NF2 patients were enlarged by 268% for L3, 233% for L4, 257% for L5, 285% for S1, and 207% for S2 (p<0.001 for all levels). Compared to healthy controls, schwannomatosis patients showed no difference in DRG volume for all levels (p>0.05). ROC analysis of DRG volume (L3 to S2) as a diagnostic marker for discrimination of NF2 from schwannomatosis showed an AUC of 0.90. Further, schwannoma formations of the lumbosacral plexus involving or originating from a DRG were found in 5/14 NF2. In contrast, while schwannomas of the lumbosacral plexus were also found in 7 Schwannomatosis patients, all of these were located at least >2mm either proximal or distal to the DRG.

Conclusions: Pathological alterations in DRG structure may play a crucial role in the development of areflexia and sensory loss in NF2. Moreover, our ROC analysis indicated that DRG hypertrophy might serve as a highly accurate and easily investigated diagnostic marker in the discrimination of the two tumor syndromes. This observation could be useful, if vestibular schwannomas are not present at the time of initial diagnosis or NF2 mutations in blood-DNA are missing. It remains to be solved whether DRG hypertrophy in human NF2 occurs prior to development of vestibular schwannoma.

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Platform Talk: Use of an NF2 Genetic Severity Score to Incorporate Genotype into Routine Patient Care

Monday, 5 November, 15:50 – 16:10

Dorothy Halliday, Oxford Centre for Genomic Medicine, Nuffield Orthopaedic Centre; NF2 Unit, Neurosciences, Oxford University Hospital NHS Foundation Trust, Oxford, United Kingdom

Background: Genotype information in NF2 is important diagnostically to delineate NF2, mosaic NF2 and schwannomatosis, as the three conditions have phenotypic overlap. Published work has delineated clear differences in phenotype between severe truncating NF2 mutations and milder missense mutations. Truncating mutations however at the beginning and end of the NF2 gene cause a less severe phenotype than mutations more centrally. In addition, the high rate of mosaicism in de-novo disease is important; as a patient mosaic for a truncating mutation may have a more severe phenotype than a patient with a constitutional missense mutation.

The validated UK genetic severity score draws together the published data on genotype phenotype correlation, to create a simple 1-3 score where 1 represents patients with Mosaic NF2 with no mutation detected in blood; and 2A-mild, 2B-moderate and 3-severe represents those with different types of NF2 mutation (constitutional or mosaic) detected in blood. The score was devised to allow categorisation of patients into likely phenotypic severity groups, based on their genotype.

Methods: Using the UK NF2 severity score we assigned all 154 patients cared for by the Oxford and South-West of England NF2 team a genetic severity. We have used this score in natural history and interventional service evaluations, approved by the Oxford University Hospitals NHS trust.

Results: In a retrospective review, we demonstrated a clear trend towards later age of deafness for patients in group 1 compared to group 3. We reviewed ophthalmological findings and showed that various eye findings including development of optic atrophy, retinal abnormalities and cataract were related to the genetic severity and that group 3 patients developed visual impairment at a younger age. We showed that psychological measures of distress in patients with NF2 were increased, and that the level of distress was not related to severity of disease. We have shown that NF2 patients with impaired balance respond well to physiotherapy intervention and that the level of response was not related to genetic severity.

Conclusions: By incorporating genotype information routinely into natural history studies we aim to further develop prognostic data on NF2 based on genotype, to better facilitate patient management decisions.

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Platform Talk: Smile Reanimation Using the Lengthening Temporalis Myoplasty in Patients with Facial Paralysis

*Monday, 5 November, 16:10 – 16:30*

Andre Panossian, MD, Beverly Hills, United States

**Background:** In patients with neurofibromatosis (NF1 or NF2), dysfunction of the facial nerve may happen directly by tumor involvement or secondary to resection of facial tumors. The lengthening temporalis myoplasty (LTM) is a relatively new technique for dynamic lip reanimation. It utilizes cranial nerve V and is, therefore, relatively spared during resection of facial tumors. In this setting, the temporalis is used in an orthograde fashion as opposed to a turnover flap to attach to the oral commissure for smile activation. There are several potential advantages that LTM may have over other methods.

**Methods:** A single-surgeon retrospective review was performed between 2012 and 2017, examining patients undergoing smile reanimation using LTM. Patient demographics, diagnosis, operative time, smile excursion, laterality, complications, and length of stay were recorded. Photos and video were obtained for all patients both pre- and postoperatively.

**Results:** A total of 29 patients underwent smile reanimation between 2012 and 2017 using LTM. Twenty-two patients underwent unilateral and 7 patients underwent bilateral procedures. Only 2 patients had a diagnosis of NF1 and one with NF2. The remainder had other diagnoses resulting in facial nerve palsy. Average operative times for unilateral LTM was 248 minutes, and bilateral LTM was 418 minutes. Smile excursion averaged 8.9 mm (range 5-17 mm). There were no failures but approximately 15 patients (52%) required at least one revision to re-establish movement due to tendon avulsion and/or adhesions. An additional 3 patients demonstrated temporal hollowing, requiring fat injection.

**Conclusions:** Lengthening temporalis myoplasty produces decreased cheek bulk, shorter operative times, ease of on-table adjustments and revisions, and outpatient surgery over other techniques. However, revision rates for LTM are still high, and the procedure requires a nasolabial fold incision. Patients who have undergone LTM also lack spontaneity due to its function as a masticatory muscle. In some cases, patients may require extended therapy postoperatively. However, the incorporation of LTM in patients undergoing extensive facial neurofibroma resection may help achieve more aggressive debulking while preserving the ability to animate the lower face.
Late Inactivation of Smarcb1 and Additional Nf2 Loss Are Necessary for Schwannoma Development in Schwannomatosis Patients

Monday, 5 November, 16:45 – 17:10

Jeremie Vitte, PhD, Department of Head and Neck Surgery, University of California, Los Angeles, United States

Germline mutations of the SMARCB1 gene are found in schwannomatosis patients developing benign tumors of cranial and peripheral nerves in their adulthood. SMARCB1 germline mutations are also the hallmark of rhabdoid tumors (RTs), malignant pediatric tumors mostly developing in brain and kidney, and leading to a familial cancer predisposition syndrome. The mechanisms by which SMARCB1 germline mutations predispose to RTs versus schwannomas are still unknown. To understand the origin of these two types of SMARCB1-associated tumors, we generated different tissue- and developmental stage-specific conditional knockout mice carrying Smarcb1 and/or Nf2 deletion. We demonstrated the existence of a time window in neural crest cells where early loss of Smarcb1 was necessary to initiate tumorigenesis in the cranial nerves and meninges with typical histological features and molecular profiles of human rhabdoid tumors. Importantly, the induction of Smarcb1 loss alone at later developmental stages in the Schwann cell lineage was not tumorigenic and additional biallelic Nf2 gene inactivation was necessary to initiate schwannoma development, thus generating the first mouse model developing schwannomas with the same underlying gene mutations found in schwannomatosis patients.

Correlation of the Tumor-Suppressive Function and the Confirmation of NF2

Monday, 5 November, 17:10 – 17:35

Tina Izard, PhD, Scripps Institute, US

We recently determined the crystal structure of lipid-bound neurofibromin 2, the protein responsible for neurofibromatosis type 2 (NF2). Since neurofibromin 2 is a member of the ezrin, radixin, moesin family of cytoskeletal proteins, it seemed likely that neurofibromin 2 would function similarly to ERM proteins. Our new structural and functional data demonstrated the correlation between the tumor-suppressive function of neurofibromin 2 and its conformation. We show that membrane attachment is necessary for inhibiting cell proliferation and what lessons can be learned from the various neurofibromin 2 structures with respect to NF2.

Parallels Between Nerve Regeneration and Tumour Formation in the Peripheral Nervous System

Monday, 5 November, 17:35 – 18:00

Alison C Lloyd, PhD, MRC Laboratory for Molecular Cell Biology, University College London, Gower Street, London, WC1E 6BT

Peripheral nerves have remarkable regenerative properties and there has been great progress in understanding the multicellular processes involved. Underlying this regenerative capacity is the regenerative nature of the major glial cell type of the PNS, the Schwann cell. Mature, adult Schwann cells dedifferentiate to a progenitor-like state following an injury and these cells have multiple roles in orchestrating the multicellular response required to regenerate a nerve. Tumours that arise in the PNS, in both NF1 and NF2, arise mostly from Schwann cells, which appear to be mimicking many of the roles of these progenitor-like Schwann cells in the injured state. However, whereas an injury usually resolves, these tumours resemble an unrepaird wound and therefore increasing our understanding of the resolution of the injury response is an important approach for the development of novel therapeutic approaches for the treatment of these tumours. In this talk, I will discuss our current understanding of how Schwann cells orchestrate the regeneration of peripheral nerves and the implications of these findings for the biology of NF1 and NF2.

References:


Background: Schwannomatosis is the third major form of neurofibromatosis family of genetically inherited peripheral neuropathies that appear with age. They can be caused by chronic axonal damage resulting in axonopathies characterized by neuronal degeneration. The origin of these axonopathies in NF1, NF2 and schwannomatosis is not understood. Schwannomatosis is mainly characterized by chronic neuropathic pain and the formation of multiple peripheral schwannomas. Recently, LZTR1 gene was identified as a new candidate gene for schwannomatosis (Piotrowski et al., and Hutter et al., 2014). The LZTR1 gene encodes a member of the BTB-kelch protein superfamily called Leucine-zipper-like transcriptional regulator 1 (LZTR1). It is not understood whether LZTR1 germline mutations in humans are causative for both chronic pain and schwannoma development, or whether additional somatic mutations (e.g. in the NF2 gene encoding for the tumour suppressor protein merlin) are necessary for tumour formation. Nothing is known about the expression, subcellular localization and function of LZTR1 except that it was suggested to be localized to the Golgi network, where it might stabilize the Golgi complex (Nacak et al., 2005). In the current study, we aim to delineate the molecular mechanisms underlying neuropathic pain associated with Schwannomatosis.

Methods: Subcellular Fractionation, flow cytometry, Immunofluorescence, mouse and human Schwann cell culture, protein biochemistry, lentiviral knock-down, electron microscopy, Seahorse metabolic assay

Results: LZTR1 is specifically found to be expressed in the unmyelinated fibres/c-fibres of the peripheral nervous system, Schwann cells and DRG neurons (dorsal root ganglion). Using subcellular fractionation of HeLa cell lysates expressing recombinant myc-tagged LZTR1, we found that LZTR1 is a membrane protein localized to mitochondria and mitochondrial associated ER membrane (MAM). Not only that, knock-down of LZTR1 in Schwann cells showed mitochondrial dysfunction with an augmented mitochondrial Ca2+ levels and respiration; and decreased reactive oxygen species production.

Conclusions: data suggest that LZTR1 is a protein associated with MAMS possibly regulating calcium homeostasis which could indirectly contribute to neuropathic pain states by modulating voltage-activated Ca2+ channels and pumps present in the endoplasmic reticulum and mitochondria.

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Merlin/ERM Proteins Regulate Growth Factor-Induced Macropinocytosis and Receptor Recycling by Organizing the Plasma Membrane:Cytoskeleton Interface

Monday, 5 November, 18:20 – 18:40

Christine C. Chiasson-Mackenzie, PhD, Department of Pathology, Massachusetts General Hospital/ Harvard Medical School; Center for Cancer Research, Massachusetts General Hospital, Boston, United States

**Background:** The architectural and biochemical features of the plasma membrane are governed by its association with the underlying cortical cytoskeleton. The neurofibromatosis type 2 (NF2) tumor suppressor, merlin, and closely related membrane:cytoskeleton linking protein ezrin, organize the membrane:cytoskeleton interface, a critical cellular compartment that both regulates and is regulated by growth factor receptors. An example of this poorly understood interrelationship is macropinocytosis, a process of nutrient uptake and membrane remodeling that can both be triggered by growth factors and manage receptor availability. A newly appreciated role for macropinocytosis in tumorigenesis and evidence that cells use macropinocytosis for uptake of therapeutics has sparked interest in exploiting macropinocytosis for drug delivery. Because NF2 mutations are associated with the development of benign schwannomas and meningiomas that reflect the persistance of normal signaling rather than aberrant activation of oncogenic signaling, drug delivery mechanisms that favor the targeting of NF2-mutant cells are desperately needed.

**Methods:** We used high-powered confocal imaging together with cell biology and biochemical approaches to investigate the relationship between ErbB signaling and membrane:cytoskeleton organization in NF2-mutant cells. We utilized extracellular vesicle uptake as a proof of principle of the therapeutic potential of exploiting macropinocytosis in NF2-mutant cells.

**Results:** We show that merlin-deficiency primes the membrane:cytoskeleton interface for growth factor-induced macropinocytosis via a mechanism involving increased cortical ezrin, altered actomyosin and stabilized cholesterol-rich membranes. These changes profoundly alter EGFR trafficking in merlin-deficient cells, favoring increased membrane levels of its heterodimerization partner ErbB2 and increased recycling. Our work suggests that merlin-deficient cells exploit macropinocytosis for receptor recycling. Finally, we provide evidence that the macropinocytic proficiency of NF2-deficient cells can be employed for therapeutic uptake.

**Conclusions:** This work provides new insight into fundamental mechanisms of macropinocytic uptake and processing, and suggests new ways to interfere with or exploit macropinocytosis in NF2-mutant and other tumors. Our discovery that NF2-deficiency facilitates growth factor-induced macropinocytosis and therapeutic uptake in Schwann and meningioma cells sets the stage for advancing this therapeutic strategy.

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1Department of Pathology, Massachusetts General Hospital/ Harvard Medical School, 2Center for Cancer Research, Massachusetts General Hospital, Boston, United States
Platform Talk: Risk of Contralateral Breast Cancer and Survival in Neurofibromatosis Type 1: A Five Country Cohort Study

Gareth Evans, MD. Genomic Medicine, University of Manchester, Manchester, United Kingdom

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant condition caused by mutation/deletion of the NF1 gene. Although nervous system tumours have long been associated with NF1 recent evidence has demonstrated an increased risk of breast cancer.

Methods: We obtained details of NF1 patients with breast cancer from five population based cohorts in Europe. Risk of contralateral breast cancer and death were assessed by Kaplan-Meier analysis using a delayed entry method for those who were ascertained by the NF center after breast cancer diagnosis. A control group of 335 women screened for familial breast cancer was used to assess relative survival.

Results: 143 women with breast cancer diagnosed at a median age of 47.2 years (range 26.9-84.4 years). There were 1208 years of clinical follow up from breast cancer diagnosis (mean=8.45 years). Thirteen women had developed contralateral breast cancer at a median age of 53.7 years (range=28.5-81.7) with a rate of 11.3 per 1000 years and cumulative risk to 20 years was 29.7%. Five and 10-year all cause survival in unscreened NF1 women was 72% (95%CI=63.0-82.2) and 58.7% (95%CI=48.5-71.0) and breast cancer specific 10-year survival was 73.5% (95% CI 62-82%) compared to the control familial population of 89.5% (95% CI 84.4-93%). There were no breast cancer deaths in the 27 women in Padova who had undergone breast screening. NF1 invasive breast cancers were less likely to be stage 1 (22% vs 61%) p<0.0001. There was also a higher rate of HER2+ cancers at 24.2% vs 11% p=0.02. 30-year all cause survival was only 18.2% (95%CI=5.97-55.6%).

Conclusions: NF1 women have a substantial contralateral breast cancer incidence and poor survival. It is likely that breast screening starting in the early 30’s will improve survival.

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Spectrum of Mutations in the NF1 Gene in Polish Patients with Neurofibromatosis Type I and Neurofibromatosis-Noonan Syndrome

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**Background:** Neurofibromatosis type I (NF1) and neurofibromatosis-Noonan syndrome (NFNS) are included to the RASopathies. Both are caused by the presence of a pathogenic variant in the *NF1* gene, are inherited in an autosomal dominant manner and display high phenotypic variability.

The aim of our study was to assess the spectrum of mutations identified in NF1/NFNS patients and an attempt to assess the genotype-phenotype correlation.

**Methods:** One hundred fifteen (115) and 23 NF1/NFNS patients, respectively, were included in the analysis. The identification of point mutations was based on next generation sequencing (60), Sanger sequencing on RNA / cDNA (42) or DNA (35) template and MLPA technique (88). Potentially pathogenic variants were tested *in-silico* using prediction algorithms and available databases (HGMD, LOVD) and were analysed in families.

**Results:** The analysis of the *NF1* gene allowed to identify a pathogenic / potentially pathogenic variant in 92% and 95% of NF1 and NFNS patients, respectively. The analysis of genotype-phenotype correlation showed that the missense mutations (including p.Arg1809Cys and p.Leu1196Phe variants) occurred more frequently in NFNS than NF1 patients (9/23; 39% vs. 21/98; 21%). In NF1 patients, most of the identified variants were loss-of-function mutations (77, 79%), including the splicing ones (30, 31%). The distribution of mutations depended on the applied technique. In case of cDNA sequencing, splicing mutations were quite common (30%) including 8 (23%) mutations identified in exons as the missense or nonsense variants. In contrast, the NGS-based analysis revealed the presence of missense and nonsense variants in 17 (34%) and 9 (18%) patients, and 18 of them might affect splicing as shown by *in-silico* analysis.

Large deletions encompassing several exons or the entire *NF1* gene were identified in 9 (10%) probands. One (deletion of exons 39-42) was identified by RNA/cDNA analysis in a patient with no splicing mutation and the MLPA revealed only the presence of exons 40-42 deletion.

**Conclusions:** The spectrum of mutations identified in the NF1 and NFNS patients was different suggesting that the specific variants were associated with dysmorphism and other features typical of Noonan syndrome. The NGS analysis of the *NF1* gene should include at least *in-silico* analysis for splicing alterations.


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Acknowledgment: The work was supported from the National Science Centre (2013/09/B/NZ2/03164) and Institute of Mother and Child intramural grants.
Genetic Variants of the Androgen Receptor and Phenotype of Neurofibromatosis Type 1

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**Background:** Neurofibromatosis type 1 (NF1) is a genetic disorder with variable expressivity. Among young adults with NF1, men tend to develop more subcutaneous neurofibromas (SCNF) than women, suggesting an influence of androgens on this phenotype. This is not the case for cutaneous neurofibromas (CNF). In addition, patients with SCNF are at higher risk for presence of internal neurofibromas and for mortality. Previous studies had shown that genetic polymorphisms in the Androgen Receptor (AR) gene on chromosome X can modulate the risk of androgen-sensitive diseases in men. The most studied is a coding CAG repeat in exon 1, for which the presence of a short allele (<20 repeats) has been associated with an increased risk of prostate cancer, whereas the presence of a long allele (> 24) may confer an increased risk of testicular cancer. We studied the influence of two common polymorphisms of the AR gene (including the CAG repeat) on NF1 phenotype in men with NF1.

**Methods:** In 190 men with NF1, the size of CAG and GGN repeats in AR exon 1 was assessed by fragment analysis. NF1 patients were classified according to their age and the presence of at least two SCNF or CNF at examination.

**Results:** 58 men aged 18 to 30 years with at least 2 SCNF (cases) were compared to 85 men over 30 without SCNF (controls). There was a significant difference in the distribution of CAG repeats between groups, with an excess of short alleles (<20 repeats) in controls (28% vs. 9% in cases) and an excess of long alleles (> 24) in cases (26% vs. 9% in controls) (p=0.0004). The presence of a short allele appears to protect against the occurrence of SCNF (OR=0.24, 95% CI 0.09-0.67), while the presence of a long allele is associated with an increased risk (OR=3.36, 95% CI 1.32-8.56). No difference in the distribution of GGN repeats was observed between the two groups.

Concerning CNF, no difference in the CAG or GGN repeat size was found between men aged 18-30 and at least 2 CNF and men over 30 without CNF.

**Conclusions:** Our study shows an association between the number of CAG repeats in exon 1 of the AR gene and the risk of early-onset SCNF in men with NF1. The mechanism responsible for this association remains to be clarified. This result points AR as a potential modifier gene in NF1.

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**Drug-Target Explorer: An Interactive Tool for Examining Chemical-Biological Interactions**

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**Background**: Phenotypic high-throughput screens are often utilized in modern drug discovery pipelines. Such screens are conducted with an array of molecules, ultimately measuring a biological change within a disease model. For example, a screen might test drugs in NF1 wild-type and mutant cell lines to find molecules that are selectively toxic to NF1 cells. While these screens can yield valuable hits, they also present challenges including identifying the target(s) that mediate the effect seen in the screen, characterizing "hits" with a polypharmacologic target profile, and contextualizing screen data within the large space of drugs and screening models. To address these challenges, we developed an application that enables exploration of the chemical-biological interaction space.

**Methods**: Data from ChEMBL, PubChem, DrugBank, Klaeger et al. 2017, and the Drug-Gene Interaction Database (DGIdb) for over 280,000 small molecules were downloaded. Drug-target interaction data points were quantified for each interaction. In addition, when quantitative data were obtained, summarized potency metrics were calculated. Each molecule was annotated with a name and chemical structure, and every target was annotated with protein & gene identifiers. To enable exploration of this database, an interactive web interface was developed using the R Shiny platform and cheminformatics R packages.

**Results**: The app enables the end user with a specific query molecule to search a database of experimentally-derived drug-target interactions. The database can be queried using drug names or structures. A chemical similarity parameter allows the user to expand their search to other structurally related molecules. The structure of the query molecule is compared to every database molecule, and similar molecules and targets are presented in interactive tabular and network-based forms for in-depth exploration. The app also performs enrichment analysis on the target lists and allows the user to evaluate structure-activity relationships for drug response data. Finally, if a user has a target of interest, they can search for molecules that bind that target and explore the resulting data interactively.

**Conclusions**: The Drug Target Explorer is a multifunctional platform for exploring chemical space as it relates to biological targets, and may be useful at several steps along the drug development pipeline including target discovery, structure-activity relationship, and lead compound identification studies.

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**Identifying Resources and Gaps in Preclinical to Clinical Translation in NF1**

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**Background**: Neurofibromatosis type I (NF1) is an autosomal dominant genetic condition characterized by peripheral nervous system tumors, including plexiform neurofibroma (pNF) that can undergo malignant transformation (MPNST). Other than early results with selumetinib, the identification of effective treatments for these tumors has remained elusive. Recent studies using in vitro and in vivo models of NF1-related tumors have identified several candidates, but it is unclear which molecule-model combinations have been tested, which models are highly- or under-utilized, and which models align with clinical outcomes.

**Methods**: To identify resources and clarify gaps, we compiled a dataset of clinically tested drugs in NF1-related tumors and an array of in vitro and in vivo models currently available with the goal of identifying gaps in preclinical pipelines. These data include the results of NF1 based clinical trials from ClinicalTrials.gov, model data curated from PubMed, and drug screening data across a variety of models of NF1-related tumors. Additional curation was performed to define and standardize the nomenclature and features of available models.

**Results**: Curation of clinical trial data revealed that 57 pharmacologic clinical trials for NF1 related tumors have been or are being conducted. 27 are ongoing, 10 are completed and did not meet efficacy endpoints (10/15), and 15 are completed with no results published. Most interventions have been tested only once. Curation of NF1 models identified 45 in vivo animal models (5 species), and 87 cell lines (3 species), but few of these appear to be readily acquirable. While several in vitro models of MPNST exist, there are fewer readily available in vitro models of pNF, OPG and other NF1 related tumors. Finally, it is unclear which model features are required to successfully predict clinical outcomes.

**Conclusions**: There are several research tools available for NF1 research predominantly focused on pNF and MPNST. However, there are challenges in translating preclinical discoveries to clinical trials. There are opportunities to fill these gaps by developing and systematically assessing in vitro and in vivo tumor models for their predictive value for clinical performance. Freely disseminating tumor models would further fill gaps in the NF1 drug development pipeline. There is also a need to publish unreported preclinical and clinical trial results, and use these data to assess the performance of preclinical models in predicting clinical outcomes.

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The NF1 Gene in Early Modern Humans

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Background: Increasing data suggest that many unique characteristics of modern humans reflect the presence and impact of the ubiquitous eukaryotic Supergene, NF1, originally identified from instances of gene mutations accounting for Von Recklinghausen disease or the NF1 syndrome.

Methods: From genetic and anthropological vantage points, keying off a publication of a Cro-Magnon person with an apparent NF1 mutation, plus our general respect for NF1 mutant phenotypic elements involving adverse effects on social skills, learning and musicality and, as well, positive effects including minimization of obesity, diabetes mellitus, alcoholism and opiate addiction, we have documented the persistent and expanding presence of NF1 alleles in multiple lineages of ancient and modern humans.

Results: In this brief presentation we document multiple bases for pursuing a unique role of NF1 as a genetic mechanism for “finessing” multiple elements of the modern human phenotype. As demonstrated here, expansion of the cranial vault associated with NF1 variants is an example.

Conclusions: As we learn more about how wildtype NF1 enhances modern humans we will both improve the clinical management of those bearing the mutant alleles and improve the health and well-being of the general population as well.

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Establishment of Neurofibroma Cells and Dedifferentiated Fat (DFAT) Cells from Neurofibromas of NF1 Patients

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Background: Neurofibroma is a critical symptom in NF1 patients, and surgical resection is the standard and only available treatment for neurofibromas. To develop therapeutic strategies for NF1-associated neurofibromas, we are attempting to develop in vitro models that recapitulate the pathological and clinical properties of neurofibromas.

Methods: Neurofibroma cells and dedifferentiated fat (DFAT) cells were established from NF1 patient tumors. Tissue samples were obtained during tumor resection surgery at our hospital from patients who met the NIH clinical diagnostic criteria for NF1. Whole-blood specimens were also obtained for gene analysis. All patients provided written informed consent. The institutional review board of our university approved this aspect of the study. Tumor tissues were dissociated in DMEM containing collagenase. The neurofibroma cells at the bottom of the tube and the floating stromal adipocytes were collected separately after centrifugation. To establish DFAT cells, the stromal adipocytes were placed in a culture flask filled with 20% FBS-DMEM, and then the flask was inverted and incubated at 37 °C in a humidified atmosphere of 5% CO2. The stromal adipocytes floated up through the medium and adhered to the ceiling of the flask. After 1 week, the cells were firmly attached to the ceiling and had dedifferentiated. Those DFAT cells can be passaged as well as the neurofibroma cells.

Results: We established neurofibroma cells and DFAT cells from NF1-associated neurofibromas. We performed flow cytometry analysis and found that those cells derived from NF1 patients were expressed SOX10, S100, and CD90, all of which are expressed in Schwann cells. We identified the NF1 mutations in the patients by next-generation sequencing. Peripheral blood specimens from patients 1 and 2 were positive for c.1466A>G, p.Tyr489Cys and c.3213_3214delAA, p.Ser1072Hisfs*16 mutations of NF1, respectively. We also identified NF1 mutations in the cells that we had established from tumors. In the tumor specimen of patient 1, we identified an additional somatic mutation, c.6772C>T, p.Arg2258X of NF1 gene.

Conclusions: We established neurofibroma cells and DFAT cells from neurofibromas of NF1 patients. DFAT cells exhibit multipotent ability of differentiation into a variety of cell types. These cells may prove useful for studies of the pathophysiology of NF1-associated neurofibromas as well as cell-based drug screening for facilitating the development of new treatments.

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EVI2B Gene Assessment in NF1 Tumors and Leukemia’s

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**Background**: Neurofibromatosis Type 1 (NF1) is an autosomal dominant disease affecting the development and growth control of a variety of tissues. NF1 represents a major risk factor for development of malignancies, particularly malignant peripheral nerve sheath tumors, optic gliomas, and leukemia. The NF1 gene encodes a transcript estimated at 11 to 13 kb containing 61 exons. Three genes, EVI2A, EVI2B, and OMPG, are embedded within intron 27b of the NF1 gene. These genes are transcribed in the direction opposite that of the NF1 gene. Little is known about the function of these genes. Both EVI2A and EVI2B encode putative transmembrane proteins. The mouse homologs (Evi-2a and Evi-2b; ecotropic viral integration sites) are associated with viral insertions involved in leukemia in mice and its relation to NF1 symptoms is unknown. It has been already identified that Evi2b as a direct target gene of C/EBPa, a transcription factor critical for myeloid differentiation. It is possible that these genes are related to the leukemia observed in NF1 patients, although there are no data confirming this association. Expression of this gene is altered by viral integration and this altered expression may predispose cells to myeloid disease. These genes might act as a modifier in the NF1 phenotypic variations. Therefore we investigated EVI2A and EVI2B gene in NF1 tumors and leukemia.

**Methods**: We analyzed 10 NF tumors, 20 leukemia and 3 NF1-leukemia by PCR based technics. DNA samples were sequenced to detect variations in each exon. The pathological status of tumor tissues was confirmed by routine pathological examination. Standard immunohistological procedure was performed for EVI2B protein and S100 in tumor samples to prove the existence of Schwann cells.

**Results**: We observed viral integration for EVI2B gene in 15 out of 20 leukemia and in all 3 NF1-leukemia patients. We did not observe any integration in NF1 patients. Moreover we do not detected any integration in EVI2A gene in none of the patients. The immunofluorescence staining results demonstrated intact protein localization in cell membranes of NF1 tumors.

**Conclusions**: No integration in EVI2A gene were detected in none of NF1, leukemia’s and NF1-leukemia’s patients. The integrations in EVI2B were discovered only in leukemia’s and NF1-leukemia’s but not in NF1 patients. This shows that EVI2B is a candidate effector gene for leukemia’s but not for NF1 disease. This study was supported by TÜBİTAK Project No: 107S96.

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LARP6, a RNA-Binding Protein Involved in Translation and Stability of Collagen mRNA, Is a New Partner of Neurofibromin (Nf1): Molecular Studies and Functional Implications

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Background: To improve our understanding of the molecular functions of Nf1, we have performed a two-hybrid screen on a human brain cDNA library using the SecPH domain of Nf1 as a bait. LARP6 was identified as an interactor. LARP6 is a RNA-binding protein involved in posttranscriptional regulation of type I collagen mRNA. In specific conditions, type I collagen synthesis can be upregulated several hundred fold. This process is predominantly achieved by LARP6 which stabilizes and stimulates translation of mRNA. For this purpose, LARP6 binds a secondary stem-loop structure (termed 5’ SL) in the 5’ untranslated region (5’UTR) of mRNA.

This interaction is of high interest since type I collagen is overproduced in neurofibroma and by Nf1 deficient osteoblasts resulting in non-mineralized collagenous matrix and bone fragility.

Methods: Interactions between Nf1, LARP6 and domains of these proteins were tested by co-immunoprecipitations and two-hybrid technics. SecPH, LARP6 and its Nt-LAM-RRM domain were produced and purified and gel mobility shift assays were conducted to visualize the formation of complexes with collagen mRNA 5’S.L.

Primary human lung fibroblasts were transfected by SecPH and collagen synthesis was monitored by western-blot.

Results: Nf1 and LARP6 interact independently of the presence of collagen mRNA. This interaction implies the Nt-LAM domain of LARP6 and the SecPH domain of Nf1

Two complexes, C1 and C2 are formed between LARP6 or its Nt-LAM-RRM domain and 5’S.L. SecPH was shown to abolish their formation and to induce the formation of a new complex, Cx. We are currently working on Cx to know if it contains SecPH.

Synthesis of type I collagen by human lung fibroblasts seems to be affected by the overproduction of SecPH. These results have to be confirmed by silencing of Nf1 in these cells and overproduction of a SecPH mutant affected in its interaction with LARP6.

Conclusions: Nf1 interacts with LARP6 and its SecPH domain affects the complex formed between LARP6 and the 5’ SL region of type I collagen mRNA. In addition, SecPH seems to affect type I collagen synthesis by human lung fibroblasts. We now want to go further in the experiments to demonstrate that these two results are linked and to unravel the precise molecular mechanism involved. This work will tell us if LARP6 can constitute a new therapeutic target for phenotypes associated with excessive collagen production in Nf1.

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Functional Predictions and Characterization of Neurofibromin Architecture

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Background: Neurofibromatosis type I (NF1) is caused by heterozygous loss-of-function variants in the NF1 gene encoding neurofibromin which serves as a tumor suppressor that inhibits RAS signaling and regulates cell proliferation and differentiation. While, the only well-established functional domain in the NF1 protein is the GAP-related domain (GRD), most of the identified non-truncating disease-causing variants are located outside of this domain, supporting the existence of other important disease-associated domains. Identifying these domains may reveal novel functions of NF1.

Methods: By implementing inferential statistics combined with machine-learning methods, we developed a novel NF1-specific functional prediction model that focuses on nonsynonymous single nucleotide variants (SNVs). The model enables annotating all possible NF1 nonsynonymous variants, thus mapping the range of pathogenic non-truncating variants at the codon level across the NF1 gene.

Results: The generated model demonstrates high absolute prediction value for missense and splice-site variations (area under the ROC curve of 0.94) outperforming 14 other established models.

By reviewing the entire dataset of nonsynonymous variants, two novel domains (Armadillo type fold 1 and 2) were identified as being associated with pathogenicity (OR 1.86; CI 1.04 to 3.34 and OR 2.08; CI 1.08 to 4.04, respectively; P<0.05). Specific exons and codons associated with increased pathogenicity were also detected along the gene inside and outside the GRD domain.

Conclusions: Exons and codons with higher and lower likelihood to be associated with NF1 were detected using a novel model, enabling better prediction of pathogenicity for variants in NF1 gene, as well as revealing novel NF1-associated domains in addition to the GRD.

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Expression of VEGF and VEGFR Isoforms in Nerve Tumors of Neurofibromatosis Type 1: A New Oncogenesis Pathway Relying on an Autocrine/Paracrine Activation Loop in Tumor Cells?

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Background: Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive sarcomas which most often arise from deep neurofibroma (NF) and occur in up to 10% of neurofibromatosis type 1 (NF1) patients. It has been suggested that angiogenesis induced by VEGF (vascular endothelial growth factor) through its receptor VEGFR could play a key role in the development of these tumors.

Methods: We have studied the expression of the different isoforms of VEGF and VEGF receptor (VEGFR) using immunochemistry (IHC, VEGFA and VEGFR1) and real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) (VEGF A/B/C/D and VEGFR1/2/3) in tumor cells (Schwann cells or fibroblastic cells), in a series of 13 MPNST and 26 deep NF (17 plexiform NF and 19 diffuse NF) from NF1 patients. Micro-vascular density (number of capillaries in 20 fields X400) and endothelial cells activation (percentage of pAKT+ and pERK+ endothelial cells in 10 capillaries) were quantified in tissue sections.

Results: IHC study showed higher positive staining for VEGFR1 in MPNST and plexiform NF in comparison with diffuse NF (p=0.05) and for VEGFA in MPNST only (p=0.01). qRT-PCR showed overexpression of VEGF and VEGFR isoforms transcripts in MPNST, especially VEGFC (p=0.03) and VEGFR2 (p<0.001). Micro-vascular density was significantly higher in NF than in MPNST (p=0.0025), with no difference regarding the expression of endothelial cells activation markers.

Conclusions: As already reported in cell lines, tumor cells of MPNST co-express VEGF and VEGFR isoforms. The expression appears quantitatively more important than in deep NF but is paradoxically associated to a reduced micro-vascular density. This may suggest that the VEGF VEGFR pathway could play an oncogenic role, rather by autocrine/paracrine activation of tumor cells than by stimulation of angiogenesis in the tumor environment.

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A. Expression of VEGFA and VEGFR1 in representative examples of plexiform NF and MPNST samples, showing the co-expression of both markers in tumor cells (arrowheads: controls on vessels; N: necrosis; immunostainings with monoclonal antibodies developed with DAB or AEC, x100 or x200 original magnifications).

B. Heat-map showing the quantitative expression of VEGF and VEGFR isoforms in a subset of tumors.
Identification of a Therapeutic Time Window That Improves Vision in an NF1-Deficient Optic Pathway Glioma Mouse Model

Miriam Bornhorst, *Children's National Health System*

**Background:** Optic pathway gliomas (OPGs) are glial tumors that arise early during development in 15-20% of patients with Neurofibromatosis Type I (NF1). While chemotherapy is often used for patients who have growing tumors and/or vision loss associated with the tumor, a minority of patients have improvement in their vision following treatment. Thus, a new approach to treatment is needed to improve outcomes in this patient population.

**Methods:** In our laboratory, we have a *Nf1*-deficient conditional knockout mouse model that develops optic pathway gliomas around 60 days of age (P60). These mice have associated axon degeneration, loss of retinal ganglion cells, and evidence of vision loss on behavioral testing, thus making this a good model to use in order to identify the optimal therapeutic time window that improves or prevents vision loss. In this study, we treated NF1-deficient mice with a Mek-inhibitor during different developmental time windows and measured response to treatment in terms of nerve cellularity, axon integrity, and retinal ganglion cell loss.

**Results:** We found that response to treatment is dependent on when the therapy is initiated during OPG development, and the best response occurred when therapy was initiated early during this process.

**Conclusions:** This study suggests that the timing of therapy initiation is critical for optimal outcomes in NF1-associated OPGs.


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ASD-like Social Behavior Deficits in Mouse Models for Neurofibromatosis Type 1 and Legius Syndrome

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**Background:** Neurofibromatosis type 1 and Legius syndrome are two related RASopathy disorders, stemming from mutations in the RAS–MAPK pathway, in the *NF1* and *SPRED1* genes respectively. Neurofibromin is a RAS-GAP protein, negatively regulating activation of RAS, and SPRED1 interacts with neurofibromin. Both disorders have similarities, including neurological problems that span cognitive deficits and increased incidence/reports of autism spectrum disorder (ASD), however the presentation is very different. Mouse models for these disorders, *Nf1+/-* and *Spred1-/-* mice, exhibit cognitive deficits consistent with human phenotypes, but little is known about the ability of these models to recapitulate the ASD-like symptoms present in patients, and what the molecular mechanisms underlying such phenotypes are.

**Methods:** Here we examined social behaviours in *Spred1-/-* and *Nf1+/-* mice, to ask if social deficits are observed in these mouse models, and whether any observed deficits depend upon RAS–MAPK signalling.

**Results:** *Spred1-/-* mice displayed abnormal social behavior in the automated tube test compared to wildtype controls, and also impairments in nesting behaviour. Studies in *Nf1+/-* mice also demonstrated social deficits in the tube test. Social deficits in *Spred1-/-* adult mice could be reversed in by acutely inhibiting the RAS-MAPK pathway with MEK inhibitors. Expression of *Spred1* mRNA was seen in both inhibitory and excitatory neurons in the mouse brain, similar to the known localisation of *Nf1* in these cell types, indicating that further investigation of the relative contribution of these cell types to social behaviour in mouse models for RASopathy disorders is warranted.

**Conclusions:** These findings indicate that social deficits relevant to ASD can be modelled in *Spred1-/-* and *Nf1+/-* mice, and suggest that RAS–MAPK pathway over-activation can underpin these deficits.

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Funding: Children's Tumor Foundation Young Investigator Award (SCB), FWO postdoctoral fellowship (HB), KU Leuven Opening the Future
Targeting NF1-Dysregulated Tumors Using a Synthetic Lethal Approach

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Neurofibromatosis type 1, a genetic disorder caused by loss of the gene neurofibromin 1 (NF1), predisposes patients to the development of aggressive, NF1-deficient neurological tumors, including malignant peripheral nerve sheath tumors (MPNSTs), highly resistant sarcomas with a 5-year survival of 16-38%. While there are clinical trials investigating new therapies for NF1-deficient MPNSTs, such as MEK inhibitor therapy, there still exists a critical need for novel therapeutics. Our group addressed this clinical unmet need by developing a yeast-based screening platform to identify compounds that are synthetic lethal with NF1 loss. Yeast lacking the NF1 homolog IRA2 (NF1 mutant) were screened with drug-like compounds representative of a >300,000 compound library. Compounds were considered a hit if they inhibited the growth of NF1 mutant cells at concentrations that had no effect on wild-type control strains. Our first screen, carried out in collaboration with Dr. Nancy Ratner at the University of Cincinnati Drug Discovery Center, also included testing compounds in NF1 wild-type and mutant MPNST cell lines, and resulted in the identification of our first lead compound UC1. Using a high-copy suppressor screen, we identified CDK9 as UC1’s target. In a second screen carried out in our yeast platform, we identified several other lead compounds, including Y100 and Y102. Y102 treatment resulted in the accumulation of reactive oxygen species and the autophagy marker p62, as well as an altered mitochondrial phenotype in NF1-dysregulated cells. Treatment with Y102 also resulted in accumulation of the mitophagy marker BNIP3L/Nix, and increased localization of lysosomes to the perinuclear region of the cell. Together our findings suggest that Y102 prevents lysosomal-directed mitochondrial clearance. Currently, we are implementing proteomic strategies to identify cellular targets of Y102 as well as Y100. I hypothesize that NF1-deficient cancers can be selectively targeted with the proposed mitophagy modulator Y102 and that modulation of autophagy/mitophagy is an effective therapeutic strategy in cancers with NF1 loss. We expect that this work will result in the identification of new targets and therapeutic leads for aggressive neurological cancers driven by NF1 loss like MPNSTs.

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Characteristics of NF1-Inactive Periosteal Derived Cells

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Background: The periosteum plays an important role in osteogenesis, both during development and fracture repair. Periosteal derived cells (PDCs) were shown to be an important source of mesenchymal stem cells (MSCs) during this process. MSCs are primarily characterized by their tri-lineage differentiation potential, to the adipogenic, chondrogenic and osteogenic lineage. Recently we found a somatic NF1 mutation in cells cultured from the pseudarthrosis site from all resections taken during the first intervention and in 60% of later surgeries removing pseudarthrosis tissue. The same somatic mutation was found in PDCs from periosteum proximal and distal to the pseudarthrosis site in three extensively sampled individuals (2 tibiae and 1 radius). Therefore we tested the functional effect of NF1 inactivation on PDCs, since we suspect them to be involved in the development of the NF1-related pseudarthrosis.

Methods: Tri-lineage differentiation was induced to the adipogenic, chondrogenic and osteogenic lineage. Differentiation was quantified using staining and qPCR. Also, VEGF secretion in the cell culture medium was determined.

Results: A pilot experiment uncovered diminished osteogenic differentiation in the NF1-inactive PDCs. Chondrogenic and adipogenic differentiation were also reduced in these cells. Strikingly though, PPARG expression was increased in undifferentiated NF1-inactive PDCs and small lipid droplets were observed in the cytoplasm of these cells. Furthermore, VEGF secretion was increased in the NF1-inactive PDCs compared to controls.

Conclusions: NF1 inactivation diminishes the MSC characteristics of PDCs. Tri-lineage differentiation was reduced in these cells, as was VEGF secretion. The extent of the effect of NF1 inactivation on PDCs is being investigated in ongoing functional characterizations. Of special interest to us is the unexpected finding of lipid droplets and increased PPARG expression in undifferentiated NF1-inactive PDCs.

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NF1 Heterozygosity Fosters De Novo Tumorigenesis But Impairs Malignant Transformation

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Background: NF1 biallelic inactivation in Schwann cell lineage and melanocytes trigger the development of a characteristic set of benign tumors and hamartoma (neurofibromas, café-au-lait-macules) present in >99% of NF1 patient by the age of 20. Strikingly, progression to malignant skin cancer was rarely if ever reported. However, recent sequencing efforts revealed that the NF1 gene is frequently mutated in malignant tumors such as melanomas and squamous cell carcinomas. This is puzzling because NF1 patients are not typically associated with these malignancies even though they already have a predisposing NF1 mutation in all cells. We reasoned that individual from the general population are actually more prone to certain malignant tumors than NF1 patient and hypothesized that the NF1+/− cells from the microenvironment is impairing certain malignant transformation in NF1 patients.

Methods: We monitored the kinetic of benign tumor onset and benign to malignant conversion rate in mice with NF1+− and NF1−− background in two mouse models. In addition, we performed retrospective analysis of published clinical data of benign and malignant tumor commonly associated with NF1 patients as well as those with high frequency mutation for the NF1 gene in the general population.

Results: Both the NF1−− and NF1+− mice develop papilloma (benign) but the later develops it slower and further progress to squamous cell carcinoma (malignant) in the two steps carcinogenesis model. Similarly, PLP-CreERT2; NF1f/f mice develop plexiform neurofibroma (benign) faster than their PLP-CreERT2; NF1f+ littermates and only the later spontaneously develop MPNST more robustly. This is corroborated by the clinical data where NF1 patients develop much more benign tumors than advanced malignant/metastatic tumors; and non-NF1 patient rarely develop the benign NF1-related tumor sporadically but do spontaneously gets NF1 inactivation in malignant tumor not frequently observed in NF1 patient.

Conclusions: NF1 heterozygosity has an antagonistic role in tumor initiation and malignant transformation. It suggests that NF1 patients would develop cancer much more frequently if it was not due to the protective effect of the NF1 heterozygosity on cancer progression. It predicts that identifying the cells and factors responsible for the malignant transformation impairment could potentially be translated into a general anti-cancer strategy for NF1 patients and beyond. JPB and CPL are CTF YIA. This work was also supported by the NCI and the US DoD.

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Reprogramming Vestibular Schwanoma Cells: Generation of Patient-Specific NF2(+/−) and (−/−) Induced Pluripotent Stem Cells (iPSC)

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**Background:** The development of bilateral Vestibular Schwanomas (VS) in NF2 patients can lead to total hearing loss, representing one of the most aggressive manifestations of this disease. There is the necessity to understand the formation of these tumors in order to develop new therapies to prevent their formation and growth. In our laboratory, we have expertise in culturing Schwann cells from both NF1 and NF2-related tumors. However, primary cultures are perishable and can only be used for a limited number of experiments. To overcome this problem, we have recently generated NF1(+/−) and NF1(−/−) induced pluripotent stem cells (iPSC) from plexiform neurofibromas and differentiated them up to Schwann cells. The objective of this project was to establish a similar iPSC-based model for VS of NF2 patients.

**Methods:** Tumor samples were kindly provided by NF2 patients from the Spanish Reference Centre (CSUR) on Phakomatoses HUGTiP-ICO. We selected three VS from three independent NF2 patients, one of them being a mosaic, with known constitutive and somatic mutations. Tumors were dissociated and plated in Schwann Cell Media. Reprogramming was performed using Sendai virus containing the 4 Yamanaka factors. iPSC clones were genotyped for NF2 germline and somatic mutations. Selected iPSC clones were further characterized for pluripotency and differentiation potential.

**Results:** We obtained iPSC clones from the three VS reprogrammed with a different efficiency. We obtained NF2(+/−) iPSC clones from two tumors. From the VS excised from a mosaic patient, we obtained NF2(−/−) and NF2(+/+). NF2(+/−) and (+/+) iPSC clones exhibited the typical morphology and growth pattern of iPSC colonies. However, all NF2(−/−) iPSC colonies exhibited an aberrant growth pattern and morphology, difficulting their culture and expansion. These results are consistent with recent findings pointing to NF2 as a growth restricting gene in pluripotent stem cells. Nonetheless, some of these clones have been characterized for pluripotency and differentiation potential.

**Conclusions:** As far as we know, this is the first time NF2(−/−) iPSCs have been obtained directly from VS of NF2 patients. This resource constitutes a non-perishable source of cells for disease modelling and for assaying new therapeutic strategies for VS.

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Mutational Spectrum by Phenotype: Patients with Clinical Suspicion of RASopathy and Children with Multiple Café-Au-Lait Macules Tested with a Custom NGS Panel

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Background: The RASopathies are a clinically defined group of hereditary syndromes caused by germline mutations in genes that encode components of the RAS/MAPK pathway. These disorders include Neurofibromatosis type 1 (NF1), Legius syndrome and Noonan syndrome, among others, and are caused by mutations in more than 20 genes. Many of these conditions exhibit numerous overlapping phenotypic features, especially during childhood, making clinical diagnosis difficult. The sequencing capacity provided by NGS-based panels allows the genetic testing of all RASopathy genes in a single assay facilitating a genetic diagnostics of patients with inconclusive clinical presentations, to provide a timely appropriate management for each disorder.

Methods: I2HCP v2.2 custom panel was validated for its use in the genetic testing of RASopathies using a set of 29 samples with known mutations in RASopathy genes. Once validated, I2HCP v2.2 was used to analyze 48 children with a clinical suspicion of a RASopathy. In addition, 94 unrelated children with multiple café-au-lait macules (CALMs) with or without other symptoms non-related to NF1 were genetically tested and also 87 patients fulfilling NIH clinical criteria for NF1. NGS-based test was performed from gDNA. NF1 and SPRED1 gene analysis were complemented by MLPA and cDNA analysis.

Results: The sensitivity and specificity of I2HCP v2.2 in RASopathy gene testing was greater than 99%. We identified pathogenic variants in 22 out of 48 patients with clinical suspicion of RASopathy, with mutations in the NF1 gene accounting for 10% of the cases in this group. Furthermore, 48% of children with only CALMs carried an NF1 pathogenic variant and 4% a SPRED1 pathogenic mutation. When in addition to CALMs other clinical manifestations were considered, mutations in the NF1 gene represented from 43% to 80% of the individuals, depending on each clinically defined group. Finally, the NF1 mutational spectrum in children with clinical suspicion of NF1 was different from the one in patients fulfilling NF1 NIH clinical criteria.

Conclusions: The I2HPC NGS-panel has been validated for its use in the genetic testing of patients with clinical suspicion of a RASopathy. In 10% of these patients we identified a pathogenic mutation in the NF1 gene. The I2HPC genetic testing strategy could also be applied to children with only CALMs as a clinical manifestation of NF1. At least in 50% of these children a mutation in the NF1 or SPRED1 genes could be identified.


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A New Mouse Model of Neurofibroma, Atypical Neurofibroma, and Peripheral Nerve Sheath Tumors

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Background: Plexiform neurofibromas (PN) are histologically benign peripheral nerve sheath tumors that occur in 25-50% of patients with the autosomal dominant tumor predisposition syndrome neurofibromatosis type 1 (NF1). PN can cause substantial morbidity and are believed to serve as precursor lesions for atypical neurofibromas (ANF). Many ANF show CDKN2A (Ink4/Arf in mouse) loss, and may histologically show atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) characteristics. ANF themselves may be precursor lesions to malignant peripheral nerve sheath tumors (MPNST), a highly aggressive soft tissue sarcoma with no effective medical therapy.

Methods: In an attempt to develop an accurate model of neurofibroma to ANF to GEM-PNST we generated a mouse model based on Joseph NM, et al., Cancer Cell. 2008, in which 26% of genetically engineered mice developed (GEM)-PNST (Nf1+/-;Ink4a/Arf-/-), and our previously described neurofibroma model (DhhCre;Nf1+/fl/fl) Wu J., et al., Cancer Cell. 2008. We generated cohorts of DhhCre; Nf1+/fl/fl; Ink4a/Arf+/- and DhhCre; Nf1+/fl/fl; Ink4a/Arf-/- mice.

Results: DhhCre; Nf1+/fl/fl; Ink4a/Arf+/- and DhhCre; Nf1+/fl/fl; Ink4a/Arf-/- mice showed poor survival versus Ink4a/Arf controls. Each cohort developed superficial GEM-PNST, which histologically were Grade 2/3 GEM-PNST (40%). Histology showed areas of GEM-PNST (superficial) arising from ANF. Each cohort also developed paraspinal tumors, half of which showed neurofibroma histology, and half ANF histology. While most DhhCre; Nf1+/fl/fl; Ink4a/Arf-/- mice died rapidly and of unrelated disease, half of DhhCre; Nf1+/fl/fl; Ink4a/Arf+/- mice died of NF-tumor-related pathologies.

Conclusions: We have developed a new model of progression from neurofibroma to GEM-ANF to GEM-PNST. DhhCre; Nf1+/fl/fl; Ink4a/Arf+/- mice may be useful as a preclinical therapeutic model of neurofibroma transition to ANF and GEM-PNST.

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Identification of Silvestrol-Related Rocaglates with Better Bioavailability and High Potency Against Malignant Peripheral Nerve Sheath Tumors

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) frequently overexpress eIF4F components, and the eIF4A inhibitor silvestrol effectively suppresses MPNST growth. However, silvestrol has suboptimal drug-like properties, including a bulky structure and poor oral bioavailability. Our objectives are to identify potent silvestrol-related rocaglates and to determine their bioavailability, anti-tumor effects, and mechanisms of action.

Methods: NF1+/+ STS26T and NF1-/- ST8814 MPNST and 697 and silvestrol-resistant 697-R leukemic cells were treated with various concentrations of each rocaglate. Cell proliferation assays, flow cytometry, Western blots, and pharmacokinetic (PK) analysis were performed. A quantifiable, orthotopic NF1-deficient MPNST mouse model and immunohistochemistry were conducted to assess antitumor effects.

Results: Among 10 silvestrol-related rocaglates lacking the dioxanyl ring examined, didesmethylrocaglamide (DDR) and rocaglamide (ROC) had potent growth-inhibitory activity comparable to silvestrol in MPNST cells. Structure-activity relationship analysis revealed that the dioxanyl ring in silvestrol was dispensable while the C-8b hydroxyl group was essential for cytotoxicity. DDR and ROC arrested MPNST cells at G2/M and significantly increased the sub-G1 fraction. Accordingly, these rocaglamides induced cleavage of caspases 3 and 7 and poly(ADP-ribose) polymerase, while decreasing total protein levels of these apoptotic markers, consistent with translation inhibition. Additionally, DDR and ROC reduced the levels of mitogenic kinases AKT and ERK1/2. Unlike silvestrol, DDR and ROC inhibited proliferation of silvestrol-resistant 697-R leukemic cells, which over-express the MDR1 multidrug transporter, at IC50 values similar to silvestrol-sensitive 697 cells, suggesting that these rocaglamides may be more bioavailable. PK analysis confirmed that ROC had 50% oral bioavailability. Importantly, ROC, when administered intraperitoneally or orally, potently suppressed the growth of luciferase-expressing NF1-/- ST8814-Luc MPNST xenografts with no overt toxicity. Treated tumors had abundant phospho-histone H3 labeling and more cleaved caspase 3-positive cells, consistent with G2/M arrest and indicative of increased apoptosis, respectively.

Conclusions: The more favorable drug-like properties and potent anti-tumor effects suggest that ROC and DDR have potential to become viable MPNST treatments.

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Support: CancerFree Kids, the US Department of Defense, and National Cancer Institute, NIH
Differential NF2 Gene Status in Sporadic Vestibular Schwannomas and its Prognostic Impact on Tumour Growth Patterns

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Background: The great majority of sporadic vestibular schwannomas (VSs) are due to the inactivation of the NF2 gene. Some tumours remain stable for years, whereas others grow relatively fast. However, the biological background of these phenotypical heterogeneities is largely unknown.

Methods: A total of 282 patients with sporadic VSs were included in a multi-center study. Bidirectional sequencing was conducted to detect microlesions in the NF2 gene. To identify large/exonic deletions, we used a commercial MLPA kit for the analysis. Immunoblot analysis, Immunohistochemistry and Immunofluorescence were conducted to evaluate the expression levels of merlin proteins.

Results: We found age-dependent differences in the clinical parameters of sporadic VSs. Young patients were characterized by progressive tumour behaviours, including earlier onset of initial symptoms, shorter symptom duration and larger tumour size. An increased rate of “two-hits” of both NF2 alleles, usually by mutation and allelic loss, was observed in young cases compared to older, and this correlated with the loss of protein and mRNA expression. Following the loss of NF2 expression, schwannoma cultures demonstrated increased proliferation rates.

Conclusions: We have identified a correlation between the NF2 status and the growth patterns of sporadic VSs. The treatment decision-making, microsurgery or “wait and scan” strategy, should be carried out according to the tumour’s genetic background.

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Developmental Origin and Spatiotemporal NF1 Loss of Heterozygosity Give Rise to Different Types of Cutaneous Neurofibroma

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Background: Dermal or cutaneous neurofibromas (cNF), a Schwann cell tumor of the peripheral nerves in the skin, affect more than 95 percent of adults with Neurofibromatosis Type 1 (NF1) and are a major source of emotional, physical and social distress as NF1 patients can have thousands of these tumors covering most of their skin. Thus, patients with NF1 often identify these tumors as their greatest burden. To date, there is no available medical treatment for cNF, no known way to prevent them from developing. The major barriers that impede progress in this field are the lack of accurate models of these common cNF tumors for drug evaluation and a limited understanding of their pathogenesis as well as the identity of specific cell of origin that directly gives rise to cNF. In this regard, mouse models are important tool for elucidating the molecular mechanism and preclinical drug screening. Ironically, none of the numerous NF1 mouse models developed so far recapitulate cNF.

Methods: We take advantage of genetic labeling for cell lineage tracing to identify mouse neural crest Cre lines that are expressed in the sub-population of Schwann cell lineage that give rise to cNF when NF1 is deleted.

Results: We discovered that a Homeobox B transcription factor serve as the lineage marker to trace the developmental origin of cNF neoplastic cells that completely recapitulates human neurofibromatosis, generating a novel mouse model that spontaneously develops both cutaneous and plexiform neurofibroma. In addition, we discovered that the modulation of the Hippo pathway acts as a modifier to promote neurofibromagenesis, suggesting that dampen the Hippo pathway may serve as part of the comprehensive treatment approach for neurofibroma.

Conclusions: This study provides insights into the developmental origin of cNF, the most common tumor in NF1, and generates the first mouse model that faithfully recapitulates both human cutaneous and plexiform neurofibroma. This novel mouse model has begun to yield vital clues to neurofibroma pathogenesis and now opens the doors for deciphering the evolution of cNF to identify potential effective therapies.

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Funding: J.P.B and C.P.L are recipients of the YIA from Children’s Tumor Foundation. This work was supported by funding from the National Cancer Institute, the US Department of Defense, the Giorgio Foundation, the Neurofibromatosis Therapeutic Acceleration Program and the NF1 Research Consortium Fund.
Mechanotransduction and NF1 Loss Partner in Crime: A “Mature” Story

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Background: In several tumor models, the potent activation of Ras/Raf/ERK signaling by a mutated Ras oncogene drives the initiating steps of cell transformation. In NF1, neurofibromin loss leads to a modest decrease of RAS-GTPase activity, which in turns leads to increased Ras activation. Thus, Ras activation alone does not trigger transformation of NF1−/− Schwann cells (SCs) (1), but requires further molecular hits to drive neoplastic progression (2). Mouse models of NF1 revealed that Ras-mediated transformation of SCs probably relies on a step-wise process that integrates circuits of amplification signals from the local niche, whose the major component is the extracellular matrix (ECM), whose mechanical and biochemical properties provide environmental cues influencing cell behavior (3). We have proposed the hypothesis that the ECM contributes to neurofibroma development and progress towards malignancy. Our hypothesis is sustained by the following observations i) in pancreatic and breast cancer progression models, loss of tensional homeostasis helps Ras oncogenic transformation (4); ii) Neurofibromas are composed of a fibrotic milieu; iii) our previous data show that primary NF1−/− SCs formed 3D colonies in stiff Matrigel and showed FAK/Src axis hyperactivation.

Methods: We settled a new 3D cellular model in vitro of soft and stiff matrix that we investigated by immunofluorescence and biochemical means.

Results: In the present report, we confirm our preliminary data in vitro in a 3D experimental model using immortalized SCs from plexiform neurofibroma. In the effort to elucidate how these cells transduce mechanical signals, we found a FAK-dependent activation of AKT, which disrupts the degradation complex and activates the oncogenic transcription factor beta-catenin. RHO/YAP transduction axes, in a collagen rich, stiff matrix further potentiate this process. Further, SCs build up a platelet growth factor autocrine loop and as carcinoma cells, they express a collagen linker enzyme—lysyl oxidase (5)— and secrete increased amounts of metalloproteinases MMP-2 and 3 and IL-8.

Conclusions: These findings point towards an important role of the fibrotic process not only in sustaining, but also in triggering neurofibroma progression.

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Genomic Variants Enriched in Patients with Plexiform Neurofibroma

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**Background:** Bi-allelic NF1 gene mutations characterize benign plexiform neurofibroma (PNF) Schwann cells (SC). Although no other genomic changes have been identified to explain PNF tumorigenesis, clinical studies imply that formation of PNF may require additional mutations.

**Methods:** We performed the Whole-Exome Sequencing (WES) using sorted SCs and their matched fibroblasts (FBs) from 8 NF1 patients. Rare non-synonymous germline and somatic variants were predicted by GATK, MuTect1, VarScan2, Strelka, and SomaticSniper. We also re-analyzed independent dermal neurofibroma (DNF) and PNF sequencing data sets to verify our findings. We measured genomic instability of PNF tumors and DNF SC in tissue sections and cultured cells, and used cell culture to define how loss of the DNA repair protein ATM affects SC and SC precursors.

**Results:** We identified possibly pathogenic variants in 47 genes, in addition to the known driver mutations in NF1. None of them have previously been evaluated in the context of the NF1 tumorigenesis. Somatic variants in SC were present at low read number, in few tumors, and were largely missense alterations of unknown significance of uncertain relevance to tumorigenesis. Importantly, we identified frequent, probably damaging germline variants in 16 genes, including ATM, many of which showed decreased gene expression. Phenotypes relevant to the DNA repair-related gene ATM were present at elevated levels in a subset of neurofibromas, and of neurofibroma SC. In mice, the Atm gene loss promoted SC precursor self-renewal, and increased tumor formation in vivo. Thus, genetic alteration of ATM may modify NF1 pathogenesis driven by NF1 mutations.

**Conclusions:** Recent studies identify ATM germline risk alleles for many sarcoma types. Our finding of increased mutational burden in ATM-mutant SC, not fibroblasts, suggests that, in SC with biallelic NF1 mutations and elevated RAS-GTP, the effects of ATM variants are enhanced. The DNA comet assay also suggests that DNA damage repair capacity of SC from some DNF is reduced. Genes, including ATM, showing common germline variants in neurofibroma are potential modifiers of tumor formation and/or transformation to malignancy, and may contribute to clinical variability. The DNA comet assay also suggests that DNA damage repair capacity of SC from some DNF is reduced.

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This work was funded by NIH-R37-NS083580 (NINDS Javits Neuroscience Investigator Award to NR) and an Innovation Award from Cincinnati Children’s Hospital (NR)
Are Reading and Motor Skills Related to Sequential Learning and Motor Adaption in Children with Neurofibromatosis Type 1?

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**Background:** About half of children with Neurofibromatosis type 1 (NF1) have learning disabilities (Cutting & Denckla, 2003), including deficits in reading and/or motor learning. In the case of neurodevelopmental disorders (APA, 2013), reading and motor difficulties have been related to a deficit in the procedural learning system (model of Nicolson & Fawcett, 2007), within which two learning processes have been distinguished: sequential learning and motor adaptation (model of Doyon and Benalli, 2005). The present study aims to better characterize learning disabilities in reading and motor skills from the rationale provided by these models.

**Methods:** Twenty-three children with NF1 (10;2 ± 1;6 years, 16 girls) participated in the study. Parents and children gave their informed consent prior to the experiment, approved by the local ethics committee. Reading and motor skills were respectively evaluated with the Alouette test (Lefavrais, 2005) and the French M-ABC motor test (Soppelsa & Albaret, 2004). Two experimental tasks were conducted, one requiring a motor adaption, namely writing trigrams in conventional (from left to right) versus unconventional directions, and one requiring a sequential learning, namely learning a new ideogram following the correct sequence of strokes.

**Results:**
- **Motor adaptation.** A cluster analysis completed with an ANOVA revealed two categories of children: a first cluster including 12 children whose performance was not different between the two writing directions, and a second cluster including 11 children in whom this difference was significant ($p < 0.001$). The M-ABC score was lower for the cluster 2 than for the cluster 1. The Alouette scores did not differ between the clusters.

- **Sequential learning.** The analyses revealed two categories of children: a first cluster including 13 children in whom the learning phase was correct, and a second cluster including 10 children in whom the sequential errors were greater. The difference between the clusters was not significant for the scores obtained in the Alouette and M-ABC tests.

**Conclusions:** A link between the performance in the adaption task and motor skills was evidenced in NF1 children. No link was observed between reading skills and performance in both tasks. This finding runs counter the reference to the model of Nicolson & Fawcett (2007) concerning learning disabilities in reading and motor skills in children with NF1.

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Disclosure of Interest: J. Danna has a conflict with: None. This work has benefited from support from the French Government, managed by the French National Agency for Research (ANR), under the project title DYSTAC-MAP (ANR-13-APPR-0010), M. Jover: None Declared, S. Ducrot: None Declared, J.-L. Velay: None Declared, J.-M. Albaret: None Declared, F. Audic: None Declared, Y. Chaix: None Declared
Combining Cre/loxP and CRISPR/Cas9 Technology for In Vivo Studies of Myeloid Cell Function in MPNST

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Background: NF1 haploinsufficiency is a hallmark of Neurofibromatosis Type 1. NF1 +/- myeloid cells are key players in neurofibroma development, but the mechanisms driving myeloid cell function in malignant peripheral nerve sheath tumors (MPNSTs) are poorly understood. Using mouse models, we have shown that NF1 +/- bone marrow can accelerate MPNST initiation, which is characterized by elevated levels of tumor-infiltrating mast cells and CD11b+ myeloid cells. Importantly, mast cells are also enriched in human NF1-associated MPNSTs, suggesting they may be attractive therapeutic targets. While our recent findings underscore the importance of NF1 +/- myeloid cells in MPNST biology, further work is needed to understand the significance and molecular mechanisms of these immune cells.

Methods: To further define the population of myeloid cells influencing MPNST initiation, we have developed unique mouse models that combine CRISPR/Cas9 tools with Cre-locP-driven, lineage-specific cell depletion. We recently established technology to generate CRISPR-induced MPNSTs in wild type mice by injecting the sciatic nerve with Adenovirus expressing Cas9 and guide RNAs to NF1 and p53. We are combining this CRISPR/Cas9 somatic tumorigenesis approach with Cre-locP control of immune cell populations to determine the role of myeloid and mast cells in MPNST formation. Using lineage-specific Cre drivers, we are generating MPNSTs in mice where myeloid or mast cells are conditionally or temporally depleted.

Results: Using mice with Cre-inducible diphtheria toxin (LSL-DTA) crossed to a mast cell-specific promoter (Mcp5-Cre), we have successfully depleted mast cells in multiple tissues, including CRISPR/Cas9-generated MPNSTs. Deletion of mast cells was sufficient to slow MPNST initiation in this mouse model. Flow cytometry analysis of MPNSTs from Mcpt5-Cre; LSL-DTA mice showed that mast cell deletion alters the immune cell infiltrate of the tumors.

Conclusions: By combining CRISPR/Cas9 somatic tumorigenesis technology with sophisticated Cre-locP control of the tumor microenvironment, we are able to differentially manipulate tumor cells and the surrounding stroma to investigate mechanisms of myeloid and mast cell function in MPNSTs. These experiments have identified a fundamental role for mast cells in MPNST initiation, and current studies are investigating the mechanisms influencing mast cell and monocyte function in NF1-associated MPNSTs.

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Targeting the Serotonin the 5-HT6 Receptor/mTOR Complex to Reverse Cognitive Deficits in Neurofibromatosis Type 1

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Background: Among the 14 serotonin receptor subtypes, the 5-HT, receptor has emerged as a promising target for the treatment of cognitive impairment associated with several neuropsychiatric disorders, including Alzheimer’s disease and schizophrenia: 5-HT6 receptor blockade consistently enhances mnemonic performance in a broad range of procedures in rodents and there is preliminary evidence for pro-cognitive properties of 5-HT6 receptor antagonists in human.

Results: Using mice with Cre-inducible diphtheria toxin (LSL-DTA) crossed to a mast cell-specific promoter (Mcp5-Cre), we have successfully depleted mast cells in multiple tissues, including CRISPR/Cas9-generated MPNSTs. Deletion of mast cells was sufficient to slow MPNST initiation in this mouse model. Flow cytometry analysis of MPNSTs from Mcpt5-Cre; LSL-DTA mice showed that mast cell deletion alters the immune cell infiltrate of the tumors.

Conclusions: By combining CRISPR/Cas9 somatic tumorigenesis technology with sophisticated Cre-locP control of the tumor microenvironment, we are able to differentially manipulate tumor cells and the surrounding stroma to investigate mechanisms of myeloid and mast cell function in MPNSTs. These experiments have identified a fundamental role for mast cells in MPNST initiation, and current studies are investigating the mechanisms influencing mast cell and monocyte function in NF1-associated MPNSTs.

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Characterization of Vestibular Schwannomas in NF2 Using a MALDI Mass Spectrometry Imaging Combined with Microproteomic Approach

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Background: Among the pathways identified controlling schwannoma growth in in-vitro and in-vivo models, none as demonstrated sufficient clinical relevance to validate its clinical use. Therefore, new potential therapeutic targets are needed. The aim of our study was to identify the protein expression and networks involved in NF2 vestibular schwannomas using an unbiased, large scale proteomic approach.

Methods: An in-vitro approach was used to identify the intracellular proteins and secreted proteins from matched primary cultures of mouse Schwann cells harboring either a NF2 competent or NF2−/− status. Then paraffin embedded tissue sections from NF2 schwannomas and non-NF2 vestibular nerves (Tumor Bank protocol CSTMT234) were digested in-situ using FASP-trypsin Digestion. Molecular images are generated from thin tissue section in order to determine the spatial localization of digested peptides. Thanks to unsupervised statistical analysis, we then generated hierarchical clustering of homogeneous molecular regions. According to these regions, microproteomic gave access to large scale protein identification and relative quantification. Statistical analysis was then carried out using ANOVA (threshold p value 0.05) and presented as heatmap and Venn diagram.

Results: A first selection of ten schwannomas operated on NF2 patients and two control non-NF2 vestibular nerves were selected. At the present time, microproteomic analysis were realized on 2/10 tumor tissues. The results on the 8 other tumors are expected for the final presentation. A total of 971 were identified. 626 proteins are common between the two patient and two panel of proteins are specifically overexpressed in each patient. One panel is associated with cellular division process whereas the other one correspond to enzyme regulation. A total of 355 intracellular proteins were overexpressed in the NF2−/− Schwann cells compared to WT cells. DAVID ontology analysis highlighted an enrichment of proteins involved in ribosomes and mRNA translation (p value 9.3E-24) which might be the consequence of mTORc activation in these NF2−/− Schwann cells. A comparison between proteins overexpressed in tumoral tissue and NF2−/− cell line is in process.

Conclusions: This first report demonstrates the possibilities raised by MALDI MSI and combined micro-proteomic approach to identify numerous modifications of protein expression secondary to the loss of the tumor suppressor gene NF2 even from paraffin embedded tissues.

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Increase of Inward Cationic Currents (Ih) with Lamotrigine Partially Corrects Deficits in Habituation Learning in a Drosophila Model of NF1

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Background: Neurofibromatosis Type 1 (NF1) is caused by heterozygous mutations in the gene NF1, encoding the Ras GTPase-activating protein neurofibromin. NF1 individuals present with high (>50%) prevalence of learning difficulties and autism spectrum disorder (ASD). Research in the NF1 mouse model has shown that learning deficits are caused by increased activity of Ras signaling through the classic MAPK pathway. Inhibiting Ras activity with lovastatin reversed these deficits in mice but was not sufficient to significantly improve cognitive impairments in individuals with NF1 in long-term clinical trials.

Recently, it has been shown that attenuation of inward cationic currents (Ih) through the potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1 (HCN1) contributes to learning deficits in the NF1 mouse model. Increase of Ih current with the HCN channel agonist, lamotrigine restored these deficits (Omrani et al., 2015, Mol. Psy.). Whether Ih currents and Ras-MAPK are converging or independent pathways downstream of NF1, and if both need to be corrected to reverse cognitive deficits remains to be investigated.

Methods: We use Drosophila, a powerful model, to investigate the role of neurofibromin (Drosophila NF1) and its downstream effectors. Drosophila genetics allows to dissect the functional relationship between Ih currents and Ras-MAPK by genetic interaction experiments and assess cognitive phenotypes and drug rescue experiments with high efficiency.

Results: We found that decreased Drosophila NF1 expression, increased Ras-MAPK signaling as well as decreased Ih currents cause deficits in habituation, a fundamental form of learning and a prerequisite for higher cognitive functioning. Genetic interaction between Drosophila NF1 and Ih confirmed the involvement of these two proteins in shared cellular process. Increasing Ih currents with lamotrigine partially restored the habituation deficits of Nf1 knockdown flies, providing a suitable system for future combinatorial drug testing.

Conclusions: Our research provides a novel approach to identify targets and treatment strategy to alleviate cognitive defects associated with NF1. Using habituation, a fundamental form of learning and a prerequisite for higher cognitive functioning that can be measured in both patients and flies we increase the translatability of our research. Our model thus offers an unprecedented throughput and flexibility, ideal for pre-clinical testing.

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Disclosure of Interest: M. Fenckova: None Declared, L. Blok: None Declared, M. Coll-Tane: None Declared, M. Voet, van der: None Declared, A. Schenck has a conflict with: The research was funded by European Union’s FP7 large-scale integrated network Gencodys (HEALTH-241995) and DCMN Radboudumc junior research fellowship.

Radioprotective Effect of the Combination of Statins and Bisphosphonates in Cells Derived From Neurofibromatosis Type 1 (NF1)

Nicolas Foray, Inserm

Background: The major molecular radioprotection approaches are generally based on the reduction of the number of radiation-induced DNA damage via anti-oxidant drugs. However, an increasing body of evidence suggests that radiosensitivity is rather caused by unrepaired DNA double-strand breaks (DSB) than induced ones. Furthermore, the number of DSB produced immediately after irradiation does not necessarily condition the number of unrepaired residual DSB. By analyzing hundreds radiosensitive cell lines, the nucleo-shuttling of the ATM protein has been shown to condition the cellular response to radiation. Since the combination of bisphosphonates (zoledronate) and statins (pravastatin) (ZOPRA) results in an enhanced diffusion of ATM in the nucleus, the ZOPRA treatment may lead to a better radioprotection of cells. Here, we investigated such hypothesis in human skin fibroblasts derived from NF1 patients.

Methods: The effect of ZOPRA treatment was analyzed on a collection of 40 cell lines derived from NF1 patients by using immunofluorescence against the major DSB biomarkers.

Results: The ZOPRA treatment appeared to significantly reduce the number of unrepaired DSB and the level of genomic instability specific to NF1 cells. It appeared that statins and bisphosphonates contribute in a supra-additive manner to such effect.

Conclusions: The use of statins and bisphosphonates may be an interesting approach to reduce the radiation-induced risk specifically linked to NF1 syndrome. Further clinical investigations are needed to confirm the hypothesis and ask whether ZOPRA may also contribute to increase the life span of NF1 patients.

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Radiobiological Characterization of Neurofibromatosis Type I: Impact of Neurofibromin Protein on the ATM-Dependent DNA Damage Repair and Signaling Pathway. A Novel Mechanistic Model.

Nicolas Foray, Inserm

Background: Neurofibromatosis type 1 (NF1) is a syndrome characterized by high occurrence of benign and malignant brain tumours and is caused by mutations of neurofibromin. While NF1 was shown to be associated with radiosensitivity, few studies have dealt with the molecular and cellular response of NF1 cells to ionising radiation.

Methods: Here, we examined the ATM-dependent signaling and repair pathways of the DNA double-strand breaks (DSB), the key-damage induced by ionizing radiation (medical X-rays), in skin fibroblasts from NF1 patients.

Results: Neurofibromin mutations are associated with an abnormal abundance of neurofibromin molecule in cytoplasm, independently of irradiation. After irradiation, quiescent NF1 cells showed abnormally low rate of recognized DSB assessed by H2AX immunofluorescence. Irradiated NF1 cells also showed a delayed nucleo-shuttling of phosphorylated forms of the ATM kinase, caused by a specific binding of ATM to mutated neurofibromin in cytoplasm. Lastly, NF1 fibroblasts showed abnormally high MRE11 nuclease activity suggesting high genomic instability. A similar analysis of 40 cell lines derived from NF1 patients consolidate these results.

Conclusions: Our results showed that NF1 belongs to the group of syndromes associated with low but significant defect of DSB signalling and delay in the ATM nucleo-shuttling associated with radiosensitivity and high-cancer proneness. Our findings are therefore consistent with a sequestration of ATM in cytoplasm due to a more frequent formation of the ATM-neurofibromin complexes. The formation of such complexes prevents the diffusion of ATM into nucleus after irradiation and impairs DSB repair and signaling. This mechanistic model about NF1 syndrome and raised the potential radiation-induced risks specifically linked to the NF1 syndrome.

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Tyrosine Nitration Regulates Neurofibromatosis Type 2-Associated Schwannoma Energy Metabolism and Cell Survival

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Background: Neurofibromatosis type 2 (NF2) is a genetic disorder of the nervous system caused by inactivation of the merlin tumor suppressor gene. NF2 patients develop bilateral vestibular schwannomas (VS) for which there is no effective drug treatment. Production of the oxidant peroxynitrite occurs in pathological conditions and leads to tyrosine (Tyr) nitration of proteins. Although Tyr nitration is found in multiple tumor types, its role in tumorigenesis is unknown. Our goal is to discover nitrated proteins that support VS growth as new potential tumor-specific therapeutic targets, enabling the development of safe pharmacological strategies for NF2 schwannomas.

Methods: To prevent Tyr nitration, isogenic human wild-type and merlin-deficient Schwann cells (WT- and MD-HSC) were cultured with and without scavengers and inhibitors of peroxynitrite formation and Tyr nitration. Cell survival and metabolic parameters were assessed using biochemical and molecular methods, and extracellular flux analysis. Nitrated proteins in VS from NF2 patients were identified by immunoprecipitation with anti-nitroTyr antibodies followed by mass spectrometry analysis.

Results: VS and MD-HSC showed significantly increased levels of peroxynitrite and Tyr nitration compared to WT-HSC. Notably, prevention of tyrosine nitration selectively decreased MD-HSC survival. A study of the MD-HSC metabolic phenotype revealed a shift in energy metabolism compared to WT-HSC characterized by decreased levels and activity of mitochondrial oxidative phosphorylation (complex I to IV), decreased mitochondrial oxygen consumption and membrane potential, and increased glycolysis and glutamine dependency. Prevention of Tyr nitration reversed the metabolic phenotype of MD-HSC back to that of WT-HSC. We previously identified nitrated Heat shock protein 90 (Hsp90) as a key inhibitor of mitochondrial activity in tumor cells. Nitrated Hsp90 was present in VS and MD-HSC but not in WT-HSC, representing an excellent target for drug development. We also identified 5 additional nitrated proteins in VS related to pathways deregulated in NF2.

Conclusions: Nitration induces a metabolic reprogramming and supports survival of human NF2-associated schwannoma cells. Identification of the signaling pathways regulated by specific nitrated proteins that promote schwannoma growth could provide additional novel targets for the treatment of NF2 and possibly other tumor types as well.

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Genomic Structural Alterations as a Driving Force in MPNST Development

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Background: Malignant peripheral nerve sheath tumors (MPNST) are soft tissue sarcomas with bad prognosis and lack of curative treatments. NF1 patients have an 8-13% lifetime risk of developing an MPNST. MPNST may arise from a preexisting benign plexiform neurofibroma (PNF), often after the formation of a pre-malignant distinct nodule termed atypical neurofibroma (aNF).

Methods: We generated genomic structure (SNP-array), mutational (exome), transcriptomic (RNA-seq, microarray) and epigenomic (DNA methylation) data from a set of 15 MPNSTs, and also collected available data on MPNST, plexiform and atypical neurofibromas. We performed an integrative bioinformatic analysis this data to infer the mechanisms of MPNST development.

Results: Regarding the genomic structure, PNFs have no structural alterations except those affecting chromosome 17q involved in the somatic inactivation of NF1. In addition to mutation of both NF1 alleles, aNFs present additional recurrent losses of the CDKN2A/B locus, involving the 9p arm. Contrasting with these nearly-normal karyotypes, the MPNSTs studied have hyperploid and highly rearranged genomes with somatic copy number alterations (SCNA) affecting most chromosomes. However, MPNST genome structure is highly stable. In contrast to SCNAs, MPNSTs have a low number of point mutations, with no clear recurrently affected genes. Most point mutations appear to be acquired after the genome reorganization.

This collective data suggest a model for MPNST origin, with a first progression towards a proliferative cell with reduced senescence due to the loss of NF1 and CDKN2A/B, followed by a number of random catastrophic events of genomic alteration and the selection of a viable stable genomic combination.

Furthermore, SCNA have a profound impact on gene transcription levels and create regions with an accumulation of over- and under-expressed genes, transcriptional imbalances (TI). TIs mostly capture passenger gene expression but allow identification of genes with SCNA-independent expression regulation. The analysis of these genes provides insight into the biology of MPNSTs.

Conclusions: Gross genomic structural alterations are a driving force in MPNST biology and their genomic stability suggest a catastrophic event mediated by loss of senescence capacity as a probable origin.

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PARP Inhibition in Combination with Cytotoxic Chemotherapy as a Therapeutic Option for MPNSTs

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas that occur at increased frequency in individuals with neurofibromatosis type 1 (NF1). These tumors have limited treatment options and a high propensity to metastasize leading to a dismal overall survival. Cytotoxic chemotherapy is basis of first line therapy for metastatic MPNSTs. This alone, however, is very unlikely to result in a durable response. Soft tissue sarcomas including MPNSTs have been shown to have mutations and copy number aberrations in DNA repair genes, suggesting the potential for response to PARP inhibition. Given these data, we decided to assess the response of MPNST cell lines to PARP inhibition.

Methods: We utilized several different MPNST cell lines, including 2 human-derived NF1-associated (96.2 and MP642), 1 human-derived sporadic (STS 26T) and murine Nf1/Tp53-mutant NPcis MPNST cells (JW23.3). Proliferation and cell death were evaluated in vitro using the IncuCyte Live Cell Analysis System (Essen BioScience) in the presence of a PARP inhibitor or drug vehicle. Next, we examined proliferation and cell death in the presence of a PARP inhibitor in combination with cytotoxic chemotherapy or drug vehicle.

Results: We observed decreased proliferation in all cell lines examined as assessed by measurement of cell confluence over 48 hours. Additionally, we observed an increase in cell death as measured by fluorescence.

Conclusions: Taken together this data suggests that a PARP inhibitor in combination with cytotoxic chemotherapy is a potential therapy for soft-tissue sarcomas. Future work is aimed at assessing response to this combination in vivo using both cell lines and patient derived xenografts developed in our lab and will be presented this fall.

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This work was funded by Dr. Hirbe’s start up funds. The PARP inhibitor was supplied by AstraZenica. Dr. Hirbe is supported by the Francis Collins Award through NTAP.
Whole Exome Sequencing Leads to the Identification of TRIM23 as a Potential Suppressor of Metastasis in NF1-MPNST

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas that occur at increased frequency in individuals with neurofibromatosis type 1 (NF1). These tumors have limited treatment options and a high propensity to metastasize leading to a dismal overall survival. While previous studies have employed a variety of discovery approaches to discover genes associated with MPNST pathogenesis, little is known about the genetic events leading to metastasis of these tumors.

Methods: Whole exome sequencing was performed on resection and biopsy materials from two patients with NF1-MPNST. Samples from patient one included primary tumor, a lung metastasis, and a bone metastasis. Samples from patient two included primary tumor, and two different lung metastases. To further explore one interesting target, we used shRNA-mediated knock down of Trim23 expression in Nf1/Tp53-mutant NPcis MPNST cells (JW23.3) to explore the role of Trim23 in MPNST pathogenesis. Subcutaneous and left ventricular (LV) tumor injections were performed to assess the role of Trim23 in growth of a primary tumor and metastasis, respectively. Injections were performed in C57BL/6 ALBINO immunocompetent mice. All human and animal studies were performed under active protocols approved by the Institutional Review Board and the Institutional Animal Care and Use Committee, respectively.

Results: First, we identified the NF1 mutations in each patient as well as copy number loss of CDKN2A across all primary and metastatic samples consistent with these genes serving as drivers of progression. Second, we have identified mutations in TRIM family members enriched in the metastatic lesions. Third, we found that loss of nuclear TRIM23 expression was associated with poor overall survival in humans (p=0.026). Finally, we identified and pursued a member of the TRIM family, TRIM23, with both in vitro and in vivo models, and have demonstrated that decreased expression of Trim23 leads to increased tumor burden and decreased overall survival in a mouse model of MPNST growth and metastasis.

Conclusions: Collectively, the ability to track the molecular evolution of MPNST in two individuals with metastatic disease offers new insights into the genetic events important for metastatic progression and metastasis. Additionally we have identified TRIM23 as a protein that may play a role in MPNST progression. Current work is aimed at better understanding the pathway by which TRIM23 affects MPNST progression.

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Defining the Role of Endoglin in Malignant Peripheral Nerve Sheath Tumor Progression

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**Background**: Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive sarcomas with poor prognosis commonly related to neurofibromatosis type 1 (NF1). Recent data demonstrate that tumor-microenvironment communication plays a crucial role in the progression of these tumors. While soluble factors have been described as the main cell-cell communication mechanism in this crosstalk, the role of secreted exosomes (30-100nm extracellular vesicles) in this scenario is completely unknown. Our previous data characterizing MPNST-derived exosomes highlighted endoglin, a TGF-β co-receptor with an important role in angiogenesis, as one of the top candidates secreted by MPNST cells. Here, we aim to analyze the role of endoglin in MPNST progression and to examine its potential use as a new biomarker and therapeutic target for these tumors.

**Methods**: Exosomes from plasma of NF1 patients in different stages were isolated by ultracentrifugation methods. Endoglin levels were tested in plasma circulating exosomes by ELISA and in human peripheral nerve sheath tumors by immunohistochemistry. Endoglin knockdown was performed in the MPNST cell line STS26T and its influence on gene expression and signaling pathways was analyzed by RNA-Seq and validated by qRT-PCR and Western blot. Moreover, the anti-human and -mouse endoglin antibodies, TRC105 and M1043, respectively, were tested in vivo.

**Results**: Endoglin levels were significantly increased in plasma-circulating exosomes and in peripheral nerve sheath tumors along the progression of the disease. Mechanistically, endoglin knockdown resulted in the downregulation of the BMP and MAPK/ERK signaling and the impairment of several pathways related to pro-angiogenic behavior in MPNST-derived cell lines. Endoglin knockdown also led to the reduction of primary tumor growth and metastasis in vivo. Finally, our data show that blocking tumor and host endoglin function using anti-human and –mouse endoglin antibodies TRC105 and M1043, respectively, significantly reduced MPNST tumor growth and metastasis in vivo.

**Conclusions**: Overall, our data suggest that both tumor and host-derived endoglin play an active role in MPNST progression and support the use of this protein as a new promising biomarker and a potential target to block the progression of these tumors. Moreover, the analysis of exosomal endoglin levels could serve as a novel readout of MPNST malignancy and treatment response using liquid biopsy.

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**Predicting In Vitro Drug Efficacy in Neurofibromatosis via Integration of Protein and Drug-Target Networks**

**Sara Gosline, Sage Bionetworks, Seattle, United States**

**Background:** The development of diverse cell line models of NF1, NF2, and schwannomatosis-related tumors has expanded the ability to profile and model the response of these tumors to various drugs. By coupling high throughput drug screening data with molecular data such as gene expression data or somatic variants, computational approaches can be used to better guide drug development. Common approaches applied to large compendium of cancer cell lines and drug screens - such as the pharmacogenomic scans of Cancer Cell Line Encyclopedia from the Broad Institute and the Cancer RX Gene dataset from the Sanger Institute - identify statistically significant correlations between genomic features of a cell line and its resulting response to a compound of interest. However, these techniques fail in cases where there are not hundreds of cell lines and drugs to compare and are therefore not applicable to rare diseases such as NF.

To overcome the paucity of samples in the modeling of NF cell line data we supplement cell-line-specific gene expression data with quantitative measurements of drug-targeting experiments and protein-protein interaction data to build a network model of drug efficacy.

**Methods:** We collected high throughput drug screening experiments and gene expression data from 1883 published cell lines to test the ability to predict in *vitro* drug efficacy. We used metaViper to identify master regulators that are differentially active between two sets cell lines and integrated these proteins with published protein-protein interaction data (from STRING) and drug-target data derived from our previously-published Drug Target Explorer. We then use the Prize Collecting Steiner Forest (PCSF) algorithm to identify the specific protein-protein interactions that link the master regulators to drugs.

**Results:** We applied this approach to both cancer cell lines and plexiform neurofibroma cell lines. Preliminary results suggest that this approach can not only identify previously tested drugs that can give rise to a differential *in vitro* response but also identify novel, untested drugs.

**Conclusions:** While still in development, network integration and subsequent reduction provides a flexible way to characterize the diverse changes that occur across a set of cells using gene expression data. Moving forward we aim to study the robustness of this network approach and apply it to other NF cell line models.

**A Pan-NF Knowledge Portal**

**Sara Gosline, Sage Bionetworks, Seattle**

**Background:** Recent developments in neurofibromatosis (NF) research have led to a deluge of data derived from diverse experimental assays including cellular imaging, drug dosage experiments, clinical trials, patient surveys and high-throughput approaches such as automated drug screens, DNA sequencing, and gene expression measurements. While some data are being shared via publicly funded data repositories such as the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/), many datasets remain unpublished or hidden in supplemental files of respective publications. The lack of a central, well-curated, and harmonized data repository for NF data makes it difficult to integrate the results of NF studies across the community, ultimately impeding the progress of NF research.

Here we introduce a pan-NF knowledge portal that includes all work funded by Children’s Tumor Foundation (CTF) and Neurofibromatosis Therapeutic Acceleration Program (NTAP) for the past three years. To supplement this work we have also collated other published NF datasets and described ongoing experiments that will be released in the future. This portal represents a milestone in a collaboration between CTF, NTAP and Sage Bionetworks to collect and curate research funded by the respective organizations.

**Methods:** Projects supported by CTF and NTAP uploaded results of their experiments by pre-defined milestones to the Synapse collaborative platform (www.synapse.org). These data were then annotated with a pre-defined metadata dictionary aligned with industry standards to enable facile searching and indexing. This dictionary was then used to build an elastic search-powered web portal to enable users to query and sort the data, view response summaries, and access data.

**Results:** This web portal is currently under development and will launch November 1, 2018 at http://nf.synapse.org with the ability to browse ongoing projects funded across the NF community, as well as data from completed projects. The knowledge portal will be open to all for new submissions for any interested NF researchers.

**Conclusions:** As more data becomes publicly available the pan-NF knowledge portal will serve as a central gathering area for scientists and clinicians interested in studying the disease. Ongoing work includes expanding the knowledge portal to incorporate new types of data as well as building interactive tools to explore specific data sets.

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Identification of Key Candidate Genes and Pathways in NF1 by Integrated Bioinformatical Analysis

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Background: Neurofibromatosis type 1 (NF1) is one of the most frequently hereditary diseases in the world. This study aimed to identified some differentially expressed genes (DEGs) and the involved pathway in NF1 by bioinformatics analysis.

Methods: Four datasets (GSE14038, GSE41747, GSE60082 and GSE1482) of expression microarray were reanalyzed after downloaded from GEO database. Differentially expressed genes (DEGs) were obtained by the ‘limma’ data packet in ‘R’ software. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were analyzed on the DAVID database, and a DEG-associated protein-protein interaction (PPI) network was constructed using STRING and Cytoscape software.

Results: A total of 147 DEGs (including 87 up and 58 down-regulated genes) were identified. GO enrichment analysis showed that those DEGs were mainly enriched in ‘regulation of adiponectin secretion’ and ‘regulation of chemokine biosynthetic process’. KEGG pathway enrichment analysis revealed that the DEGs were mainly enriched in ‘Transcriptional misregulation in cancer’, ‘Toll-like receptor signaling pathway’. Based on the PPI network, MFAP5, EFEMP1, ADH1B, NEGR1, COL3A1, TAC1, COL28A1, SYNPO2 were screened as hub genes.

Conclusions: This bioinformatics analyses identified some key genes which promote the development of NF1. Especially, some key DEGs like MFAP5 and EFEMP1 could be used as a potential biomarker in diagnosis for NF1.

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Figure A&B : The heatmap of the different expression genes
Figure C : GO term enrichment analysis of the differentially expressed genes identified in NF1
Figure D : KEGG enrichment analysis of the differentially expressed genes identified in NF1
Figure E : Protein-Protein-Interactions network analysis of the differentially expressed genes identified in NF1
Phase 0 Trial Investigating the Intra-Tumoural Concentration and Activity of Sorafenib in Neurofibromatosis Type II

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Background: Drug therapies for Neurofibromatosis type 2 (NF2) patients, suffering from schwannomas, meningiomas and ependymomas, are needed. The aim for this study was to establish the pharmacokinetics (PK) and pharmacodynamics (PD) of the PDGFR/Raf inhibitor Sorafenib (Nexavar) in tissue samples from NF2 patients (primary endpoint), as well as to test potential blood biomarkers.

Methods: In this phase 0 trial five NF2 patients received 400mg Sorafenib twice daily (Maximum Tolerated Dose, MTD) for 11 days in-between two tumour biopsies. PM was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and PD by western blotting and immunohistochemistry in peripheral schwannomas (PS) and blood.

Results: Sorafenib was detected in plasma (3316.9 ng/ml-20792.2 ng/ml), and in peripheral schwannoma (PS) samples (1425.89ng/g-6242.06ng/g) from all patients. In respect to PD western blotting on PS samples demonstrated non-significant changes of pPDGFR, pAKT, cyclin D1, and cleaved caspase 3 in all patients; non-significant changes of pERK in four patients and significant reduction in one patient (1/5); non-significant changes of ps6 in three patient (3/5) and significant reduction in two (2/5). All results from PBMC, pPDGR, pERK/ERK, pAKT/ACT were not significant. Immunohistochemistry results were in line with above. Side effects were mostly mild to moderate. A rash in one patient was classed as severe.

Conclusions: In this study we confirmed the usefulness of Phase 0 trials for drug development in NF2 patients. The trial was, however, negative for the primary outcome and patients experienced several side effects.

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Genetic Ablation of Pak1 Extends Lifespan and Reduces the Size of Tumor Bearing Tissue in a Genetically Engineered Mouse Model of Neurofibromatosis Type 2 (NF2)

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Background: Although it has been well established that Neurofibromatosis Type II (NF2) is caused by loss of heterozygosity of the NF2 gene, which encodes the protein Merlin, relatively little is known about how Merlin functions as a tumor suppressor. One of the few known functions of Merlin is as a negative regulator of the group A serine/threonine p21 activated kinase, PAK1. PAK1 is a known oncogene which serves as a critical signaling node regulating cell proliferation, evasion of apoptosis, and DNA damage repair and is commonly amplified in a variety of human malignancies including up to a third of breast cancers. PAK1 has significantly increased basal activity in Merlin deficient Schwann cells and we hypothesized that Merlin is a critical negative regulator of PAK1 in Schwann cells and that loss of Merlin leads constitutive activation of PAK1, which in turn drives oncogenic transformation and tumor growth. We therefore wanted to test whether or not genetic disruption of Pak1 would be protective against the prototypic pathologies observed in our genetically engineered mouse model of NF2, Nf2lox/lox; Postn-Cre (NF2 GEMM).

Methods: In IACUC approved studies, we crossed our NF2 GEMM animals with Pak1 deficient animals and observed the mice over the period of 15 months to assess for the development of sensorineural hearing loss, tumor formation, and overall survival. We also assessed the potency and growth inhibitory effects of a new PK1 small molecule inhibitor in a murine Nf2 deficient schwannoma cell line.

Results: In the context of our NF2 GEMM, mice genetically deficient in Pak1 but not Pak2 have significantly prolonged overall survival and are significantly protected from sensorineural hearing loss with a reduction in the size of tumor bearing tissue. The PK1 small molecule inhibitor NVS-PAK1-1 potently inhibits PK1 activation at submicromolar concentrations in vitro and reduces the proliferation of an Nf2 deficient schwannoma cell line.

Conclusions: Genetic deletion of Pak1 but not Pak2 extends the average lifespan and reduces the size spinal dorsal root ganglia in our NF2 GEMM. Total deletion of Pak1 was well tolerated in Cre-negative animals. The reduced tumor burden and longer lifespan observed in these mice supports the further development of the PK1 specific inhibitor NVS-PAK1-1 for preclinical and clinical trials.

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Disclosure of Interest: E. Hawley has a conflict with: Funding for this work was provided by the US Department of Defense through the NFRP. S.-J. Park: None Declared, L. Jiang: None Declared, J. Chernoff: None Declared, D. W. Clapp: None Declared
The Tumor Suppressor Protein Merlin Mediates Macrophage Activation and Myelin Phagocytosis

Dario-Lucas Helbing, Morrison Laboratory

**Background:** It has been shown that macrophage infiltration into NF2-associated schwannoma is commonly observed and that this inflammatory microenvironment has an impact on tumor development and progression. Because both Schwann cells and Macrophage activities for example cytokine production and myelin clearance are critical for proper nerve de- and regeneration processes, we wanted to test whether the Merlin (NF2) protein also plays a role in control of macrophage function in the context of the peripheral nervous system (PNS) function and dysfunction.

**Methods:** We used the mouse macrophage cell line RAW 264.7 and primary mouse peritoneal macrophages as an in vitro model to study the effect of macrophage polarization on Merlin protein level. Consequently we knocked down Merlin to investigate the cytokine profile of Merlin deficient macrophages via qPCR. Furthermore we used sciatic nerve explants as a described model system of ex vivo Wallerian degeneration to address the role of Schwann cell intrinsic Merlin in myelin clearance after a nerve injury.

**Results:** We show that Merlin protein level is downregulated in activated M1 macrophages. After Merlin knockdown the proliferation of RAW264.7 macrophages is elevated while we did not observe any significant changes in cytokine production per se. We show that Merlin deficient macrophages react stronger to proinflammatory stimuli in terms of a higher expression of proinflammatory cytokines. In a myelin phagocytosis assay Merlin deficient macrophages displayed elevated engulfment of myelin as well as increased lysosomal abundance suggesting enhanced myelin phagocytosis and processing. qPCR analysis revealed that Merlin deficiency leads to increased mRNA levels of proinflammatory cytokines after myelin stimulation. In agreement with the observed Merlin deficient macrophage phenotype, an ex vivo nerve degeneration assay, carrying a Schwann cell specific Merlin knockout also exhibited enhanced clearance of myelin.

**Conclusions:** Our results show that Merlin can regulate the activation of macrophages and therefore cytokine production in these innate immune cells. We furthermore found that Merlin can regulate phagocytosis in macrophages and Schwann cells which is of high interest for understanding peripheral nerve de- and regeneration and maintenance as well as schwannoma development. Our data imply that manipulation of phagocytosis and autophagy might be a possible therapeutic opportunity for schwannoma which should be tested in future studies.

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Merlin Regulates p53 and YAP via Direct Binding to the Tumor Suppressor ASPP2

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**Background:** Schwannomas are benign peripheral nerve tumors that are characterized by biallelic inactivation of the NF2 tumor suppressor gene, resulting in loss of function of the NF2 gene product, Merlin. In Schwann cells in vitro, Merlin mediates contact inhibition of growth, in part by activating the growth suppressive HIPPO pathway. However, there is no consensus on the mechanism by which Merlin acts as a tumor suppressor. Merlin has no intrinsic catalytic activity; its function is mediated by the proteins with which it interacts. Previously, we used a global proteomic screening technique, proximity biotinylation, to perform a census of Merlin interactions in living Schwann cells. We found that the Merlin interactome predominately consists of components of cell junctions; focal adhesions, adherens junctions and tight junctions, including actin binding proteins and associated signaling molecules. Cell junctions are now seen as signaling complexes that regulate growth in response to intra- and extracellular mechanical forces.

**Methods:** We designed a powerful set of protein interaction techniques to explore the functional relationships among the proteins defined by our interactome analysis and to fully define the role that Merlin plays in mechanosensing.

**Results:** We identified a set of proteins that bind Merlin directly and mediate signaling in response to mechanical forces. This includes ASPP2, a tumor suppressor that interacts with a range of oncopgenic signal transduction molecules; including p53, Ras, NF-kB and YAP. We have found that Merlin inhibits ASPP2 transcriptional regulation of both p53 and YAP.

**Conclusions:** Our data suggest that the Merlin-ASPP2 interaction plays a critical role in multiple oncopgenic signaling pathways. Loss of Merlin in NF2 results in disruption of ASPP2s ability to regulate a key node in onco-regulatory signaling network, causing the dysregulation of critical signaling systems for p53, YAP and Ras that contribute to tumor growth and progression.

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Development of a Preclinical NF1-MPNST Platform Suitable for Precision Oncology Drug Discovery and Evaluation

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Background: One of the most common malignancies affecting adults with neurofibromatosis type 1 (NF1) are malignant peripheral nerve sheath tumors (MPNSTs). 8-13% of individuals with NF1 will develop MPNSTs during young adulthood. There are few therapeutic options, and the vast majority of people with these cancers will die within 5 years of diagnosis. Despite advances in our understanding of the pathobiology of these tumors and the identification of seemingly-promising therapeutic targets using a single mouse MPNST model system (NPCis mice and derivative cell lines), no investigational agents have demonstrated efficacy following translation to human clinical trials. We hypothesize that one of the major reasons that rationally-chosen drugs underperform in human clinical trials is that the preclinical models used to discover and evaluate promising therapeutic agents do not capture the genetic heterogeneity of the human cancer. To optimize clinical translation, we aim to develop a collection of MPNST Patient Derived Xenografts (MPNST-PDXs) for the NF community, which more fully represent the spectrum of genetic heterogeneity seen in the human condition.

Methods: PDX lines were generated from 5 different NF1 patients in each case by implanting a small piece of tumor on the dorsal surface of a nude mouse. These tumors were then serially passaged in vivo. Whole exome sequencing, RNA sequencing, and copy number analysis was performed on the PDX lines, and compared to their parental MPNSTs. Blood samples were obtained as germline DNA controls.

Results: To date, we have generated 5 clinically annotated NF1-MPNST PDX lines that we have histologically and genomically characterized. These lines have also been labeled with GFP-luciferase to allow for the monitoring of tumor burden in live animals in preclinical drug studies. We are in the process of creating 5 more lines.

Conclusions: A collection of histologically and genomically characterized NF1-MPNST PDX lines are now available for pre-clinical testing. Future work with continue to expand this collection.

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This work was funded by the Francis Collins Scholar Award through NTAP and a pre-clinical grant through the NF Research Initiative (NFRI) at Boston Children's Hospital.
NF1 Minipigs Exhibit Spontaneous Loss of Heterozygosity and Attain Clinically Relevant Plasma Concentrations of MEK Inhibitors

Sara H. Isakson, University of Minnesota, Minneapolis

Background: Neurofibromatosis Type I (NF1) is a genetic disease characterized by changes in skin pigmentation and development of nervous system tumors throughout the body. While symptoms vary widely between individuals, nearly all patients develop benign neurofibromas (NFs) and café au lait macules (CALMs). Traditional mouse models provide some insight into the pathogenesis of NF1, but do not accurately model the spectrum of disease seen in human patients. We have developed a porcine model of NF1 that exhibits hallmarks of the syndrome, including NFs and CALMs.

Methods: Using transcription activator-like effector nucleases (TALENs) and homology-dependent repair (HDR) constructs, we generated NF1+/− (NF1) minipigs harboring a premature termination codon found recurrently in human patients. A cohort of NF1 minipigs was examined for phenotypes associated with NF1 syndrome, and cells were isolated from lesions for genetic and biochemical analysis in vitro. A separate cohort of juvenile WT and NF1 littermates was enrolled in pharmacological studies to evaluate the potential differences in drug metabolism and target inhibition that may be present in NF1 patients. We evaluated pharmacokinetics and pharmacodynamics of MEK inhibitors that have shown promise in patients with NF1-associated nervous system tumors.

Results: We have observed full penetrance of CALMs, a phenotype that has not been demonstrated in other animal models. A subset of NF1 minipigs also develop NFs that closely resemble those seen in human patients. Primary Schwann cells cultured from NFs and melanocytes cultured from CALMs exhibit spontaneous loss of heterozygosity of the remaining wild type allele, with variable corresponding levels of Ras hyperactivity. A single oral dose of Selumetinib resulted in clinically relevant plasma concentrations in all minipigs. Moreover, pharmacodynamic analysis of MEK inhibition in peripheral blood mononuclear cells revealed significant suppression of ERK phosphorylation, a downstream effector in the Ras/MAPK pathway. Characterization of NF1 minipig tumors and pharmacologic analysis of NF1-targeted therapies is ongoing.

Conclusions: We have shown that NF1 minipigs develop CALMs and NFs with spontaneous LOH and can be dosed successfully with MEK inhibitors. Our results suggest that NF1 minipigs and the cell lines generated from their tissues will be useful in answering prevailing questions in the field of NF1 and may facilitate development of new therapies for NF1-associated nervous system tumors in humans.

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Disclosure of Interest: S. Isakson: None Declared, T. Rizzardi: None Declared, A. Coutts: None Declared, D. Carlson: None Declared, M. Kirstein: None Declared, J. Fisher: None Declared, J. Vitte: None Declared, N. Ratner: None Declared, S. Fahrenkrug: None Declared, M. Giovannini: None Declared, C. Moertel: None Declared, D. Largaespada has a conflict with: DL is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., (a subsidiary of Recombinetics, Inc.) and Genentech, Inc. is funding some of his research. This abstract describes collaborative research between Surrogen and The University of Minnesota. This work was supported by the Children's Tumor Foundation Synodos for NF1 Award, the National Institutes of Health under Award Number T32OD010093 (SHI), the American Cancer Society Research Professor Award (DAL), and the Children's Cancer Research Fund. A. Watson: None Declared
Muscle Stem Cell Intrinsic Role of Merlin in NF2 Associated Muscle Atrophy

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Background: NF2 patients often present a slow but progressive distal muscle atrophy and paresis in later stages of the disease. In a previous study we showed that Merlin-deficient neurons after sciatic nerve crush injury showed a proper re-innervation concomitant with no difference in muscle weight and a regular architecture of muscle fibers. Therefore we started to investigate the role of Merlin in muscle stem cells (MSCs) after injury and during in vitro myogenesis.

Methods: Murine MSCs were isolated via FACS and microarray analysis was performed to evaluate Merlin expression during aging. Furthermore quantitative real time-PCR of a differentiation time course of primary MSCs derived myoblasts was performed to assess Merlin expression during in vitro differentiation. We also examined the effect of Merlin levels on differentiation and proliferation of myoblasts by using ectopic overexpression and siRNA mediated knockdown. Additionally we performed immuno blot and Immunofluorescence analysis to assess Merlin’s effect on crucial signaling pathways involved in myogenesis. To further investigate the functional relevance of Merlin in MSCs during regeneration of the skeletal muscle (SkM) in vivo we injured the tibialis anterior (TA) muscle of Pax7-creER;Nf2flx/flx with cardiotoxin. The regeneration of the TA was analyzed 7 days post injury (dpi). Additionally MSC numbers were quantified using the MSC marker Pax7.

Results: Strikingly only Merlin isoform 2 expression increased during differentiation of myoblasts. Knockdown of Merlin expression during myogenic differentiation resulted in changed fusion index and myotube diameter. Moreover we could show that loss of Merlin results in deregulation of a major signaling pathway during in vitro myogenesis. To further investigate the functional relevance of Merlin in MSCs in vivo investigated the regeneration of the TA muscle of Pax7-creER;Nf2flx/flx mice after injury. Loss of Merlin expression in adult MSCs affected the regeneration and MSC numbers at 7 dpi.

Conclusions: This is the first study that could show that Merlin isoform 2 plays a crucial role in MSCs and during myogenic differentiation. Furthermore our study suggests that Merlin is important for the regeneration of the SkM after injury and MSC function. Moreover we could already identify one crucial signaling hub involved in MSC activation and maintenance of muscle mass, to be regulated by Merlin in MSCs. This pathway could be one potential target for the treatment of muscle atrophy in NF2 patients.

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Targeting the Hyaluronan-Rich Peripheral Nerve Sheath Tumor Microenvironment to Improve Drug Efficacy and Delivery

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Background: Malignant Peripheral Nerve Sheath Tumors are aggressive soft tissue sarcomas that manifest at a high rate in individuals with the genetic cancer predisposition syndrome Neurofibromatosis Type 1. Although many therapeutic avenues have been explored, little improvement has been seen in the poor prognosis. Surgical resection and nonspecific chemotherapeutics remain the only standard of care. Evidence is mounting that a desmoplastic reaction involving high deposition of the glycosaminoglycan Hyaluronic Acid (HA) in the Extra-Cellular Matrix (ECM) can lead to poor drug penetrance and efficacy. Human MRI examples display large regions of poor uptake of contrasting agent, and a human tissue microarray shows 100% positivity of HA deposition through all samples, including plexiform neurofibromas. Breaking down this physical barrier is a promising potential avenue to improve drug penetrance, perfusion, and efficacy.

Methods: To accurately model high grade Peripheral Nerve Sheath tumors (PNST), we implemented our previously described genetically engineered mouse (GEM)-PNST consisting of Dhh-Cre; Nf1fl/fl; PTEN fl/fl (Keng V., et al., Cancer Research. 2012). This model manifests multi-focal, 100% penetrant peripheral nerve sheath tumors with a median lifespan of 18 days post birth. These tumors also display elevated HA levels in the ECM as well as collapsed and sparse vasculature. This model is amenable for treatment with a human pegylated hyaluronidase (PEGPH20) from Halozyme (San Diego, CA) to deplete HA and improve drug delivery.

Results: The GEM-PNST model, when treated with PEGPH20, displayed a dose-dependent decrease in tumor HA levels with no obvious toxicities to the animals. Taking advantage of the natural fluorescence of the broad-spectrum chemotherapeutic doxorubicin, sections cut from animals that had received PEGPH20 treatment before doxorubicin showed a quantifiable increase in perfusion. Furthermore, CD31 staining suggests an increase in blood vessel patency post-PEGPH20 treatment. Dual treatment of PEGPH20 with doxorubicin slightly improved longevity of these animals as compared to the monotherapy. Preliminary results also show an exciting increase of life when PEGPH20 is combined with the targeted MEK inhibitor PD0325901.

Conclusions: PEGPH20 shows promising therapeutic benefit in targeting physical barriers in MPNSTs. Improved drug delivery and efficacy will open avenues to further drug combinations in this currently incurable malignancy.

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Disclosure of Interest: B. Keller: None Declared, A. Watson: None Declared, K. Williams: None Declared, S. Scully: None Declared, R. Williams: None Declared, L. Anderson: None Declared, J. Knight: None Declared, C. Forster: None Declared, K. Choi: None Declared, M. Carlson: None Declared, N. Ratner: None Declared, P. Provenzano: None Declared, D. Largaespada has a conflict with: DL is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., and Genentech, Inc. is funding some of his research. The business of all these companies is unrelated to the contents of this abstract. Other authors have no conflict of interest to disclose. Supported by W81XWH-15-1-0114 DOD Grant awarded to D.A.L and PPP
Synergism between Topo I and mTOR inhibitors in Malignant Peripheral Nerve Sheath Tumors Affecting NF1 Patients

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are very aggressive and often metastatic soft tissue sarcomas, which are often found in patients who have neurofibromatosis type 1 (NF1). Currently, surgical excision is the only curative therapy for MPNST, although many patients have unresectable or metastatic tumors at diagnosis and the recurrence rate after surgery is high. Chemotherapy regimens are only partially effective and associated with significant toxicity that can severely reduce the quality of life. Therefore, identification of new genetic dependencies and drugs with anti-tumor activity will be critical to advance MPNST therapy.

Methods: To identify effective compounds, we assessed the response of primary NF1-mutant zebrafish MPNST cells that had been implanted into embryos as a novel system to assay drug activity in vivo. Primary MPNSTs were harvested from sox10:mCherry; nf1a+/-;nf1b-/-;p53m/m zebrafish and the cells were mechanically dispersed. Approximately 100-120 MPNST cells were implanted into the pericardial cavity of embryos at 2 days post-fertilization (dpf). Twenty-four hours after implantation, the fluorescent tumor cross-sectional area was imaged. The embryos were arrayed in 96-well plates and were incubated for 4 days in either vehicle or each individual drug. Quantitative assessment of the cross-sectional area of remaining fluorescent tumor cells was performed at 7 dpf and the fish were raised in the absence of drug and monitored to assess the durability of the response. The compounds showing drug response in the embryonic implantation assay were evaluated in human MPNST cells.

Results: After testing a series of drugs, we identified inhibitors of topoisomerase I and mTOR as the most effective single agents in inducing apoptosis in MPNST cells. Furthermore we show that the topoisomerase I inhibitor irinotecan and the mTOR kinase inhibitor AZD2014 act synergistically induce cell death in human NF1-mutant MPNST cell lines by isobologram analysis. Mechanistic studies implicate mTOR inhibition and DNA damage induced by inhibiting topoisomerase I in synergistically blocking phosphorylation of 4E-BP1, leading to arrest of protein synthesis and tumor cell death.

Conclusions: Co-inhibition of topoisomerase I and mTOR induces synergistic cell death in NF1-associated MPNSTs. Our study provides compelling preclinical evidence that this drug combination is highly active against MPNST at dosages tolerated by normal tissues in vivo.

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Genome-Wide Mapping Reveals New Nuclear Functions for YAP in Schwann Cells

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Background: The Hippo pathway regulates cell number and organ size during development through the control of the YAP transcriptional regulator. Significantly, the pathway has emerged as a major driver of tumorigenesis in many human cancers. We have previously shown that YAP function is required for NF2-null Schwann cell survival, proliferation, and tumor growth in vivo. Upon extracellular stimuli such as cell-cell contact, the Hippo pathway negatively regulates YAP through cytoplasmic sequestration. At low cell density, YAP is nuclear and functions as a transcriptional activator of genes involved in cell proliferation and survival.

Methods: Using genome-wide approaches such as ChIP-seq.

Results: We identified that in addition to its role as an activator, YAP can act as a transcriptional repressor through recruitment of EZH2, a member of the Polycomb repressive complex (PRC2). YAP co-localizes with EZH2 on the genome to transcriptionally repress a broad network of genes mediating a host of cellular functions, including a number of central regulators of cell cycle progression and contact inhibition of cell proliferation.

Conclusions: This work unveils a broad and underappreciated aspect of YAP function as a transcriptional repressor and suggests that Ezh2 inhibition might be a viable therapeutic approach in the treatment of NF2.

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Targeting a Novel RABL6A-RB1 Pathway Suppresses MPNST Pathogenesis

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are deadly sarcomas that arise in ~10% of patients with neurofibromatosis type I (NF1). There are no effective chemotherapies for MPNSTs, which are the leading cause of death in NF1 patients. In most MPNSTs, the retinoblastoma (RB1) tumor suppressor pathway is inactivated by hyperactivation of CDK4/6 kinases, commonly through loss of cell cycle inhibitors such as p27. RABL6A is an oncogenic GTPase that inhibits RB1 signaling, but its role in MPNST has not been studied. We hypothesized that RABL6A drives MPNST pathogenesis through inactivation of RB1.

Methods: RABL6A and p27 expression in human MPNSTs and cell lines were determined by immunohistochemistry (IHC) and western blotting. RNAi was used to silence RABL6A in MPNST and normal human Schwann (NHSC) cells. Effects of RABL6A loss on MPNST biology and RB1 signaling were measured by cell proliferation/survival assays, western blotting, and orthotopic xenograft tumor studies in mice. Dose response curves and in vivo drug treatments assessed effects of RABL6A expression on MPNST responses to RB1 targeted drugs.

Results: Tissue microarray analyses showed dramatic upregulation of RABL6A coincident with p27 loss in human MPNSTs compared to patient-matched plexiform neurofibromas. Likewise, RABL6A was upregulated in human MPNST lines compared to primary NHSCs. In vitro cell-based assays showed that RABL6A is essential for MPNST cell proliferation and survival. Loss of RABL6A caused significant MPNST cell death and G1 phase arrest concurrent with p27 upregulation and accumulation of active, hypo-phosphorylated RB1. Conversely, RABL6A overexpression enhanced MPNST cell proliferation and RB1 phosphorylation. We tested if MPNST viability would be reduced by drugs targeting the RB1 pathway and found that a selective inhibitor of CDK4/6 kinases, palbociclib (PD0332991), killed MPNST cells in a RABL6A-dependent manner. Ongoing mouse studies are evaluating the in vivo efficacy of palbociclib against MPNST tumors that express varying levels of RABL6A.

Conclusions: Our data uncovered RABL6A as a new oncogenic driver of MPNST proliferation and survival. RABL6A activity promoted p27 downregulation, inactivation of RB1, and increased response to RB1 targeted drugs. These findings establish a critical role for RABL6A in MPNST pathogenesis and identify RABL6A-RB1 signaling as a novel, clinically relevant target for MPNST therapy using FDA-approved CDK4/6 inhibitors.

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Delivering on the Vision of Bench to Bedside: A Funding Community Effort to Develop Effective Therapies for Neurofibromatosis Type 1 Tumors

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Background: In recent years drug discovery research in rare diseases has proved to be a valuable strategy for companies to enrich their pipeline and diversify their portfolio of therapeutic areas. This has been sustained in large part by the opportunities created by regulators for additional revenue and market exclusivity. However, much of the funding for rare disease research is still provided by the not-for-profit organizations, including federal agencies and private medical foundations. The current analysis showcases the collaboration between funders of neurofibromatosis type 1 (NF1) research to develop effective therapies for NF1-associated tumors. The authors use the example of the investigation of MEK (mitogen-activated protein kinase kinase) inhibitors for NF1-associated tumors as a case study to highlight the unique research and funding landscape that contributed to the ultimate launch of several promising clinical trials for various NF1 tumors leading to the results of the SPRINT study involving the use of MEK inhibitor selumetinib in inoperable plexiform neurofibromas. By presenting an analysis of the grants from the National Institutes of Health (NIH), the Department of Defense (DoD) Congressionally Directed Medical Research Program (CDMRP), and major philanthropic organizations that have funded NF1-MEK research over the last 10 years, the authors highlight the value of having a collaborative funding strategy that would result in a natural flow of research that has directly contributed to the development of effective therapies for a rare tumor syndrome.

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Habitation in Children with Neurofibromatosis Type 1 Measured by Electroencephalography

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Background: The purpose of this research is to explore the electroencephalographic (EEG) markers of habitation in children with neurofibromatosis type 1 (NF1). Studies on animal models of NF1 have identified deficits in habitation, a simple form of learning that is conserved across species and crucial for the development of higher cognitive functions (Larkin et al., 2010; Wolman et al., 2014). However, habitation deficits in humans with NF1 have not yet been investigated. Repetition suppression (RS), the reduction of brain activity in response to the repeated presentations of a single stimulus, is considered the neurophysiological equivalent of habitation and can be measured using EEG. In this study, we looked at RS in the time-frequency domain through the variation of event-related spectral power. We hypothesized that habitation deficits would be seen in the NF1 group reflected by lower RS compared to controls.

Methods: A sample of 40 participants (20 NF1 and 20 controls) between 4 and 18 years of age will be recruited. To date, EEG recordings were performed on 8 participants with NF1 and 8 controls at CHU Sainte-Justine in a soundproof room with a 128-electrodes EEG system. The task presented was composed of thirty pseudowords repeated six times each, which allowed us to observe auditory RS in a previous study using a similar design (Knoth et al., 2018). Timeframes of 500 ms for each presentation of the pseudowords were analyzed separately in the time-frequency domain (Morlet decomposition). Repetition effects were observed through the variation of spectral power between the first two presentations of the pseudowords at the left temporal region of interest for each frequency band of interest (i.e. Theta, Alpha 1 and 2).

Results: Preliminary analyses were conducted using the data of 16 participants (8 NF1, 8 controls) between 4 and 16 years of age. Mixed-design ANOVA (groups x repetitions) analyses revealed a tendency for an interaction between repetitions and groups (F(1, 14) = 3.60, p = 0.079) in the Alpha 1 band (8-10Hz), suggesting that while the control group shows repetition suppression between the first and second presentation of a pseudoword, the NF1 group shows repetition enhancement.

Conclusions: The study of repetition suppression alterations in NF1 could help us understand learning disabilities in NF1. Also, EEG markers of habitation promise to be highly relevant translational measures of basic learning mechanisms for clinical trials aimed at learning disabilities in NF1.

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Characterization of a Novel Patient Specific Neurofibromatosis Type I (NF1) Rat Model

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Background: Although multiple NF1 mouse models exist, there is a need for additional preclinical models to test new therapeutics. As rats are a traditional animal model for pharmacological, toxicological, and neurological studies, using this model organism to express NF1 patient alleles will allow examination of the efficacy and safety of pharmacological modulators as well as cognition and behavior. Therefore, we created an NF1 rat model with the patient missense allele c.3827G>A, p.R1276Q associated in humans with spinal NF1.

Methods: Two CRISPR guides and a dually compatible repair template were designed to target exon 28 of rat Nf1 embryos were injected with ribonucleoprotein complexes of guide RNA and Cas9 protein along with a single-strand DNA repair template and transferred to pseudopregnant recipients. Pups were genotyped by tail biopsy ten days postpartum. Animals were euthanized upon tumor mass ulceration or inhibition of normal movement. Tissues were collected for histology and evaluated by a board certified veterinary pathologist.

Results: Three of seven pups born were positive for CRISPR activity from which independent NF1 knock-out (P1220fs*1223) and missense knock-in (R1276Q) colonies were established. Unmated heterozygous NF1 R1276Q female rats do not display any spontaneous tumors at least out to 5 months of age; however, of four females that were mated, all rapidly developed tumors within two weeks of pregnancy. Heterozygous NF1 P1220fs*1223 females also develop tumors post-mating but, spontaneous tumors have also been observed in an unmated female. Homozygous mutant offspring have not yet been detected in litters born from crossing of heterozygous NF1 mutant rats, although a homozygous mutant pup was detected at embryonic day 9.5 following Cesarean section.

Conclusions: Two novel mutant NF1 alleles, patient mutation c.3827G>A, p.R1276Q and deletion c.3661_3674del, p.P1220fs*1223, have been generated. These alleles are embryonic lethal, although statistical significance has not yet been reached.

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Generation of Patient-Specific Neurofibromatosis Type I (NF1) Rodent Models

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Background: Animal models are critical for preclinical studies of therapeutics. While previously limited to null and conditional knockout alleles, advances in genetic engineering technologies have allowed the creation of personalized animal models of NF1. Models being developed represent different types of mutations occurring in patients including nonsense, missense, insertions, deletions, splicing, and frameshifts. These models recapitulate missense mutations associated with familial spinal NF (p.Gly848Arg and p.Arg1276Gln), as well as the less severe genotype-phenotype correlations associated with p.delM992 and p.Arg1809Cys.

Methods: Mouse and rat NF1 genes are targeted by pronuclear microinjection or electroporation of CRISPR/Cas9 and Cpf1 reagents with repair templates to generate founder animals. Both traditional targeting vectors and CRISPR reagents are used to modify mouse NF1 in embryonic stem cells. Founder animals and chimeras are outcrossed to establish germline transmission and independent colonies. Mutant alleles are tested for function by assessing viability when homozygous, as well as tumor formation when placed in the proper genetic context.

Results: Mouse models developed to date include c.2041 C>T (p.Arg681X), c.2542G>C (p.Gly848Arg), c.2393_2408del16 and c.2919_2920insTT. Additional model development is currently underway in the mouse including c.5425C>T (p.Arg1809Cys), c.1466A>G (p.Tyr489Cys), c.2970-2971delAAAT (p.delM992), c.2446 C>T (p.Arg816X), and c.499_502delTGGTT. Rat models created to date include the c.3827G>A (p.Arg1276Gln) mutation and a 14bp deletion model (c.3661_3674del).

Conclusions: Patient-specific NF1 alleles have been successfully generated in rodents using ES cell and CRISPR based approaches. The c.2041 C>T; p.Arg681X and c.2393_2408del16 mouse models are embryonic lethal when homozygous, whereas the homozygous c.2542G>C; p.Gly848Arg mice are viable with no gross phenotype despite neurofibromin levels. Creating alleles from different mutation classes will allow in vivo testing of gene-based therapeutics such as nonsense suppression, antisense-mediated splicing modulation, gene replacement via cDNA, or nuclease-mediated gene editing approaches.

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The Role of Hippo Signalling in Merlin Null Schwannomas and Meningiomas

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Background: Loss of the NF2 gene product Merlin is the most common cause of meningiomas and schwannomas. Merlin loss leads to increased activity of the Hippo pathway co-transcriptional activators YAP/TAZ which drive tumour phenotypes in numerous cancers. Previous studies in schwannoma have shown that Merlin loss leads to increased degradation of the Hippo pathway kinase LATS1/2 resulting in nuclear accumulation of YAP/TAZ. This study aims to identify novel Hippo pathway targets in Merlin negative meningiomas and schwannomas.

Methods: Primary meningioma and schwannoma cells were cultured following surgical resection and informed patient consent. Cultured cells were subjected to drug inhibition and lentivirus mediated knockdown of YAP or TAZ, and analysed for protein expression and proliferation by Western Blot and immunocytochemistry. An RNA sequencing analysis was conducted on the sciatic nerves of Schwann cell conditional knockout mice that were either WT, NF2-/- or NF2-/-/YAP-/-.

Results: Merlin negative meningiomas and schwannomas display a greater proportion of nuclear YAP/TAZ compared to meningiomas with functional Merlin, and are also more proliferative following Ki-67 analysis. Both chemical inhibition (verteporfin) and lentivirus mediated knockdown of YAP or TAZ, and analysed for protein expression and proliferation by Western Blot and immunocytochemistry. An RNA sequencing analysis was conducted on the sciatic nerves of Schwann cell conditional knockout mice that were either WT, NF2-/- or NF2-/-/YAP-/-.

Conclusions: This study has shown that Merlin negative meningiomas exhibit increased YAP/TAZ activity and as a result are more proliferative. Inhibition or knockdown of YAP/TAZ can partially ameliorate the elevated proliferation seen in these tumours. In addition, differentially regulated genes following loss of Merlin and YAP may reveal novel mechanisms by which Merlin negative meningiomas and schwannomas proliferate. Overall, Merlin/YAP dependent genes as well as YAP/TAZ inhibition may lead to new therapies for Merlin negative meningioma and schwannoma.

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Modeling an MPNST-Like State in Immortalized Human Schwann Cells for High-Throughput Drug Screening

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Background: Neurofibromatosis Type 1 (NF1) patients are predisposed to development of many types of cancers and aggressive benign tumors. The most highly penetrant of these are tumors of Schwann cell in origin and located in the peripheral nervous system. These include plexiform neurofibromas and atypical neurofibromas, which are precursors of malignant peripheral nerve sheath tumors (MPNST). MPNSTs are the most deadly of the soft tissue sarcomas, with 5-year survival rates as low as 20 percent. Loss of function mutations in Polycomb Repressive Complex 2 (PRC2) genes, such as SUZ12, are commonly identified in NF1-associated and sporadic MPNSTs. This is highly suggestive that perturbation of epigenetic homeostasis plays a role in malignant transformation of neurofibromas. Dysregulated chromatin remodeling caused by the loss of PRC2 in MPNSTs likely confers novel vulnerabilities, that if identified could be exploited therapeutically.

Methods: To identify new therapeutic targets in an MPNST context, our lab created NF1 and SUZ12 deficient immortalized human Schwann cells (HSC1lambda) for high-throughput drug screening. Using CRISPR/Cas9, we designed guide RNAs targeting NF1 and SUZ12. We isolated clones and screened for cells that were wildtype (WT) or had heterozygous or homozygous deletions of NF1, and/or SUZ12. The cells were subjected to an epigenetic compound library in a drug screening experiment.

Results: Isolated clones were verified to harbor loss of function mutations in NF1 and SUZ12 as well as reduced (or loss) of NF1 or SUZ12 protein expression via immunoblotting analysis. High-throughput drug screening revealed several compounds, some of which fell into mechanisms, that showed selective inhibition of SUZ12^-/-;NF1^-/- cells compared to NF1^-/- or WT cells. Two of these compounds were vorinostat and azacitidine, an HDAC and DNA methyltransferase inhibitor respectively.

Conclusions: Testing cells of an appropriate genetic background is fundamental in assaying drug compounds that could have clinical relevance. Our cells reflect such a need by recapitulating an MPNST genotype whereby the epigenetic machinery has been disrupted. Our initial drug discoveries of an HDAC and DNA methyltransferase inhibitor offers confidence that MPNSTs harbor specific vulnerabilities which can be targeted. The top compounds from our screens will be tested further in human MPNST cell lines and some may move forward to animal models with the overall goal of informing future clinical trials.

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Disclosure of Interest: A. Larsson: None Declared, S. Finnerty: None Declared, M. Sokolowski: None Declared, R. Williams: None Declared, K. Williams has a conflict with: K.B.W. is supported by Children's Tumor foundation Young Investigator Award., D. Largaespada has a conflict with: DL is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., and Genentech, Inc. is funding some of his research. The business of all these companies is unrelated to the contents of this abstract. Other authors have no conflict of interest to disclose. This work was supported by the NF Research Initiative Pre-clinical MPNST Research Grant Program, The National Institutes of Health (1R01-NS086219), the American Cancer Society Research Professor Award (to DAL), The Children's Cancer Research Fund, and The Children's Tumor Foundation Synodos for NF1 Award.
JHU395, A Nervous Tissue Penetrant Glutamine Antagonist, Restricts Growth of Malignant Peripheral Nerve Sheath Tumor Through Inhibition of Biosynthesis

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Background: Malignant peripheral nerve sheath tumor (MPNST) is a sarcoma associated with nerve tissue that occurs in ~10% of patients with neurofibromatosis type I (NF1). When incompletely surgically resected at diagnosis the 4-year event-free survival is <30%, and improved treatments are needed. Recent evidence suggests that perturbing glutamine utilization warrants further exploration as a potential therapeutic strategy for MPNST. Our group described JHU395, a nervous system penetrant glutamine antagonist (GA). JHU395 delivers active GA preferentially to nervous tissue which may result in less gastrointestinal toxicity than was observed with competitive glutamine inhibitors in past clinical trials. The primary goal of this study was to evaluate JHU395 in preclinical models of MPNST.

Methods: We investigated glutamine antagonism on growth of MPNST cells in culture and on growth of murine flank MPNST. JHU395 was administered orally for 14 days to mice bearing inoculated tumors derived from the NPcis (NF1+/-;p53+/−) genetically engineered model of MPNST. Tumors were measured every other day and tumor volume was calculated as the primary endpoint. GA was detected in tumor and plasma using a previously validated bioanalytical method. Targeted liquid chromatography-mass spectrometry (LC-MS)-based metabolomics and MS-based flux analysis with stable isotope labeled 15N₂- or 13C₅-glutamine were performed on MPNST cells and murine tumors.

Results: Compared to immortalized Schwann cells, growth of MPNST was preferentially inhibited by GA (IC₅₀=8 micromolar versus >30 micromolar). JHU395 delivered GA to MPNST cells with >4-fold higher cell-to-plasma ratio compared to native GA and maintained ~2-fold higher tumor-to-plasma levels in vivo. Mice treated with JHU395 had mean tumor volume >40% smaller compared to vehicle controls with limited signs of toxicity. Targeted metabolomics of GA-treated MPNST cells demonstrated multiple effects on glutamine-dependent metabolites. In vivo flux analysis showed that JHU395-treated tumors decreased glutamine-derived nitrogen flux into several pathways including de novo nucleotide synthesis.

Conclusions: JHU395 inhibits growth of MPNST with effects on multiple biosynthetic pathways. Nervous tissue penetrant glutamine antagonism is a feasible and effective therapeutic approach for MPNST. Future studies will investigate how JHU395 affects driver pathways in MPNST and evaluate JHU395 in combination with other antitumor agents.

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Disclosure of Interest: K. Lemberg: None Declared, Y. Wu: None Declared, L. Zhao: None Declared, J. Alt: None Declared, A. Gadiano: None Declared, R. Rais has a conflict with: Dracen Pharmaceuticals, P. Majer has a conflict with: Dracen Pharmaceuticals, J. Blakeley: None Declared, B. Slusher has a conflict with: Dracen Pharmaceuticals
Schwannomatosis Schwannomas Harbor Distinct DNA Methylation Profiles

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Background: Schwannomas are characteristic manifestations of NF2 and schwannomatosis syndromes. However, the majority of schwannomas are solitary and sporadic. It is unclear whether and to what extent sporadic and syndrome-associated schwannomas or their histologic subtypes represent distinct biological groups. Clinically, although schwannomatosis schwannomas are considered benign, the majority of patients experience unmanageable pain; however, the underlying mechanism of this pain is not well understood. There is increasing evidence for DNA methylation profiling being able to distinguish biologically relevant tumor subgroups, even within the same cellular lineage and histopathologically similar tumors.

Methods: In this study, we used Illumina Methylation EPIC arrays for methylome-based characterization of 88 schwannomatosis schwannomas, in comparison to 90 sporadic schwannomas and 14 NF-2 schwannomas. We performed unsupervised hierarchical clustering selecting 30,000 probes that showed the highest median absolute deviation (MAD) across all beta values.

Results: Three different clustering sets were utilized to obtain the most refined differentiation. Schwannomatosis schwannomas formed 3 distinct methylome-based subgroups, which were fully distinct from sporadic schwannomas and NF-2 schwannomas. Additionally, we performed copy number analysis using the DNA methylation data to infer gross chromosomal deletions or gains among the sporadic and syndromic schwannomas, in addition to schwannomas with hybrid features. Methylation subgroups were further correlated with clinical parameters including age, gender, anatomic location, tumor size, germline mutation status (LZTR1/SMARCB1), and 22q LOH, in addition to the histopathologic features associated with each tumor and pain. Furthermore, RNA sequencing was performed to examine gene expression profiles associated with the 3 methylome subgroups and the data was integrated with DNA methylation profiles to establish the biological relevance of hypo- and hyper-methylation of the top varying CpG sites.

Conclusions: Our findings suggest that schwannomatosis schwannomas form 3 distinct epigenetic subgroups and they are distinct from sporadic schwannomas.

Memory Systems and Neurofibromatosis Type 1 (NF1): What is Impaired, What is Spared?

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Background: Previous studies focusing on the cognitive profile of children with Neurofibromatosis type 1 (NF1) have shown difficulties regarding visuo-perceptive capacities, executive functions and attention, motor coordination, as well as linguistic abilities. Few studies have investigated memory function of this population. A working memory deficits is frequently reported. However, results concerning semantic memory, verbal and non-verbal episodic memory, perceptive capacities, executive functions and attention, motor coordination, as well as linguistic abilities. Few studies have investigated memory function of this population. A working memory deficits is frequently reported. However, results concerning semantic memory, verbal and non-verbal episodic memory, perceptive capacities, executive functions and attention, motor coordination, as well as linguistic abilities.

Methods: 18 children with NF1 were recruited in the local NF1 referral center. They were examined by a neuropsychiatrist and the neuropsychological assessment of memory was administered by a neuropsychologist. We compared them to 18 typically developing children (TD) of same age (8-12 years) using a t-test and repeated measures ANOVA. The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki. We obtained written informed consent from the parents and their children.

Results: We observe a significant difference between NF1 and TD children in verbal working memory but not for the visuospatial sketchpad. We also found a significant difference concerning verbal anterograde memory (encoding process) but not for the visual anterograde memory. Regarding semantic memory, we showed a significant difference for general knowledge. Contradictory with our expectations, the children with NF1 experienced difficulties evoking personal memories but were improved by cueing. No difference was found regarding procedural memory.

Conclusions: These results support a dissociation between memory systems in children with NF1. These alterations may have an impact on the acquisition of academic knowledge. Moreover, attentional and executive abilities could explain children with NF1’s memory profile. The specificities of their memory profile must be taken into account in the clinical follow-up of these children for the understanding of their learning disorders and their care.

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Understanding the Role of NF1 in Schwann Cell Differentiation and Tumor Formation through the Analysis of an In Vitro iPSC-Based Differentiation Model

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Background: Neurofibromatosis type 1 patients develop dermal and plexiform neurofibromas (PNF). It is well established that these tumors arise through the bi-allelic inactivation of the NF1 gene in Schwann cells (SC) or in their precursors. We generated isogenic NF1(-/-) and NF1(+/+) induced pluripotent stem cell (iPSC) lines from PNF-derived primary cells to have an imperishable cellular model for PNFs. We established an iPSC differentiation protocol to obtain SC through a Neural Crest Stem Cells (NCSC) stage and compared control NF1(+/+) with PNF-derived NF1(+/-) and (-/-) iPSCs. The goal of this work is to gain insight into the role of the NF1 gene in the NCSC-SC differentiation axis and on the identity of cells composing PNFs.

Methods: The NCSC-SC in vitro differentiation process was monitored by immunofluorescence and RT-qPCR analysis using a set of markers at different time points representing distinct differentiation stages: pluripotent stage, NC stage, and 7 (SC precursor), 14 (immature SC), and 30 days (mature SC) stages. We characterized the distinct differentiation stages by: RNA-Seq and Western blot.

Results: RNA-seq analysis of the in vitro NCSC-SC differentiation model using control NF1(+/+) iPSCs confirmed that the established protocol is robust and reproducible. Differential expression analysis among the distinct stages allowed the identification of tens to hundreds of genes differentially expressed in each stage. We selected some genes of each stage and are currently characterizing them in PNFs, nerves and their primary cultures. NF1(-/-) iPSC lines maintain a high proliferation rate during the in vitro SC differentiation process compared to NF1(+/+), altering their differentiation potential. Presently, we are comparing the differential expression of the NF1(-/-) iPSCs with the NF1(+/+) iPSCs. In addition, preliminary results from Western blot indicate the relevance of neurofibromin downregulating the cAMP-PKA and Ras-MAPK pathways at different stages of the SC differentiation process.

Conclusions: Several differential expressed genes have been identified in the distinct NCSC-SC stages. Their in vivo relevance is being analyzed in PNFs and nerves. Alterations in the SC differentiation process due to the lack of neurofibromin are being characterized by RNA-seq and pathway activation. Preliminary results point to a role of neurofibromin in the negative regulation of cAMP-PKA and Ras-MAPK at different stages of the SC differentiation process.

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Processing of Visual and Vocal Emotions in Children and Adults with Neurofibromatosis Type 1, and the Way They Combine with Attention to Determine Aggressive Behavior

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Background: Social competence seems compromised in NF1 and is frequently attributed to weaken reading of emotional information on faces. However, understanding social information also requires collecting and processing cues beyond facial expressions, and also involves attention. Except that little is known about visual and vocal emotion processing in adults with NF1, no study compared directly children and adults with NF1 in order to uncover any age-related changes. This is surprising since children with NF1 will some day grow up and will have to cope with their difficulties. Here, we compare performance of children and adults with NF1 in tasks assessing emotions and tried to unravel the way emotions combine with attention to determine aggressive behavior.

Methods: 114 participants were distributed in 4 independent groups (controls: 27 children and 30 adults; NF1: 27 children and 30 adults). Patients and their respective controls were matched in age and IQ level. They were asked to complete a task of facial expression recognition, a task of attribution of apprehending visually presented social interactions, and a task of vocal prosody perception. They also completed a task assessing two attention processes, namely inhibitory control and divided attention. Finally, questionnaires were used to assess aggressive behavior.

Results: Independently of IQ level and gender, performance was lower in children with NF1 than their controls. However, adults with NF1 did not differ from their controls. This pattern of normalization of performance from childhood to adulthood in NF1 patients was observed in all three tasks. The processing of each basic emotion also followed the same pattern. A regression analysis showed that aggressive behavior in NF1 was explained by a combination of weaken inhibitory control and good perception of vocal emotions.

Conclusions: Children with NF1 have difficulties in perceiving and understanding emotions. However, adults with NF1 have no signs of such impairments. This may be due either to the known physiopathological changes that take place within the brain from childhood to adulthood in NF1, or to the development of coping strategies. The results also suggest that an adequate perception of vocal emotions combined with failures to inhibit distracting information is predictive of patients' aggressive behavior. These results underscore the importance of taking simultaneously into account several types of psychological processes when trying to understand social behavior.

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Genomics of MPNST (GeM) Consortium

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are associated with a 15% lifetime risk in people with NF1. Current treatment is ineffective in most cases. Prior research has identified key molecular pathways, but the rarity of cases has prohibited a comprehensive molecular analysis of a large number of these tumors. Our goal is to correlate multi-omic datasets and make such data widely available to the scientific community to advance research on MPNST therapeutics.

Methods: In Spring of 2017, the NF Research Initiative at Boston Children’s Hospital founded the multi-institutional, international Genomics of MPNST (GeM) Consortium with the goal to collect and characterize more than 100 NF1-related and sporadic MPNSTs and related neurofibroma. Our hypothesis is that selection of well-characterized cases along with comprehensive analysis will identify novel correlations between molecular markers and clinical/pathologic features of MPNSTs, potentially leading to identification of new therapeutic targets. Analytical methods include whole genome sequencing (WGS) of tumor and paired normal specimens, multiregional targeted panel of oncogenes for deep sequencing, RNA-Seq, epigenomic chip analysis, chromatin immunoprecipitation (ChIP)-Seq, and immunohistochemical studies of tissue microarrays. This data is correlated with histological features assessed through a central pathology review. Clinical features are collected through collaboration with the International MPNST Registry at Washington University School of Medicine in St. Louis.

Results: The GeM Consortium includes 13 founding sites, a Steering Committee for governance and Working Groups (Oncology and Pathology, Genomics and Informatics, and Data Use and Publications) to manage specific aspects of specimen collection and data analysis. Based on retrospective samples collected at the various sites, more than half of the total goal of 100 tumors is now available. Sage Bionetworks will manage and enable cloud-based collaborative analysis and sharing of de-identified data.

Conclusions: Our GeM Consortium effort will provide insight into tumor heterogeneity, progression to malignancy, and evolution of primary tumor lesions over time and with treatment. This will help advance research for MPNST treatment.

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Linking the Mechanosensing Ability of NF1+/-, NF2+/-, and Healthy Fibroblasts to Their Force-Generating Capabilities

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**Background:** Cells sense the physical properties of their extracellular environment and translate them into biochemical signals. In NF1 and NF2, the tumor-initiating cells are believed to be cells with gene deficiency (e.g., NF1-/- Schwann cells). However, haploinsufficient stromal cells (e.g., NF1+/- fibroblasts) are suspected to play a role in the development of tumors, and this may be due to altered mechanosensing and force-generating behaviors. Little work has been invested to study the mechanobiology of these cells. Here we studied the mechanosensing ability, motility, and force-generating capability of healthy, NF1+/-, and NF2+/- fibroblasts.

**Methods:** Nano- to micro-grooved substrates were seeded with fibroblasts in regular culture media at a density of 5000 cells/cm². Thirty-six hours after plating, we characterized their orientation as $S = \theta$, where $\theta$ is the angle between the cell’s major axis and the microgroove. Traction force microscopy was performed after encapsulating fluorescently labeled fibroblasts within mechanically well-defined hydrogels with co-embedded fluorescent microbeads. Microbead displacements were captured with confocal microscopy and computationally translated into traction stresses. To elucidate mechanotransduction mechanisms in haploinsufficient NF1 and NF2 fibroblasts, we studied mechanosensing and force-generating behaviors with inhibitors of factors associated with common mechanotransduction pathways (i.e., Y27632, ROCK inhibitor; SFF-304, CD44 inhibitor; and FRAX486, PAK1 inhibitor).

**Results:** Alignment generally decreased with increasing groove width and decreasing groove depth. NF2 fibroblasts generally aligned less with the microgrooves than both healthy and NF1 fibroblasts (Figure 1). Similar trends in migration speed between the three cell lines were also observed; whereas, mean squared displacement was effected for the NF1 cells more strongly in a microgroove width-dependent fashion. Immunohistochemical staining revealed abnormal shape and a poorly organized actin cytoskeleton in NF2 fibroblasts, in addition to smaller and less well-aligned focal adhesions. Traction force experiments revealed that NF2 fibroblasts also produced significantly lower traction force (10-25 nN) than healthy or NF1 fibroblasts (30-40 nN).

**Conclusions:** Here we present a comparative study of alignment, migration, and contractile behavior of healthy, NF1, and NF2 fibroblasts. These results will be important in determining the biomechanics of tumor development in NF1 and NF2.

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Reliability of Functional Outcome Measures in Adults with Neurofibromatosis Type 2

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Background: People with neurofibromatosis type 2 (NF2) may have hearing, balance, dizziness and/or visual impairments that lead to functional challenges. Functional challenges may be amenable to medical, surgical or physical interventions and there is a need for robust functional outcome measures in this patient group to assess treatment efficacy, track disease progression and assist with clinical decision making. This study is the first stage of evaluation of a core group of functional performance outcome measures in adults with NF2, and aims to determine intra-rater and inter-rater reliability of the four square step test (FSST), the modified test of sensory integration and balance (mCTSIB) and a modified nine-hole peg test (m9HPT). We also aimed to ascertain how closely objective and subjective measures align through correlating the functional performance outcome measures against the validated disease specific NF2 quality of life questionnaire (NFTI-QOL).

Methods: Twenty-nine ambulant adults with NF2 aged 16 years and over were included in this observational study conducted in a tertiary referral outpatient clinic. Median age 45 years (range 18-73), 18 females, 11 males. Participants were video-recorded performing three functional outcome measures. Three raters from the Neurofibromatosis centre multi-disciplinary team independently scored the measures to determine inter-rater reliability. One rater scored the measures a second time on a separate occasion to determine intra-rater reliability. Participants also completed a disease specific quality of life questionnaire (NFTI-QOL) and a computerised dynamic visual acuity test.

Results: Inter-rater reliability and intra-rater reliability scores (intra-class coefficient, ICC) were similar for each outcome measure. Excellent rater agreement (ICC r ≥ 0.9) was found for the FSST and mCTSIB. The m9HPT correlated highly with subjective perceived balance and walking difficulties identified in the NFTI-QOL questionnaire. Standard error of measurement and minimal detectable change for each outcome measure were calculated and deemed acceptable.

Conclusions: The FSST and mCTSIB are potentially useful outcome measures for monitoring NF2 treatment in clinical practice and for research purposes. They will undergo further metric evaluation in this disease, including assessment in multi-centre and longitudinal studies.

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Identification of Rac1 by an shRNA Library Screen and Genetic Proof of Concept That Disruption of Rac1 Prevents Plexiform Neurofibroma Formation in a Mouse Model of Neurofibromatosis Type 1

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Background: Neurofibromatosis Type 1 (NF1) is a highly penetrant autosomal dominant genetic disorder where mutations in the tumor suppressor gene NF1 leads to decreased neurofibromin. The most debilitating manifestation is the presence of complex multi-lineage Schwann cell-derived plexiform neurofibromas (PN). Historically, little clinical success has been achieved targeting PN through surgery or chemotherapies. We performed an shRNA library screen of patient-leads to decreased neurofibromin. The most debilitating manifestation is the presence of complex multi-lineage Schwann cell-derived plexiform neurofibromas

Methods: Twenty-nine ambulant adults with NF2 aged 16 years and over were included in this observational study conducted in a tertiary referral specialist outpatient clinic. Median age 45 years (range 18-73), 18 females, 11 males. Participants were video-recorded performing three functional outcome measures. Three raters from the Neurofibromatosis centre multi-disciplinary team independently scored the measures to determine inter-rater reliability. One rater scored the measures a second time on a separate occasion to determine intra-rater reliability. Participants also completed a disease specific quality of life questionnaire (NFTI-QOL) and a computerised dynamic visual acuity test.

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Identification of Rac1 by an shRNA Library Screen and Genetic Proof of Concept That Disruption of Rac1 Prevents Plexiform Neurofibroma Formation in a Mouse Model of Neurofibromatosis Type 1

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Background: Neurofibromatosis Type 1 (NF1) is a highly penetrant autosomal dominant genetic disorder where mutations in the tumor suppressor gene NF1 leads to decreased neurofibromin. The most debilitating manifestation is the presence of complex multi-lineage Schwann cell-derived plexiform neurofibromas (PN). Historically, little clinical success has been achieved targeting PN through surgery or chemotherapies. We performed an shRNA library screen of patient-derived Schwann cell lines to identify novel therapeutic targets to disrupt PN formation and progression.

Methods: An shRNA library screen of human kinases and Rho-GTPases was performed in NF1- and paired NF1 competent immortalized Schwann cell lines. Following sequencing, candidates were identified. We previously developed a novel mouse model of NF1 wherein a neural crest specific Postn-cre targeted lox-flanked NF1 that replicate the PN found in patients. Additional cohorts of mice were generated with single or biallelic deletion of Rac1(NF1lox/P0ostn-Cre-; NF1lox/Rac1lox/P0ostn-Cre-; NF1lox/Rac1lox/P0ostn-Cre- respectively). Mice were aged for 9 months and peripheral nerves were harvested and fixed in formalin. Peripheral nerve size was measured and tumors were identified through blinded analysis of hematoxylin and eosin and Masson’s Trichrome (collagen) stained slides. Mast cell infiltration was quantified with toluidine blue staining.

Results: Rho family members, including RAC1, were identified as candidates through an shRNA library screen. Genetic disruption of Rac1 in the Schwann cell lineage resulted in the prevention of tumour formation in NF1lox/P0ostn-Cre- mice, as observed by peripheral nerve size and histological analysis (0.67±0.06 mm vs 1.35±0.19 mm; p<0.001). We observed an average of 14.8 ±2.65 tumors per mouse in the NF1lox/P0ostn-Cre- cohort compared to 0 tumors in the NF1lox/Rac1lox/P0ostn-Cre- (+p<0.0001). Single allele loss of Rac1 did not decrease peripheral nerve size, but decreased the average number of tumors per mouse (5 ±0.93; p<0.001). Furthermore, loss of Rac1 in Schwann cells decreased the number of infiltrating mast cells found within the peripheral nerve (6.4 ±0.8 vs 35.4 ±2.9; p<0.0001).

Conclusions: Following an shRNA library screen, Rac1 was identified as a candidate to modulate PN formation. Biallelic deletion of Rac1 in vivo prevented PN formation. We demonstrate that a candidate identified in an shRNA library screen can translate to an biological effect in a mouse model of PN.

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Potential Diagnostic and Prognostic Biomarkers for Neurofibromatosis Type 1 and Related Cancers: Preliminary Results of Expression Profiling of Serum Circulating miRNAs in an Italian Study Cohort.

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Background: MicroRNAs (miRNAs) are small noncoding RNA molecules that function as major players of posttranscriptional gene regulation in several biological processes. MiRNA aberrant expression profile has been characterized in many human cancer types. The miRNA role in mediating tumorigenesis in many disorders, including neurofibromatosis type 1 (NF1), remains largely unexplored. NF1 is the most frequent hereditary tumor syndrome (1:3500 individuals) caused by loss-of-function mutations in the \textit{NF1} gene. The NF1 hallmark is the development of peripheral nerve sheath tumors either benign (dermal and plexiform neurofibromas) or malignant (MPNSTs). Few studies reported deregulated miRNAs in MPNSTs and only one indicating the involvement of circulating miRNA in NF1 has been published. Recently, we performed a pilot study to identify circulating miRNA signature in an Italian cohort of NF1 patients, as novel biomarker for diagnosis, prognosis and monitoring of disease.

Methods: The study cohort included 336 familial/sporadic NF1 patients (41% females) and 300 age/gender-matched healthy individuals. All individuals have been enrolled at 2\textsuperscript{nd} Division of Neurology, Neurofibromatosis and Rare Diseases Center of the University Hospital, University of Campania Luigi Vanvitelli. The patients’ age at the time of NF1 diagnosis was 14±12.7 years (range 2-75 years). Serum from 40 NF1 patients and 100 healthy controls was analyzed by miRNA sequencing (Illumina HiSeq2000 platform). qRT-PCR will be performed using the TaqMan MicroRNA assays kit. Statistical and bioinformatics analysis were performed. Deregulated miRNAs have been validated by qRT-PCR assay.

Results: Our preliminary results identified two deregulated miRNA markers (MiR-801 and miR-214) for which significantly different serum concentrations were found in NF1 patients compared to healthy controls. Our data confirm the results published in Chinese NF1 population (Weng Y. et al. Med Oncol. 2013. 30(2):531). Genome-wide serum miRNA expression analysis is ongoing in the entire study cohort.

Conclusions: Our pilot study identified two potential serum markers for early detection of NF1 patients at risk of developing malignant tumors. Such markers may be useful as diagnostic tool to support the diagnosis of NF1 and for timely recognition of NF1-related cancers.

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MRI Multimodal Multivariate Signature of NF1: A Multicentric Study

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Background: Several studies have inquired brain differences between NF1 and healthy children (HC) beyond the macroscopic differences evident to a visual assessment of magnetic resonance imaging (MRI) to understand the neural signature of this pathology. However, these studies have focused on single MRI modality, overlooking the fact that subtle brain abnormalities in NF1 are bound to involve different tissues and have different micro/macrostructural characteristics.

Methods: To overcome this limitation we designed a multicentric study including a multimodal MRI protocol: T1-weighted, diffusion weighted images (DWI) and resting state functional MRI. We included 42 HC and 38 NF1. All procedures were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. We derived whole-brain indexes of brain macro/micro structure (i.e. fractional anisotropy, FA, mean diffusivity, MD, grey matter volume, GMV) and activity/connectivity at rest (i.e. fraction of low amplitude frequency fluctuations, fALFF; local correlation and global correlation) and used them to discriminate between HC and NF1. Our new approach used features reduction coupled with supporting vector machine. The main aim of the pipeline was to achieve a good accuracy while keeping only the most discriminative features, to have an interpretable model of the multimodal signature of NF1-associated brain modifications.

Results: The best performance was achieved using MD (accuracy = 0.79 95%CI[.77 - .81]), with the model using GMV, MD and FA in combination reaching a similar accuracy (0.79 95%CI[.76 - .80]). GMV and FA in isolation significantly discriminated between the groups, but with lower accuracy. Of the functional indexes, only local correlation significantly discriminated between the two groups, although with low accuracy (0.66 95%CI[.62 - .68]). The most discriminative clusters for the structural modalities were located in regions known to be involved in the physiopathology of NF1 (GMV in the thalamus, striatum, hippocampus and occipital lobe; FA in the cerebellum, brainstem and internal capsule; MD in cerebellum, thalamic radiation and superior corona radiata).

Conclusions: Our results show that NF1 lead to a set of diffuse and diverse macro/microstructural abnormalities in the brain, with only minor effect on brain connectivity at rest. Our method can be expanded in the future by adding different MRI modalities that will deepen our understanding of NF1 physiopathology.

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A CRISPR/Cas9 Based Synthetic Lethality Screen in NF1-deficient Human Schwann Cells

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**Background:** Synthetic lethality describes a situation in which alterations in two or more genes simultaneously lead to the loss of cell viability, while the same alterations in either gene alone are tolerated. In this way, tumor-specific mutations can not only drive tumor progression, but also produce vulnerabilities that can be therapeutically exploited to selectively kill tumor cells, thus greatly expanding the current armamentarium of anti-cancer drugs. We hypothesize that NF1−/− cells of the Schwann cell lineage may be selectively vulnerable to the loss of function of another gene(s) that is not essential to similar NF1+/- or NF1+/- cells. We designed a genome-wide CRISPR/Cas9 KO screen for synthetic lethality in NF1−/− Schwann cells. We aim to uncover and exploit second-site targets that when disrupted, in conjunction with NF1 knock out, result in cell death.

**Methods:** NF1+/+ and isogenic NF1−/− immortalized human Schwann cells were generated via CRISPR/Cas9 targeting of exon 10 in the NF1 gene. The cells were characterized and expanded into isogenic cell lines. These cell lines were then engineered to stably express Cas9. The levels of Cas9 expression and activity were determined in cell lines. Finally, cells were transduced with a sgRNA-expressing lentiviral library targeting 19,114 human genes. Negative selection was applied by passaging the isogenic cell lines for several doublings. Genomic DNA was extracted from post-selection populations and guide sequences were PCR amplified.

**Results:** Individual cells, and pooled isogenic populations of cells, exhibited comparable and relatively uniform levels of Cas9 nuclease as assessed by flow cytometry and immunoblotting. Cas9 activity was verified using TIDE algorithm analyses after single gRNA tests, prior to infection with the pooled lentiviral library. Using DNA from isogenic cell lines following expansion we have prepared the library for Next-Generation Sequencing (NGS), which is currently in progress.

**Conclusions:** NGS data will be used to determine the frequency of sgRNA guides in NF1−/− and NF1+/+ cells post-selection. We expect certain guides to be under represented in the NF1−/− cells compared to NF1+/+ cells, identifying candidate synthetic lethal genetic interactions with loss of NF1. Genes associated with such guides will be targeted to expand the repertoire of anti-tumor therapeutics by indirect targeting of non-druggable RASopathies, such as NF1, through the identification of the second-site synthetic targets that may be druggable.

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Disclosure of Interest: J. Nikrad has a conflict with: J.A.N. was supported by National Science Foundation Graduate Research Fellowship # 00039202. D. Largaespada has a conflict with: This work was supported by The National Institutes of Health (1R01-NS086219), the American Cancer Society Research Professor Award (to DAL), The Children's Cancer Research Fund, and The Children's Tumor Foundation Synodos for NF1 Award. K. Williams has a conflict with: This work was supported by the Children's Cancer Research Foundation Emerging Scientist Award and Children's Tumor Foundation Young Investigator Award to K.B.W.
The Secretomes of Painful Versus Nonpainful Human Schwannomatosis Tumor Cells Differentially Influence Sensory Neuron Gene Expression and Sensitivity

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Background: Schwannomatosis (SWN) is a multiple tumor syndrome in which patients develop benign tumors along peripheral nerves throughout the body. However, the first symptom with which schwannomatosis patients often present, prior to discovery of tumors, is pain. This pain can be debilitating and is often inadequately alleviated by pharmacological approaches. As a consequence, patients frequently resort to multiple surgeries to remove tumors in search of pain relief. Schwannomatosis-associated pain can be localized to the area of a given tumor, but in many patients is widespread or nonspecific. Moreover, not all schwannomatosis tumors are painful, and the occurrence of pain is often unrelated to tumor size or location. We hypothesize that some individual schwannomatosis tumors, but not others, secrete factors that act on nearby nerves to augment nociception by producing neuronal sensitization or spontaneous neuronal firing.

Methods: To determine the effects of the SWN secretome on sensory neurons, primary mouse dorsal root ganglion (DRG) neurons were incubated in conditioned medium (CM) derived from: 1) painful SWN cells 2) non-painful SWN cells and 3) normal human Schwann cells. After a 48 hour incubation with CM, the DRG neurons were tested for their ability to be excited by an ascending series of KCl concentrations, using fura 2-based calcium imaging as a readout. Protein-level analysis of cytokines secreted into CM collected from painful vs. non-painful SWN cell lines was performed using the Human XL Cytokine Array from R&D Systems.

Results: Conditioned medium collected from the painful schwannomatosis tumors, but not that from nonpainful schwannomatosis tumors, sensitized DRG neurons, causing increased sensitivity to depolarization by KCl, and also upregulated the expression of pain-associated genes in the DRG cultures. Multiple cytokines were also detected at higher levels in conditioned media from painful tumors.

Conclusions: Taken together our data demonstrate a differential ability of painful versus nonpainful human schwannomatosis tumor cells to secrete factors that augment sensory neuron responsiveness, and thus identify a potential determinant of pain heterogeneity in schwannomatosis. Deciphering the mechanism(s) of action of these factors on nociceptive pathways may help identify rational targets for pain therapy in patients with schwannomatosis.

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Targeted Next-Generation Sequencing for Differential Diagnosis of Neurofibromatosis Type 2, Schwannomatosis, and Meningiomatosis

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Background: Clinical overlap between neurofibromatosis type 2 (NF2), schwannomatosis, and meningiomatosis can make clinical diagnosis difficult. Hence, molecular investigation of germline and tumor tissues may improve the diagnosis.

Methods: We present the targeted next generation sequencing (NGS) of NF2, SMARCB1, LZTR1, SMARCE1, and SUFU tumor suppressor genes, using an amplicon-based approach. We analyzed blood DNA from a cohort of 196 patients, including patients with: NF2 (N=79), schwannomatosis (N=40), meningiomatosis (N=12), and no clearly established diagnosis (N=65). Matched tumor DNA was analyzed when available. Forty-seven NF2-/SMARCB1-negative schwannomatosis patients and 27 NF2-negative meningiomatosis patients were also evaluated.

Results: A NF2 variant was found in 41/79(52%) of NF2 patients. SMARCB1 or LZTR1 variants were identified in 5/40(12.5%) and 13/40(~32%) patients in the schwannomatosis cohort. Potentially pathogenic variants were found in 12/65 (18.5%) patients with no clearly established diagnosis. A LZTR1 variant was identified in 16/47(34%) of NF2/SMARCB1-negative schwannomatosis patients. A SMARCE1 variant was found in 3/39(~8%) of meningiomatosis patients. No SUFU variant was found in the cohort. NGS was an effective and sensitive method to detect mutant alleles in blood or tumor DNA of mosaic NF2 patients. Interestingly, we identified a four-hit mechanism resulting in the complete NF2 loss-of-function combined with SMARCB1 and LZTR1 haploinsufficiency in two-thirds of tumors from NF2 patients.

Conclusions: Simultaneous investigation of NF2, SMARCB1, LZTR1, and SMARCE1 is a key element in the differential diagnosis of NF2, schwannomatosis, and meningiomatosis. The targeted NGS strategy is suitable for the identification of NF2 mosaic in blood and for the investigation of tumors from these patients.

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Estimation of Frequency of Pathogenic Variation in NF1, SPRED1 and MAPK1/3 in Unaffected Populations Using Public Genomic Databases

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Background: The RASopathies are a group of disorders caused by mutations in predominantly RAS-MAPK pathway genes. Neurofibromatosis type 1 and Legius syndrome are caused by mutations in NF1 and SPRED1, tumor suppressors that downregulate RAS-RAF signaling. MAPK1/3 are protein kinases that act downstream of the RAS-RAF-MEK axis; germline variation is not known to be associated with genetic disorders. Germline variation in these genes in the general population is unknown.

Methods: We downloaded germline variants in the 4 genes from the 3 largest public databases: Exome Aggregation Consortium, excluding The Cancer Genome Atlas samples (ExAC_non-TCGA), Exome Sequencing Project (ESP) and 1000 Genomes (1K), and analyzed these variants computationally (InterVar) and by review of the literature. The variants were classified according to the ACMG/AMP guidelines.

Results: The majority of variants were benign, likely benign or variants of unknown significance (VUS). For NF1, we identified 15 pathogenic (P) or likely pathogenic (LP) variants in 16 out of 53,105 ExAC_non-TCGA samples (frequency 0.00030; 1/3,320), close to previous estimates of NF1 prevalence (1/2,000 - 1/4,000). Given that ExAC excludes individuals with severe pediatric disease, the frequency of NF1 P/LP variation in this db could be lower than in the general population. We also identified 3 P/LP NF1 variants in 3 out of 6,258 individuals from ESP (frequency 0.00048; 1/2,086). There were no P/LP NF1 variants in the 1K database (2,504 samples). We did not observe P/LP variation in SPRED1 in any of the databases. In ExAC_non-TCGA, we identified 4 loss-of-function (LOF) variants in the gene, however, none of them were reported in the literature and due to the lack of other supporting information, these variants were classified as VUS. We did not observe P/LP variation in MAPK1/3. There were 3 LOF variants in MAPK3 and a modest number of rare missense variants with high CADD (>30) and REVEL (>0.6) scores in both genes.

Conclusions: We show that frequency of P/LP NF1 variation in the general population is 1/2,086 – 1/3,320 and is similar to previous estimates. We did not observe P/LP variants in SPRED1 or MAPK1/3, however we identified a number of bio-informatically deleterious mutations in these genes. Future work focuses on phenotype spectrum and penetrance of NF1 and other RASopathies in large, exome-sequenced populations linked to clinical records.

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Deep Targeted Resequencing of the LZTR1, SMARCB1 and NF2 Genomic Loci for Non-Coding or Mosaic Mutations in Schwannomatosis

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**Background:** We hypothesized that a portion of schwannomatosis cases without SMARCB1 or LZTR1 coding mutations in blood by Sanger sequencing have (i) other rare causal variants in the non-coding regions of the SMARCB1 or LZTR1 loci or (ii) constitute low-level mosaic form of NF2.

**Methods:** Patients: 77 schwannomatosis individuals without LZTR1, SMARCB1 or NF2 coding mutations in blood. The study was approved by MUG, UAB IRBs and USAMRMC HRPO.

DNA-seq: Targeted enrichment with SeqCap EZ Choice (Roche) for the entire LZTR1, SMARCB1 and NF2 genomic sequence, deep (~1000x, min. >300x) massively parallel paired-end sequencing (2x75 nt, Illumina NextSeq 500), followed by Sanger sequencing verification on gDNA or cDNA.

RNA-seq: alternative transcript isoform analysis in primary Schwann cells RNA.


Identification of mosaic NF2 cases: verification of presence of NF2 mutations previously found in tumor by deep resequencing (>3500x) of blood DNA.

**Results:** We identified 2 deep intronic SMARCB1 mutations, c.500+883T>G and c.500+887G>A, that lead to out-of-frame missplicing of intron 4.

Seventeen patients carried SMARCB1 3’UTR mutations or rare variants: c.*82C>T (14/77), c.*70C>T (2/77) or c.*17C>T (1/77). Functional analysis demonstrated that c.*82C>T and c.*17C>T, but not c.*70C>T, negatively affect transcript levels.

We also identified an alternative LZTR1 transcript isoform (c.1942+342_c.1943-262ins117) that is present at ~10% level of the primary transcript.

Bioinformatic analysis indicates that this isoform results in truncated, non-polyadenylated transcript of unknown function.

Additionally, we identified two cases carrying low-level mosaic NF2 mutations through targeted resequencing for mutations previously identified in a schwannoma: c.179G>A (p.Trp60*) at 2.58% (125 out of 4836 reads) and c.686del (p.Gly229Alafs*22) at 0.35% (14 out of 3999).

**Conclusions:** A novel SMARCB1 region predisposing to deep intronic mutations was identified. Next generation deep coverage sequencing allows diagnosing mosaic NF2 cases previously missed by Sanger sequencing. SMARCB1 3’UTR mutations negatively affect transcript levels. An alternative LZTR1 transcript isoform was identified and further clarification of its functional consequences is needed.
Establishment of a Comprehensive Clinically-Annotated Nerve Sheath Tumor Bank from Patients with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is a common genetic disorder characterized by a predisposition to the development of nerve sheath tumors, including cutaneous and plexiform neurofibromas (PN), and malignant peripheral nerve sheath tumors (MPNST). The development of nonsurgical therapy for PN has been limited by: 1) lack of cell culture based models, 2) limited number of animal models, and 3) limited access to primary tissue from patients with NF1. Although there is progress in the development of animal and cell culture models, the limited availability of primary patient tissue remains unaddressed. Comprehensively characterized banked tissue allows for the most efficient and targeted use of specimens in collaborative NF1 research efforts.

Methods: Through collaboration with our Comprehensive Neurofibromatosis Center, neuropathologists and surgeons, we have created and expanded a local biospecimen repository for the purpose of banking blood fractions and tumor tissue from patients with NF1 undergoing surgical resection of tumors, and generating cell culture and xenograft models to propagate primary human tissue. Patients are consented to an IRB-approved research study. Tissue is collected according to Standard Operating Procedure on the day of surgery. H&E of each banked sample is reviewed by the study neuropathologist for quality control. Banked specimens undergo comprehensive genomic characterization using RNAseq and whole exome sequencing (WES), and data are available through Synapse (https://www.synapse.org/#!Synapse:syn4939902/wiki/235907). A fully annotated clinical database accompanies the bank, and includes NF1-associated symptoms and findings, tumor characteristics, and outcome data. We have implemented an internal review process for researchers outside our institution to request access to specimens and accompanying de-identified clinical information.

Results: We have established a biorepository of high quality, well-annotated nerve sheath tumor tissues and blood fractions. Since inception in January 2016, over 150 unique samples have been banked, and include neurofibroma (n=58), MPNST (n=6), blood fractions, and xenograft (n=5) specimens, from 56 unique patients. Four researchers from outside institutions have requested access to our specimens.

Conclusions: Our biospecimen repository represents a high-quality, robustly-characterized and valuable resource for ongoing scientific efforts in the NF1 research community.

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Tyk2 Promotes Malignant Peripheral Nerve Sheath Tumor Progression through Inhibition of Cell Death

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Background: Neurofibromatosis Type 1 (NF1) is a common autosomal dominant cancer predisposition syndrome, occurring with an estimated incidence of 1 in 2500 individuals. One of the most common malignancies affecting patients with NF1 is the malignant peripheral nerve sheath tumor (MPNST), an aggressive sarcoma that affects 8% to 13% of individuals with NF1. NF1-associated MPNSTs (NF1-MPNSTs) tend to develop in young adults and carry a dismal prognosis. Despite aggressive treatment with surgical resection combined with radiotherapy and chemotherapy, most patients die within 5 years of diagnosis. Prior genomic studies in our lab identified tyrosine kinase 2 (TYK2) as a frequently mutated gene in MPNST. Additionally, TYK2 protein overexpression was observed in 60% of MPNST cases examined. Thus we hypothesized that TYK2 plays a role in MPNST pathogenesis.

Methods: We used shRNA-mediated knock down of Tyk2 expression in Nf1/Tp53-mutant NPCis MPNST cells (JW23.3) to explore the role of Tyk2 in MPNST pathogenesis. Proliferation, cell death and migration were evaluated in vitro using the IncuCyte Live Cell Analysis System (Essen BioScience). Subcutaneous and left ventricular (LV) tumor injections were performed to assess the role of Tyk2 in growth of a primary tumor and metastasis, respectively. Injections were performed in C57BL/6 ALBINO immunocompetent mice. All human and animal studies were performed under active protocols approved by the Institutional Review Board and the Institutional Animal Care and Use Committee, respectively.

Results: Knockdown of Tyk2 significantly increased cell death in vitro and in vivo in a subcutaneous tumor model. These effects were accompanied by a decrease in levels of activated Stat3 and Bcl-2 as well as an increase in levels of cleaved caspase-3. In addition, control cells treated with ABT-199, a Bcl-2 inhibitor, exhibited increased cell death, while there was less of an effect of the drug on Tyk2 knockdown (Tyk2-KD) cells, suggesting that the phenotype in the Tyk2-KD cells is partially driven by Tyk2 actions on Bcl-2. Finally, Tyk2-KD cells demonstrated impaired migration in an in vitro wound assay and impaired metastatic potential as evidenced by decreased tumor burden and increased overall survival following LV tumor injections in vivo.

Conclusions: Taken together, these data illustrate the importance of TYK2 in MPNST pathogenesis and suggest that the TYK2/Bcl-2 pathway may be a potential therapeutic target for MPNSTs.

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This work was partially funded by a grant from the Doris Duke Foundation. Dr. Hirbe is supported by the Francis Collins Award through NTAP.
Injury Signals Promote the Development of Cutaneous Neurofibroma in Mouse Model of NF1

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Background: Cutaneous neurofibromas (cNF) constitute a nearly ubiquitous symptom of NF1. There are no therapies currently approved for cNFs aside from surgical resection, and the cellular origin and pathogenesis of cNF remain largely unknown. We have recently developed a novel NF1 GEM model that recapitulates the development of cNF and allows in vivo tracing of NF1 mutant cells throughout development. We found that microscopic cNFs are present in the skin of young individuals several months before becoming macroscopically detectable, suggesting their slow evolution into full-blown cNFs. Since inflammatory environment seems to be critical for NF growth and several case reports suggest that skin trauma promotes tumorigenesis in NF1 patients, we seek to understand whether and how skin injury facilitates development of cNFs in the mouse.

Methods: Full-thickness skin incisions were made at the thoracic level on 3-month-old Prss56Cre, R26tdTom, Nf1flox/− (Nf1-KO) mutants and control littermates. Animals were sacrificed at different time points after injury and their skin was compared with that of uninjured Nf1-KO and ctrls using histologic, transcriptomic and proteomic approaches.

Results: By 7 months after surgery all injured Nf1-KO mice developed typical diffuse cNFs at the wound site along with numerous smaller lesions all over the back skin. Remarkably, mutant wounds contained dense aggregates of Nf1−/− Schwann cells as early as 7 days post-injury, accompanied by prominent inflammatory cell and fibroblast infiltration. Thus, extracellular cues from the wound microenvironment likely cooperate with cell-intrinsic mechanisms to reprogram the mutant SC to a highly proliferative, invasive phenotype. To identify the underlying molecular pathways, we combined genome-wide transcriptome profiling with analysis of secreted factors from the wound site and distant skin of Nf1-KO and control mice. Results of these analyses will be discussed.

Conclusions: Our observations support the idea that skin injury accelerates the progression of micro-cNFs to cNFs. Surprisingly, this effect was also observed at a long distance from the wound site, suggesting the involvement of systemic factors, possibly related to inflammation. The identification of such molecules might open new avenues for impeding cNFs growth. Currently, surgical ablation is the ultimate option for treatment. However, in view of our observations, such interventions might actually facilitate tumor growth, raising doubts as to the benefit of these treatments.

TRAPpping the Metabolic Adaptations of NF1-Associated Tumors

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Background: We have previously found that dysregulation of Ras/ERK transduction axis impacts on tumor metabolism through phosphorylation and thus activation of the mitochondrial chaperone TRAP1, which mediates inhibition of mitochondrial respiration and leads to succinate-dependent stabilization of the pro-tumorigenic transcription factor HIF1alpha (1). These findings have raised the attention on the potential contribution of TRAP1-mediated metabolic changes in Neurofibromatosis type 1 (NF1)-associated tumors, where hyper-activation of Ras/ERK signaling is crucial.

Methods: We have used DNSCs (mouse embryonic day 13.5 dorsal root ganglia (DRG)/Nerve root sphere cells), the cells of origin of neurofibromas in a NF1 mouse model (2), in order to test the impact of TRAP1 modulation on tumor onset and growth. Furthermore, we have exploited computational approaches based on chaperone internal dynamics in order to identify regions of the protein that might be targeted by selective inhibitors. We have tested the identified compounds for their capability of acting as TRAP1 allosteric inhibitors.

Results: Inhibition of TRAP1 expression in Nf1-deficient DNSCs strongly impairs tumor development; furthermore, TRAP1 silencing is associated with significant metabolic changes of tumor cells, as several glycolytic markers are repressed. In addition, increased TRAP1 expression leads to exacerbation of the glycolytic shift and to a faster neurofibroma progression toward MPNST (Malignant Peripheral Nerve Sheath Tumors) when a concomitant loss of the tumor suppressor gene p53 occurs. We have fished out and tested a set of putative TRAP1 allosteric inhibitors, finding that all these molecules inhibit TRAP1 ATPase activity in a highly selective way and with a potency very similar to that displayed by classical Hsp90-family inhibitors. At a cellular level, these lead compounds are able to reverse completely the inhibition of SDH activity exerted by TRAP1 in both mouse and human MPNST.

Conclusions: Our work sheds light on TRAP1-mediated metabolic adaptations in neurofibromas, which could provide selective advantages to NF1 tumor cells. The identified TRAP1 inhibitory lead molecules are useful tools for the comprehension of TRAP1-mediated metabolic changes and their possible implication in malignant progression of neurofibromas and could be exploited as new anti-neoplastic drugs for NF1-associated tumors.
Development of an Antisense-Mediated Exon Skipping Therapeutic Strategy for Neurofibromatosis Type 1

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Background: Antisense oligonucleotide (AON) therapies -exon-skipping- may have potential for the development of new therapeutics towards the cause of the NF1 disease instead of its consequences, as has been done for other genetic diseases. Our project aims to further the research in antisense therapy for NF1 by investigating the potential of exon-skipping for mutations in exons 36 or 37, in both in vitro and in vivo models. Exons 36 and 37 of NF1 gene meet the conditions for the application of this approach because: i) the reading frame is restored through both exons skipping, as observed via in silico analysis (ExonMine tool) (Fig1); ii) both exons encode part of the SEC14 and the entire Pleckstrin Homology (PH) domains thus not compromising the central GTPase-activating protein-related domain (GRD) (Fig2); iii) it will allow further investigation of their possible role in RAS suppression; iv) NF1-hallmark features have been reported in patients with mutations in such exons.

Methods: The project will be developed in 3 stages: 1 – AONs design and synthesis. candidate AONs will be analyzed with different bioinformatic tools to select the most promising ones; 2 - In vitro testing of human and mouse AONs. The human and mouse NF1 gene expression, and neurofibromin function will be evaluated in treated/untreated cells; 3 - In vivo mouse testing of AONs. Animal procedures will be done following the European guidelines.

Results: Expected results: The removal of both exons will produce alterations in the 3D structure of NF1 protein, but Phyre2 predictions pointed to the production of a shorter but potentially functional neurofibromin protein. This way, we propose to establish the proof–of–concept that antisense exon-skipping therapy can be applied to NF1 patients to rescue the function of neurofibromin.

Conclusions: This, will potentially benefit many other patients bearing mutations in these two exons since more than 100 mutations are described for these exons, some of them being recurrent. Future therapies based on exon-skipping will be somewhat individual-specific, therefore being included in the so-called personalized medicine therapies. Nonetheless, since different individuals may present different nucleotide mutations in the same exon(s), a drug application may be generalized if successful neurofibromin expression, following excision of such exons, is achieved.

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Restoring Functional Neurofibromin by Protein Transduction

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Background: In Neurofibromatosis 1 (NF1) germ line loss of function mutations result in reduction of cellular neurofibromin content (NF1+/−, NF1 haploinsufficiency). The Ras-GAP neurofibromin is a very large cytoplasmic protein (2018 AA, 319-kDa) involved in the RAS-MAPK pathway. Aside from regulation of proliferation, it is involved in mechanosensoric of cells.

Methods: We investigated neurofibromin replacement in cultured human fibroblasts showing reduced amount of neurofibromin. Full length neurofibromin was produced recombinantly in insect cells and purified. Protein transduction into cultured fibroblasts was performed employing cell penetrating peptides along with photochemical internalization. This combination of transduction strategies ensures the intracellular uptake and the translocation to the cytoplasm of neurofibromin.

Results: The transduced neurofibromin is functional, indicated by functional rescue of reduced mechanosensoric blindness and reduced RasGAP activity in cultured fibroblasts of NF1 patients or normal fibroblasts treated by NF1 siRNA.

Conclusions: Our study shows that recombinant neurofibromin is able to revert cellular effects of NF1 haploinsufficiency in vitro, indicating a use of protein transduction into cells as a potential treatment strategy for the monogenic disease NF1.


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Tumor Evolution Revealed by Deep Sequencing of Primary and Locally Recurrent NF1-Associated Malignant Peripheral Nerve Sheath Tumors (MPNST)

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Background: Malignant peripheral nerve sheath tumors (MPNST), a heterogeneous soft tissue sarcoma arising from the neural crest lineage, affects 15% of patients with Neurofibromatosis Type I. Despite surgical and radiotherapy/chemotherapy, these tumors recur locally in 40-45% of cases resulting in high morbidity. The genetic characteristics of these recurrent tumors are poorly understood. Furthermore, prior studies on MPNST typically involve sequencing to depths of 75-100x exome or 30-50x whole genome, which may limit detection of genetic alterations in tumors that are impure, aneuploid, or heterogeneous.

Methods: In order to evaluate the genetic heterogeneity of MPNSTs and genetics of local tumor recurrence, we performed deep whole genome sequencing (120x) and deep exome sequencing (900x) on sets of matched primary and locally recurrent tumors from two individuals with NF1-associated MPNSTs. Matched normal tissue or blood from each individual was sequenced at standard depth. Tumors were obtained as frozen tissue collected by the Neurofibromatosis and Allied Disorders Tissue Bank at Massachusetts General Hospital.

Results: We characterized the impact of sequencing depth on detection of nucleotide variation and structural variation based on subsampling of the original deep sequencing data. Such analysis reveals insights into the degree of tumor heterogeneity and the optimal sequencing strategy in MPNSTs. Our results show that MPNSTs may be tetraploid, and that tetraploidy may occur as an early genetic event during tumor development. We showed that local recurrence of MPNST shows genetic variation that suggests a genetic precursor prior to that of the initial MPNST in one individual.

Conclusions: We show the relevance of deep sequencing for genetic evaluation of MPNSTs which are known to be heterogeneous tumors. Our data agrees with prior sequencing studies that show that MPNSTs have a relatively low burden of single nucleotide variation. In addition, we detected a high degree of structural variation including copy number changes and polyploidy. Paired primary and locally recurrent tumors suggest potentially early tumor precursors that remain present after the initial tumor resection and contribute to local recurrence.

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The Evaluation of Telomer Length and Telomerase Activity in NF1 Tumors

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease that affects 1 in 3000 people worldwide. The disease is developed due to occurrence of mutations in NF1 gene. NF1 gene is coding cytoplasmic protein which is negative regulator of RAS proteins. The loss of neurofibromin results in activation of RAS cascade and cell proliferation. For this reason NF1 gene is categorized as tumor suppressor gene. It is clinically characterized by cafe-au-lait spots, Lisch nodules, axillary and inguinal freckling, multiple peripheral nerve tumors, bone lesions, and a predisposition to malignancy. Variations in NF1 mutations may not correlate with the variations in clinical phenotype. This unclear genotype–phenotype correlations is assumed to be due to modifier genes. One of these candidates is telomer length and telomerase enzyme activity. Telomeres are repetitive nucleotide sequences located at the ends of chromosomes and protect them from fraying and sticking to each other. The length of telomereres are shortening in each cell division. Nevertheless this shortening can be prohibited by the enzyme telomerase which adds a species-dependent telomere repeat sequence to the 3' end of telomereres. However, telomerase activity usually diminished after birth in somatic cells. Researches done in last years have shown the importance of telomer and telomerase activity and they are causally connected to human disease. However, the number of research on this concept for NF1 patients is very few.

Methods: We analyzed DNA and tumors from 10 NF1 patients by quantitative PCR based technics. DNA samples were isolated from blood samples and sequenced to detect variations in each exon. Telomere length measurement were also done using the DNA samples. The pathological status of tumor tissues was confirmed by routine pathological examination. Telomerase activity were evaluated from proteins isolated from acquired tumor samples.

Results: Considering the preliminary results, higher telomerase activity is measured in some NF1 tumors and also variatons in telomere size were detected. For better analysis we enlarged our sample number. We cannot present the detailed data of the study because of the limitation of the space.

Conclusions: These primary data indicate that telomere length may play an important role in NF1-associated tumor’s progression and could provide information about the telomere-targeted therapeutic approaches for treatment of telomere dysfunction in the clinic.

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CRISPR Based Engineering of NF1-Associated MPNST-Like Cells

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**Background:** Neurofibromatosis Type 1 syndrome (NF1) is an autosomal dominant cancer predisposition syndrome, due to loss of function mutations in the *NF1* gene, encoding neurofibromin. Most patients develop benign Schwann cell (SC) tumors including dermal neurofibromas and plexiform neurofibromas that can progress to malignant peripheral nerve sheath tumors (MPNSTs). Neurofibromas develop after somatic loss of the one *NF1* wild-type allele that these patients carry. MPNSTs have biallelic defects in the *NF1* gene and also in other genes, including *SUZ12* or *EED*, resulting in defective polycomb repressor complex 2 (PRC2). CRISPR/Cas9 engineered human cells allow study of the effects of the stepwise accumulation of *NF1* and *SUZ12* mutations on Schwann cell physiology.

**Methods:** In order to generate NF1-associated MPNST-like cells, we introduced somatic mutations into the human *TERT* and murine *Cdk4* immortalized human Schwann cell line, HSC1λ, using CRISPR/Cas9 and co-transposition enrichment methods we developed. We created and utilized useful *NF1* and *SUZ12* specific single guide RNA (sgRNA) constructs and made derivative clones defective in *NF1* and/or *SUZ12*. Molecular and phenotypic studies were carried out on these cells.

**Results:** An analysis of mutant clones shows complete loss of histone H3K27 trimethylation after biallelic loss of *SUZ12*. The mutant clones also have an increase in H3K27 acetylation. We are in the process of characterizing these cell lines using several transformation assays along with control isogenic cell lines. We have also generated additional control cell lines by reintroducing the knocked out genes via lentiviral transduction for both the edited HSC1λ and MPNST cell lines. We are in the process of generating RNAseq and proteomics data for these cell lines.

**Conclusions:** We successfully generated HSC1λ *NF1* and/or *SUZ12* knockout cell lines. *SUZ12* edited HSC1λ cell lines lack detectable *SUZ12* protein, show reduced EZH2 protein, and all histone H3K27 trimethylation, similar to MPNST cell lines with PRC2 loss. Based on the protein expression data, we anticipate the RNAseq and proteomics will uncover additional changes between the isogenic pairs of edited and unedited cell lines and shed light on mechanisms relevant to tumor progression and therapy.

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Disclosure of Interest: M. Sokolowski: None Declared, K. B. Williams has a conflict with: KBW is supported by the Children's Tumor Foundation Young Investigator Award, R. Williams: None Declared, S. Finnerty: None Declared, D. A. Largaespada has a conflict with: Supported by The National Institutes of Health (1R01-NS086219), the American Cancer Society Research Professor Award (to DAL), The Children's Cancer Research Fund, and The Children's Tumor Foundation Synodos for NF1 Award. David A Largaespada is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., and Genentech, Inc. is funding some of his research. The business of all these companies is unrelated to the contents of this abstract.
Organoid Models of Cutaneous Neurofibromas for High-Throughput Drug Screenings

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**Background:** Cutaneous neurofibromas (cNF) are heterogeneous benign tumors that develop in Neurofibromatosis type 1 (NF1) patients. Therapeutic options are limited to surgical removal of lesions. In order to support drug screening efforts and identify effective therapies, we set out to develop organoid models of cNF from surgically removed tumors.

Organoids recapitulate most features of the tissue they are generated from, including cell heterogeneity, microenvironment and drug response. We routinely generate organoids from primary tumor samples in a format suitable for automation and high-throughput drug screenings (Phan et al, 2017). Our approach does not necessitate of cell expansion in vitro and allows us to obtain a drug sensitivity profile within 5-6 days, preventing senescence and/or accumulation of genetic changes that can occur with prolonged times in culture. Our method is particularly suited to sustain growth of heterogeneous primary cells (Soragni et al, 2016; Phan et al, 2017), thus is applicable to cNFs which typically contain a mixture of Schwann cells, mast cells and fibroblasts.

**Methods:** We discovered that ANP expressing C. novyi (ANP-C. novyi-NT) can protect mice from cytokine release and mortality through reducing the levels of tumor lysis and direct toxic effects of the bacteria. Tools for managing these complications have been limited. To mitigate this dose-limiting toxicity, we thus created a novel bacterial strain that expresses the anti-inflammatory peptide atrial natriuretic peptide (ANP).

**Results:** We have obtained cNFs from n=6 patients enrolled in the study. cNF tumors were processed individually when their size was greater than 0.5 cm with yields between 0.3 – 6.5 Mio cells/cNF. We optimized culturing conditions by testing three different base media and two cytokine cocktails intended to support the growth of Schwann cells, fibroblasts and mast cells. We successfully observed growth of all cell types in a subset of conditions tested. We also compared the histology of the cNF of origin to that of the reconstituted organoids by staining for SOX10, S100, NGFR, NCAM1, SOX2, cytokeratin and CD34. A comparison of the transcriptomes of the organoids and corresponding cNF is in progress.

**Conclusions:** We developed and characterized miniaturized 3D organoid models for human cNFs. Our approach to process cNFs and generate organoids allowed us to establish pre-clinical models suitable for future high-throughput drug screenings.

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Reducing Mortality from C. Novyi-NT-Induced Cytokine Release Syndrome via Bio-Engineering of a Novel ANP-Expressing Strain

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**Background:** Neurofibromatosis Type 1 (NF1) is a RASopathy that represents a major risk for the development of malignancies, particularly malignant peripheral nerve sheath tumors (MPNST). To date, surgery is the only treatment modality proven to have survival benefit for MPNSTs and even when maximal surgery is feasible, these tumors are almost never curable. We have previously shown that a single dose of the “therapeutic” oncolytic spore-forming bacterium Clostridium novyi-NT (C. novyi-NT) can result in robust and reproducible antitumor responses in MPNSTs. However, when very high doses of spores are injected into very large tumors, a massive infection occurs and the animals die within a few days with severe cytokine release due to a combination of tumor lysis and direct toxic effects of the bacteria. Tools for managing these complications have been limited. To mitigate this dose-limiting toxicity, we thus created a novel bacterial strain that expresses the anti-inflammatory peptide atrial natriuretic peptide (ANP).

**Methods:** A gene cassette encoding the ANP of 28-amino acid fused with a signal peptide at the N-terminus was optimized for C. novyi codon usage and stably integrated into the C. novyi-NT genome using group II Intron targeting and bacterial conjugation. Selected C. novyi-NT clones were characterized for ANP expression and tested in vivo for toxicity and antineoplastic efficacy.

**Results:** We discovered that ANP expressing C. novyi (ANP-C. novyi-NT) can protect mice from cytokine release and mortality through reducing the levels of circulating catecholamines. There was also a noticeable reduction of tissue damage in mice treated with ANP-C. novyi-NT compared to controls. In vitro and in-vivo studies revealed that catecholamines orchestrate the immunodysregulation via a self-amplifying loop in immune cells. Direct pharmacologic inhibition of catecholamine synthesis with metyrosine protected mice from the lethal complications of cytokine release resulting from oncolytic bacteria without affecting the tumor eradication efficacy.

**Conclusions:** We successfully created a next generation C. novyi strain that can reduce treatment-induced fatal toxicity without impairment of the therapeutic response. Furthermore, we identified catecholamines as essential driver mechanism for toxicity that can be pharmacological targeted resulting in superior animal survival.

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Geranylation Inhibitors Show Selective Toxicity on Nf2-Null Cells, Which is Driven by Activated Rac1 Translocation to the Nucleus

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Background: The NF2 gene product, Merlin, remains one of the least understood tumour suppressors so far. First discovered as a structural protein it soon turned out to be a regulator of multiple proliferation and migration cascades, such as Pak and Rac signaling, receptor tyrosine kinase signaling, mTor signaling and many others. Still, the exact mechanisms by which Merlin regulates these pathways remain mostly unclear, and targeted therapy of NF2 has not succeeded so far due to abundance and redundancy of such cascades. In our study we focus on the pathologic activation of Rac1 in the Nf2-deficient cells and a new cytotoxic function of this activated protein in the absence of membrane anchoring.

Methods: Cell lines: MEF Nf2fl/fl (provided by Dr. Giovannini), MEF Nf2−/−, SC4-9 (provided by Dr. Giovannini)

Fluorescent microscopy, Fluorescence resonance energy transfer measurement, immunoblotting, immunoprecipitation, xenograft studies

Results: In order to probe the vulnerability of Nf2-null cells to small molecules, we performed a synthetic lethal screen using a library of small bioactive compound library in isogenic Nf2+/+ and Nf2−/− mouse embryonic fibroblasts, seeking compounds that selectively kill Nf2-deficient cells. Nine compounds showed significant selectivity for NF2-deficient cells; of these, lovastatin proved to be the most effective. Further investigation revealed that the effect of lovastatin was due to inhibition of geranylation, and that a GGTase type I was involved. We then showed that GGTase I target Rac1 was responsible for Nf2-null cell death in the presence of statins and GGTase I inhibitors (zoledronic acid and GTI248). We also showed that Rac1, when removed from the cell membrane by statins and GGTase I inhibitors translocates to the nucleus in both Nf2-deficient and wild-type cells, but FRET measurements showed that only in Nf2-null cells Rac1 is activated in the nuclei. Finally, we proved that Rac1 activation in NF2-deficient cells upregulates SAPK/JNK-Stat5-Jun cascade and leads to cell death. In vivo, xenograft studies using SC4-9 (Nf2-deficient mouse schwannoma) cells revealed that simvastatin and zoledronic acid significantly slowed tumor progression.

Conclusions: Our studies indicate that the two FDA-approved agents, simvastatin and zoledronic acid, might be useful in treating NF2-related schwannomas.

We also show a new cytotoxic function of activated Rac1 when this protein is detached from cell membrane, which provides a new potential therapeutic target for NF2.

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Cancer Stem Cells as Targets for MPNST Tumorigenesis and Relapse

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Background: Malignant peripheral nerve sheath tumor (MPNST) is a type of soft tissue sarcoma that arises from the neural crest lineage. The complete MPNST clearance is limited by surgical resectability, and the local recurrence is 40–45% with high morbidity. Cancer stem cells (CSCs) have been reported in multiple solid tumors such as glioblastoma. Targeting this population provides a new strategy to prevent tumorigenesis, relapse and even metastasis.

Methods: We use cisNf1+/−:Trp53+/− (cisNP) spontaneous mouse models and recombined Schwann cell progenitor (Nf1−/−:Trp53−/−) driven nude mouse allograft models. Two different transgenes, Nes-TK-GFP and Nes-CreERT2-eGFP-DTR, driven by the same endogenous rat Nestin promoter with the second intron, were harnessed to label the putative CSCs. Lineage tracing by BrdU or EdU/Brdu sequential labeling was performed to characterize the quiescent CSC population in multiple models. Ganciclovir or diphtheria toxin treatment is applied to allograft tumors with Nes-TK-GFP or Nes-CreERT2-eGFP-DTR, respectively, to evaluate the role of CSCs in tumorigenesis. Using Nes-CreERT2-eGFP-DTR transgene, we performed a serial tumor transplantation by injecting sorted primary GFP+ or GFP- MPNST cells into the sciatic nerve to functionally differentiate their abilities to seed and sustain a new tumor. We further characterized sorted GFP+ and GFP- tumor cells by RNAseq.

Results: A quiescent (BrdU-;Ki67+) cell population exists in MPNSTs after longterm BrdU pulsing. In mice with the Nes-TK-GFP transgene, 1) ganciclovir treatment eliminates the dividing cells expressing the herpes simplex type 1 thymidine kinase (TK) and significantly decreases the tumor growth in both spontaneous and allograft models; 2) the EdU and BrdU sequential labeling assay after chemotherapy regimes in MPNST allografts shows that GFP+ cells can continuously repopulate the tumor after chemotherapy. In the allograft model with Nes-CreERT2-eGFP-DTR transgene, 1) diphtheria toxin treatment shrinks the primary MPNST significantly; 2) the serial transplantation demonstrates that sorted GFP+ cells can continuously initiate new tumor and keep a homeostasis, while the counterpart GFP- tumor cells fail at the tested cell number with orthotopic sciatic nerve transplantation.

Conclusions: We identified a quiescent cell population in mouse MPNSTs, and CSCs can maintain the MPNST as a hierarchy with CSCs residing at the apex. Targeting MPNST CSCs combined with chemotherapy inhibits the tumorigenesis and relapse.

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Identification of Candidate Synthetic Lethal Genetic Interactions with NF1 Loss Using Cancer Dependency Maps

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Background: Neurofibromatosis Type 1 (NF1) syndrome is an autosomal dominant genetic disorder observed in roughly 1:2500 births. This disorder predisposes patients to cancer and is caused by mutations in the tumor suppressor gene NF1. NF1 encodes neurofibromin, a negative regulator of RAS signaling pathway. Individuals with NF1 syndrome develop benign and sometimes malignant tumors of the peripheral nervous system originating from the Schwann cell lineage after the loss of wild-type (WT) copy of the NF1 gene. The genetic basis of NF1 syndrome makes it ideal for using synthetic lethal approaches to uncover unique vulnerabilities in NF1 deficient cells.

Methods: Cancer cell line mutation and copy number data for the NF1 gene were downloaded from cBioPortal. AVANA CRISPR screen 2018Q2 release of 436 cell lines was downloaded from depmap.org portal. Cancer cell lines with deleterious NF1 mutations and/or homozygous loss of NF1 were identified from the mutation data. We then performed enrichment analysis using BROAD Institute Avana CRISPR screen with 436 cancer cell lines and compared the NF1 mutant cell lines to NF1 wild type cell lines.

Results: To deduce differentially essential genes between NF1 mutant and NF1 WT cells we calculated z-scores for each gene normalizing by the genes variation across samples and the deviation observed within the sample. Preliminary results suggest that there may be genes that are essential in the WT compared to NF1 mutant samples. CRISPR screen results will also be compared to shRNA screens performed in the same cell lines.

Conclusions: The CRISPR screen did not show any essential genes enriched in the absence of NF1. This could be due to the complexities of the cancer cell lines studied. Further exploration will be needed to determine synthetic lethal genes in the absence of NF1.

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Disclosure of Interest: N. Temiz has a conflict with: NAT consults for Celculty, Inc. Celculty business is unrelated to the contents of this abstract. K. Williams: None Declared. D. Largaespada has a conflict with: DL is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., and Genentech, Inc. is funding some of his research. The business of all these companies is unrelated to the contents of this abstract.
Prediction of Functional Prognosis in Japanese NF2 Patients by Phenotype-Genotype Correlation

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Background: Prediction of mortality with neurofibromatosis type 2 (NF2) has been reported possible due to recent clinical-genotype analysis. However, prediction of functional prognosis with NF2 has not been enough discussed. Estimation of functional deterioration is extremely important for the proper understanding of their life planning. The aim of this study is to enable prediction of their functional prognosis based on clinical-genotype correlation analysis.

Methods: A cohort of 48 patients analyzing their full clinical and genetic data, with follow-up of 18.0±10.4years, were used to assess predictors of functional outcomes. NF2 alteration are detected by Sanger sequence and multiple ligation-dependent probe amplification with germline DNA. We analyzed their functional outcome focusing on hearing, swallowing, and ambulation. Kaplan–Meier survival and Cox regression analyses were used to evaluate predictors of functional disability. The study protocol was approved by the Institutional Review Board of our hospital (G10026), and informed consent was obtained from all patients.

Results: An NF2 alteration was identified in 30 / 48 (62.5 %) including truncating mutation (13: 27.1 %), large deletion (4: 8.3 %), splice site mutation (8: 16.6 %), and missense mutation (5: 10.4 %). Somatic mosaicism and undetected case were found in 18 (37.5 %). Consequently, it was established that the patients with "somatic mosaicism or undetected case" and "onset age-25" had clearly different functional prognosis compared to other NF2 patients.

Conclusions: Clinical-genotype correlation analysis enables prediction of functional prognosis in NF2 patients, allowing adequate lifelong disease management and social integration planning.

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Disclosure of Interest: Y. Teranishi: None Declared, S. Miyawaki has a conflict with: Research grant from Japan Intractable Diseases (Nanbyo) Research Foundation, H. Hongo: None Declared, S. Dofuku: None Declared, A. Okano: None Declared, S. Takayanagi: None Declared, H. Nakatomi: None Declared, N. Saito: None Declared

Development of Small Molecule Inhibitors of LIM Kinases, New Therapeutic Targets to Treat Neurofibromatosis Type I

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Background: LIMK1 and LIMK2 (LIM kinases or LIMKs) are kinases, playing a crucial role in cytoskeleton dynamics by regulating both actin filament and microtubule remodelling. They have been shown to be involved in cancer development and metastasis, resistance of cancer cells to microtubule targeted treatments, neurological diseases, viral infection, and Neurofibromatosis type I and type II. LIMKs have thus recently emerged as new therapeutic targets.

Methods: Based on molecular modelling and docking experiments, we have synthesized a library of small molecule inhibitors of LIM kinases. We have tested their activity in vitro, and in cellulo on actin filament dynamics, as well as their cytotoxicity on different cell lines.

Results: We have synthesized more than 130 small molecule inhibitors of LIM kinases. Over fifty molecules are very active in vitro on purified LIMKs, with Ki under 50 nM. These molecules are also very active in cellulo on actin cytoskeleton dynamics, they even show better activity than the reference inhibitors LIMKi3 and LX7101. Moreover, these compounds exhibit low cytotoxicity.

Conclusions: We want to go further into SAR (Structure Activity Relationship) in order to increase chemical originality and molecule stability. We also want to further characterize the biological activity of our compounds (microtubule remodelling, cell cycle progression, cellular migration, kinase selectivity, and pharmacokinetics). Our ultimate goal is to perform pre-clinical assays on the three most promising compounds.

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Evaluating Modified Diets and Dietary Supplement Therapies for Reducing Muscle Lipid and Improving Muscle Function in Neurofibromatosis Type 1 (NF1)

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**Background:** The musculoskeletal manifestations of NF1 can have a significant impact on quality of life, particularly during childhood. NF1 has been associated with muscle weakness, hypotonia, and fatigue, and murine models have suggested an underlying metabolic myopathy. Our prior published research has shown that the \( Nf1Prx1^{-/-} \) mice, which show increased lipid and weakness in the limb muscles, can be partially rescued by a medium-chain fatty acid (MCFA) enriched diet and L-carnitine supplementation.

**Methods:** We are currently undertaking a study where a variety of dietary interventions are being trialed using the same \( Nf1Prx1^{-/-} \) mouse model. Key outcomes include a comparison of MCFAs and L-carnitine alone versus the combination; modeling cheat days with less frequent dosing; testing the advantages of a “mitochondrial cocktail”; and examining alternative MCFA sources such as coconut oil and triheptanoin. In addition, we will also perform a time course study where phenotypic reversion after the cessation of dietary intervention will be measured.

**Results:** This study is currently supported by a Children’s Tumor Foundation Drug Discovery Initiative Grant (2018-2019). Key experimental outcomes include muscle-lipid profiles using LC-MS, *in situ* physiology of isolated muscles, and tissue histology.

**Conclusions:** This study will be critical for guiding future clinical trials for dietary and nutraceutical interventions to improve NF1 muscle function.

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NF1 Funding: A State of the Field

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**Background:** A collective of funders with different strategies and focus areas exists for the NF1 community, and is composed of both public as well as private not for profit groups. Given the multiple funders with different priorities, and multiple areas of research focus for neurofibromatosis, it is of interest to examine the funding landscape to ascertain what research topics have been of priority for neurofibromatosis type 1 (NF1). Plexiform neurofibroma, MPNST, glioma, cognitive, skeletal, quality of life, and muscular effects are key ailments and issues associated with NF1. Funding distribution across these areas is explored.

**Methods:** The number of grants awarded by focus area and their associated funding amounts were extracted from reporting sites for the Congressionally Directed Medical Research Program (CDMRP) and National Institutes of Health (NIH, via RCDC), and Dimensions for Funders (a database of publicly and privately funded research projects worldwide), in addition to websites of known funders not appearing on Dimensions for Funders. Data from 2008-2017 was extracted using a query for NF1 as well as specific queries for plexiform neurofibroma, cutaneous neurofibroma, MPNST, glioma, cognitive, skeletal, quality of life, and muscular, followed by manual inspection of grants data to filter out redundancies and false positive results.

**Results:** The NIH (all institutes) and CDMRP provided the largest funding for NF1 research, having provided aggregate funding of $174 million and approximately $73 million, respectively. The role of private not for profit funders for NF1 research has become more prominent since 2008, as exemplified by the Children’s Tumor Foundation, the Neurofibromatosis Therapeutic Acceleration Program, and NF research initiative, which together have provided funding in excess of $40 million dollars across more than 180 grants. Funding for plexiform neurofibromas, MPNST, and glioma research represent the greatest expenditures for NF1 research, followed by cognitive and quality of life areas. Funding for cutaneous neurofibroma, skeletal, and neuromuscular have received overall less funding.

**Conclusions:** Skeletal, cutaneous neurofibroma, and neuromuscular are areas of NF1 research with comparatively less funding. Private not for profit funders complement critical federal funding programs by providing seed funding and supporting less represented areas. A notable effort has been made across NF1 funders to coordinate and integrate funding to maximize value and efficiency.

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An Efficient Operational Model for Funding NF1 Research

Sharad Verma, Neurology, Johns Hopkins University, Baltimore, United States

Background: While public agency support for funding of the NF1 research community is mission critical, the processes entail longer grant review cycles, by necessity have less flexibility, and may be susceptible to uncertain funding conditions. The Neurofibromatosis Therapeutic Acceleration Program (NTAP) at Johns Hopkins was created in 2012 with a mission of accelerating the development of therapies for plexiform and cutaneous neurofibromas by identifying research bottlenecks and deploying enabling strategies and resources. Part of NTAPs mission includes balancing careful grant review with swift and flexible funding mechanisms. This study analyzed the elements contributing to efficient grant review and processing.

Methods: A systematic review of all NTAP proposals between 2013-2015 and 2016-2017 was conducted. Based on a starting point of receipt of a new application to an endpoint of obtaining a fully executed agreement, the processes between these points (internal scientific review, external peer review, analysis of peer reviewer feedback, follow-up discussions with investigators, return of a final decision, and contracting processes) were evaluated.

Results: The average cycle time for establishment of a fully executed agreement from receipt of a new application was 26.4 weeks during 2016-2017, vs 40 weeks for 2013-2015 (34% reduction). Improvements to the review process, in particular the targeted completion of an internal review for a new proposal within 10 business days of receipt, and early engagement with peer reviewers, reduced the review cycle time by 53% (12 weeks in 2013-2015 vs 6.4 weeks in 2016-2017). The cycle time to obtaining a fully executed agreement following the return of a final decision was reduced by 30% (28 weeks in 2013-2015 vs 20 weeks in 2016-2017), and was driven by the establishment of a consistent communication process between the scientific, financial, and administrative functions, and proactive engagement with investigators and institutional contracting offices. This model has enabled NTAP within its first 5 years to issue 35 fully executed agreements, which have yielded 56 publications and presentations.

Conclusions: A ‘streamlined’ operating model has been developed that is conducive to the swift review and approval of submitted grants. This model is feasible owing to the smaller number of grants and narrow topic focus. The elements described may be incorporated into granting and award processing to accelerate research efforts in the NF1 community.

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Comprehensive Spectrum of NF1 Binding Partners

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Background: NF1 is a large multi-domain protein with several known binding partners. Previously, reagents for studying NF1 and affinity of NF1 antibodies has been limited.

Methods: We have created a tandem affinity purification (TAP) tag composed of a TEV cleavage site followed by a Strep II Tag and a 6X His Tag cloned in frame to the full-length mNf1 cDNA (separately to both the 5' and 3' end).

Results: We show that the clones are still functional and express His-Tag, NF1 and can alter (correct) p-ERK/ERK ratios. We have used them in both stable and transient transfections to pull down NF1 via Strep-Tactin®XT beads along with other interacting partners that were identified through MS. We were able to identify 1560 different proteins; 15 overlap with reports in the literature or BioGrid database; 56 also show differential protein expression between wild type and NF1 deficient cells.

Conclusions: These interacting proteins represent many families and complexes: 14-3-3, annexins, keratins, tubulins, Ras-related proteins, proteasome subunits, and DNA repair proteins. In efforts to begin validating and mapping targets, we have also already generated clones with this TAP tag at the 3' end of the human GRD and CSRD sequence as well as various NF1 patient specific mutations.

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Analysis of Patient-Specific NF1 Variants Leads to Functional Insights

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Background: Neurofibromatosis type 1 (NF1) is caused by pathogenic variants in the NF1 gene that encodes neurofibromin. NF1 has several predicted functional domains, with the best characterized and most significant being the GAP-related domain (GRD). The GRD functions by binding GTP-bound Ras and stimulating intrinsic Ras GTPase activity to return Ras to its inactive GDP-bound state. The fact that missense mutations in other areas of the protein can also cause disease with divergent phenotypes suggests that other domains have alternative and critical functions. We hypothesize that some variants lead to increased levels of Ras-GTP by altering function of the NF1 protein outside the GRD.

Methods: We previously described an approach to determining the functional consequences of NF1 genetic variants through analysis of transfected mouse Nf1 cDNA. We created a new panel of approximately 30 mutant mNF1 cDNAs from multiple protein domains representing NF1 patient variants with different clinically relevant phenotypes, and assessed their ability to produce mature neurofibromin and restore NF1 activity in NF1-/- cells.

Results: We see great variation in NF1 protein levels produced by different cDNAs, suggesting that some variants lead to protein instability or enhanced degradation. When expressed at high levels, some of these proteins are still able to repress Ras activity, indicating that the NF1 phenotype may be due to protein instability. However, high levels of NF1 protein do not always reflect repressed Ras activity, implying that while some mutant proteins are produced and stable, they are not functional. Further, we see great variation in Ras activity resulting from different cDNAs. This variation typically falls between wild type to null levels, but also includes a third category with Ras activity levels that are elevated above the null levels, suggesting dominant negative activity. Titration assays with these mutations indicate that some mutant proteins are able to impair wild type function.

Conclusions: We find evidence that NF1 mutations have a variety of effects on NF1 protein levels (stability) and Ras activity levels. We also provide the first data indicating dominant negative activity of specific neurofibromin variants.

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Multi-omics Profiling for Biomarker Discovery in Neurofibromin (NF1) Deficient Cells

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Background: Loss of NF1 is an oncogenic driver. In efforts to define pathways responsible for the initial development of neurofibromas and other cancers, we evaluated transcriptomic and proteomics changes in a non-malignant NF1-null cell line.

Methods: We created NF1-null HEK293 cells using CRISPR/Cas9 technology and compared them to parental cells via both RNA-seq and proteomics analysis.

Results: We found 1222 genes and 142 proteins to be differentially expressed. We integrated the analysis to identify 8 transcripts/proteins that are differentially regulated in both analyses. Metacore Pathway analysis was able to identify altered expression of the Neurogenesis NGF/TrkA MAPK-mediated signaling pathway. Next, we compared our data set to other published studies that involve analysis of cells or tumors that are deficient for NF1 and found that 141 genes recur in our sample and others; only thirteen of these genes recur in two or more studies. We validated a few genes (ETV4, LAMB3, SPRY4, SFN, and KRT8) and proteins (KRT8, PDCD4, LBR, MARCKS, and SFN) of interest via qRT-PCR or Western blot respectively. In addition, we “rescued” the null cell line by transfection of mNf1 cDNA and validated that KRT8, LBR, and 14-3-3 sigma expression changes dependent on neurofibromin. Finally, we also examined HEK293 NF1 null cells protein levels after a dose response of 48 hour selumetinib treatment; PDCD4 expression changes with selumetinib treatment: but KRT8, 14-3-3 sigma, and LBR do not respond to selumetinib indicating that they are independent of this Ras effector pathway.

Conclusions: Some of the transcripts/proteins we have identified, such as KRT8, could potentially be used as biomarkers. Further, we have newly implicated 14-3-3 sigma, a gene family already known to modulate rasopathy phenotypes such as Noonan Syndrome, in NF1.

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Adaptive Signaling Response to MEK Inhibition in NF1-Associated Malignant Peripheral Nerve Sheath Tumor

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**Background:** About 50% of MPNST arise in patients with neurofibromatosis type 1 and are one of the most difficult types of soft tissue sarcoma to manage. NF1 is a tumor suppressor gene essential for negative regulation of RAS activity and is the most common genetic alteration in MPNST (86%). Despite much effort, there has been little advancement in overall patient survival, and thus novel therapeutic approaches are needed. The concept of pharmacologic MEK inhibition has been applied to models of MPNST. However, the preclinical responses to single agent MEK inhibitor (MEKi) have been partial at best and suggest a need for a better understanding of the role of ERK and other effector signaling pathways. A complex interplay of upstream signaling or parallel pathways may characterize NF1-driven tumorigenesis and inhibiting more than one RAS effector pathway may be necessary for complete anti-tumor effect.

**Methods:** Expression of NF1, endogenous activity of ERK and PI3K/AKT signaling by immunoblotting; isoform-specific RAS activity using active Ras immunoprecipitation assay; and transcription of representative RAS-ERK signature genes by real-time PCR, were performed and evaluated in seven human MPNST cell lines. Experiments included determination of sensitivity of MPNST cells to MEKi and identification of actionable alterations in signaling that underlie adaptive resistance; characterization of in vitro MEKi-resistant models using genomic, proteomic and biochemical approaches to identify mechanisms of resistance. The anti-tumor efficacy of combination strategies was further examined.

**Results:** We found that N-RAS activity is predominant in human MPNST cells. NF1-associated MPNST cells demonstrate variable responses to the allosteric MEKi trametinib. Relief of negative feedback and signaling adaptation to MEK inhibition result in compensatory activation of parallel pathways, including PI3K/AKT. The combination of MEKi plus PI3K/mTORi effectively inhibits MPNST cell growth. Additionally, HGF/c-Met signaling is elevated in a MEKi-resistant model and c-Met inhibitor (METi) is active against MEKi resistance in MPNST.

**Conclusions:** In summary, our ongoing work demonstrates that NF1 critically regulates NRAS signaling in MPNST and has begun to unravel the complicated networks which are adaptively changed in response to MEK inhibition. We have identified one mechanism of acquired resistance to MEK, and found that combination strategies using MEKi+PI3K/mTORi or MEKi+METi may delay or prevent MEKi resistance in MPNST.

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**Disclosure of Interest:** J. Wang: None Declared, C. Pratilas has a conflict with: We are grateful to Neurofibromatosis Therapeutic Acceleration Program (NTAP); Hyundai Hope on Wheels; Children’s Cancer Foundation and DHART SPORE whose support made the research possible.

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EZH2 is Unable to Regulate Transcription in the Absence of SUZ12 or EED

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**Background:** Genetic mutations affecting chromatin modifiers are recurrent in cancers. In Malignant Peripheral Nerve Sheath Tumors (MPNST), the Polycomb Repressive Complex 2 (PRC2), which plays a crucial role in gene silencing, is inactivated through recurrent mutations in EED and SUZ12 core subunits but mutations in PRC2’s main catalytic subunit EZH2 are never found. This is in contrast to myeloid and lymphoid malignancies, which harbor frequent loss-of-function mutations in EZH2.

**Methods:** We investigated whether the lack of mutations affecting EZH1/2 reflects a neo-functionalization of EZH2 outside of the PRC2 complex as suggested in other pathologies or simply the redundancy between the two enzymatic subunits.

**Results:** Here, we show that in the absence of SUZ12, EZH2 remains bound to EED but loses its interaction with all other core and accessory PRC2 subunits but mutations in PRC2’s main catalytic subunit EZH2 are never found. This is in contrast to myeloid and lymphoid malignancies, which harbor frequent loss-of-function mutations in EZH2.

**Conclusions:** This work suggest that context-dependent redundancies explain part of the tumor-type specific patterns of mutation affecting PRC2.

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Genetically Engineered Minipigs Model Major Clinical Features of Human Neurofibromatosis Type 1

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**Background**: We have developed a Neurofibromatosis Type 1 (NF1) minipig that harbors a known human *Neurofibromin* 1 (NF1) premature termination codon, resulting in a minipig model that replicates the broad spectrum of disease that develops in NF1 patients.

**Methods**: We have generated a cohort of animals and applied the criteria used for diagnosing NF1 patients in our model. We have aged animals and applied imaging technology to follow NF1-associated phenotypes. We have also conducted pharmacological studies in our NF1 swine to look at the pharmacokinetics and pharmacodynamics of currently used drugs in the treatment of NF1, including a variety of MEK inhibitors, currently in clinical trials for NF1.

**Results**: The NF1 minipig meets the National Institute of Health’s diagnostic criteria for NF1. The NF1 boars are fertile and the NF1 mutant allele is transmitted at a Mendelian rate with no reduction in fitness of offspring that inherit this allele. To date, we have observed 100% penetrance of café au lait macules, a phenotype that occurs in nearly every NF1 patient, but has never been demonstrated in any other animal model. The NF1 minipig develops skin lesions over time, that histologically resemble human dermal neurofibromas. One in seven NF1 minipigs that underwent magnetic resonance imaging was confirmed to have an optic pathway glioma (OPG) that histologically resembled OPGs seen in NF1 patients. Additionally, we have observed other NF1-associated phenotypes including Lisch nodules, tibial dysplasia, white matter decompaction, hypopigmentation, and freckling of the skin. We have demonstrated that oral administration of the MEK inhibitor PD0325901 results in clinically relevant plasma levels of the drug and inhibition of Ras signaling.

**Conclusions**: The FDA has emphasized the need for development and testing of new therapies in large animal disease models, in addition to rodent models, prior to human studies. We envision this large animal model of NF1 will become a standard in the evaluation of the safety and efficacy of new drugs prior to Phase I clinical trials and aid in the discovery of effective treatments and cures for patients with NF1. Further, an NF1 minipig may enable researchers to better understand the biological and genetic mechanisms underlying this complex disease, detect NF1-related tumors earlier, identify biomarkers, discover novel drug targets, and test new drugs and combination therapies for safety and efficacy.

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Funding provided by the Children’s Tumor Foundation, NF1 Synodos

Disclosure of Interest: A. Watson has a conflict with: Employee of Recombinetics, S. Isakson: None Declared, A. Rizzardi has a conflict with: Employee of Recombinetics, A. Coutts has a conflict with: Employee of Recombinetics, D. Carlson has a conflict with: Employee of Recombinetics, M. Kirstein: None Declared, J. Fisher: None Declared, J. Vitte: None Declared, K. Williams: None Declared, G. E. Pluhar: None Declared, S. Dahiya: None Declared, B. Widemann: None Declared, E. Dombi: None Declared, T. Rizvi: None Declared, N. Ratner: None Declared, L. Messiaen: None Declared, A. Stemmer-Rachamimov: None Declared, S. Fahrenkrug has a conflict with: Employee of Recombinetics, D. Gutmann: None Declared, M. Giovannini: None Declared, C. Moertel has a conflict with: Consultant, Recombinetics Inc., D. Largaespada has a conflict with: Employee of Recombinetics
An Innovative Pig Model of Neurofibromatosis Type 1 (NF1) That Mimics the Human Disease

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Background: Loss of the NF1 tumor suppressor gene causes the autosomal dominant condition, neurofibromatosis type 1 (NF1). Children and adults with NF1 suffer from pathologies including benign and malignant tumors to cognitive deficits, seizures, growth abnormalities and peripheral neuropathies. NF1 encodes neurofibromin, a Ras-GTPase activating protein, and NF1 mutations result in hyperactivated Ras signaling in patients.

Methods: Existing NF1 mutant mice mimic individual aspects of NF1, but none comprehensively models the disease. We describe a novel Yucatan miniswine model bearing a heterozygotic mutation in NF1 (exon 42 deletion) orthologous to a mutation found in NF1 patients.

Results: NF1+/ex42del miniswine phenocopy the wide range of manifestations seen in NF1 patients, including café au lait spots, neurofibromas, axillary freckling, and neurological defects in learning and memory. Molecular analyses verified reduced neurofibromin expression in swine NF1+/ex42del fibroblasts, as well as hyperactivation of Ras, as measured by increased expression of its downstream effectors, phosphorylated ERK1/2, SIAH and the checkpoint regulators, p53 and p21. Consistent with altered pain signaling in NF1, dysregulation of calcium and sodium channels was observed in dorsal root ganglia expressing mutant NF1.

Conclusions: Thus, these NF1+/ex42del miniswine recapitulate the disease and provide a unique, much needed tool to advance the study and treatment of NF1. In this presentation, we will provide the latest update on the progression of this innovative model.

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Pharmacologic Vulnerabilities of NF1-Associated Tumors and Malignancies: A Small Molecule Screen Identifies Compounds Capable of Selectively Killing NF1 Deficient Human Schwann Cells and Controlling Tumor Growth In Vivo

Kyle Williams, Department of Pediatrics, University of Minnesota, Minneapolis, United States

Background: Treatment options for the both plexiform neurofibromas and MPNSTs are limited. While recent clinical trials with MEK inhibitors for treatment of problematic plexiform neurofibromas have been extremely encouraging, treatment has historically relied on surgical resection and broad-spectrum chemotherapy. The genetic basis of NF1 syndrome makes it a top candidate for using pharmacogenomic and synthetic lethal genetic approaches to uncover unique vulnerabilities harbored by NF1 deficient cells.

Methods: Given that both plexiform neurofibromas and MPNSTs arise within the Schwann cell lineage, we have developed a drug discovery pipeline to identify targeted therapeutics for treating NF1-related neoplasia, including MPNSTs. Using CRISPR/Cas9, we have created immortalized human Schwann cell lines that are deficient for the NF1 gene. Pairing these with isogenic wild-type cell lines yield an outstanding tool for identifying synthetic lethal interactions.

These isogenic cell lines were utilized for several synthetic lethal screens designed to identify effective therapeutics and new targets specific to cells lacking NF1. These included: 1. A large-scale screen (~12,000 compounds) for drugs that preferentially kill NF1 deficient cells. 2. Synthetic lethal screens using genome-wide RNAi and CRISPR/Cas9 approaches to knockdown/out expression of additional genes.

Results: Our small molecule screening efforts resulted in identification of ~20 compounds showing selective lethality towards NF1 deficient cells. We moved 3 clinically interesting candidates forward for testing in vivo, utilizing both a genetically engineered mouse (GEM) model (DhhCre; NF1fl/fl; Ptenfl/fl) and a human MPNST xenograft (S462TY) model. These compounds include the cardiac glycoside digoxin, a PP2A inhibitor (LB-100), and a parthenolide derivative (DMAPT) that inhibits NF-kB signaling. All three were effective in prolonging the life of the GEM model. However, only LB-100 and digoxin could both control tumor growth and rapidly shrink the size of established human MPNST xenografts.

Conclusions: Large scale pharmacogenomic screens leveraging synthetic lethal interactions with NF1 have proven useful to identify therapeutics of clinical interest for the treatment of NF1-related neoplasia. Digoxin is of particular interest given its well understood safety profile and that is has shown synergy with MEK inhibition for treatment of metastatic melanoma in recent human clinical trials.

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Disclosure of Interest: K. Williams has a conflict with: KBW is supported by the Children’s Tumor Foundation Young Investigator Award and the Children’s Tumor Foundation Emerging Scientist Award. S. Finnerty: None Declared, B. Keller: None Declared, R. Williams: None Declared, S. Rathe: None Declared, A. Watson: None Declared, J. Hawkinson: None Declared, G. Georg: None Declared, C. Moertel: None Declared, D. Largaespada has a conflict with: DL is the co-founder and co-owner of several biotechnology companies, specifically NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. He consults for Surrogen, Inc., and Genentech, Inc. is funding some of his research. The business of all these companies is unrelated to the contents of this abstract. This work was supported by The Children's Tumor Foundation Synodos for NF1 Award and the American Cancer Society Research Professor Award (to DAL).
Interruption of the AG-Exclusion Zone is the Major Mechanism of 90 NF1 3’ Splice Site Mutations Outside the Canonical AG Di-Nucleotides: Lessons to Learn for Intronic Variants of Unknown Significance

Katharina Wimmer, Division of Human Genetics, Medical University Innsbruck, Innsbruck, Austria

Background: Systematic application of RNA-based NF1 mutation analysis for nearly 20 years in our laboratories shows that 30% of NF1 mutations affect mRNA splicing. Two thirds of these mutations do not involve the canonical di-nucleotides GT-AG of the splice sites. As their effect on mRNA splicing cannot unequivocally be predicted, they are considered potentially splicogenic variants of unknown significance (VUS) when identified by gDNA-based mutation analysis methods. Elucidating the mechanisms by which non-canonical splice mutations alter splicing will help to develop strategies to select those (VUS) with a high potential to affect splicing for further more labor intensive RNA analyses.

Methods: Through performing mini-gene experiments the mechanism of action of the NF1 mutation c.1722-11T>G was uncovered. This finding was evaluated in 89 additional intronic NF1 3’ splice site (3’ss) mutations outside the canonical AG-di-nucleotides.

Results: Mini-gene experiments revealed that the pyrimidine to purine (Y>R) transversion c.1722-11T>G although expected to affect splicing by weakening the poly-pyrimidine tract (PPT) caused skipping of the downstream exon mainly by the creation of a novel AG dinucleotide in the region between the natural 3’ss and the branch-point, a region known as the AG-exclusion zone (AGEZ). Evaluation of 89 additional intronic NF1 (3’ss) mutations showed that in total 49/55 of the single-nucleotide variants (SNVs) located between position -35 and -4 created a novel AG in the AGEZ while only 26 of them were used as novel 3’splice site instead of the natural one. Another 24 mutations were SNVs at position -3. With a single exception, they all were Y>R transversions. Finally, all but two of eleven deletions, insertions and delins including two or more nucleotides removed two or more Y from the PPT and/or created an AG in the AGEZ.

Conclusions: Taken together, 90% (81/90) of the non-canonical NF1 3’ss mutations follow one of these motives: (i) creation of an AG in the AGEZ, (ii) removal of ≥2Y in the AGEZ or (iii) Y>R transversion at position -3. In order to test whether these three motives can be used to distinguish splicogenic from splice-neutral variants also in other genes, we applied them to a cohort of 49 previously analyzed neutral variants. We identified only two of eleven deletions, insertions and delins including two or more nucleotides removed two or more Y from the PPT and/or created a novel AG in the AGEZ.


Hippo Signaling-Mediated Schwann Cell Reprogramming Drives Malignant Peripheral Nerve Sheath Tumorigenesis

Natalie Wu, Cincinnati Children’s Hospital Medical Center, Cincinnati

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive Schwann cell (SC)-derived sarcomas with strong metastatic proclivity and resistance to radiation and chemotherapy. Molecular events driving SC-to-MPNST transformation are incompletely understood.

Methods: Here, we analyze the transcriptome profiles from two cohorts of MPNST and benign neurofibroma patients and show that human MPNSTs exhibit elevated expression of HIPPO-TAZ/YAP activity, as confirmed by histological analysis. To investigate the function of TAZ and YAP activation in MPNST, we developed a mouse model with TAZ/YAP hyperactivity in SCs.

Results: TAZ/YAP hyperactivity in mouse SCs caused by LATS1/2 loss potently induces high-grade nerve-associated tumors with full penetrance. Lats1/2 deficiency reprograms SCs to a cancerous, progenitor-like phenotype and promotes hyper-proliferation. Conversely, disruption of TAZ/YAP activity alleviates tumor burden in Lats1/2-deficient mice and inhibits human MPNST cell proliferation. Moreover, genome-wide profiling reveals that TAZ/YAP-TEAD1 directly activates oncogenic programs including PDGFR signaling. Co-targeting TAZ/YAP and PDGFR pathways inhibits tumor growth.

Using single-cell transcriptomics to characterize cellular composition and heterogeneity in early-stage and full-blown MPNST-like tumors, we identify cell-cell communication networks between SC-derived tumor cells and cancer-associated stromal cells within the tumor microenvironment. Transformed SC-derived tumors exhibit neural crest and mesenchymal cell signatures as Lats1/2 mutant tumors progress into malignancy. Moreover, a small-scale compound screen using Lats1/2 mutant tumor cells identifies potential epigenetic regulation of MPNST-like tumor growth.

Conclusions: This work establishes a previously unrecognized convergence between Lats1/2-TAZ/YAP signaling and MPNST pathogenesis. Taken together, our findings suggest hyperactivation of TAZ/YAP as an oncogenic signaling hub that promotes SC transformation and identify a HIPPO-PDGFR dependent pathway for MPNST pathogenesis and potential therapeutic intervention.

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Runx1 and Runx3 Cooperatively Repress Pmp22 to Drive Neurofibromagenesis

Jianqiang Wu, Cincinnati Children’s Hospital, Cincinnati

**Background:** Neurofibromatosis type 1 (NF1) patients are predisposed to develop neurofibromas but the underlying molecular mechanism(s) of neurofibromagenesis are not fully understood. The Runx-related transcription factor family of genes (Runx1, Runx2, & Runx3) have been shown to be involved in cancer development in a context-dependent manner.

**Methods:** We used mouse models to define the role of RUNX transcription factors in neurofibroma formation. We combined transcriptome profiling, chromatin immunoprecipitation sequencing (ChIP-seq), and assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) to identify Runx target genes. We used cell culture with the CRISPR-Cas9 approach to confirm the specificity of the Runx/Pmp22 binding sites in Schwann cells (SCs).

**Results:** Dual genetic deletion of Runx1(Rx1) and Runx3(Rx3) in SCs and Schwann cell precursors (SCPs) using desert hedgehog-Cre (DhhCre) significantly delayed neurofibromagenesis and prolonged mouse survival. Combined transcriptome profiling, ChiP-seq, and ATAC-seq, we identified 14 highly plausible direct targets of Rx1/3. Comparing these 14 genes to those overexpressed in neurofibroma tumor-initiating cell microarray data, we identified a single gene, peripheral myelin protein 22 (Pmp22) specifically and/or regulated in neurofibromagenesis, and increased mouse survival. Combined transcriptome profiling, ChiP-seq, and ATAC-seq, we identified 14 highly plausible direct targets of Rx1/3. Comparing these 14 genes to those overexpressed in neurofibroma tumor-initiating cell microarray data, we identified a single gene, peripheral myelin protein 22 (Pmp22) specifically and/or regulated in neurofibromagenesis, and increased mouse survival. A subset of Pmp22 in mouse neurofibroma SCs decreased cell proliferation. Mouse neurofibroma SCs with homozygous deletion of 5 putative Runx binding sites in the Pmp22 gene decreased proliferation and increased Pmp22 protein expression versus controls. Mechanistically, Rx1/3 regulated alternative Pmp22 promoter usage and reduced post transcriptional expression Pmp22. Finally, pharmacological inhibition of Runx/core binding factor beta (Cbf-β) activity with a Runx/Cbf-β interaction inhibitor, Ro5-3335, significantly reduced neurofibroma volume in vivo.

**Conclusions:** We identified a novel signaling pathway involving the oncogenes Rx1 and Rx3 regulation of Pmp22 in neurofibroma. Suppression of Pmp22 expression plays a role in neurofibroma initiation and/or maintenance. Targeting disruption of Runx/Cbf-β interaction might provide a novel therapy for neurofibroma patients.

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Supported by NIH-R01-NS097233 to JW and R37 NIH-R37-N083580 to NR to support KC.

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Novel Function of Enhancer of Zeste Homolog 2 (EZH2) in Malignant Peripheral Nerve Sheath Tumor (MPNST)

Xiyuan Zhang, Pediatric Oncology Branch, National Cancer Institute, Bethesda, United States

**Background:** Polycomb Repressive Complex 2 (PRC2) contains methyltransferase EZH2 which is tightly associated with inactive gene promoters through its methylation of histone H3 on lysine 27 (H3K27). Previous genomic studies of malignant peripheral nerve sheath tumors (MPNSTs), a potentially devastating outcome for patients with neurofibromatosis type 1 (NF1), discovered mutations, homozygous deletions, or heterozygous allelic loss in key genes of PRC2 (EED and/or SUZ12). This study investigates the expression levels and function of EZH2 in MPNSTs, given the genetic loss in PRC2 partners.

**Methods:** Analysis of RNA-sequencing results from 16 MPNST tumors and 5 MPNST cell lines identified differentially expressed genes in this disease. STS-26T (PRC2-wildtype) and T265 (SUZ12-null) cell lines were identified as appropriate models for downstream studies. Modified T265 cells were constructed to stably express the SUZ12 gene and the effects of PRC2-reassembly on cell growth and EZH2 function were studied. Chromatin immunoprecipitation followed by DNA sequencing (ChiP-seq) was used to identify the binding sites of EZH2 on the genome, as well as the histone markers of H3K27me3 (PRC2 target), H3K4me3 and H3K27ac (activation markers).

**Results:** By comparing the gene expression levels of MPNST tumors and cell lines to non-cancerous normal tissues, EZH2 is commonly up-regulated in MPNST tumors (FDR = 4.16E-11), and cells (FDR = 6.36E-0.8). Furthermore, the overexpression of SUZ12 in T265 cells reassembled the PRC2 complex and slowed the cell growth. Results of ChiP-seq revealed that T265 cells lose 95% of their EZH2 binding sites at PRC2 targets due to the loss of SUZ12 when compared to STS-26T cells. Surprisingly, a subset of PRC2-independent EZH2 peaks are present in T265 cells and in STS-26T cells. Paradoxically these EZH2 peaks co-localized with activation markers. Finally, these common PRC2-independent EZH2 peaks had a significantly higher average score than other peaks (p<0.001) and they exhibited a significant enrichment (58%) at the promoters of the associated genes.

**Conclusions:** The results of this progressing study indicate a potentially new epigenetic function of EZH2 at genomic loci that are independent of PRC2 in MPNST cells, which is a unique tumor system to study the role of EZH2 in a cell without functional components of the PRC2 complex. This mechanistic study may have therapeutic implications for rationally designed epigenetic targeting in MPNST.

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Beta-III-Spectrin Promotes Tumor Cell Survival in NF1-MPNST

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Background: The most common malignancy affecting adults with the neurofibromatosis type 1 (NF1) cancer predisposition syndrome is the malignant peripheral nerve sheath tumor (MPNST), a highly aggressive sarcoma that typically develops from benign plexiform neurofibromas. Prior work from our laboratory has demonstrated that beta-III-spectrin is highly expressed in MPNSTs, but not plexiform neurofibromas suggesting that beta-III-spectrin could play an important role in the progression of these tumors.

Methods: We used shRNA-mediated knock down of beta-III-spectrin expression in N11/Tp53-mutant NPcis MPNST cells (JW18.2 shLacZ vs JW18.2 shSPTBN2) to explore the role of beta-III-spectrin in MPNST pathogenesis in vitro and in vivo. Subcutaneous and left ventricular (LV) tumor injections were performed to assess the role of beta-III-spectrin in growth of a primary tumor and metastasis, respectively. Injections were performed in C57BL/6 ALBINO immunocompetent mice. All human and animal studies were performed under active protocols approved by the Institutional Review Board and the Institutional Animal Care and Use Committee respectively.

Results: Knockdown of beta-III-spectrin significantly increased cell death in vitro and in vivo in a subcutaneous tumor model. Additionally, in the metastasis model, knockdown of beta-III-spectrin led to a decreased tumor burden and increased overall survival. Knockdown of beta-III-spectrin was also associated with mis-localization of the mGluR1 glutamate receptor, a G-protein coupled receptor that can affect cell survival. Additionally, beta-III-spectrin –deficient cells had decreased levels of EAAT4, a glutamate transporter.

Conclusions: Collectively, these data suggest that beta-III-spectrin promotes cell survival in MPNSTs through mGluR1 and that this pathway may be able to be exploited for therapeutic purposes.

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This work was funded by a SARC career development award to Dr. Hirbe. Dr. Hirbe is also supported by the Francis Collins Award through NTAP.
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# POSTERS: Clinical Science

**SUNDAY, 4 NOVEMBER 2018 (17:25 – 18:55)**

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Background: Neurofibromatosis (NF1) is an autosomal dominant disease, with an estimated prevalence of 1/3000. Approximately 50% of the cases are inherited. NF1 show hugely variable expressivity, with symptoms age dependent and associated with great morbidity throughout life. This disorder is associated with an 8-15 year reduction in average life expectancy, primarily due to malignant neoplasms and cardiovascular problems. Nevertheless, individuals with NF1 usually lose their medical care once they turn 18. A multidisciplinary team created in 2016, follows 83 pediatric NF1 patients with a median age of 11 years old. 37.5% has family history, some of them with more than one family member affected. In order to provide optimal care services for affected families we explored the care need of adults with NF1. Recommendations for management of NF1-related clinical problems in adults are based on consensus from populations and multi-institution cohorts literature and the collective expertise of the authors.

Methods: Every adult patient diagnosed with clinic NF1 will have an initial evaluation by the genetic team. This initial evaluation will contemplate an assessment of clinical problems, directly and indirectly related to NF1. Accordingly to this first evaluation a routine screening will be planned by a specialized NF1 clinic, including genetics, neurology, neurosurgery, ophthalmology, orthopedics, vascular and plastic surgery, oncology, nephrology, psychology and/or prenatal care, as needed. Concerns will be categorized: neurofibromas, tumor risk (including malignant peripheral nerve sheath tumor and breast cancer), hypertension and vasculopathy, bone health, psychiatric and neurologic conditions and pregnancy. We are partners with a patient association, to create educational material and awareness in the general population. We are preparing our candidacy to ERN Genturis (European Reference Network) to improve access to high-quality European healthcare for our patients.

Results: Close collaboration between NF1 clinicians facilitate a uniform approach to the diagnosis and management of NF1 and its complications, with less work absenteeism. Close collaboration with society and European networks improve quality of life and access to new therapeutics and medical investigation, respectively.

Conclusions: We want to evaluate the clinical and psychological impact of this multidisciplinary team in patients and their families. We hope that this approach will have medical and social value to their care.

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NF1 Splice Site Variant Demonstrates Highly Concordant Phenotype of Spinal Neurofibromatosis in 3 First-Degree Family Members

Kara Anstett, Neurology, NYU Langone Health, New York, United States

Background: Neurofibromatosis Type 1 is highly variable in expression, and is not uncommon for family members to differ greatly in presentation. However, with the advent of genetic testing, multiple phenotype correlations have been identified. Here we present a family with a highly concordant burden of spinal disease with predominant cervical lesions and a NF1 splice variant.

Methods: The clinical history and imaging of 3 individuals within one family were reviewed.

Results: Case 1: The proband is a male diagnosed with neurofibromatosis (NF) at 25 after imaging revealed nerve sheath tumors consistent with paraspinal plexiform neurofibromas (NFs) extending from cervical to sacral spine, with compression and deformation of the cervical spine and compression at the cauda equina root level. His other NF features include faint, typical café au lait (CAL) macules, inguinal freckling, a small plexiform NF to the abdomen, and palpable nodules to the back of the neck.

Case 2: The proband’s mother was evaluated at age 53, and found to have multiple CAL macules, unilateral inguinal and bilateral axillary freckling, dermal NFs to the face, chest, and abdomen, subcutaneous masses to the forearm, back and thigh, and a possible plexiform NF to the abdomen. After a motor vehicle collision at 54, she developed posterior neck pain down the right arm to the fingers, and tingling and weakness to the right hand. Imaging revealed multiple spinal NFs from cervical to lumbar spine with severe spinal canal stenosis, cord compression, and cord edema at C1-C2 level, a C4-5 level NF resulting in compression and displacement of the cord with edema.

Case 3: The proband’s sister was evaluated at 18 and was found to have multiple faint, typical CAL macules and skin fold freckling, with no evidence of peripheral NFs. Her history was significant for scoliosis, short stature, and pes planus. At 19, spinal imaging revealed numerous paraspinal NFs extending from the bilateral neuronal foramina through the entire spine, with stenosis and cord deformity most notable at the cervicomedullary junction, C3-C4, and C5-C6 levels.

Genetic testing in the proband identified the novel pathogenic variant NF1 IVS50+1G>C. Another pathogenic variant has been reported at this position (Griffeths et al 2007).

Conclusions: This case demonstrates a highly concordant spinal NF1 phenotype with significant cervical burden in 3 first degree relatives. This may provide evidence of a phenotypic correlation for this splicing variant.

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Late Onset and Slowly Progressive Neurofibromatosis 2 Phenotype in a Family with a Splice Site Variant

Kara Anstett, Neurology, NYU Langone Health, New York, United States

Background: Neurofibromatosis type 2 (NF2) is a benign tumor predisposition syndrome with average age of onset is between 18-24 years, with nearly all individuals developing bilateral vestibular schwannomas (VS) by 30, and average age of death at 36. In recent years, mild and late onset presentations have been identified, though these are primarily de novo, mosaic presentations. Here we present a 3 generation family with a late onset, chronic stable presentation, with a confirmed germline mutation in NF2 in multiple family members.

Methods: Review of the clinical history and genetic testing results of the proband and family members was performed.

Results: A 24 year old male of with history of noise exposure presented with complaints of hearing changes and fullness in his right ear. At the time, his mother was known to have a history of single vestibular schwannoma (VS). An audiogram showed normal hearing with 100% speech discrimination and brain IAC MRI demonstrated bilateral VS, with the right filling the IAC and the left measuring approximately 6mm, confirming a clinical diagnosis of NF2. Total spine imaging did not reveal any involvement. The proband has been followed conservatively for 15 years, and serial imaging of the brain IAC shows slowly progressive VS with minimal increase in size, with the most recent imaging at 38 showing a left VS measuring 15 x 18 mm and the right VS 6 x 11mm, and a possible small meningioma. Spine imaging continues to demonstrate no evidence of schwannomas. Audiograms continue to demonstrate normal hearing and excellent speech discrimination, though the proband has subject hearing complaints with background noise.

Since the proband’s diagnosis, multiple maternal family members have been diagnosed with stigmata of NF2. His mother was diagnosed with a contralateral VS in her early 60s, and his grandmother had a large meningioma resected and was doing well at 83, with no evidence of VS or other stigmata of NF2. The sister of his grandmother was also diagnosed with bilateral VS and a meningioma, and was doing well at 90.

Genetic testing of NF2 in the proband revealed the novel pathogenic variant c.240G>A (p.Lys80=), which results in missplicing at intron 2. This variant was confirmed in the proband’s affected mother and maternal grandmother.

Conclusions: This family demonstrates an intrafamilial correlation of a splicing variant in NF2 resulting in a late onset and slowly progressive presentation of Neurofibromatosis Type 2.

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Profiling the Reading Abilities of School-Age Children with Neurofibromatosis Type 1

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Background: Reading difficulties are one of the most significant challenges for children with neurofibromatosis type 1 (NF1). Although impairments in basic word reading and the comprehension skills of children with NF1 are frequently reported, the underlying cause of these impairments is not well understood. Primary aims of this study were 1) to provide a detailed description of the reading subskills of children with NF1 and 2) to investigate the profile of impaired readers with NF1. An additional exploratory aim was to explore the relationship between reading ability and other potential cognitive and behavioural risk factors for reading difficulties.

Methods: Children with NF1 (n=60) aged 7 to 12 years were compared with unaffected children (n=36). All children completed a cognitive assessment including a detailed literacy battery. Group differences were examined using independent-samples t tests or equivalent nonparametric tests. Poor readers (≤ 1 SD below mean) were classified according to their type of reading impairment (i.e. phonological, surface, mixed). A hierarchical multiple regression was conducted to establish predictors of reading ability in children with NF1.

Results: Children with NF1 performed significantly poorer than controls on all literacy measures including letter-sound knowledge, receptive vocabulary, blending, reading accuracy (regular, irregular and nonword), reading fluency and comprehension (all p≤.001), except for letter identification (p=.06). Of the 49 children with NF1 classified as poor readers, 20 (41%) had a primary phonological impairment, 24 (49%) had mixed and 5 (10%) could not be classified. Children with mixed dyslexia displayed the most widespread and severe reading impairments. Children with NF1 who had stronger working memory (p<.01) and receptive language (p<.05) and displayed less inattentive behaviours (p<.05) had better word reading.

Conclusions: The majority of children with NF1 display widespread deficits across reading subskills. Those children with weaknesses in working memory, receptive language and attention are at greatest risk of reading difficulties. It is recommended that children with NF1 receive comprehensive assessment of their literacy skills, so they can receive appropriate, targeted intervention.

Evaluation of Pulmonary Findings in a Large Single-Center Cohort of Patients with Neurofibromatosis Type 1 (NF1) Using Multi-Detector Computed Tomography

Maxim Avanesov, Diagnostic and Interventional Radiology and Nuclear Medicine, University Medical Center Hamburg-Eppendorf, Hamburg

Presented by: Dr. Johannes Salaman

Background: The prevalence and the characteristics of NF1-associated lung disease remain unclear. To dates knowledge is based on case reports and few studies with small patient populations. An additional limitation of the majority of previous studies was the use of plain X-ray only. In this large single-center cohort study different lung manifestations in NF1 patients were assed using multi-detector computed tomography (MDCT). Smoking history and patients age as potential cofounders for lung pathologies were included for evaluation.

Methods: In this retrospective, institutional board approved study, 71 patients with NF1 (33±14 years, range 2-68 years, 56% females) were evaluated for the presence of distinctive lung manifestations including reticulations, ground glass opacifications, consolidations, thickened interlobular septa, pleural and pericardial effusion, emphysema, honey combing, tree-in-bud sign, and the number of pulmonary nodules and cysts. All patients underwent a PET/CT (Gemini GLX PET/CT system with a 16 MDCT, Philips®, The Best, Netherlands). Two experienced radiologists in thoracic imaging (both 6 years) read all MDCT studies in consensus. Patients were divided into 4 subgroups based on their smoking history (smoker vs non-smoker) and their age (≤30 years vs. >30 years). All patients gave written informed consent for the use of their data.

Results: 17 patients (24%) were smokers and 36 patients (51%) were >30 years old. In the total study group, pulmonary cysts, nodules, and emphysema were the most common pulmonary findings (25(35%), 23(32%), and 22(31%)). Paraseptal emphysema was the predominant type of emphysema (20(28%) followed by centrilobular emphysema (7(10%)). No panlobular emphysema, honey combing, tree-in-bud sign and pericardial effusion were present in the study group. Significantly more pulmonary cysts were observed in patients >30 years compared to those ≤30 years (19(53%) vs. 6(17%), p<0.05). In contrast, there was no significant difference of any investigated pulmonary findings between smokers and non-smokers. There was no significant correlation between the age and the number of pulmonary cysts (Spearman’s rho: 0.14, p=0.49).

Conclusions: Pulmonary cysts, nodules, and paraseptal emphysema were the most common pulmonary findings in NF1 patients with 35%, 32%, and 28% and their presence was independent of the smoking history. In contrast, the presence, but not the total number of pulmonary cysts were associated with increased age.
Analysis of NF1 Symptoms in Familial and Sporadic Cases

Süksüye Ayter, Medical Biology and Genetics, TOBB University of Economics and Technology, Faculty of Medicine

Background: Neurofibromatosis type 1 (NF1) is the most common neurogenetic disorder, affecting in 3,000 - 3,500 individuals worldwide. Clinic presentations of NF1 are highly variable. Typical manifestations are cafe-au lait spots, freckling, peripheral nerve sheath tumors (benign: Neurofibromas; malignant: Neurofibrosarcomas) and other malignancies (intracranial astrocytomas, gastrointestinal stromal tumors, pheochromocytomas, and juvenile mononcytic leukemia. The 15% of developing tumors in NF1 patients are malignant. NF1 is caused by mutations of the NF1 gene and 50% of patients represent sporadic NF1 which occurs in the absence of a family history of the disease and usually results from a new mutation in the germ cell of one of the parents. Although NF1 is a single-gene disorder with autosomal-dominant inheritance, its clinical expression is highly variable and unpredictable. NF1 patients have the highest known mutation rate among all human disorders, with no clear genotype–phenotype correlations. Such a clinical variability could be described by presence of modifier gene effects.

Methods: We investigated the presence of different symptoms percentage in 241 NF1 patients (121 sporadic and 120 familial) who were seen in Hacettepe hospital which is a reference center for genetic diseases in Turkey. For statistical analysis *SPSS (Statistical Package for Social Sciences) - Windows 20* program was used. Spearman's and Chi – square test were used for statistical analysis.

Results: The percentage of occurrence of axillary freckling, inguinal freckling, lisch nodules, dermal neurofibromatosis, optic glioma, skeletal dysplasia and scoliosis were approximately the same in both sporadic and familial cases. However, hypertension (80%), MPNST (75%), Astrocytoma, Rhabdomyosarcoma and Epilepsi (66,7%) are detected significantly higher in sporadic cases. On the other hand Hamartoma (61,5%) and learning disability (62,5%) are observed more in familial cases. We also compared the coexistence of different number of the NF1 symptoms. No significant statistical differences were detected.

Conclusions: Such a significant difference of symptoms occurrence between familial and sporadic cases could be explained by the effect of possible modifier genes.

Quality of Life in Adults with NF1 and NF2 Attending a Specialized Neurofibromatosis Clinic in Toronto, Canada

Carolina Barnett, Medicine, Neurology, University Health Network, Toronto, Canada

Background: NF1 and NF2 are multisystemic disorders associated with reduced quality of life (QoL). There is scarce data on QoL of Canadian NF patients. We studied QoL in NF1 and NF2 patients attending a specialized NF clinic in Ontario, Canada. We also assessed which clinical and personal factors are associated with reduced QoL. We hypothesized that patients with NF1 and NF2 have lower QoL compared to healthy Canadian population. Furthermore, we hypothesized that the disease visibility and pain would be associated with reduced QoL in NF1

Methods: Cross-sectional study conducted at The Elizabeth Raab NF clinic (Toronto, Canada). Patients with NF1 and NF2 completed generic QoL measures: SF-36 and EQ5D and PROMIS-pain interference questionnaires. Disease-specific questionnaires were the Peds-QoL NF1 module and the NF2-QoL. We collected clinical data including Ablon’s visibility index, plexiform neurofibroma, and malignant peripheral nerve sheath tumors. We compared SF-36 scores between NF1, NF2 and normative Canadian population using t-tests and ANOVA. We also assessed how the different EQ-5D, Peds-QoL, and NF2-QOL dimensions were affected. We analysed correlations between the clinical factors and QoL scores through Pearson correlation coefficients and we used regression models to adjust for confounding.

Results: 162 adult patients with NF1 and 22 with NF2 participated. NF1 and NF2 patients had lower scores in most components of the SF-36 compared to the general Canadian population, worse for NF2 compared to NF1 patients. Using the EQ5D dimensions, 66% of NF1 and 73% of NF2 patients had pain; 61% of NF1 and 68% had anxiety/depression. There was a strong correlation between pain interference and SF-36 PCS, and EQ5D scores in NF2 patients (r: -0.90, p<0.001), and slightly lower for NF1 patients (r:-0.70, p<0.001). Multivariable models in NF1 showed that pain interference was a common predictor of reduced QoL for both generic and disease-specific measures. Visibility Index was correlated with reduced EQ5D utility scores, but not with SF-36 or Peds-Qol scores.

Conclusions: We found that patients with NF1 and NF2 have reduced quality of life compared to Canadian healthy population. Pain is highly prevalent in NF1 and NF2 and is a main driver of reduced QoL in NF1 and NF2. Additionally, there is high prevalence of anxiety and depression. These findings support multidisciplinary approach in the care of adults with NF, including mental health and pain management.

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Evidence of Small Fibre Neuropathy in Neurofibromatosis Type 1

Carolina Barnett, Medicine, Neurology, University Health Network, Toronto, Canada

Background: Large fibre neuropathy is a rare manifestation of Neurofibromatosis Type 1 (NF1) and small fibre neuropathy has not been previously reported. We studied the prevalence of small fibre neuropathy in patients with clinically definitive NF1.

Methods: All patients had a detailed history and neurological examination. Patients underwent standard nerve conduction studies—for large fibre testing, and small fibre tests: quantitative sensory thresholds, laser imaging Doppler flare, intraepidermal nerve fibre density and corneal nerve fibre length.

Results: Fifty-two patients completed the study, thirty-one (60%) were female and the mean age was 33.0 ± 12.3 years. Only four (8%) patients had abnormal nerve conduction studies. Small fibre tests were frequently abnormal: sensory thresholds in seven (13%), laser Doppler flare in ten (19%), intraepidermal nerve fibre density in eleven (22%) and corneal nerve fibre length in twenty-seven (52%). Patients with distal sensory symptoms had significantly lower intraepidermal nerve fibre density (6.7±3.0) compared to asymptomatic patients (9.2±4.1, t.test p=0.02). There was a moderate correlation between pain intensity and corneal nerve fibre length (r=-0.42, p=0.05).

Conclusions: This study shows that small fibre neuropathy is common in patients with NF1 and, in the absence of other causes for neuropathy, we propose that it can be considered a manifestation of NF1.

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Characteristics and Predictors of Quality of Life in Children with Neurofibromatosis Type 1 (NF1)

Belinda Barton, The Children's Hospital at Westmead, Sydney

Background: NF1 is associated with a range of physical, cognitive and psychological manifestations that can impact an individual’s health related quality of life (QOL). We examine the QOL of children with NF1 and cognitive impairments, and predictors of self-reported QOL. It was hypothesised that children would report poor QOL and psychosocial rather than cognitive functioning would predict QOL.

Methods: Children with NF1 (8-15 years) and visuospatial learning and/or attention impairments enrolled in the NF Clinical Trials Consortium STARS lovastatin trial were eligible. At baseline, children completed the Generic Pediatric Quality of Life Inventory (PedsQL), questionnaires assessing social-emotional functioning and cognitive tests assessing IQ, memory, attention and executive functioning. Parents rated their child’s QOL and social-emotional functioning. PedsQL data were compared to normative data using one sample t-tests. Intra-class correlation (ICC) coefficients were calculated to assess child-parent agreement for QOL. Correlations and multiple regression were conducted to identify predictors of QOL.

Results: Both child (n=135) and parent reports indicated that children with NF1 had significantly poorer QOL for all PedsQL scales when compared to normative data (all p<.001). Lowest QOL was found for school functioning. There was fair to moderate agreement between child and parent report of QOL (ICC .401 to .586). There were no significant associations between self-reported psychosocial QOL and age, IQ, or cognitive performance. There were moderate to strong associations between child reported psychosocial QOL and their ratings of social stress, depression, anxiety, sense of inadequacy, interpersonal relations, hyperactivity, and attention problems. Regression indicated that only social stress and attention problems were significant, predicting 62% of the variance in child psychosocial QOL.

Conclusions: Children with NF1 and cognitive impairments experience poor QOL across both physical and psychosocial domains. Study findings highlight the significant impact on QOL of children feeling socially excluded and stressed when interacting with their peers, and being unable to maintain their attention. Interventions that target social stress (e.g. building resilience) and attentional difficulties may improve the QOL of children with NF1.

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Radius Architecture in 10 Children with Neurofibromatosis 1 (NF1)

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**Background:** Neurofibromatosis type 1 (NF1) is associated with abnormal muscle and bone development as well as increased fracture risk. A previous study performed peripheral quantitative computed tomography (pQCT) on non-dysplastic tibias of NF1 patients and found altered tibial geometry (PMID:19118659).

**Methods:** To assess whether musculoskeletal abnormalities also affect the non-weight-bearing upper extremities, we performed forearm pQCT (Stratec XCT-2000) in 10 children and adolescents (aged 5 ½ to 16 years, mean 12.4 years, 7 girls) with NF1. The metaphysis ('4% site') and diaphysis ('65% site') of the radius were analyzed on the non-dominant forearm. Clinical data were reviewed from medical charts. Results were expressed as z-scores using age- and sex-matched reference data. One sample t-test (significant p value: < 0.05) was performed for significance of differences from 0, the expected mean results in a healthy population.

**Results:** All children had classical NF1. None had bone fractures or tibial bowing. 1 had dystrophic scoliosis. Mean height z-score in this population was -1.0 (standard deviation (SD): 1.5), mean weight z-score was -0.5 (SD: 1.5). Lumbar spine areal BMD (by dual-energy X-ray absorptiometry) was significantly below the result expected in healthy individuals, with mean z-score of -2.0 (SD: 0.8; p < 0.001). Trabecular and cortical volumetric BMD were both normal, with mean z-scores of -0.4 and +0.5, respectively. However, bone cross-sectional area was low at both sites: mean z-score of -1.3 (SD: 1.7) at the metaphysis and -2.4 (SD: 1.0) at the diaphysis. Muscle cross-sectional area at the 65% site was also low (mean z-score -1.7, SD: 1.3) (p < 0.05 for each measure). Calcium levels were high-normal, vitamin D, and collagen type I telopeptide and propeptide levels were normal.

**Conclusions:** This is the first cross-sectional pQCT study of the upper limb in NF1. We show that the radius of children with NF1 is narrower even after correction for height. The small radius cross section indicates deficient periosteal bone growth. Forearm muscles were also small, suggesting that inadequate muscle development may cause the bone size deficit. Our results align with results obtained at the lower extremity, indicating that the musculoskeletal disturbances in NF1 are at least in part independent of weight bearing.

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Nerve Repair in the Management of Malignant Nerve Tumors

Allan Belzberg, Neurosurgery, Johns Hopkins University, Baltimore, United States

**Background:** Advances in treatment modalities such as radiation and chemotherapy have not impacted the natural history of malignant peripheral nerve sheath tumors. En-bloc gross total resection with negative margins remains the best prognostic indicator for long term survival.

Surgery often requires taking major nervous structures resulting in significant morbidity.

Nerve repair is commonly used for traumatic nerve injury but has not been widely employed in the treatment of malignant nerve tumors. Concerns include: 1) Post-operative radiation is thought to severely compromise nerve grafts. 2) Long gaps after surgical resection limit the ability of nerve regeneration. 3) Tendon transfers offer almost immediate function. 4) Neural regeneration may influence cancer growth.

The recent development of novel neural reconstruction techniques, including the use of nerve transfers, offer the surgeon alternative methods for neural repair and regeneration.

**Methods:** 10 patients with malignant tumors affecting peripheral nerves underwent surgery with nerve grafting and or nerve transfers. Five of these were patients with MPNST. Careful attention was paid to identifying results from nerve transfers versus nerve grafting. The use of adjuvant therapy including chemotherapy and radiation therapy was determined to be delivered pre-operative or posy-operative

**Results:** The results from the 10 patients in this series are summarized and compared to what is in the literature. The data will be presented in detail.

**Conclusions:** Nerve transfer surgery provides a method for neural regeneration where the surgical intervention is remote to the tumor resection. Peripheral nerve repair offers a valuable surgical adjunct to the management of malignant peripheral nerve sheath tumors.

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Surgery for Painful Schwannomas in Patients with Schwannomatosis

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Background: Diffuse or local severe pain is the most commonly reported symptom in patients with Schwannomatosis (SWN). Surgery is currently the only effective intervention. However, pain may not improve or may recur following surgery. Predictors of optimal post-surgical outcomes are lacking.

Methods: SWN patients undergoing surgery for painful schwannomas were retrospectively reviewed (January 1 2000 to June 30, 2017). Surgeries for each lesion were considered independently. Demographics, prior surgeries on lesion of interest, and duration of pain for the presenting lesion were documented. Individual patient tumors were categorized into “sustained pain relief” (group 1), “pain relapse” (group 2), and “no pain relief” (group 3). Chi-squared analysis was used to assess categorical predictors of outcome. Cox regression analysis was used to assess predictors of pain-free survival.

Results: Eighteen patients underwent 32 surgeries. Seven of 14 patients (14/32 lesions, 44%) were in group 1 (median follow-up 68.5 months, range 1-276 months). All 14 lesions in group 1 presented with local pain of median duration 4.5 months (0.5-26 months). A gross-total resection (GTR) was achieved in 12/14 lesions (86%). Five of 14 patients (13/32 lesions, 41%) were in group 2. Median pain-free period was 11 months (range 4-122 months). Ten of 13 lesions (77%) had presented with diffuse/neuropathic pain of median duration 6 months (range 3-7 months). None had prior surgeries. A GTR was achieved in 4/13 (31%). Four of 14 patients (5/32 lesions, 16%) were in group 3. Three of five (60%) lesions presented with diffuse/neuropathic pain of median 18 months duration (range 2-100 months). Two lesions had prior surgeries. None had GTR and no patient had postoperative deficits. GTR and local pain on presentation (p < 0.01) were significant predictors of postoperative pain relief.

Conclusions: Surgery can offer sustained pain relief for many patients. Presentation with local pain and the possibility of achieving GTR may be predictive of postoperative pain relief. Given the risk of neurological deficit associated with surgery, these factors must be considered for clinical decision-making and patient counseling. Larger multi-center studies are needed to validate our findings.

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Electrophysiological Studies in Support of Surgical Decision Making in Schwannomatosis

Allan Belzberg, Neurosurgery, Johns Hopkins University, Baltimore, United States

Background: Schwannomatosis (SWN) is a rare neurogenetic syndrome, characterized by multiple peripheral and spinal schwannomas. Pain and neurological dysfunction are common. In well-selected patients, surgery is currently the only successful definitive treatment option. Electrophysiological studies (EDx) are useful adjuncts in the surgical decision-making for nerve tumors in general, but their role in the management of patients with SWN is unknown.

Methods: An institutional retrospective review (January 2006-July 2017) of patients with SWN undergoing EDx was conducted. The metrics evaluated included demographics, known genetic testing results, known tumor burden, prior surgeries, comorbidities potentially affecting EDx results, medications, presence of symptoms (pain, numbness, or motor), reason for EDx referral, and details of EDx testing. Impact of EDx on clinical decision-making was also evaluated.

Results: Eighteen of 85 patients with a diagnosis of possible or definite SWN had EDx evaluation (10 male; median age 46, range 21-65 years). Patients were referred for EDx when the etiology of their pain (9/18, 50%), numbness (3/18, 16%), or weakness (6/18, 34%) was not clear based on clinical evaluation and MRI. Fourteen of 18 patients (78%) had an EDx abnormality in the clinically-symptomatic nerve(s). EDx showed abnormalities in the region of pain (83%) and motor dysfunction (89%) frequently, but was less likely to reveal abnormality in the region of symptomatic numbness (33%). EDx abnormalities were attributed to underlying tumor affecting the nerve in 5/14 patients (36%). Seven of 18 patients (39%) also had an EDx abnormality in clinically-silent nerves, 4 of these (57%) were attributed to tumor. In 15/18 (83%), EDx directly influenced decision-making: confirming the surgical target in 6 cases or favoring non-operative management in 9.

Conclusions: The yield of EDx is high in patients with SWN and can assist with clinical decision-making. SWN tumors, whether symptomatic or silent, can result in EDx abnormalities. Given our limited understanding of the pathophysiology of pain and neurological dysfunction in SWN, these findings are instructive, but need to be explored via larger prospective studies.

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Supplementary Services to Persons with NF and Their Families - the Frambu Model

David K. Bergsaker, National Advisory Board for Rare Disorders, Frambu Resource Centre for Rare Disorders, Siggerud, Norway

**Background:** Frambu Resource Centre for Rare Disorders is a centre of interdisciplinary expertise for more than 400 rare disorders, including NF1, NF2, Schwannomatosis and Legius’ syndrome. The centre offers courses, counselling, research and up-to-date information. Our services are funded by the government as a supplement to the public health care system and are free of charge. Frambu is one of nine centres working with rare disorders in Norway, and a part of the Norwegian National Advisory Unit on Rare Disorders.

**Methods:** Frambu’s mission is to give people with NF of all ages the possibility to live a life according to their abilities, wishes and needs. Developing and spreading knowledge are primary ways to achieve our mission.

Frambu offers courses about NF every year:

Age-specific courses aimed at persons with NF and their families. Meeting others in similar situation and sharing experiences.

Camps for siblings.

Courses for grandparents.

Summer camps for youth and adults with rare disorders. Opportunity to experience and master social and physical activities independently in safe and supporting environments.

Courses aimed at professionals. Also available by video conference and on-line streaming.

E-learning course for teachers working with students with NF.

We offer information and counselling individually or in groups by attending meetings at Frambu, in home communities, or by video conference. Individual counselling to persons with NF, their families and support network incl professionals working in the local communities.

Frambu offers communication and documentation services. Anyone can contact Frambu with concerns about NF by phone, e-mail or social media. We collect and develop high quality transdisciplinary knowledge about the NF diagnosis. Our knowledge is shared through our courses, written material on our website or in print, short video films and podcasts.

**Results:** Frambu develops new information on NF through experience and research, currently Health survey of adults with NF1, follow up after 5 years.

**Conclusions:** Through courses, information, counselling, communication, documentation and research we aim to empower persons with NF, their families and professionals working with NF. For more information about Frambu and our services, visit www.frambu.no.

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Reading for Meaning: Impact of Attention Processes in Reading Comprehension Skills in French NF1 Children

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Background: Despite recent advances in our understanding of the neurobiological aspects of the cognitive deficits in NF1 (Chaix et al., 2017), there is a pressing need to advance current knowledge of the learning disabilities associated with this condition. Especially, the causal relationships between learning problems (that occur in 50 % of NF1 children) and cognitive profiles have to be more investigated. In particular, while difficulties in reading comprehension have been commonly associated with a lack of attention abilities in non-NF1 children, little is known about this in NF1 children. The purpose of this study is to examine which attentional skills are involved in reading comprehension and which attentional tests are the best appropriate ones to explore this process.

Methods: A multicenter, cross-sectional study was conducted on two groups of 75 children (72♀-78♂) with or without NF1 (8–12yo), matched for age, sex, handedness, and reading level (thus forming a continuum from good to poor readers in both NF1 and non-NF1 groups). [Excluded: mental retardation, neurological/psychiatric disorder]. Attentional skills were assessed combining (1) a parental questionnaire (CBCL, Achenbach 2001) and (2) performance-based assessment (CPTII, Conners 2000). Reading comprehension (text and sentences) was assessed through a standardized reading comprehension test (ORLEC, Lobrot 1973).

Results: The correlations between questionnaires and performance-based measures were low on NF1 and non-NF1 children. For both groups, the performance-based scores were associated to the text and sentences comprehension ability (p=0.0235 and p=0.0164 respectively), while indirect questionnaire scores were associated only to sentences comprehension (p=0.0263).

Conclusions: Our study shows that attention capabilities greatly influence reading text and sentence comprehension for NF1 and non-NF1 children. The attentional predictors of a good reading comprehension include little attention difficulties and an efficient and swift selective attention. However, indirect observer-rated (questionnaires) and direct performance-based measures of attention did not assess the same thing, both on NF1 and non-NF1 children. Thus, no single instrument capture all aspects of attention function. Children with NF1 should therefore benefit from a multimodal assessment of attention skills.

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[Grant Clinical Research Hospital Program from French Ministry of Health (PHRC2008, No. 0811301), & Occitanie Region (APRTC No. 09004813)]
Cognitive and Behavioral Disorders in Children with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with a prevalence of 1 in 2000-3000 live births. 80% of children with NF1 experience cognitive and behavioral difficulties. However, IQ scores are within normal range or only slightly lower compared with unaffected controls. Additionally, a high prevalence of attention deficit hyperactivity disorder (ADHD) (38%) and autism spectrum disorder (29%) is also reported. The last systematic review on behavior of children with NF1 was in 2012. The study aim was to synthesize recent relevant work regarding this issue.

Methods: Systematic review of literature. Relevant articles were identified using PubMed, PsycINFO, and Scopus and a manual search of reference lists. Inclusion criteria: Children with NF1 aged 6–17 years, studies published from 2012 to 2016, empirical studies using quantitative methods, studies published in peer-reviewed journals, and clinical trials (if they reported data for all participants).

Results: The study revealed deficits in phonological skills and receptive language, poor performance in visuospatial learning. Motor skills were also associated with poorer reasoning and working memory indexes. Disagreement persists about the precise IQ profile of NF1 children. Poor general performance and deficient planning skills are correlated with academic difficulties. Attention deficits have been related to worse performance in cognitive tasks and executive function, as well as poor social and emotional functioning. Among other findings, children presented weaker recognition of child and adult faces in low-intensity conditions, high prevalence of autistic traits, co-occurrence with ADHD, and presence of T2-hyperintensities. Benefits of using statins for cognitive problems are not clear, however there is improvement of ADHD symptoms with Methylphenidate.

Conclusions: Future research on NF1 should evaluate those aspects of the disorder for which findings remain inconclusive. Particularly in language, attention, academic performance, and executive functions. More studies on motor skills are needed to clarify the interdependency between these skills and neurocognitive performance. In addition, the use of neuroimaging techniques could provide a better understanding of the mechanisms involved in cognitive and social functioning. In regard to therapy, research on interventions could be conducted in combination with the use of statins and methylphenidate.

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**FIGURE: Prisma algorithm**

Records identified through database search and other sources (n = 188)

Records after duplicates removed (n = 156)

Full-text articles assessed for eligibility (n = 66)

Studies included in qualitative synthesis (n = 30)

Records excluded (based on title and abstract) (n = 90)

Full-text articles excluded, with reasons (n = 36)

• Age of population: 25
• Outcomes: 11
Genotype-Phenotype Correlation in Neurofibromatosis Type 1: A Systematic Review

Juan Sebastián Botero-Meneses, Neuroscience research group “NEUROS”

Background: Neurofibromatosis type 1 is a heterogeneous genetic disorder, considered to have an autosomal dominant pattern of inheritance, complete penetrance, and widely variable inter and intrafamilial expression with mainly neurologic and cutaneous manifestations. There is no current consensus on genotype-phenotype correlation.

Methods: Systematic review of literature. Relevant articles were identified using PubMed, Embase, Lilacs, and a manual search of reference lists. Inclusion criteria: Patients with NF1 with clinical or genetic diagnosis, studies published from 2001 to 2018, case reports, case series, cross sectional, were considered; languages: English, French, Portuguese, and Spanish.

Results: After thorough examination, 62 articles met inclusion criteria. The study showed there are two types of genotypes common to most of the NF1 population: intragenic mutations and deletions. What is known today is that patients with deletions tend to have more severe. Individuals with large deletions in the NF1 coding region present dysmorphic features, cardiovascular manifestations, joint laxity, large hands and feet, a high number of dermal neurofibromas, and a variable range of learning disabilities. Considering the opposite, patients with in-frame intragenic mutations have milder phenotypes with only multiple café-au-lait spots and freckling, but no other clinical manifestations. Other findings are that there is not a clear relationship between physical findings regardless of the type of mutation.

Conclusions: In spite of some studies that have presented evidence of association in probands, there is still no proved genotype-phenotype correlation in NF1. This suggests that for discordant phenotypes with similar genotype there are still others factors that must be considered such as epigenetics, smaller genetic alterations or even environmental factors.

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Figure. Prisma Algorithm
Adult Patients with Neurofibromatosis Type 1 (NF1) and High-Grade Glioma: A Case Series and Review of the Literature

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Background: There have been no prior studies on outcomes for high grade glioma (HGG) in adult NF1 patients. This is the largest case series to date.

Methods: An institutional review board-approved single-center retrospective review of 220 medical records over the past 30 years identified 12 adult patients (18 or older) with NF1 complicated by new diagnosis of HGG.

Results: The median age at diagnosis was 40.5 years and the average KPS at diagnosis was 80. All HGGs were astrocytomas, located in the brain in 11 patients, and spinal cord in 1 patient. Pathology included 2 glioblastomas, 3 anaplastic astrocytomas, and 7 tumors with pilocytic features but pleomorphism, high mitotic index, or other genetic features prompting an anaplastic designation. Patients were treated with various combinations of surgery, chemotherapy (temozolomide, procarbazine, carboplatin, etoposide), bevacizumab, and/or radiation. The patients with GBM had poor outcomes: the first progressed at 2 months and died at 5 months, and the other died within 1 month. Three patients had radiographic evidence of leptomeningeal disease at diagnosis. Median overall survival was 40 months (range 1-68 months) not including 2 patients who were lost to follow up and 2 without progressive disease to-date. Three patients suffered from stroke during treatment, including one with comorbid moyamoya syndrome and one with premorbid coarctation of the aorta. One patient developed rapidly advanced radiation leukoencephalopathy, and 2 suffered from pulmonary embolism or deep veinous thrombosis.

Conclusions: Although rare, HGG in NF1 patients may not progress or respond to therapy in the same manner as in the general population. This may be attributed in part to a higher predilection of NF1 patients for HGG with pilocytic histopathology and high mitotic index or pleomorphism. Accurate discrimination of this subclass from true pilocytic tumors, which are common in NF1 patients, is important as more aggressive management may be indicated. Treatment paradigms may need to be reexamined in NF1 patients, who were highly susceptible to complications of chemotherapy and radiation.

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The Role of the Physiotherapist in Neurofibromatosis 2

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Background: Physiotherapy for Neurofibromatosis 2 (NF2) patients is challenging. Within the framework of a specialist multidisciplinary team, it is important to evaluate physiotherapy standards and provision constantly. NF2 varies in its impact on each individual as they manage their condition on a daily basis. Physiotherapy practices should be updated across service centres so that patients receive excellent quality patient care. By keeping abreast of newly published guidelines and relevant research and through cross-centre communication, the role of physiotherapists in monitoring, maintaining and improving patient’s symptoms can become more clearly defined and evidence based.

Methods: A review of the role of the physiotherapist and how it relates to:
1) The Multidisciplinary team
2) Genetic Severity
3) Referral criteria
4) Physiotherapy skill base/NF2 issues
5) Outcome Measures
6) Evaluation of Physiotherapy

Results: First reports of the outcomes of physiotherapy within the NF2 population are encouraging and suggest that it is comparably effective for patients of varying genetic severity. However, further work is required in order to delineate best practice for physiotherapy within this genetically and phenotypically variable condition. Patients should be referred for an initial physiotherapy assessment at the earliest opportunity and should be able to access standardised pathways of physiotherapy services wherever they are. Outcome measures should include both self-reported as well as physiotherapist-assessed data. It is also clear that more work is required in developing physiotherapy in NF2 via continuous education and improved communication with colleagues.

Conclusions: The role of a specialist physiotherapist in NF2 is diverse role and ever-changing. In order to respond efficiently to the patients’ needs throughout their lifetime we suggest a physiotherapy program, which should be tailored to each individual, consisting of the following pathway: baseline assessment, follow up, monitoring and regular recording of outcome measures. By cross-centre collaboration amongst physiotherapists as well as with other care professionals, we can evaluate service standards and formulate care pathways that include physiotherapy.
Typical Café-au-Lait with Atypical Additional Features: 15q14 Deletion in the NF1 Clinic

Emma M. Burkitt Wright, Complex NF1 Service, Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust; Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom

**Background:** 15q14 deletions are rarely reported. Many affected individuals have ventricular septal defect (VSD), other congenital anomalies and autistic spectrum disorder. Previous studies suggest MEIS2 as the gene within this region likely to account for these features. SPRED1 is a contiguous gene, co-deleted in a number of these patients. We report such a patient, with a phenotype initially very suggestive of NF1.

**Methods:** A four year old boy was seen in the paediatric neurology clinic for developmental delay. Café-au-lait (CAL) macules were noted and he was referred to the NF1 clinic.

**Results:** Clinical findings
An antenatally diagnosed single high muscular VSD had resulted in neonatal cardiac failure, prompting early surgical closure. Acute deterioration at 8 months led to further surgery, with a slow recovery. Delayed development was initially attributed to critical illness. Brain MRI demonstrated Chiari I malformation but no other specific features. Motor milestones were modestly delayed, speech more delayed, and assessments for autism were instituted.

Five typical CAL and freckling were present, prompting consideration of NF1. The severity of congenital heart disease and degree of speech delay were atypical, but craniofacial and other features, including relative macrocephaly (OFC 85th, height 25th, weight 80th centile), were reminiscent of those seen in NF1.

**Molecular results**
Chromosomal microarray, NF1 and SPRED1 analysis were performed, with no sequence changes identified, and no copy number anomalies at 17q11.2. A heterozygous 6.7Mb deletion of 15q14 including MEIS2 and SPRED1 was found. Heterozygous loss of SPRED1 was confirmed on MLPA.

**Conclusions:** This patient’s complex phenotype appears attributable to the identified 15q14 deletion, emphasising the value of copy number analysis in patients with multiple CAL but an otherwise atypical phenotype for NF1. MEIS2 deletion is likely to be aetiologically important in the patient’s neurodevelopmental features and VSD, but haploinsufficiency for SPRED1 or other genes may also be implicated. Further analysis of other individuals with loss of function/deletion of MEIS2 and contiguous genes may help to clarify this. Whilst he has significant developmental impairment, this patient appears unlikely to be at high risk of additional NF1-associated sequelae, as his CAL are due to SPRED1 deletion.

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Half a Century of ‘Watson Syndrome’

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**Background:** In 1967, Watson (10.1136/jmg.28.11.752) reported three families with an autosomal dominant condition reminiscent of NF1 with a high prevalence of pulmonary stenosis (PS) (Watson G (1967) Arch Dis Child 42:303-7). Mutations in NF1 are the molecular cause. c.3827 C>G, predicting p.(Arg1276Gln) being found in the largest of these families (10.1038/ejhg.2012.221). This kindred now includes 21 affected individuals, and a clinical re-evaluation of the family has been undertaken.

**Methods:** Patients were reviewed in person and via medical records. Sanger sequencing of NF1 exon 22 was performed.

**Results:** The matriarch of the family died aged 85. A heterozygous NF1 c.3827C>G variant was seen in 10 members of different branches of her family, and absent in many unaffected people, consistent with pathogenicity. PS appears to be present only in the siblings reported in 1967, with one additional individual (born to a father with the family mutation but no PS) having pulmonary atresia fatal at 8 days. Mild intellectual disability was present in people with the variant, consistent with the original description of Watson. Café au lait (CAL) macules were present, but less prominent with age. No individual had developed multiple documented neurofibromas. Noonan-like facial features including significant ptosis were present in several individuals, one of whom required repeated surgery for this.

**Conclusions:** The description of ‘Watson syndrome’ is now historic, as these patients are known to have a variant of NF1, due to mutations that are considered hypomorphic. The distribution of PS in this large family suggests further genomic factors may be important in its causation (affected siblings sharing more alleles than more distantly related family members), though shared intrauterine influences could also contribute.

The large family reported here demonstrate mild intellectual disability and CAL as consistent features of their NF1, whilst PS has not been observed in the many affected individuals born to the third and fourth generations. This intrafamilial variability emphasises that techniques such as whole genome analysis of informative families may help to dissect this and other polygenic traits in NF1 and other disorders in the future.
**Multiple Genetic Sources of Ras-MAPK Pathway Dysfunction May Lead to a Severe Neurodevelopmental Phenotype**

**Emma M. Burkitt Wright. Complex NF1 Service, Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust; Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom**

**Background:** Neurofibromatosis type I (NF1) is frequently associated with developmental features. Neurofibromin is a negative regulator of the Ras-MAPK signal transduction pathway. Mutations of many other genes of this pathway also cause neurodevelopmental disorders. Only very few patients with more than one mutation affecting Ras-MAPK pathway function are known.

**Methods:** We report longitudinal data and medical photography on a further such patient, with an unusually severe phenotype.

**Results:** Clinical findings
A 12 month old boy came to the NF1 clinic with 10 café-au-lait macules and bowing of right tibia and fibula. He later sustained a fibular fracture. Macrocephaly was present (99th centile), with increased prominence of the extraaxial fluid spaces. The severity of developmental delay was greater than expected in NF1, with hypotonia and ongoing feeding difficulties that were also unusually severe. He had a birthweight of 4.58kg (96th centile) and neonatal hypoglycaemia that required intravenous dextrose. He was noted to have multiple capillary malformations (CM), the largest under his chin and another over his right knee. His father had a similar CM, and also an arteriovenous malformation (AVM) of his right hand. Other family members also had CMs but no documented AVM or associated health problems. At age 4, he was severely developmentally impaired, non-verbal and non-ambulant, but continuing to make slow developmental progress.

Molecular results
Genetic testing demonstrated a de novo NF1 c.6754A>T p.(Lys2252Ter) mutation and paternally inherited RASA1 c.1253+2T>C novel splice variant. Microarray analysis was normal.

**Conclusions:** The co-existence of two related genetic conditions in an individual is rare, but reported previously in patients with Noonan syndrome (NS) and NF1. Severe phenotypic effects have been present in some of these patients. Biparental inheritance of NF1 is likely to be lethal, whilst severe phenotypes have been observed in patients with two mutations associated with NS, or NS and NF1. Study of these rare patients has potential to further understanding of the effects of Ras-MAPK signalling in vivo.

Combined de novo NF1 and inherited RASA1 mutations appear a plausible explanation for this patient’s severe phenotype. The role of additional genomic variants in the pathogenesis of NF1-associated sequelae such as pseudarthrosis or intellectual disability requires further exploration.

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Decreased White Matter Integrity of Fronto-temporal White Matter Tracts in Children with NF1 Compared to Age-Matched Controls

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**Background:** Learning challenges often seen in children with NF1 include decreased working memory, executive dysfunction, and inattention, neurocognitive functions localized, in part, to the frontal lobes, and frontotemporal circuitry. White matter (WM) tracts in adults with NF1 show differences in diffusion tensor imaging (DTI) compared to controls, with fractional anisotropy (FA) values reflective of decreased organization of the WM in patients with NF1.

**Methods:** FA was measured in 17 children with NF1 and 27 age-matched control subjects on 3T MR imaging. Measurements were obtained at the bilateral cingulate gyri, and frontal and temporal WM, using ROI method. Two-tailed student t-test was used with p values <0.05 judged as significant. Analysis was performed on the group as a whole and breaking the group into three age groups: 17 mos–5.9 years; 6 years–11.9 years; 12 years–18 years.

**Results:** There were differences in FA between patients with NF1 and controls in the WM of the cingulate (NF mean: 0.55 [SD: 0.1], control mean: 0.59 [SD: 0.87], p= 0.04), frontal (NF mean: 0.48 [SD: 0.87], control: 0.56 [SD: 0.66], p<0.0001) and temporal lobes (NF mean: 0.38 (SD: 0.06), control: 0.41 (SD: 0.04), p = 0.0005). This difference was largely driven by the youngest group (17 mos–5.9 years) with cingulate NF mean: 0.49 (SD: 0.1), control mean: 0.55 (SD=0.1), p=0.01; frontal WM: NF mean: 0.4 (SD: 0.07), control mean: 0.54 (SD: 0.11), p <0.00001; temporal WM, NF mean: 0.35 (SD: 0.05), control mean: 0.41 (SD: 0.43), p = 0.006. The 6-11.9 year-old group demonstrated significant differences in the WM of the frontal (NF mean: 0.49 [SD: 0.08], control mean: 0.56 [SD: 0.09], p = 0.04) and temporal lobes (NF mean: 0.36 [SD: 0.68], control mean: 0.41 [SD: 0.05], p = 0.02, while the 12-18 year-olds had no differences.

**Conclusions:** Children with NF1 have lower FA values of the frontotemporal WM compared to age-matched controls indicating decreased myelination or less organized WM. These differences could be implicated in the cognitive dysfunctions seen in children with NF1. The difference is more apparent at younger ages than the older ages. The difference in WM organization may be due to alterations in oligodendroglial lineage dynamics, which have implications not only in cognition, but also in tumorogenesis. Further interrogation of other WM tracts and in NF-associated neoplastic processes are indicated.

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Diffusion MRI Tractography Reveals Decreased White Matter Integrity in Patients with NF1 Compared to Age-Matched Controls

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**Background:** Automated Fiber Quantification (AFQ), a method of quantitative analysis for diffusion MRI tractography has shown utility in assessing white matter tract (WMT) micro-organization. Compared to conventional region of interest methods, AFQ offers a unique advantage with its efficient, automated platform for analysis of the entire WMT, not just focal regions of manual analysis that are susceptible to interrogator bias. We used AFQ to examine FA of WMTs implicated in attention and executive function, cognitive domains known to be impaired in many patients with NF1.

**Methods:** Twenty-four children with NF1 (age mean = 9.9 years, 14 male) and 24 age- and sex-matched controls (age mean = 9.9 years, 14 male) underwent diffusion MRI at 3T using a dual-spin diffusion weighted sequence (25 directions, b=1000 s/mm2, 1x b=0 volumes) We used AFQ to segment and extract diffusion metrics of bilateral anterior thalamic radiations (ATR), inferior fronto-occipital fasciculi (IFOF), cingulate gyri, superior longitudinal fasciculi, and uncinate fasciculi. Independent T tests were used to evaluate differences in mean tract-FA across 30 nodes/WMT between NF1 patients and controls with a p value of 0.05 deemed significant.

**Results:** Patients and controls did not differ in relative head motion. Significant differences in FA were found between the NF1 and control groups in the bilateral ATR and IFOF, while only trends toward significant differences were seen in the bilateral cingulate gyri superior longitudinal fasciculi, or uncinate fasciculi.

**Conclusions:** AFQ revealed significantly reduced white matter organization in NF1 patients compared to age-matched controls in the WMT of the bilateral anterior thalamic radiations and inferior fronto-occipital fasciculi, areas implicated in executive function and inhibitory control. The current methodology (AFQ) represents improvement over previously used methods in both efficiency and integrity of the results. It promises to be a powerful method by which to investigate white matter microstructure in NF1. Investigations using larger samples and interrogation of other WMTs with associated functional outcomes are warranted.

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Patient Views Regarding Cutaneous Neurofibromas and Treatment

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Background: A characteristic feature of neurofibromatosis 1 (NF1) is the cutaneous neurofibroma, which affects more than 99% of adults. Although these tumors do not have malignant potential, they have significant negative effects on quality of life. Current treatment of cutaneous neurofibromas has been limited to surgical excision or destruction using a laser, electrodessication, or radiofrequency ablation. These treatments only target a subset of cutaneous neurofibromas and can result in scarring; in addition, these tumors can recur from residual tumor cells left after treatment. There is a strong unmet need for the development of effective medical therapies. Currently, there is a lack of information concerning how patients assess morbidity related to cutaneous neurofibromas and how they view current and potential cNF treatments. As therapies become available for cutaneous neurofibromas, it is critical to understand the patient perspective to guide the design of clinical trials.

Methods: A survey for adult patients with NF1 was created by the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) cutaneous neurofibroma working group, which is composed of an international group of healthcare providers and patient representatives. The survey was designed to collect basic demographic information, details about the patient’s cutaneous neurofibromas (e.g., burden, location), views on morbidity related to specific aspects of cutaneous neurofibromas (e.g., body location, size, number, itch), and views regarding current and potential future cutaneous neurofibroma treatment (e.g., meaningful response, treatment modalities, side effects, and outcome measures). The survey link will be distributed via the Children’s Tumor Foundation Patient Registry email blast to adults (≥18) with NF1.

Results: Results will be available in advance of the 2018 NF Conference.

Conclusions: This information will guide the design and implementation of patient-centered treatment of cutaneous neurofibromas.

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Dysfunctional Coping is Related to Impaired Skin-Related QoL and Psychological Distress in Patients with Neurofibromatosis Type 1

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Background: Low skin-related quality of life (QoL) is usually associated with low levels of self-confidence and self-esteem and is frequently associated with high levels of anxiety and depression symptoms. The way patients cope with a physical disease impacts significantly on their psychosocial adjustment to the disorder and on their emotional functioning. The current study explored how coping strategies, skin-related QoL, psychological distress, and self-esteem interact in a sample of unselected individuals with Neurofibromatosis type 1 (NF1).

Methods: 72 adult patients with NF1 completed the following questionnaires: Coping Orientation to the Problems Experienced (COPE), Skinex-29, Padua Skin-Related QoL questionnaire (PSRQ), State Trait Anxiety Inventory-X2 (STAI-X2), Depression Questionnaire (DQ), and Rosenberg Self-Esteem Scale (RSES). The K-modes algorithm was used to identify clusters of patients based on four variables: sex, global severity of NF1, number and distribution of cutaneous neurofibromas. Individuals of different clusters were compared with regard to their scores on measures; correlations between scores were analyzed within each cluster separately.

Results: Two main clusters were identified. Individuals of Cluster A had a larger number and a more widespread distribution of cutaneous neurofibromas compared to those of Cluster B; Clusters were comparable with respect to sex and global severity of NF1.

Patients of Cluster A scored higher than those of Cluster B on the PSRQ “Interpersonal impairment”, “Negative feelings and emotions”, and “Physical distress and Impairments” scales; conversely, they showed lower scores in the PSRQ “Positive feelings and emotions” scale. Consistently, patients of Cluster A scored higher than those of Cluster B on the Skinex-29 “Emotions” and “Functioning” scales. No differences between Clusters emerged with respect to the other measures.

The COPE “avoidance strategies” scale was significantly correlated with the PSRQ “Physical distress and Impairments” scale, the Skinex-29 “Symptoms” and “Functioning” scales, the STAI-X2, the DQ and the RSES only in Cluster A.

Conclusions: Our data suggest that patients with a large number and a widespread distribution of cutaneous neurofibromas have reduced skin-related QoL; in addition, the higher is the use of dysfunctional coping, the more impaired are skin-related QoL, psychological distress and self-esteem.

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Body Image and Quality of Life in Adult Neurofibromatosis Type 1 Patients

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Background: Type 1 Neurofibromatosis (NF1) is an autosomal dominant genetic disease mainly characterised by the presence of multiple café-au-lait spots and cutaneous or subcutaneous neurofibromas, which may cause disfigurement of the body. This descriptive cross-sectional study aims to evaluate emotional impact and quality of life in adult NF1 patients and its possible relationship with body image.

Methods: 100 adult patients with NF1, who were attended at the Spanish National Reference centre for Phakomatoses, participated in this cross-sectional survey. Socio-demographic data and sources of social and psychological support were collected by an ad-hoc questionnaire. Outcome measures were: Quality of life evaluated with EuroQoL-5D questionnaire; emotional impact with the Hospital Anxiety and Depression Scale (HADS), and body image, through an adaptation of the Hopwood's Body Image scale.

Results: Altered Body image appears to be related to impaired quality of life and associated with the presence of increased depression and anxiety in adults with NF1, being more significant in women. Changes in body image depend on the age of appearance and severity of NF1 skin manifestations.

Conclusions: Our findings suggest that the way adult NF1 patients experience their body image is a predictor of quality of life. Moreover, these experiences may lead to anxiety and depression symptoms. These results are concordant with previous reports from Granström et al, 2012. More research is needed in Spanish-speaking patients.

Implications for Clinical Practice: Knowing how patients with NF1 experience changes in body image would guide genetic counselling and the implementation of specific psychotherapeutic and psychosocial interventions for this population.

NF1 and Breast Cancer a Retrospective Analysis and a Prospective Study

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Background: Neurofibromatosis type 1 is a complex and heterogeneous multiorgan disease. It is a tumor predisposing condition with a general risk of developing a neoplasia estimated to be 5-15% higher than in the general population. In recent years an increased risk of developing breast cancer (BC) compared to the general population has been estimated for NF1 women.

Methods: We conducted a retrospective study in our cohort of NF1 patients, collecting clinical and anamnestic data in a dedicated database, in order to establish the incidence of BC in our population. If available, data on histotypes and immunophenotypic factors were also collected. Prospectively, we are currently working on drawing up a clinical/instrumental protocol for BC prevention in NF1 female patients based on the literature and our centre clinical experience data and supported by the district guidelines on NF1 follow up (http://malattierare.maronenegr.it/images/downloads/PDTA/PDTA_schede/nf1.pdf).

Results: Our clinical cohort is composed by 491 adult patients (190 males and 301 females), referred for multidisciplinary follow-up at our institution. The mean age of patients is 40 years old, ranging from 18 to 76. The mean age of the female cohort is 41 years, ranging from 18 to 72. Among the 239 women for whom an updated follow up is available, 23 women (9.6%) developed a breast problem that necessitated further investigation and in 18 of these (7.5%) a BC was diagnosed. The mean age of this latter group is 51 years old (ranging from 36 to 64 years). Eight of them developed BC before 50 years, 5 between 50 and 60 years and the remaining 5 after 60 years. From February 2018 to all NF1 women followed in our institution we offer a dedicated screening consisting of annual breast ultrasound for women under the age of 40 and annual mammography for those over 40 years old. In specific cases (familiarity, breast tissue peculiar characteristics or suspicious lesions) further clinical and/or instrumental examinations are prompted up (e.g. MRI, oncological evaluation).

Conclusions: Our findings confirm the literature data regarding the higher risk for NF1 women to develop BC than the general population. The implementation of the dedicated BC screening in a single centre will increase specificity and sensitivity of these diagnostic procedures, by creating a multidisciplinary experienced equipe and also by providing standardized data and characteristic on breast tissue images in NF1 women.

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Mutational Spectrum of NF1 Identified from Molecular Genetic Testing of Korean Patients with Neurofibromatosis Type 1

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Background: Neurofibromatosis 1 (NF1) is one of the most common autosomal dominant disorders in humans, occurring with an incidence of 1 in 2500 to 3000 individuals regardless of ethnicity, race, and gender. The molecular genetic testing of NF1 is hampered due to the large size of the gene, presence of pseudogenes, lack of a mutational hotspot, and the abundance in proportion of family-specific mutations in NF1 gene.

Methods: In this study 263 Korean patients from 255 families underwent molecular genetic testing. The patients who were requested for NF1 gene mutation analysis at Seoul St. Mary's Hospital, Seoul, Korea were included in this study. Molecular genetic testing for NF1 included the combination of cDNA and gDNA based sanger sequencing and multiplex ligation probe amplification.

Results: One-hundred ninety five non-duplicate mutations including 101 novel mutations (52%) and 8 different NF1 large deletions in 21 patients were identified among the 255 eligible NF1 families. Seventy-seven were frameshifting mutations, 6 were in-frame deletion/duplication mutations, 38 were nonsense mutations, 20 were missense mutations, 39 were splicing mutations, and 15 were variants of unknown significance.

Conclusions: This study provides a comprehensive characterization of the mutational spectrum of NF1 gene in Korean patients and demonstrates extremely high diversity in NF1 gene mutations. It also highlights the importance of exhaustive mutation analysis scheme for molecular characterization of NF1 mutations.

Prevalence of Optic Pathway Glioma in Children with NF1 Screened by MRI – a Single Centre Experience

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Background: Children with Neurofibromatosis type1 (NF1) are at risk of developing optic pathway glioma (OPG). Due to possible vision loss screening is indicated. Ophthalmologic examinations are undisputed, whereas screening by magnetic resonance imaging (MRI) remains controversial. Over the last two decades routine MRI screening of paediatric NF1 patients was performed at the Expertise Centre for Neurofibromatosis, Medical University of Vienna (MUV), Austria.

Methods: Children aged 0-20 years with clinical or genetic diagnosis of NF1 followed at the Department of Pediatrics and Adolescent Medicine, MUV, between 1995 and 2015 were included. Chart review was performed and imaging reports were analysed for descriptive statistics.

Results: 171 patients (53% female) were included, of whom 151 received a MRI. 28.5% fulfilled criteria for OPG, 8.6% had abnormalities of the optic nerve (e.g. tortuosity, widening of optic sheets). 10.6% of patients were symptomatic (revealing OPG in 12/16 cases), the others were screened asymptatically. Symptomatic patients were significantly younger at diagnosis (median 4.2 years), and almost all patients developed their OPG before the age of 8 years.

14/43 (32.6%) OPG patients received chemotherapy. Visual and radiologic outcome were assessed, but not meaningfully evaluable due to low numbers.

Conclusions: OPG was far more common in our study population screened by MRI then previously reported. Those numbers may be influenced by the fact that our NF1 centre is closely associated with a high accrual paediatric neuro-oncology centre, but the number of asymptomatic patients clearly outweighs. Further validation is needed to assess the value of MRI screening for clinical decision making.

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Clinical Analysis of 325 Patients with NF in Argentine

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Background: Neurofibromatosis are complex, multisystemic and rare diseases with many clinical features in adult patients.

The purpose of this study was to find clinical differences between familial and sporadic cases and analyze our results.

Methods: We retrospectively reviewed clinical reports of 325 patients with NF, in our University Hospital of Buenos Aires (NF Center) between 2014 and 2017.

The data was analyzed using statistical package: SPSS 16 BY SPSS INC. The descriptive statistics suitable for each variable were determined according to their scale of measurement and distribution. Confidence intervals were estimated for average and percentages. We used: chi square, t Student test.

Results: 325 cases with NF: 56,9% female/43,1% male. NF1:270 patients, NF2:38 p, NF3:17 p.

(83%, 12% y 5% respectively) Mortality: NF1: 1,9% > NF2: 10,5% y NF3: 0%. Affected first degree: NF1: 33,3%; NF2: 18,4% y NF3: 17,6%.

NF1 MCL: 99,3%, Lisch: 50,7%; Scoliosis: 35,8%, Bone Dysplasias: 11,4%; Dermic neurofibromas: 61,9%; Peripheral Nerve NF: 20%; Plexiform NF: 18,1%; MPNT: 3,3%; OG: 4,4%.

NF2: Bilateral Vestibular Schwannoma 94,7%, MG: 50%, Paraspinal Schwannoma: 36,8%, Ependymoma: 18,4%, Peripheral Nerve Schwannoma: 18,4%, MCL: 18,4%, Skin plaque: 10,5%.

NF3: Peripheral Nerve Schwannoma: 97%, Spinal Schwannoma: 29,4%, Unilateral Vestibular Schwannoma: 10%, Skin plaque: 5,9%.

Conclusions: 1). We've found statistically significant differences between 2 groups (familial and sporadic) in bone dysplasia, optic glioma and plexiform neurofibroma. 2). Our patients showed 4 dermatological appearance: nodular type, macular type, freckled type and combined type. 3). We classified the NF2 patients: NF2B (Bilateral Schwannoma), NF2M (Meningioma), NF2I (Intramedullary Tumor), NF2S (Spinal schwannoma), NF2D (Dermic tumor).

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Impairments in Communication and Social Interaction in Children with Neurofibromatosis Type 1: Characteristics and Risk Factors

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Background: Neurofibromatosis type 1 (NF1) is a multisystem neurocutaneous disorder with autosomal dominant inheritance. Cognitive and behavioral difficulties are considered the most common neurologic complications. Patients with NF1 have a higher incidence of autism spectrum disorder (ASD) than the general population but, were significantly less likely to receive a community-based ASD diagnosis. The aim of the study was to assess the presence of ASD-like characteristics in young children with NF1 and to identify predictors of social and communication disorders.

Methods: The cohort included 30 patients with NF1 attending the multidisciplinary NF1 clinic of a tertiary pediatric medical center from September 2015 through September 2016. The parents/caregivers completed the Social Communication Questionnaire (SCQ), Lifetime form and the Vineland Adaptive Behavior Scales, second edition (VABS II).

Results: Sixteen patients (53%) had a previous diagnosis of attention deficit/hyperactivity disorder (ADHD). There was a positive association between the presence of ADHD and a low Interpersonal Relationships score. Children with poor interpersonal relationships were more likely to be placed in a special educational setting than children with better socialization (67% vs. 5%). Language delay, documented in 12 children (40%), also correlated with a low Interpersonal Relationships score.

Conclusions: NF1, as a genetic disorder, harbors a potential impairment in neurological substrates, leading to problems in interpersonal communication and autism associated with speech/language function. ADHD appears to be more a marker than an actual independent risk factor of NF1. The early evaluation of children with NF1 for interpersonal communication problems and ASD, especially those with a speech delay or ADHD, will alert clinicians to initiate appropriate and timely treatment.

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Development and Preliminary Evaluation of a Quality-of-Life Questionnaire for Adults with Neurofibromatosis Type 1 (NF1-AdQOL)

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Background: Neurofibromatosis type 1 (NF1) is a variable and unpredictable multi-system disorder which predisposes to medical complications, cognitive impairment and disfigurement. A disease specific questionnaire can evaluate effects of NF1 from the patient’s viewpoint, enabling clinicians to identify patient concerns. Validated patient reported outcome measures are also essential in determining if treatment is impacting on quality of life (QOL). The aim of this study was to develop and validate a NF1 specific QOL questionnaire for adults.

Methods: The NF1 adult QOL questionnaire (NF1-AdQOL) was developed based on interviews with affected adults (n=8, age 18–40 years), a literature review, a survey of clinicians and piloting of the questionnaire. The domains of emotions, functioning and symptoms as conceptualised in Skindex-29, a dermatology specific QOL instrument, were applied. Adults with NF1 (n=114) were recruited from three Australian genetics clinics and completed the NF1-AdQOL, Skindex-29 and Short Form-36v2 (SF-36v2) questionnaires. Validity of the NF1-AdQOL was determined by an exploratory factor analysis, and by conducting a multi-trait multi-method matrix analysis with scores obtained from the Skindex-29 and SF-36v2 questionnaires.

Results: Factor analysis indicated that 62.7% of the common variance could be explained by three factors labelled as ‘emotions associated with cosmetic appearance’ (12 items), ‘social functioning and learning’ (11 items) and ‘symptoms’ (8 items). The NF1-AdQOL had good internal consistency (Cronbach’s alpha =0.96) and demonstrated good convergent validity with the Skindex-29. Weak convergent validity of NF1-AdQOL with some domains of the SF-36v2 indicated that these two questionnaires focus on aspects of QOL in adults with NF1 in somewhat different ways.

Results indicated overall generally healthy QOL for adults with NF1. When measured by NF1-AdQOL, the QOL of adults was lower than that assessed by the Skindex-29 and the SF-36v2.

Conclusions: The results provide preliminary evidence that the NF1-AdQOL is a reliable, valid and feasible tool to measure QOL in adults with NF1. Further evaluation is required to determine the responsiveness of the NF1-AdQOL to detect changes over time. NF1-AdQOL may be a useful secondary outcome measure in NF1 clinical trials or an aid to clinical decision-making.

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Experience and Acceptability of Breast Cancer Screening in Young Women with an Increased Risk of Breast Cancer Due to Neurofibromatosis Type 1 (NF1): Early Insights

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Background: Women with NF1 have a moderately increased risk of developing breast cancer (18% lifetime risk), and national risk management guidelines recommend breast screening with mammogram from age 40. Evidence has emerged to show the excess incidence of breast cancer risk in NF1 is highest among young women aged 30-50 years, with a 47.4% 10-year breast cancer risk from age 30. This is higher than the 10-year risk for women with certain high-risk gene mutations, including PALB2 (3.65%) and CDH1 (3.0%), and is approaching the risks for female BRCA2 carriers (6.6%). Annual breast screening with breast MRI +/- mammogram +/- ultrasound is recommended from age 30 for women with high risk gene mutations. Given this, breast screening using MRI and mammogram +/- ultrasound from age 30 is now being offered to women with NF1 at our adult NF clinic between ages 30 and 50. This prospective single centre pilot screening study seeks to evaluate this new screening modality in women with NF1.

Methods: The psychological impact of breast screening will be evaluated by patient-administered validated questionnaires at four time points to determine the short and medium term effects of screening and breast cancer discussions on anxiety, depression and cancer worry. Outcomes from breast screening will also be reviewed to determine the number of false positive and false negative breast screens in this cohort, including the frequency of further biopsies, investigations and adverse events.

Results: Recruitment and data collection is ongoing. We plan to report on the early findings from the initial screening round of our patient cohort.

Conclusions: We hope that study outcomes will inform best practice on NF1-associated breast cancer risk management nationally and internationally.

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A Clinicopathological Study of the Expression of G Protein-Coupled Estrogen Receptor 1 in a Large Series of Cutaneous Neurofibromas from Individuals with Neurofibromatosis 1

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Background: Multiple neurofibromas are a hallmark of neurofibromatosis 1 (NF1). Cutaneous neurofibromas begin to appear during puberty and increase in number and volume during pregnancy, suggesting a hormonal influence. Most cutaneous neurofibromas express progesterone receptors, but few express the classical estrogen receptors (ER). Beyond its effects on classical receptors, estrogen may have a rapid effect on cells that express the alternative receptor G protein-coupled estrogen receptor 1 (GPER-1). GPER-1 expression has been reported in some ER-negative neoplasms. Our aim was to evaluate the expression GPER-1 in a large series of NF1 cutaneous neurofibromas and to investigate their correlation with patient’s age and gender, tumor’s size, and proliferating index, through expression of Ki-67.

Methods: The present study was approved by the institutional Ethics Committee, and all the individuals signed the informed consent term. A cross-section observational study was performed with a sample of 160 cutaneous neurofibromas: 80 large and 80 small tumors from 80 NF1 individuals. GPER-1 and Ki-67 expression were investigated by immunohistochemistry in tissue micro and macroarrays and quantified using a digital computer-assisted method.

Results: The percentage of positive cells for GPER-1 in neurofibromas ranged from 0.2% to 84.8%. Large neurofibromas expressed more GPER-1 than the small ones (p=0.023). There was a high predominance of strong staining for GPER-1 in tumor cells (p<0.0001) in both groups (large and small tumors). There was no association between GPER-1 and age, gender, tumor’s size and proliferation index.

Conclusions: We show for the first time that NF1 cutaneous neurofibromas commonly express GPER-1, suggesting that estrogen may act on ER-negative neurofibromas through GPER-1. The strong intensity of GPER-1 staining and the fact that large neurofibromas have a higher percentage of GPER-1 positive cells suggest that GPER-1 may have a role in the pathogenesis of NF1 cutaneous neurofibromas. Future investigations are required to understand the impact of the activation of GPER-1 on these tumors.

High Prevalence of Periodontal Disease in Individuals with Neurofibromatosis 1

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Background: We have recently shown that individuals with neurofibromatosis 1 (NF1) present high prevalence of hyposalivation. Saliva is an important parameter for maintaining oral health, and low salivary flow rate may cause many oral alterations, including xerostomia, taste changes, difficulty in swallowing, and oral infections, such as candidiasis, caries and periodontal disease. We aimed to investigate the prevalence of periodontal disease in individuals with NF1.

Methods: This study was conducted with 42 NF1 Brazilian individuals with ages ranging from 18 to 77 years (mean age: 40). Of these, 72% (32/42) were female and 28% (10/42) were male. All participants were submitted to anamnesis, oral exam, measurement of community periodontal index (CPI), and periodontal loss of attachment index. The results were compared with a sex and age paired control group.

Results: According to the CPI 37% of NF1 individuals had gingival bleeding and 68% had periodontal loss of attachment of 4-5mm. Comparing to the control group, there was a significant statistical difference in CPI (McNemar p=0.013) and periodontal loss of attachment (McNemar p=0.001).

Conclusions: Individuals with NF1 have a high prevalence of periodontal disease.

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Lower Extremity Orthopedic Manifestations of NF1

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Background: It is hypothesized that patients with NF1 may have a higher rate of ankle valgus than previously reported. Neurofibromatosis Type 1 (NF1) is an autosomal dominant disorder that affects one out of 3000-4000 people in the United States (Gutmann, 2002; Tonsgard, 2006; Wolkenstein et al, 2009). It is associated with a defect in the NF1 gene found on the long arm of Chromosome 17. The phenotypic manifestation of the disease is highly variable and includes a wide range of physical and psychological symptoms (Krab et al, 2009; Tonsgard, 2006). About half with have significant orthopedic complications (Vitale, Guha, and Skaggs, 2002) which may include ankle valgus. Ankle valgus is an insidious defect that results in pronation of the foot and medial malleolar prominence. The literature is contradictory when reviewed for the use of orthotics as treatment so it is often hard to get insurance approval for these types of treatments. This study will evaluate the prevalence of ankle valgus in patients with NF and the associated subjective and objective measures.

Methods: Subjects will be recruited from the Division of Pediatric Hematology Oncology clinic in a large Midwest metropolitan academic and research center. The patients and their parent were approached by one of the neurofibromatosis team (MD, CNP or nurse coordinator) for permission to photograph ankles as part of their comprehensive visit. The medical record was reviewed for complaints of back, leg, hip or knee pain as well as wear patterns/age of shoes if discussed with the patient during the visit. BMI was noted. The physical exam of foot/ankle/LEs was reviewed. Pain medication use was reviewed. Patient gait was evaluated both walking and running. The presence of pronation noted. Exercise tolerance was discussed and performance compared to peers in physical education classes, and athletics.

Results: Summary of the preliminary findings will be reviewed. Case studies highlighting the importance of diagnostic imaging and proper diagnosis will be reviewed. A standard assessment for lower extremity orthopedic manifestations will be developed based on findings.

Conclusions: Ankle Valgus may be a more common orthopedic manifestation in children with NF than previously appreciated. In addition to physical exam, photographic and radiologic assessment are an important adjunct to assure correct diagnosis and treatment.

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Depressive Symptoms in Children with Neurofibromatosis

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Background: The phenotypic manifestation of Neurofibromatosis Type 1 (NF1) is highly variable and includes a wide range of physical and psychological symptoms (Krab et al, 2009; Tonsgard, 2006). About half of adults with NF Type-1 are reported to have depressive reactions or depression (Hummelvol & Antonsen, 2013), but there is a paucity of studies addressing the psychological profile and emotional functioning of children with NF1 and researchers who study children with NF often use Child Behavior Checklist (CBCL; Achenbach and Rescorla 2001) to identify behavioral/emotional problems. Surprisingly, mood questionnaires are rarely used. The Child Depression Inventory (CDI; Kovacs, 1992) includes diagnostic symptoms.

Methods: The specific aim of the proposed study is to describe the psychosocial experience of children with NF1; the individual’s psychosocial descriptors, feelings, and their perceptions of the impact of NF1 on their quality of life from a qualitative and quantitative perspective. A prospectivesample of 20 participant-parent pairs (Children aged 11-17 who have a diagnosis of NF Type 1 and one parent/guardian) will be recruited from a large Midwest metropolitan academic and research center. Once consent is signed, child participants will be interviewed. They will be prompted to describe their experience of living with neurofibromatosis and to discuss their feelings and how the disease impacts their daily life. The participant will complete two questionnaires to provide additional information about the emotional/psychosocial/behavioral health of the patients (CDI-2 SR and YSR) as will the parents (CDI-2:P and CBCL).

Results: Preliminary data will be shared. Specifically, the qualitative interview data will be analyzed and the key themes that emerged will be examined for quantitative support. Descriptive statistics of the instruments will also be analyzed and the trends between parent and child responses on two self-report measures - Child Behavior Checklist (CBCL and YSR) and the Child Depression Inventory 2 (CDI-2 and CDI-2:P) will be descriptively compared.

Conclusions: This study concurrently explores the psychosocial experience of children with NF1 from the qualitative and quantitative perspective. We will learn patient’s psychosocial descriptors, feelings, and their perception of the impact NF1 has on their quality of life. This study also examines other variables that may alter the child’s view of health.

Psychiatric Aspects of a Population of Adults with Neurofibromatosis 1 (NF1) Followed at the McGill University Health Center (MUHC)

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Background: Mental health disorders are over-represented in adults with NF1 compared to the general population. However, data on the prevalence and characterization of psychiatric disease in adults with NF1 is limited. We sought to define the prevalence and psychiatric phenotype of the population of adult NF1 patients regularly followed in our MUHC NF1 Clinic.

Methods: Data of 90 NF1 patients followed regularly in our MUHC-Adult-NF1 Clinic since its creation 3 years ago were reviewed to extract the following information: psychiatric symptoms, formal psychiatric diagnoses, ethnicity, family history, gender, age at diagnosis, developmental and learning history, visibility of lesions, burden of NF1 associated multisystem disease and comorbidities. Clinical information was recorded in a dedicated database. Statistical analysis was performed for trait distribution differences between patients with psychiatric manifestations and those without.

Results: 40% of patients received a formal psychiatric diagnosis including: depression, anxiety, obsessive compulsive disease, substance abuse, attention deficit disorder and atypical autism. Psychiatric manifestations were over-represented in patients with dysmorphic features, high number of café au lait spots (CALs), disfiguring neurofibromas requiring multiple surgeries and in those with high burden of skeletal disease. Comorbidities included cancer and chronic pain. Expert psychiatric and psychological assessment was beneficial.

Conclusions: Psychiatric disease in adult NF1 patients is underdiagnosed. Atypical autism may be misdiagnosed and over-represented in patients with NF1. A formal psychiatric diagnosis allows rational treatment and facilitates access to community services. Accurate definition of the prevalence of psychiatric disease among patients with NF1 provides the bases for better resource allocation for their care.

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Associations Between Disease Severity and Visibility on Psychological Distress and Need for Support in Adults with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is characterized by a number of physical clinical characteristics and sequelae, but the psychosocial burden in adults with NF1 has only been sparsely described. We examined 1) the prevalence of patients with impaired health-related quality of life, depression, anxiety and need for support 2) associations between disease severity, visibility as well as demographic factors and the psychosocial burden among adults with NF1.

Methods: We conducted a nationwide, cross-sectional questionnaire study inviting all adult NF1 patients followed from 1977 at the National Centers of Rare Diseases located at Copenhagen University Hospital, Rigshospitalet, and Aarhus University Hospital in Denmark. With a 54% response rate, a total of 244 completed the self-report questionnaire including standard measures of quality of life (PedsQL NF Module), depression (PHQ-9), anxiety (GAD-7), and new indicators of disease-related severity (Riccardi Scale), visibility (Ablon Scale) and need for professional support (single items). Investigating associations between disease severity, visibility as well as demographic factors and the psychosocial burden among adults with NF1, different models were examined to determine optimal fit: quality of life was examined in a linear model, depression and anxiety were examined in negative binomial models. Finally, models with and without severity and visibility were compared using chi-squared test.

Results: Among the person with NF1 (61.9% female, mean age 40.19 years (SD 14.7)), we observed impaired quality of life (mean 81; 95% CI 76.21 - 86.41), increased symptoms of depression (mild: 26%, moderate: 12% and severe: 8%) and anxiety (mild: 25%, moderate: 8% and severe: 7%) as well as need for support especially for physical, psychological, familial and work related problems. Severity and visibility of the disease were significantly associated with lowered quality of life (p<0.0001), increased symptoms of depression (p<0.0001, p=0.0024) and anxiety (p<0.0001, p=0.0174), as well as increased need for support (p<0.0001, p=0.0002-0.0244).

Conclusions: Based on nationwide Danish data, this study provides further evidence of impaired quality of life among adults with NF1 and shows that severity and visibility have a great negative impact on the psychosocial well-being of these patients.

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Disclosure of Interest: K. Doser has a conflict with: The study was supported by a grant from U.S. Army Medical Research and Materiel Command (The Neurofibromatosis Research Program), E. Wreford Andersen: None Declared, H. Berg: None Declared, S. Oksbjerg Dalton: None Declared, L. Kenborg: None Declared, H. Hove: None Declared, J. Østergaard: None Declared, J. Jepsen: None Declared, S. Asger Sørensen: None Declared, J. Mulvihill: None Declared, J. Falck Winther: None Declared, P. Envold Bidstrup: None Declared
An Investigation of Eye Movement Dysfunctions in Children with Neurofibromatosis Type 1

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Background: Today’s estimates indicate that 30-60% of Neurofibromatosis type 1 (NF1) children suffer from learning disorder, including reading disabilities, secondary to language and/or visuospatial deficits (Chaix et al., 2017) with a profound impact on their academic achievement (Krab et al., 2008). Some studies suggest a delay in the maturation of low-level vision processes in children with NF1 (e.g. saccadic system, Lasker et al., 2003; magnocellular processing, Ribeiro et al., 2012). The findings from typically developing readers suggest a strong relationship between reading ability and visual processing (Leibnitz, et al., 2017). The main goal of this study was to investigate the occurrence of perceptual, visuo-attentional and oculomotor deficits in NF1 children and their potential to explain reading behavior and reading problems in this population.

Methods: Seventeen children with NF1 (9;8 ± 1;4 years, 10 girls) and twenty-one control children (9;4 ± 0;9 years, 10 girls) participated in the study. Parents and children gave their informed consent prior to the experiment, approved by the local ethics committee. Reading and visual processing skills were respectively evaluated with the Alouette test (Lefavrais, 2005) and the DEM test (Garzia et al., 1990). We also recorded eye movements while NF1 children performed an oculomotor lateralized bisection task on words, strings of hash marks and solid lines.

Results: A link between reading skills and performance in both tasks (DEM test and ocular bisection task) was evidenced in NF1 children. Children with NF1 and Reading Delay (RD) differed from children with NF1 without RD in the Horizontal Time (HT) distribution, with a deviant density peak for the first ones. Poor NF1 readers also displayed poor results in visual attention and generation of saccadic processing (e.g., less accurate, more variability, no sensibility to the discreteness of the stimuli). Note that there was no significant correlation between DEM test performance and saccadic parameters.

Conclusions: DEM test (especially the HT) can be used clinically to distinguish NF1+RD from NF1 without RD. Analysis of eye movement patterns represents a potential way to identify differences in the cognitive processing and visuo-attentional mechanisms underlying reading in NF1 children before the occurrence of school failure. Both language and visual aspects of reading should be targeted in intervention programs.

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This work benefited from support from the French Government, DYSTAC-MAP (ANR-13-APPR-0010)
Preoperative Embolisation in the Surgical Management of Massive Neurofibromas

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**Background:** The surgical management of large neurofibromas in neurofibromatosis type 1 and its clinical segmental subtypes presents certain challenges which are specific to the nature of the diseased tissue. There is a number of patients who have had surgery in naive surgical units where surgery has had to be abandoned due to uncontrolled haemorrhage. The tissue has large tortuous thin walled vessels which respond less well to standard surgical ligation and haemostatic devices. We have sought to explore interventional radiological techniques to provide preoperative control of large feeding vessels to allow surgery to proceed.

**Methods:** Six patients with massive cutaneous neurofibromas were subjected to preoperative embolisation either the day before or the day of surgery. All patients were cannulated in a dedicated interventional radiology suite, and embolisation using coils, onyx or other suitable media was completed prior to surgery. Surgery was then performed according to agreed margins, to debulk the disease.

**Results:** It was possible to control bleeding in patients who underwent resection of large neurofibromas. Surgery was completed as planned in all cases. Transfusion volumes ranged from 0 to 10 units. Cell salvage techniques were employed to maximise the use of autologous transfusion. Hospital stay, initially in a high dependency and then ward setting, had a mean of seven days. Primary wound healing was achieved in all but one case. Delayed healing was managed with simple dressings.

**Conclusions:** Preoperative embolisation allows the surgical management of what would otherwise be unresectable massive neurofibromas with few postoperative problems. Good multidisciplinary liaison between radiological and surgical departments in the planning phase of these cases gives a safe surgical environment and good outcomes.

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Monitoring of Neurofibromas Using a 3D Real Time Multispectral Camera

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**Background:** Measure standardization and automation using imaging of neurofibroma (NF) is of major importance for monitoring therapeutic trials/follow-up. This should be achieved through the acquisition of real-time 3D sub-millimetric accuracy high quality colometric (multi-spectral) images of cutaneous NF.

**Methods:** Images (FOV 220x110 mm, exposure time 80ms, distance 35 cm, report generation in less than 2s) of a patient’s back presenting cutaneous NF were acquired with a novel user-friendly 3D Real Time Multispectral Camera (RTMC, Tridimeo, France). Patient’s photographs using different random acquisition photographs up to 8 cm variation in distance acquisition/position were taken in order to mimic the conditions of multi-temporal monitoring. NFs were automatically classified with a pixel accuracy by color spectral images. Diameters were estimated from segmented NFs surfaces (planimetric accuracy ±0.4mm). In a second time, each NF 3D image was flattened to a 2D image using a newly developed algorithm with the sake of compensating patient’s positional variations and to enable multi dates comparisons of NFs volumes and heights. Root mean square values were provided for heights and volume.

**Results:** In total 17 images of 4 cutaneous NFs with respective diameters equal to 8, 6, 4 and 3mm were monitored (Fig 1). Instrumental repeatability on 3D data (same position) was estimated with a maximum variation of 0.05 mm (rms). For all images, NF heights equaled 4.2±0.3, 2.5±0.2, 1.0±0.2 and 0.8±0.3 mm. Accuracy in height of 0.2 mm (rms) was near the nominal sensitivity of the recorder. Larger uncertainty (0.3mm rms) in estimate of the largest and of the smallest NFs respectively resulted from significant shadowing and data scarcity, volumes respectively equal 90.9±7, 30.0±3.3, 6.7±1.4 mm³ and 3.2±1.1 mm³. Uncertainties in the total volume was mainly linked to uncertainty in heights because of shadowing.

**Conclusions:** 3D RTMC is promising to monitor NF with a repeatable procedure and an automated image analysis. Automated algorithms detected a delta variation of NF until up to 0.2mm. For clinical trials assessing a new therapy efficacy, a decrease of 20% of the volume of NF is considered as a good result (favorable outcome). Our system allows high repeatability/reliability measures and should undergo further validation by being compared to physician global assessment or assessment using a caliper for targeted lesion.


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Spinal Manifestations of Neurofibromatosis Type 1 (NF1)

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**Background:** Spinal manifestations of neurofibromatosis type 1 (NF1) are well described. There is a lack of research looking into this specific aspect of NF1.

**Methods:** This was a population study of the supra-regional NF1 service for the North of England at The Royal Manchester Children’s Hospital. We identified 128 NF1 patients who had undergone an MRI scan of their spine prior to the age of 18 years out of a total number of 144 patients who had presented to the service at this age. All MRI scans were reported by a consultant radiologist.

**Results:** Male and female patients were represented equally (47% vs 53% respectively). The age range was 2-18 years. Over 60% of patients had an abnormal MRI scan of the spine. Almost one third (32%) of our patient group had some degree of kyphoscoliosis, 29% of whom required surgical fixation. 16.4% of patients were found to have spinal neurofibromas with 9.5% of them requiring surgery (1.6% of all NF1 patients). Dural ectasia and vertebral scalloping were observed in 14.8% and 11.7% respectively. Spinal cord signal change was seen in 12.5% of cases. Of these cases, the signal change progressed to spinal cord tumour in 12.5% of cases (1.6% of all NF1 patients).

**Conclusions:** Spinal pathology in NF1 is common. Kyphoscoliosis affects almost one third of patients and a significant proportion of these patients will require corrective surgery. Neurofibromas were seen less frequently. Spinal cord signal change seen in NF1 can progress to spinal tumour in a significant proportion of patients. Surgery for spinal neurofibromas or spinal cord tumours was seen in equal measures in a small section of the sample.

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NF1 and the Gastrointestinal Canal: A High Prevalence of Gastrointestinal Symptoms Correlated to Constipation

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**Background:** Gastrointestinal involvement in the NF1 population is poorly described and mostly reported as case reports. Our theory was that NF1 could be associated with a higher prevalence of gastrointestinal symptoms, predominantly leading to constipation. Thus, the purpose of the PhD. was to gain new insight into the frequency and classification of gastrointestinal symptoms in patients with NF1 and to correlate these findings with NF1 phenotype and genotype.

**Methods:** Participants were recruited from one of two Danish National Centers of Expertise for NF1. Gastrointestinal symptoms were assessed with a web-based Rome® III diagnostic questionnaire. The patients completed a supplementary questionnaire on self-perceived conception of NF1. NF1 disease severity and visibility severity were assessed by the patient’s physician. Simple and multiple logistic regressions were used and the groups were compared using odds ratio. NF1 mutational analysis was performed with targeted next-generation sequencing.

**Results:** We compared 102 4-17-year-olds, median age 10.3, and 46 of their unaffected siblings, median age 10, and 175 adults, median age 34.2 and 91 of their unaffected relatives, median age 42.0. The overall response rate was 80%.

The overall likelihood of having gastrointestinal symptoms usually attributed to either functional dyspepsia, irritable bowel syndrome or constipation was higher among the 4-17-year-olds with NF1 (odds ratio (OR) 3.58 (95% CI: 1.30-9.79) and among the adults with NF1 (OR: 3.06; 95% CI: 1.62-5.79, adjusted). The likelihood of functional constipation was higher among the 4-17-year-olds with NF1 (OR: 6.41 (95% CI: 1.45-28.24) and the among adults with NF1 (OR: 3.49; 95% CI: 1.14-10.64, adjusted).

Assessing the NF1 mutational spectrum in relation to constipation, there was a higher occurrence of missense mutations in cases compared to controls. NF1 severity and the patient’s conception of NF1 showed no statistically significant effect on the likelihood of constipation. Even though not significant, the conception of NF1 illness burden showed the strongest association with constipation (OR: 1.83 (95% CI: 0.95-3.52).

**Conclusions:** The prevalence of gastrointestinal symptoms attributed to constipation was high in children, adolescents, and adults with NF1. No clear correlation with NF1 phenotype severity or genotype was established. The high prevalence of constipation indicates that it is not functional but part of the NF1 disorder.

A Longitudinal Retrospective Study of the Association of Genetic Severity with Hearing Outcomes in Patients with Neurofibromatosis Type 2

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**Background:** We present an overview of the progression of hearing loss in patients with Neurofibromatosis type 2 (NF2), who have been seen in a highly specialised multidisciplinary clinical setting. We also explore the use of a validated genetic severity tool as a predictor of hearing outcomes and hearing deterioration.

**Methods:** A retrospective observational study of patients with NF2 followed up for a mean period of 10 years. Using optimum discriminations scores (ODS), Pure tone audiometry (PTA), and genotype data collected, patients were classified according to hearing class (American Academy of Otolaryngology), their candidacy for auditory implantation (UK National NF2 consensus) and grouped by genetic severity. Kaplan-Meier survival analysis was employed to report on the age of loss of serviceable hearing according to genotype.

**Results:** Overall, genetic severity was significantly associated with hearing classification, audiometry, as well as annual rates of deterioration. Genetic severity was also a significant predictor of the age of loss of serviceable hearing. Notably, in a clinical setting, we observed a 48 year difference in the median ages of hearing loss between severe and tissue mosaic patients.

**Conclusions:** This is the first report of long term hearing outcomes as seen in clinical practice of a large heterogeneous cohort of patients with NF2 and recommends the use of a genetic severity score in clinical practice as well as in planning future studies.

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The Development of an End of Life Decision Aid for NF2

Beatrice Emmanouil, Neurosciences, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom

Background: Provision of end of life care for NF2 patients is challenging for three principal reasons. Firstly, it is difficult to identify the triggers which lead to deterioration in order to implement a plan for end of life care due to a scarcity of mortality data for NF2 patients. Secondly, whilst there are specific guidelines for nursing care at end of life, they are not tailored to NF2. End of life pathways for other chronic neurological conditions are not directly transferrable to NF2 due to the variable phenotype of the condition and the lack of mortality information. Finally, due to the complex needs of patients with NF2, their care is provided at specialist centres which are not always local. For end of life care, this centralisation of care may result in difficulties in identifying local services which provide palliative care, engaging with those services and working with them to provide clear and useful information for the local service providers who may often turn away because the challenge of caring for such a rare condition appears insurmountable due to lack of guidance and information.

This study provides an overview of the development of a stratification tool to assess life limiting symptoms in NF2 patients. The tools intended benefit is to identify NF2 patients at risk of deterioration, assessing unmet needs and support. Specifically the tool helps identify where specialists NF2 teams need to share the patient’s pathway with local services.

Methods: Content validity has been established from literature reviews, service evaluation, and narrative analysis, to identify assessment indicators. Reliability is being reviewed during use of the tool in practice.

Results: The main areas of assessment have been identified as:

- Deterioration in performance status
- Unplanned/repeated hospital admissions
- Repeated respiratory infections
- Weight loss
- Swallow dysfunction

Persistent symptoms despite treatment

Conclusions: This tool aims to remind clinicians of indicators that are life limiting, and provide guidance so that end of life can be better managed for NF2 patients, while also building evidence and knowledge of end life symptoms over time, which can then be evaluated.

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Implementation of Updated NCCN Breast Cancer Screening Guidelines at a Large Comprehensive Neurofibromatosis Center

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Background: Because research has shown an increased risk of breast cancer among women with Neurofibromatosis Type 1 (NF1) aged 30-50 years, in September 2016, NCCN released updated breast cancer screening guidelines for women with NF1. The guidelines recommend annual mammography for women aged 30-50 and to consider MRI, breast with contrast. By January 2017, we began consistent discussion of these updated guidelines among women with NF1 in the appropriate age range (as well as with women turning 30 in 1-2 years) during clinic visits with Dr. Kaleb Yohay. This poster presents our findings since implementing the new guidelines at our clinic, a large comprehensive neurofibromatosis center in New York City.

Methods: Utilizing chart review and selecting for female patients aged 30-50 with a confirmed clinical diagnosis of NF1 and seen by Dr. Kaleb Yohay between January 2017 through June 2018, we identified approximately 49 patients with whom discussion regarding updated guidelines was documented. We then determined how many patients, based on discussion, pursued breast imaging as advised; already were receiving breast imaging for a variety of indications; or, to date, had not yet pursued evaluation.

Results: Of these 49 patients, 17 pursued breast imaging based on discussion, and in several cases, further investigation was required, all ultimately benign. At least 7 women already were receiving annual or enhanced breast cancer screening for various reasons. Many have not yet pursued screening for a variety of indications. We note that in all cases obtaining authorization for mammogram was easier than for MRI.

Conclusions: From January 2017-June 2018, we documented discussion regarding the updated guidelines with at least 49 patients and referred approximately 42 patients for imaging. Of the 17 who initiated screening post-discussion, several were noted to have findings that required further evaluation, in all cases benign. Several patients with whom discussion took place already were receiving appropriate follow up. Many patients have yet to pursue screening, for various reasons. In terms of obtaining authorization through insurance, mammogram has been easier than MRI, likely because mammography is a recommendation and MRI is a suggestion according to the new guidelines.

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Implementation of Depression and Anxiety Screening in Patients with Neurofibromatosis at a Large Comprehensive Neurofibromatosis Center

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Background: Anxiety and depression are commonly reported among patients with neurofibromatosis. To better understand the prevalence of anxiety and depression among our patients with different types of neurofibromatosis, and to identify need for referrals where warranted, we implemented two different screening tools at our large comprehensive neurofibromatosis center in New York City. To measure anxiety, we used the Screen for Child Anxiety Related Disorders (SCARED), distributed to children only (not their parents), age 8-18 years. To measure depression, we used the PHQ-9 depression screen in patients age 12 and up.

Methods: We began screening patients during their appointments with Dr. Kaleb Yohay in January 2018. Through June 2018, a total of 26 anxiety screens were collected, representing 26 unique patients. A total of 72 depression screens were collected, representing 65 unique patients (some patients had more than one visit during the period of analysis).

Results: Early data analysis has been enlightening. In terms of anxiety screens, scores ranged from 0 to as high as 35, indicating significant anxiety, sometimes of a very specific type, such as separation anxiety. Regarding depression screens, scores ranged from 0 to 19, again raising red flags. Clearly, many of our patients do in fact experience anxiety, depression, or both, sometimes to a significant extent.

Conclusions: We are still analyzing data but note that many of our patients do experience some degree of depression, anxiety, or both. Implementing the screens has helped pinpoint the specific types of anxiety and level of depression among our patients. This information is helpful in terms of formulating referrals to address significant concerns and ensure that our patients are adequately supported. Many of the patients already were connected with supportive services. In some cases, screening helped identify need for more specific interventions.

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NGS High-Throughput Sequencing In Clinical Diagnosis Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 is an autosomal dominant tumor predisposition syndrome associated with mutations on NF1, a large gene that has no mutational hotspots and whose analysis is time-consuming and quite expensive. In this study NGS with Ion Torrent was performed to improve the molecular analysis of neurofibromatosis type 1.

Methods: 238 consecutive patients, 125 pediatric and 113 adults with suspected NF1 were included in the study between 2017 and 2018. Analysis with Ion Torrent was performed using a customized gene panel including NF1 and 4 NF-related genes (NF2, SPRED1, SMARC81, LZTR). Genomic DNA libraries were prepared using Ion AmpliSeq Library Kit and sequenced on Ion Torrent PGM. Ion Torrent Server and Reporter were used for SNVs calling. Control analysis on DNA and RNA using Sanger sequencing was performed in positive and negative patients. Before NGS, MLPA method was performed to rule out large deletions/duplications in all patients.

Results: 137 patients tested positive (47 familiar; 90 sporadic cases) and 101 negative (64 pediatric, 37 adults) for NF1. Of the 137 mutations found, 42 were frameshift, 39 splicing, 30 nonsense, 22 missense and 4 in frame deletion. Ninety-five mutations were previously reported, whereas the remaining 42 were novel. NGS failed to identify 8 mutations, three of them on the same exon observed on RNA by Sanger.

However 7 patients NF1 negative were found positive for the other genes (5 LZTR1, 1 SPRED1, 1 NF2).

Conclusions: NGS is cheaper and quicker than Sanger sequencing and allows the identification of mutations in the other four genes the same sequencing run. Therefore is particularly useful to identify genetic mutation in NF1 negative patients, allowing a personalized clinical and instrumental follow-up and genetic counseling.

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Patient Derived Xenograft for Real-Time Therapy Recommendations in a Sporadic Pediatric Malignant Peripheral Nerve Sheath Tumor

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Background: Pediatric malignant peripheral nerve sheath tumors (MPNST) are rare, aggressive, locally invasive soft tissue sarcomas with dismal prognosis, especially if complete surgical excision cannot be achieved. In relapse settings, no efficacious regimens have been established. Herein, we show the feasibility and potential utility of developing orthotopic patient derived xenograft (PDX) models for deciding therapeutic interventions in real time.

Methods: We generated orthotopic PDX mice for pharmacological testing from a sporadic relapsed MPNST developed in a 14 year-old male. In parallel, genomic characterization of the tumor was performed with the aim of identifying molecular targets for potential salvage therapies. PDX models were treated with different compounds, some of them based on tumor profiling.

Results: We were able to obtain orthotopic PDX models for deciding therapeutic interventions in real time. SNP array and exome sequencing of the tumor revealed genomic alterations pointing to putative drug treatments such as a mutation in MAPK1 and homozygous deletions encompassing EED and CDK2NA/B genes. PDX models were first treated with the same therapeutic regimen received by the patient, everolimus plus trametinib. After this treatment, residual tumors were allowed to regrow in the mice model with the aim of better mimicking a future relapse scenario. PDX mice were then treated with different compounds (JQ1, palbociclib, abraxane, bevacizumab, doxorubicin plus sorafenib and gemcitabine plus docetaxel), some of them based on tumor profiling (JQ1 and palbociclib). In the meantime a lung metastatic relapse was identified in the patient. At that moment, patient was treated according to our PDX results; abraxane was used as a first line treatment, but only a partial response was observed. Doxorubicin plus sorafenib was used after but not response was seen.

Conclusions: In this report, we want to highlight the feasibility and the potential of combining tumor genomic profiling characterization with orthotopic PDX as a tool to be used in patients with recurrent MPNST. To our knowledge this is the first case for this rare, aggressive type of tumor using this approach.

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Use of 3D Printing Model for Surgical Correction of Syndromatic Scoliosis. Experience in a Universitary Hospital

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Background: Scoliosis is defined as the deformity of the spine, there is deviation in the coronal plane and vertebral rotation in the transverse plane. In those patients who are not candidates or who failed the conservative treatment, the correction plus vertebral arthrodesis, is the treatment to be performed. Currently to achieve total or partial correction, the use of pedicle screws and rod is the gold standard. The inadequate pedicle screw placement could lead neuro-vascular lesions or non-optimal fixation. We present our experience using 3D printing technology for the analysis and treatment for syndromic scoliosis in young patients.

Methods: It is a cross-sectional descriptive study; three patients were enrolled. A whole-spine radiographs and a three-dimensional CT scan of the spine was performed and saved as a DICOM file type. The application of 3D printing model on spinal deformities enhanced the analysis and study of the deformity, such as the surgical technique for the correction of spinal deformities. The use of the 3D printing models would allow the extracorporal view, preoperative planning, surgical technique training and intraoperative procedure in a precise and customized manner considering to the pattern of deformities, mainly in those cases where fluoroscopic control would not be practical neither usable due to the degree of morphological distortion. However, we recognize that the sample is limited.

Results: Despite of a few cases, using 3D printing model, we can reduce the surgical time in a 30% in all cases. Reduce the blood loss in 30% during the surgery and it was no necessary to use fluoroscopic control.

Conclusions: The utilization of 3D printing model, it was very useful, not only to plan the surgery but also to use it during the surgical procedure to guide us to the anatomical variations, when we have to correct the deformity and put the screws in the precise place. This technique, is a safe way to avoid neurological and vascular complications during the surgery.

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Optic Pathway Glioma in Children: 15 Years of Experience in a Single Institution

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Background: Optic pathway gliomas (OPG) represent approximately 3-5% of childhood intracranial tumors and are seen in 11-30% of patients with neurofibromatosis Type 1 (NF1). Although OPG are typically low-grade gliomas, the clinical course and natural history are highly variable, making treatment paradigms difficult. The aim of this study is to acknowledge the paediatric population with OPG at our institution in the last fifteen years and analyze the influence of the presence of NF1, tumor topography, type of surgery and age at diagnosis on the progression-free survival (PFS).

Methods: Retrospective analysis of clinical charts of all children diagnosed with OPG from 2001 to 2015. Data analysis was done with IBM-SPSS.

Results: Twenty-nine patients were included, eleven with NF1; age at diagnosis between 8 months and 15 years (median 3 years). The first ophthalmologic evaluation revealed visual impairment in 21/29 patients. In 7/29 patients diagnosis was established during follow-up for their NF1. Anatomical specimen was obtained in 15/29 patients (10 were pilocytic astrocytomas). On MRI, in 15/29 patients there was involvement of optic nerve and/or chiasma, in 6/29 postchiasmal structures and in 8/29 chiasma and hypothalamus. As initial treatment, 18/29 patients received carboplatin-based regimens with four (4/18) subsequent tumor surgery; 3/29 patients surgery alone and 8/29 close observation. Tumor excision was done in 11/29 patients (total-3, partial-8). Biopsy was done in 4/29. Radiotherapy performed in one patient. The median follow-up time was 91 months. Median PFS was 61 months (minimum 8, maximum 174). PFS was not statistically different in the presence of NF1, according to the age group or the topography of the tumor. Total tumor resection (3/11) was associated with higher PFS versus partial. There was one tumor-related death. Among those 21 patients with initial visual impairment, 13 remained stable or improved according to the last ophthalmological evaluation.

Conclusions: This analysis emphasizes the importance of screening OPG in children with NF1 and corroborates that visual impairment, often not recoverable after the treatment, mark this pathology. In this study, total tumor resection was associated with higher PFS. In this series of cases, PFS was not statistically different in the presence of NF1 as well as according to the age group at diagnosis or with the topography of the tumor.

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Efficacy and Tolerability of Low-Dose Carboplatin-Etoposide Chemotherapy in NF1 Patients with Progressive Low-Grade Gliomas

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Background: Optic pathway gliomas (OPGs) and infratentorial gliomas represent the most common intracranial neoplasms in neurofibromatosis type 1 (NF1) patients. Most of these tumors are low-grade gliomas (LGGs). Generally, treatment started after documented progressive disease by clinical symptoms related to lesions (visual and field acuity defects, optic pallor, hydrocephalus, endocrine dysfunction and/or presence of diencephalic syndrome). Treatments for LGGs can prevent further tumor growth, they rarely result in recovery of associated visual or neurological deficits.

The aim of our study was to evaluate efficacy and tolerability of low-dose chemotherapy treatment to control tumor growth and associated symptoms in patients with NF1 with progressing LGGs.

Methods: We analyzed response to treatment in pediatric NF1 patients with progressive LGG. Treatment consisted of carboplatin-based chemotherapy protocol 400 mg/m^2 on day 1 and etoposide 100 mg/m^2 on days 1 to 3 every 4 weeks for the first 3 courses, every 5 weeks for a further 3 courses and every 6 weeks thereafter to complete ten treatment courses. Response to treatment was assessed through eye tests (visual and field acuity when possible, and optical coherence tomography-OCT) and MRI every 4 months during treatment and every six months during follow-up.

Results: From 2012 to 2017, 14 NF1 patients with diagnosis of LGG were treated in our center. The median age was 7 years (range 3-19 years). 8 patients had OPG, 4 patients exophytic brain stem glioma, a patient LGG of left cerebral peduncle, a patient cerebellar LGG. 5 patients started chemotherapy due to worsening of visual function, 9 patients for instrumental disease progression. One patient presented clinical progression (reduction of visual acuity) during treatment that led to stop protocol and to start second-line chemotherapy treatment. One patient presented instrumental response. 2 patients presented a stable disease on MRI at the end of 10 courses of treatment and a considerable clinical improvement (visual function, torticollis). No patient reported grade 4 toxicity. Only one patient needed support with granulocyte growth factors and transfusion during treatment. Currently 10 patients still have a clinically and instrumental stable disease.

Conclusions: Low-dose chemotherapy with carboplatin and etoposide in NF1 patients with progressive LGG is an active regimen, which can produce interesting clinical benefits. In our series, this treatment has shown to be efficacious and well tolerable.

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Breast Cancer Risk in Neurofibromatosis Type 1 is a Function of the Type of NF1 Gene Mutation: A New Genotype-Phenotype Correlation

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**Background:** Neurofibromatosis type 1 (NF1) predisposes to breast cancer (BC), but no genotype-phenotype correlations have been described.

**Methods:** Our 5 y international study ascertained 78 NF1 patients with BC (NF1-BC). Their constitutional NF1 mutations were compared to the NF1 Leiden Open Variation Database (N = 3432; https://databases.lovd.nl/shared/genes/NF1).

**Results:** There were no gross relationships with mutation position, but, notably, no NF1-BC cases were observed with whole or partial gene deletions (HR 0.10; 95% CI: 0.006 – 1.63; p = 0.014, Fisher’s exact). Higher proportions of both NS (39.7 vs 30.4%) and MS (17.9 vs 11.7%) mutations were also observed (Benjamini-Hochberg adjusted p-values of 0.254 with a false discovery rate of 0.25).

As six pairs and one triplet of patients all shared mutations conferring the same effect on neurofibromin, indeed two pairs had different mutations at the DNA level, we analysed the frequency of every mutation: 45 (64.3%) of the 70 different mutations have p-values <0.05 (Fisher’s exact), while 52 (74.3%) are likely true associations when adjusted for multiple comparisons (Benjamini-Hochberg p ≤0.125) and, moreover, all have individual hazard ratios between 5.3 – 148 (CI: 1.4 – 3669). Ten (9.1%) of the 11 MS cases with known age of BC occurred at <50 y (p = 0.041; Fisher’s exact).

Eighteen cases had BRCA1/2 testing, revealing one BRCA2 mutation.

**Conclusions:** This study demonstrates that certain heritable mutation types, and indeed certain specific mutations in NF1 confer different risks of BC, which may be clinically useful.

The lack of large deletions and selective excess of MS and NS is consistent with mutations conferring a gain of function being involved in NF1-BC risk, and also that neurofibromin may function as a dimer. The observation that somatic NF1 amplification can occur independently of ERBB2 amplification in sporadic BC, supports the concept that increased BC risk in NF1 may be due to mutations that change neurofibromin function.(1)

A prospective clinical-molecular study of NF1-BC needs to be established to confirm and build on these findings, but regardless of NF1 mutation status NF1-BC patients warrant testing of other BC-predisposing genes and we encourage submission of NF1 mutation data to the LOVD.


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Peripheral Nerve Sheath Tumors of the Lower Extremity and Buttocks in Patients with Neurofibromatosis Type 1: Topography of Tumors and Evaluation of Surgical Treatment

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Background: Peripheral nerve sheath tumors (PNST) are the hallmark of neurofibromatosis type 1 (NF1). Plexiform neurofibromas (PNF) are detected in a high proportion of affected patients, can lead to severe disfigurement and are classified as precancerous. This study examines the surgical procedures that have been performed on large PNST of a defined body region.

Methods: The surgical treatment of NF1 patients with PNF of the lower extremities and buttocks (LEB) was evaluated (treatment interval 25 years). Topography of the tumors was classified according to dermatomes.

Results: 243 PNST procedures were performed on 90 patients (male: 30, female: 60). One surgical procedure was sufficient in approximately half of the patients. Duration of stay in hospital was on average 15.72 days. Malignant PNSTs were rare (n = 3). Neurological complications were rarely noted and occurred only temporarily. There were no dermatomes affected by PNF with particular frequency. About 25% of patients experienced complications, in particular longer bleeding times and delayed wound healing.

Conclusions: Surgical treatment of PNF of the LEB helps alleviate physical disability and reduce patient disfigurement. Multiple surgical procedures are often necessary.

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Certain Radiographic Changes of the Mandible Indicate a PNF of the Mandibular Nerve in Patients with NF1

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Background: Neurofibromatosis type 1 (NF1) is both a tumour suppressor syndrome and a bone disease. Several local skeletal deformities have been recorded in patients with NF1. In the craniofacial region, obvious syndrome-related alterations of the jaws are particularly conspicuous in the mandible. This study applies to the analysis of mandibular changes on radiographs.

Methods: A total of 358 orthopantomograms (OPGs) of 358 patients (mean age, 34.63 years; range, 12.57-69.13 years) were analyzed. One half of the patients (n=179) had a confirmed NF1, the other were sex and age matched controls without this genetic background.

Results: Very characteristic mandibular occur in NF1 patients and are one-sided. These changes are very prevalent in NF1 patients with PNF of the mandibular nerve. A specific but variable pattern of bone changes can be expected in these cases with high probability.

Conclusions: The radiological sign of the unilateral deformed mandible should be included in the diagnostic criteria for NF1 because it is both pathognomonic and indicates another diagnostic relevant finding. Furthermore, these findings are important for planned interventions in this region.

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Genotype-Phenotype Relationships in Japanese NF1 Patients

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**Background:** It is likely that two groups of patients showing large deletion of chromosome17q11 including the entire NF1 gene and each group has distinctive features of clinical symptoms.

**Methods:** We examined NF1 gene mutation to each patient by NGS and confirmed by Sanger sequence for the etiological result.

**Results:** Out of 216 cases of Neurofibromatosis type 1 (NF1) patients who have visited our facility and participated in this study, etiological NF1 gene mutation was found in 185 cases (85.6%). Thirteen patients out of 185 cases (7%) showed large deletion including the entire NF1 gene. Patients with large deletion of NF1 gene frequently exhibit severe phenotype of clinical features. On the other hand, small number of patients with large deletion of NF1 gene exhibit mild phenotype of clinical features, which would be associated with somatic mosaicism.

**Conclusions:** It would be underlined to take assessment for genetic counseling of NF1 patients who have large NF1 deletion whether they are mosaic condition.

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Cochlear Implant in Type 2 Neurofibromatosis: The Feasibility of a Proactive Early Rehabilitation

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**Background:** Bilateral vestibular schwannoma are pathognomonic of type 2 neurofibromatosis (NF2). As a result, the patients will inevitably develop progressive sensorineural hearing loss.

Current guidelines for treatment of vestibular schwannoma in NF2 patients are still very controversial. As yet the strategies are observation, surgery or medical therapy, although results are only preliminary for the latter; all options should consider hearing preservation as one of the main goals.

In case of failure of hearing preservation surgery, it is important to save as much cochlear nerve fibers as possible during tumor resection. Their presence allows hearing rehabilitation via cochlear implantation (CI). Whenever the nerve cannot be considered functional, the only other option left is brainstem auditory implant (ABI).

**Methods:** We evaluated 8 NF2 patients who underwent unilateral cochlear implant after surgery. Each patient was assessed by tonal threshold, speech audiometry in quiet, speech audiometry in noise with CI on and CI off and speech therapy to estimate vowel, words and phrases identification and recognition. In the 6 patients with contralateral normal hearing, we used wireless accessories to perform all tests.

We evaluated one patient by electrical auditory brainstem response (eABR) to test the functionality of the device, because of low auditory results.

**Results:** Five patients improved hearing threshold, they detected environmental sounds and human voice, but do not recognize words and phrases; overall CI helped them in background noise.

Three patients with contralateral good hearing reported subjective benefit in pinpointing sounds.

Only 2 patients achieved vowel, words and sentences recognition in quiet and sentences in background noise. One had a recurrence of schwannoma and so underwent ABI, achieving the detection of environmental sounds and human voice but not recognition.

One patient, with completed recovered post-surgical complications, showed absent auditory results (evident only in the follow up) probably because cochlear nerve was disrupted and unfit to CI.

**Conclusions:** The treatment of vestibular schwannoma in NF2 patients is still challenging, especially considering hearing preservation. In our experience cochlear implantation should always be considered prior to ABI as it provides better functional results and it is easier to fit and maintain. Moreover, CI does not rule out a subsequent ABI.

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Prenatal Diagnosis in NF1: What Should Be Offered?

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Background: In the context of prenatal diagnosis for Neurofibromatosis type 1 (NF1), what is currently assumed is that if the fetus does not carry the familial mutation, the risk of developing NF1 is the same as that in the general population. The probability of finding a NF1 gene mutation arising de novo in a family with NF1 is considered very remote.

Methods: We present the case of a 35 year-old male, diagnosed with NF1 during his childhood, who was referred to our consultation because his partner was pregnant and they wanted to perform a prenatal diagnosis. NF1 sequencing was ordered in the context of genetic counselling. The genetic test identified the pathogenic genetic variant c.6792C>A in NF1 responsible for his disease. After consultation, the couple decided not to perform the prenatal diagnosis. At their daughter’s birth, the presymptomatic genetic test showed that she did not carry the familial pathogenic variant. During her paediatric routine follow-ups, more than six café-au-lait spots were identified, suggesting NF1. The genetic test was repeated confirming the previously obtained results. Therefore, a comprehensive NF1 gene test was performed, identifying the pathogenic variant c.2990G>A.

Results: The cloning and the subsequent polymorphisms analysis of the region adjacent to the mutation, allowed us to determine that daughter’s mutation was originated in the paternal allele.

Conclusions: These results should contribute to highlight the possibility of the coexistence of two independent mutations in NF1 gene within a family.

Although a paternal origin of the daughter’s mutation does not discard the possibility of being a random event, it may suggest a higher frequency of NF1 rate mutations in this family.

More than 450 families with NF1 have been given consultation in our multidisciplinary unit of Neurofibromatosis. In three families, two or more independent mutations in NF1 gene have been identified. In addition, several cases have also been reported in the literature (Martín Santo Domingo Y et al, 2017; Upadhyaya M et al, 2003; Klose A et al, 1999).

International studies are needed in order to determine the frequency of de novo mutations in NF1 families. Some data suggest that families with NF1 have a higher risk of experiencing de novo mutations in NF1 gene. Once more data support this observation, the analysis of the whole NF1 gene should be considered in the context of prenatal screening instead of only studying the identified familial mutation.

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Transcranial Direct Current Stimulation and Cognitive Training Enhance Visuospatial Processing in Neurofibromatosis Type 1: A Pilot Study

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Background: Cognitive, social and behavioral difficulties are common comorbid complications of Neurofibromatosis Type 1 (NF1), affecting up to 70% of all children with the disorder. In animal models of NF1, an increase of synaptic GABAergic inhibition results in impaired Long Term Potentiation (LTP) and synaptic plasticity, linked to consequent cognitive impairment. We conducted a feasibility study of a transcranial Direct Current Stimulation (tDCS) intervention in children aged 11-16 years with NF1 and report the effect of anodal tDCS on working memory (WM) and a non-trained visuospatial processing Corsi blocks task.

Methods: We ran a double (participant, assessors) blind, randomised controlled trial of active versus sham tDCS on 16 children aged 11-16 years (8 active, 8 sham). tDCS (anode over left dorsolateral prefrontal cortex – DLPFC – and cathode over vertex) was delivered in conjunction with cognitive training using a spatial working memory n-back task for three consecutive days. Stimulation lasted for 20 minutes (active) or 1 minute (sham) at 1mA current. Corsi block memory span measures were obtained before the start (day 1) and after the end (day 3) of cognitive training.

Results: We found a main effect of Session, F(3,42)=8.155; p<0.001; h²=.368. The main effect of Condition and the interaction between Session and Condition were not significant. Importantly, we tested transfer effects on the visuospatial Corsi blocks task using a 2X2 mixed ANOVA with factors Condition (active, sham) and Session (pre-training, post-training). The effect of session was not significant, but crucially, there was a significant interaction between session and condition F(1,13)=5.607, p=0.034 h²=.301. Participants in the active condition achieved higher memory span following WM training.

Conclusions: While both anodal and sham group improved on mean n-back performance, anodal stimulation resulted in enhanced transfer effects on a non-trained visuospatial task. These findings suggest that repeated anodal tDCS over DLPFC combined with a challenging WM task may be an effective method to enhance domain independent performance. The results indicate the feasibility of using a safe, low-cost, easy to use intervention in children with NF1. This pilot data on effectiveness provides a basis for future planned intervention trials, including use of tDCS as an adjunct to pharmacological treatments.

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Spinal Lesions in Neurofibromatosis Type 1: Analysis of 149 Cases

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Background: Spinal abnormalities are common in neurofibromatosis type 1 (NF1). Spinal NF1 (SNF1) describes an NF1 subgroup with extensive spinal neurofibromas and limited cutaneous findings. The literature surrounding SNF1 is currently sparse. The aim of this single-centre study was to describe spinal findings in a large cohort of NF1 patients with and without SNF1.

Methods: Review of referrals to a national NF1 referral centre (May 2016 to April 2017). Inclusion criteria: adults (≥17 years) with NF1 and at least one spinal abnormality detected on an MRI spine. SNF1 was defined as the presence of bilateral spinal neurofibromas involving the cervical, thoracic and lumbosacral spine, with limited cutaneous findings.

Results: 149 patients were included of which 26 patients (17.4%) had SNF1. The median age was 37 years (range 17-78 years) with no gender discrepancy (M:F , 77:72). Back pain (52%) was the most commonly reported symptom and significantly associated with abnormal spinal curvature (p=0.048). Scoliosis (66%) of the thoracic spine (71%) was the most common abnormal curvature present. Dural ectasia (28%) was most commonly present in the lumbosacral spine (51%). Most NF1 patients (64%) had spinal neurofibromas. Neurofibromas commonly extended beyond the margins of the intervertebral foramen in the cervical (44%), thoracic (44%) and lumbar spine (47%). The SNF1 subset was significantly associated with intradural tumours (p<0.001), cord compression (p<0.001) and spondylolisthesis (p=0.037).

Conclusions: Patients with SNF1 have a high incidence of mechanical spinal column dysfunction and neurofibroma related complications. This cohort therefore requires close surveillance.

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Challenges of Spine Surgery in Neurofibromatosis and Current Operative Strategies

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Background: Neurofibromatosis poses significant challenges for the spine surgeon. Poor neurological state, increased anesthetic risk from co morbidities, dystrophic bone, dural ectasia, spinal deformities, multiple neurofibromas and poor bone fusion make spine surgery more risky and prone to failure.

Methods: The neuroradiological notes of 378 complex NF-1 patients were reviewed from 24 months of multidisciplinary team meetings (from one of two specialist centres for NF-1 in the UK). Patients who had previous spine surgery were selected. Details of the surgery performed and outcomes were collated.

Results: 16 patients had previous spine surgery. 8 patients had instrumentation and 7 patients had repeated operations. Multiple neurofibromas, deformity and presence of severe dural ectasia were associated with repeat surgery.

Conclusions: Spine surgery in Neurofibromatosis type 1 is challenging and associated with significant risks. Based on our results we have developed an operative algorithm for dealing with neurofibromas and deformity

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Transition Readiness Assessment in Adolescents and Young Adults with Neurofibromatosis Type 1 (NF1)

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Background: Neurofibromatosis type 1 (NF1) is a progressive genetic tumor predisposition syndrome which conveys significant disease morbidity and lower levels of quality of life (QOL) compared to individuals in the general population. Previous research has shown that inadequate development of health-related self-management skills is directly correlated to decreased positive health outcomes in those with complex medical conditions such as NF1. Thus, the process of healthcare transition (HCT), or the purposeful and planned movement of adolescents and young adults from child-centered care to an adult-oriented care system, is necessary both to help eliminate deficiencies in provision of care and improve positive health outcomes in adults with NF1. Improving QOL is a core goal of HCT. In order to design an informed HCT intervention for NF1, baseline transition readiness must be assessed.

Methods: An anonymous online REDCap survey was developed. The survey consists of three sections aimed at assessing baseline transition readiness: 1) demographic information, 2) transition readiness questions utilizing the validated Transition Readiness Assessment Questionnaire (TRAQ), and 3) non-validated questions targeting factors of young adult life. The recruitment goal is 100 participants with an age range of 14-26 years. Recruitment will occur through Children’s Tumor Foundation (CTF) and will consist of email invitations to NF Registry participants, flyers in Neurofibromatosis Clinic Network clinics, and private NF1 Facebook group posts by CTF volunteers. Statistical analysis will be conducted using SPSS software or a similar program.

Results: Results are anticipated in early fall 2018. Statistical analysis of demographic information, transition readiness, and factors of young adult life is anticipated to reveal areas of HCT requiring additional support and services for those with NF1.

Conclusions: Data from this study will support the development of a NF1-specific HCT intervention.

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Disclosure: This study is funded by a Children’s Tumor Foundation grant.

Vasculopathy as a First Symptom of Neurofibromatosis Type 2 in Childrens and Young Adults

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Background: Neurofibromatosis Type 2 (NF Type 2) is an autosomal-dominant tumor-prone disorder characterized by the manifestations of central nervous system lesions. Although the disease is known to be associated with eye, skin and bone lesions, patients often get first symptomatic with polyneuropathy, peripheral (plexiform) schwannomas, unknown or tumor-associated eye muscle paresis, or other symptoms which are misleading and diagnosis is failed to recognize. For example, vasculopathy, a rarely presented but well known described phenomenon in Neurofibromatosis Type 1, is especially not yet described in literature as first symptom in NF Type 2.

Methods: We reviewed 3 cases of young adults and children diagnosed with Neurofibromatosis Type 2 and who were first symptomatic with vasculopathy in the brainstem and the cerebellum. They all are under observation and treatment at our neurosurgical department.

Results: Inclusion criteria were met in 15/462 identified articles. These studies included patients with orthopedic problems (arthritis, injuries, and surgeries, N=5), brain injuries/stroke (N=4), spinal cord abnormalities (N=2), PNS abnormalities (N=2), and multiple diagnoses (N=2). Two studies also included healthy controls. Median number of subjects/study was 25 (range 10-50) and muscles studied was 6 (range 2-30). Patient age ranged from 5-99 years. Studies reported intrarater (N=11), interrater (N=4), intrasession (N=11), and intersession (N=8) reliability. Majority of the studies reported good (intraclass correlation coefficient (ICC)>0.75) to excellent (ICC>0.9) reliability, with ICC<0.75 reported in only certain muscles in some studies. Reliability of affected and unaffected muscles was reported separately in 12 studies with no difference noted in 4, and worse reliability in unaffected (N=6) or affected muscles (N=2) in remaining studies. Smallest detectable difference (SDD) was reported in four studies and ranged from 15-68%.

Conclusions: HHD was reliable in many studies involving varied ages, diagnoses, and disease phenotypes. No consistent differences in reliability between affected and unaffected muscles were noted. Based on this, a pilot trial is being developed in NF that will evaluate HHD in the most relevant weak muscle and one common muscle in all subjects. Both relative (ICC) and absolute (standard error, SDD) reliability measures will be assessed.

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Adult Brainstem Gliomas in Neurofibromatosis Type 1 (NF1)

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**Background:** The brainstem is the second location of brain tumors after optic pathways in NF1. In NF1 children, brainstem gliomas are usually indolent and have a better prognosis than their counterparts in non NF1 children. In contrast, the natural history and prognosis of adult brainstem gliomas in NF1 is nearly unknown.

**Methods:** We conducted a retrospective analysis of medical records and MRI of adult NF1 patients followed for a brainstem glioma in 8 centers over a 17 years period (2000-2017). Clinical and imaging characteristics, management and outcome were analyzed.

**Results:** Twenty five patients were included in the study (13 males and 12 females) with a median age of 32 (range 17-58). The epicenter of the tumor was located into the pons n=13 (52%), the mesencephalon n=7 (28%), the medulla oblongata n=5 (20%). On MRI, contrast enhancement was seen in 19 tumors (76%). Pathological examination was available in 13 tumors (52%) and showed a high grade astrocytoma (III or IV) in 9 tumors. Five patients were asymptomatic, 3 remained asymptomatic during the follow-up (median follow-up: 86 months, range 22-124). Twenty patients were symptomatic with a median duration of symptom of 2.5 months (range 1-10) before diagnosis. Among these symptomatic patients, 15 died from tumor progression despite treatment with radiation therapy and or chemotherapy. The median overall survival of symptomatic patients was 36 months.

**Conclusions:** Brainstem gliomas are very rare tumors in adults with NF1. Unlike children, adult brainstem gliomas seem to have an unexpected poor prognosis, suggesting the disease may be different in adulthood.

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Long-Term Results for a OneStage Surgery Technique for Patients with Craniofacial Plexiform Neurofibroma

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**Background:** Neurofibromatosis (NF) is an autosomal dominant genetic disorder, and NF type 1 (NF1) is one of the most common forms. Plexiform neurofibroma (PNF) is one of the characteristic expressions of NF1. The proper treatment for patients with craniofacial PNF is surgery. The evaluation methods for the surgical outcome of these patients are still controversial. As a consequence, a one-stage surgical technique and an appropriate evaluation method for patients with craniofacial PNF were discussed in this article.

**Methods:** This research is a retrospective study. Nine patients with craniofacial PNF were included in this study. They had undergone a one-stage surgical technique of tumor debulking and nasolabial fold reconstruction. Three methods had been applied to evaluate the surgical outcome.

**Results:** Significant improvement was observed in 8 patients. Eight patients were assessed by the relatively objective evaluation method. Obvious symmetry improvement was calculated using Mimics software in 7 patients.

**Conclusions:** The surgical technique could achieve good surgical outcomes in both functional and cosmetic terms. Additionally, the relatively objective evaluation technique based on Mimics software could be a more convincing method for evaluating the surgical outcomes of craniofacial patients with PNF.

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Novel Three-Dimensional Morphometry to Reassess Orbit Deformities Associated with Orbital-Periorbital Plexiform Neurofibroma

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Background: Orbit deformities are usually found in neurofibromatosis type 1 patients, especially those with orbital-periorbital plexiform neurofibroma (OPPN). Unfortunately, current morphometry is complicated and, in some cases, cannot be performed on the deformed orbit due to the destruction of landmarks. Herein, we present a novel three-dimensional (3D) morphometry for these orbital measurements.

Methods: We retrospectively reviewed 29 patients with OPPN, and another 29 disseminated cutaneous neurofibroma patients served as controls. All patients had undergone craniofacial CT and 3D reconstruction. New morphometry was used to measure the area of the orbital rim (OR) and superior orbital fissure (SOF).

Results: For the 29 OPPN patients, the area of the OR at the affected side was 14.18 ± 3.50 cm², while the OR at the non-affected side was 12.32 ± 1.38 cm². In addition, the area of the SOF at the affected side was 5.37 ± 5.75 cm², while that at the non-affected side was 1.27 ± 1.03 cm². The OR and SOF at the affected side are more likely to become enlarged compared with those at the non-affected side. Among the 29 OPPN patients, the novel morphometry could be performed in 19 cases (65.5%) that cannot be measured by previous morphometry.

Conclusions: The novel morphometry is convenient and reproducible, which optimizes its application in pathological cases, especially those involving deformed orbits.

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Figure: The areas of the orbital rim and SOF in NF1 patients. (A) Comparison between the areas of the orbital rims at both sides; (B) comparison between the areas of the SOF at both sides; (C) orbit measurements in different sex groups, showing no significant difference; (D) data distribution: the dots indicate patients in the OPPN group, the triangles indicate patients in the DNF group, and the dotted line indicates the separatrix. *** means statistical significance (P < 0.05). (E, F) The outline of the SOF was drawn as an irregular cycle; (G, H) the lateral and anterior view of the SOF in CAD; (I) calculation of the area of the SOF in CAD.
Computer-Assisted Planning and 3D Printing Modeling for Craniofacial Plexiform Neurofibroma Surgery

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Background: The craniofacial plexiform neurofibroma (PNF) could cause serious disfigurement in neurofibromatosis type 1 (NF1) patient. In some case, to an achieve a symmetrical result is difficult. We report a surgery plan designed by 3D printing to solve this problem.

Methods: Patients with PNF have received craniofacial computed tomography (CT) imaging and 3D reconstruction. They divided into two groups depends on whether they have selected a 3D printing plan. A 3D printing model was the shape designed by computer based on the CT imagines and medical software MIMICS. The surgery was operated by resected the shape of a 3D model on the PNF. The primary outcome was calculated by the CT and MIMICS with the symmetrical result at 3 months postoperatively.

Results: From September 2016 to June 2017, 30 patients have enrolled in our research and 12 of them have received computer-assisted and 3D printing plan. No major complications occurred. At 3 month, the patient with 3D printing achieved a better symmetrical result than the group without 3D printing.

Conclusions: The 3D printing combined with the medical digital software can help the surgery in PNF, especially in symmetry. This method also reduces the learning curve in PNF surgery.

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Functional Impairment in Neurofibromatosis Type 1: Associations with Cognitive Deficits and ADHD Symptoms

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Background: While cognitive deficits, attention deficit/hyperactivity disorder (ADHD) symptoms, and poor functional outcomes are well recognised in children with neurofibromatosis type 1 (NF1), the links between these difficulties remain unclear. Guided by a causal neurodevelopmental model, we investigated the association between cognitive deficits in attention and executive function, ADHD symptoms, and functional impairments in activities of daily living (ADLs) and quality of life (QoL). We hypothesised that (1) cognitive deficits would be positively associated with elevated ADHD symptoms, (2) cognitive deficits would predict impaired ADLs irrespective of ADHD symptoms, and (3) cognitive deficits would underlie elevated ADHD symptoms, which would subsequently moderate impaired ADLs and poorer QoL.

Methods: Baseline data from the NF Clinical Trials Consortium’s STAtin Randomized Study (STARS) were analysed. The study sample consisted of 135 children with NF1 (mean age 11.67 years, SD 2.32), who completed cognitive measures assessing attention and executive function. The Conners-3 parent questionnaire was used to rate ADHD symptomatology. Parents also completed the Behaviour Assessment System for Children second edition and Pediatric Quality of Life Inventory to assess ADLs and QoL respectively. Path analyses were conducted to determine relationships between variables. This study obtained institutional review board approval at each study site.

Results: Children with NF1 were significantly impaired on all variables measured. There were weak relationships between the executive and attention cognitive scores and ADHD symptomatology. Cognitive outcomes were also relatively poor predictors of functional impairment (ADLs and QoL). Elevated ADHD symptomatology was strongly associated with QoL as well as impaired ADLs (both, \( p < .001 \)).

Conclusions: Irrespective of cognitive deficits, elevated ADHD symptoms of inattention and impulsivity/hyperactivity in children with NF1 have a negative impact on daily functioning and quality of life. Interventions and treatments aimed at minimising ADHD symptoms in this condition would be beneficial to improve everyday functioning and general well-being.


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Phenotype of Paediatric NF2

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Background: Neurofibromatosis type 2 (NF2) presenting in childhood can be a severe debilitating condition. To further define the paediatric NF2 phenotype, we reviewed the notes of all children known to the English NF2 service. We assigned all patients into one of three severity groups depending on the NF2 mutation identified, and assessed for phenotypic trends according to severity.

Methods: We performed a retrospective observational study of 87 NF2 patients, aged under 18 years. Genetic severity was assigned based on the UK NF2 severity score, and phenotype assessed for tumour load, and intervention. Ethical approval for the study was obtained.

Results: 49 (56%) patients had inherited NF2 from an affected parent. 31% of patients were grouped into 2A (mild), 34% into 2B (moderate) and 35% into 3 (severe). Mean age at first symptom and diagnosis was 6 and 7.5 years respectively. Mean age of first MRI head was 11.5 years in group 2A and 9.8 in group 3, with mean current age 14.0 years for group 2A and 12.9 for group 3.

There was a significant trend with genetic severity for the proportion of patients with bilateral vestibular schwannoma (BVS), occurring in 15 (58%) of patients in group 2A compared to 24 (77%) of group 3, moreover 4 (19%) group 2A patients had a (VS) over 1 cm in size at first scan, compared to 9 (33%) of group 3 patients. The proportion developing intracranial meningioma significantly increased with genetic severity, with 5 (22%) in group 2A, compared to 14 (52%) in group 3. Similarly 8 (35%) 2A patients developed non-vestibular intracranial schwannoma compared to 23 (85%) in group 3. There was a significant association between severity and the development of other radiological anomalies such as cortical dysplasia, occurring in 4 (17%) 2A patients, compared to 15 (57%) group 3 patients.

51% had had an intervention by the time of last assessment, with 15 patients (27%) having 2 or more major interventions. 9 patients (35%) in group 2A had one or more major intervention compared to 15 (48%) in group 3. Mean age at first intervention was 12.1 years in group 2A compared to 9.3 years in group 3, and for starting Avastin was 14.9 years for group 2A compared to 12.7 years in group 3 patients.

Conclusions: This national cohort of paediatric NF2 adds to the understanding of the natural history of NF2, illustrating the often severe nature of NF2 when occurring in children, and demonstrates the utility of incorporating genotype into clinical assessment.

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Lack of Retinal Toxicity in Children with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PN) Treated on SPRINT: A Phase II Trial with the MEK Inhibitor Selumetinib (AZD6244, ARRY-142886)

Christopher K. Hampton, NEI, NIH, Bethesda, United States

Background: MEK inhibitors have been associated with retinal toxicities in adults with a variety of tumors and infrequently in children. The MEK inhibitor selumetinib is being used in a phase 2 trial for children with NF1 and inoperable PN (NCT01362803). We describe the monitoring for and incidence of ocular toxicity in patients treated at the NCI on this trial as of 3/31/18.

Methods: Selumetinib is given on a continuous dosing schedule (1 cycle=28 days) at 25 mg/m² PO BID (60% of adult recommended dose). Patients underwent comprehensive ophthalmologic examinations, including Optical Coherence Tomography (OCT) as tolerated. Patients were examined at a minimum at baseline, after cycles 4, 12, and then annually. Baseline OCT measurements were compared to pre-cycle 13 (preC13) measures or most recent OCT date (if patients on study for < 12 cycles).

Results: Of 34 patients enrolled at the NCI, 32 (ages 5-17) were evaluable for toxicity (had at least 1 dose of study drug and a subsequent eye exam) with a median of 24 cycles on therapy (range 7-30). Twelve patients had self-resolving grade 1 or 2 eye-related adverse events (e.g. blurred vision, eye pain, photophobia) but no retinal edema or sub-retinal fluid was found on exam. A median of 5 OCTs/patient were obtained (range 3-13). Eight patients had orbito-temporal PN (OPN) (4 OS, 4 OD, 0 OU) and 10 had a history of optic pathway glioma(s) (OPG) (2 OD, 1 OS, 3 OU, 4 with subsequent OPG regression). Two had a history of both OPN and OPG. After 12 cycles of treatment there were no observable signs of retinal toxicity clinically or by OCT. No changes were seen in the Retinal Nerve Fiber Layer thickness bilaterally (baseline: 90±15 mm OD, 84±19 mm OS; preC13: 86±14 mm OD, 85±18 mm OS; p>0.05). Macular thickness was slightly increased OD following treatment compared to baseline (baseline: 251± 24 mm; preC13: 259 ± 20 mm; p<0.05); however, given there was no evidence of retinal toxicity, this was not a clinically significant change. No change was seen in the macular thickness OS (baseline: 250±31 mm; preC13: 258±21 mm; p>0.05).

Conclusions: We did not observe retinal toxicity in this carefully monitored pediatric population. OCT monitoring did not reveal any occult retinal changes. This suggests that routine OCT monitoring for selumetinib induced retinal toxicity in the absence of visual complaints may not be necessary in this pediatric patient population. Data validation and further analyses are ongoing.

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Computerized Cognitive Training Intervention for Children with Neurofibromatosis Type 1 (NF1): Accrual and Adherence in a Multi-Site Trial

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Background: Cognitive deficits, particularly difficulties with attention and working memory (WM), are an important contributor to long-term dysfunction in patients with Neurofibromatosis type 1 (NF1). Few intervention strategies for WM have been established for patients with NF1, however. We sought to evaluate the efficacy of a computerized cognitive training program, Cogmed®, in children with NF1 as compared to children randomized to an active control condition. Here, we report on eligibility, accrual, and treatment adherence to date in our multi-site trial.

Methods: This study is a randomized, controlled trial with a pre-post test design evaluating changes in attention and WM as a function of treatment assignment. Forty-six children (47.8% male; Mean age = 10.9, Range = 8-15; 63% non-white racial/ethnic identity) have been recruited to participate. Thirty-four (73.9%) consented patients qualified for randomization, defined as evidence of verbal or visual WM performance > 1 SD below the mean, and were assigned to 25 training sessions on either a computer-based WM or reading comprehension program plus weekly contact with intervention coaches.

Results: Recruitment is open at 3 of 5 sites; across these, accrual is 119% of the expected rate. Baseline functioning is consistent with extant literature (WASI-II Full-scale IQ M = 90.5; digit span forwards = 7.7; spatial span forwards = 7.3; digit span backwards = 8.4; spatial span backwards = 7.3). Of 30 randomized patients who have completed study procedures, 21 (70%) have met treatment adherence criteria. There is no significant difference in adherence between participants assigned to WM (M sessions = 21.0) vs reading comprehension training (M sessions = 20.0). Nonadherence is unrelated to participant age, gender, or baseline cognitive characteristics.

Conclusions: The accrual rate for this study provides evidence that families of children with NF1 are motivated to participate in convenient, minimal risk treatments, which address outcomes perceived as significant. Acceptability of this approach is also indicated by the high percentage of individuals who identify as racial/ethnic minorities, who are historically underrepresented in clinical trials. Strong adherence and follow-up also suggests that using an active-control design may appeal to patients and families more than a traditional placebo-controlled approach. Outcome data on intervention efficacy and satisfaction will be forthcoming when target accrual is reached in 2019.

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Severe Neurofibromatosis Type 1 Caused by One Germline Mutation and One “Early Second Hit”

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Background: An unusually severe case of neurofibromatosis type 1 (NF1) presented at birth with left sided hemihypertrophy, ante-curved legs and large fleshy nevi on the back. NF1 was diagnosed at age 2, but later changed to possible Proteus syndrome because of the gyrate pattern on the left foot and overgrowth as well as histology of the tumor samples removed. At age 2, only 2 cafe au lait (CAL) spots were present, axillary freckling nor Lisch nodules were present, but she developed a severe scoliosis as well as spina bifida and a large soft tissue mass was present in the pelvic region, extending upwards to the adrenals retroperitoneally, and also on the dorsal side of the pelvis. Biopsy showed “myxomatous” tissue. Her main problem was diarrhea with up to 30 stools a day and bladder infections, later constant bacteriuria. Clltoral hypertrophy with abundant nerve cells was also present.

She became wheelchair dependent from age 3, but had normal mental development and no manifestations above shoulder levels. Short stature, delayed puberty, little subcutaneous fat, no freckling, few CAL and neurofibromas, but several large plexiform neurofibromas especially on the truncus. She had a normal cerebral MRI at age 9. She had alpha-interpherone treatment at age 6, without any effect on the tumor masses.

Her left leg was amputated at age 19 above the knee, as almost paralytic and much longer and heavier than the left leg. At age 22 the left kidney was removed because of bladder infections, hydronephrosis/ hydrourether and an ileostomy and urostomy was performed resulting in much improved quality of life and social function.

Conclusions: She came to a Proteus meeting in the UK in 2009, and was re-diagnosed with NF1. Mutation analysis identified a germline NF1 splice mutation c.1466A>G (p.Tyr489Ter) and an identical somatic second hit mutation in the Schwann cells from five affected tissues from different anatomical locations: c.5458C>T; p.Gln1820Ter.

She died at age 29, possibly from the abdominal mass (pathology still pending) after 6 months of wasting.

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Burden and Feasibility of Functional Evaluations and Patient Reported Outcome (PRO) Measures in SPRINT: A Phase II Trial of the MEK Inhibitor Selumetinib (AZD6244, ARRY-142886) for Children with Neurofibromatosis Type 1 (NF1)

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Background: Plexiform neurofibromas (PN) in NF1 can cause substantial morbidity. A phase I trial of selumetinib demonstrated PN shrinkage, however prospective evaluations of clinical benefit were not assessed. Our pediatric phase II trial of selumetinib (NCT01362803) includes extensive functional and patient reported outcome (PRO) measures to prospectively capture changes in PN-related morbidity, in addition to safety and restaging evaluations.

Objective: To assess the burden and feasibility of performing time-intensive functional and PRO evaluations after every 4 cycles on patients and their families.

Methods: PN related morbidities were assessed with evaluations selected based on the location of each patient’s PN and clinical symptoms at baseline, repeating every 4 cycles (1 cycle = 28 days) for the first year. We evaluated the total number of days spent at the NIH for screening and on-therapy visits during the first year. To assess the burden of functional/PRO testing specifically, we counted each evaluation performed during the first 12 cycles of therapy, repeating every 4 cycles (1 cycle = 28 days) for the first year. We evaluated the total number of days spent at the NIH for screening and on-study visits, spending a median of 5.75 hrs/visit on functional/PRO evaluations. Eleven patients were classified as having a PN-related airway morbidity, requiring interval sleep studies, of which 28/30 were successfully performed.

Results: Twenty-seven patients aged 5-17 years old with NF1 enrolled at the NCI. The median number of clinic days required for screening was 5 (range 4-8), averaging 10 hours per patient spent on functional/PRO exams. In the first year, patients were present at the NIH for a median of 18 days performing screening and on-study visits, spending a median of 5.75 hrs/visit on functional/PRO evaluations. Eleven patients were classified as having a PN-related airway morbidity, requiring interval sleep studies, of which 28/30 were successfully performed.

Conclusions: Functional and PRO evaluations required a substantial time commitment from patients and families but were generally feasible. Although functional evaluations are burdensome, given a high level of motivation and support from families, it is feasible to include them on clinical trials. Engagement of patients for the design of future clinical trials is critical to achieve highest compliance and mitigate the burden on families.

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Neurofibromatosis Therapeutics Program Nurse Care Coordinator Role

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**Background:** Neurofibromatosis (NF) therapeutics is an emerging and growing field. Patients with NF have an increased incidence of central nervous system (CNS) tumors and plexiform neurofibromas. Recent developments in the treatment of plexiform neurofibromas have significantly increased the numbers of patients seen for therapy. In order to provide high-quality care to these patients, a Neurofibromatosis Therapeutics Program (NFTP) should be developed and utilized.

**Methods:** The program at Children’s Hospital Colorado has grown over the past years and now includes a physician, nurse practitioner, nurse care coordinator, and social worker. The Nurse Care Coordinator (NCC) is the key person on the NFTP engaging in close collaboration with other disciplines that are vital in the care of the NF patient with plexiform neurofibromas and/or CNS tumors including: neuro-oncology, neurosurgery, neuroradiology, otolaryngology, orthopedics, plastic surgery, ophthalmology, neurology, dental, urology, dermatology, genetics, neuropsychology, and rehabilitation. The NCC completes triage, determines subspecialties to be seen and timing of visits, educates patients and families about treatment, and completes insurance authorization. The NCC assists with tissue collection of all plexiform neurofibromas and brain tumors to assist the laboratory with conducting epigenetic research on tissue obtained during surgery. Most importantly, the NCC ensures that patients and families maintain close contact with the NFTP regarding side effects and compliance with treatment.

**Results:** The NCC and continued development of the role within the NFTP has resulted in increased adherence to required studies and monitoring while on treatment, earlier identification and treatment of side effects, better enrollment on the tissue collection study, increased patient satisfaction from patients and families with care delivered. The NCC has improved insurance authorization of medications prescribed. Collaborating disciplines are more likely to refer patients to the NFTP due to the efficiency and ease in contacting and working with the NCC.

**Conclusions:** The NCC ensures that the NFTP continues to be a strong and vibrant area of growth with the aim to provide high quality of care for the whole child and conduct lab and clinical research.

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Dermatologic Skin Toxicity Standards of Care for Patients Receiving MEK Inhibitors

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**Background:** Children’s Hospital Colorado has treated more than 40 children on MEK inhibitors in the last two years. Due to the significant skin toxicity associated with MEK inhibitors, the Neuro-Oncology team collaborated with the Dermatology department to establish standards of practice to prevent and treat skin toxicities.

**Methods:** Children, adolescents, and young adults with Neurofibromatosis Type I were treated on MEK inhibitors for low grade brain tumors, high grade brain tumors, and plexiform neurofibromas. As treatment started, skin toxicity was carefully monitored. Photos and data regarding the different types of rash and paronychia were collected. The rashes were initially treated with standard practice used for other drug rashes, which was minimally effective. Dermatology was consulted to look at research regarding MEK inhibitor skin toxicities and to develop Neuro-Oncology MEK Inhibitor Skin Care Standards of Practice.

**Results:** Standards of Practice for skin care while taking a MEK inhibitor exist for prevention of rash, sun protection, eczematous dermatitis (scalp dermatitis and angular cheilitis), folliculitis, paronychia, and papulopustular dermatitis. Patients are given these standards as well as descriptions of each type of skin toxicity prior to starting therapy and periodically during therapy. Nurses complete skin checks via phone and electronic medical record-based email to review photos in addition to office visits as needed.

**Conclusions:** Skin toxicities are less severe overall, with an increased adherence to preventative care and earlier treatment for all skin rashes and paronychia. Instructions are clearly laid out for patients, families, and practitioners to adhere to. The next step in research is to determine if there is anything in the epigenetics of the blood sample or tumor sample collected to predict what patient will experience a more significant drug rash.

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Aesthetic Surgical Management of Head and Neck Neurofibromatosis

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Background: Neurofibroma (NF) should be removed surgically when causing dysfunction or deformity. Plastic surgery aims not only to excide NF tumor, but also to restore function and normal face contour for those patients.

Methods: We reviewed our series of 269 NF1 cases with NF on head and neck from 2010 to 2018. We performed 240 operations for 136 patients.

Results: Removal of PNF on head and neck is a huge challenge due to complicated anatomy. When lesions were small or superficial, esthetic surgical results were expected. While NF lesions infringed widely and deeply without definite demarcation, especially facial nerve was involved, the situation was much harsh for NF resection without facial nerve injury. We initially explored surgical technique to protect temporal branch of facial nerve (TBFN) in PNF resection. After operations, 10 cases acquired temporal PNFs removal and TBFN function successfully preserved simultaneously. The most difficult case to handle was orbital NF who still has eye vision, especially with sphenoid bone defects, eyeball pulsation and proptosis. For 3 such severe orbital NF cases, we removed orbital NF, repaired orbital wall, and put eyeball back into reconstructed orbital socket without visual injury.

Conclusions: Continuous developing plastic surgery techniques are helpful to achieve much more functional and aesthetic improvements for NF patients.

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Clinical Outcomes after Unplanned Resection in Patients with Malignant Peripheral Nerve Sheath Tumors

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Background: Owing to the rarity of malignant peripheral nerve sheath tumors (MPNST), tumors arising in patients with neurofibromatosis type 1 (NF1) are subjected to inappropriate excision without adequate preoperative planning. In such patients with unplanned excisions (UE), there is a concern about the increased risk of local recurrence. This study aimed to compare the clinical outcomes between patients with MPNST who underwent unplanned excisions (UE) and those with planned excisions (PE).

Methods: Between 2000 and 2017, 57 patients with MPNST who diagnosed and treated at our institutions were included in this study. There were 26 male and 21 female, with a mean age of 42 years. Thirty-four patients were associated with NF1. The mean tumor size was 9.6 cm. Forty-four patients with PE and 13 with UE at pre-referral hospitals were identified. Ten patients developed distant metastasis at the first visit. The mean follow-up duration was 59 months.

Results: The tumor in patients with PE was significantly larger (P=0.021), and its location was deeper (P<0.001) than that with UE. There was no significant difference between the two groups regarding age, gender, NF1 status, tumor site, histological grade, and the necessity of plastic reconstruction. The 5-year overall and local recurrence-free survival rates were 54% and 71% in patients with PE and 73% and 90% in patients with UE, respectively. Among 13 patients with UE, additional excisions were performed in ten, and the remaining three were treated with palliative therapy including chemotherapy and radiotherapy because they had lung metastases at the first visit. Residual tumor cells were observed in six cases of additional excised specimens. Among UE group, there was only one patient whose tumor was evaluated with MRI and/or CT before initial surgery at pre-referral hospitals. Among ten patients treated with additional excision, seven required plastic reconstructive procedures. Local recurrence occurred in one patient after additional excisions.

Conclusions: Additional excisions with adequate surgical margin at a specialized center could provide favorable overall survival and local control even after UE in patients with MPNST. However, surgeons should avoid UE for soft tissue tumors without considering the possibility of malignancy, especially in patients with NF1 because of the high incidence of MPNST.

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The Effect of Selumetinib (AZD6244, ARRY-142886) on Spinal Neurofibromas (SNF) in Patients with Inoperable Plexiform Neurofibromas (PN) and Neurofibromatosis Type 1 (NF1)

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Background: Spinal neurofibromas (SNF) in NF1 can cause progressive cord compression and clinical morbidity. Surgical outcomes for SNF can be unsatisfactory. A phase 1 study of the MEK inhibitor selumetinib in patients with NF1 plexiform neurofibromas (PN) demonstrated PN shrinkage in 17 of 24 patients, by volumetric MRI analysis (partial response = PN volume decrease ≥20%). We assessed the effect of selumetinib on SNF.

Methods: Participants of the ongoing phase 2 pediatric (NCT01362803) and adult (NCT02407405) selumetinibstudies for NF1 PN, who underwent serial spine MRI with high resolution balanced fast field echo sequence, were included in the analysis. Selumetinib was administered at the recommended pediatric (25mg/m²BID)or adult (50mg BID) dose on a continuous dosing schedule (1 cycle =28 days).We evaluated the effect of selumetinib on SNF size and shape of the spinal canal, spinal cord, and cerebrospinal fluid (CSF) at baseline and during treatment. As SNF are not amenable to MRI volumetric measurement, size changes were assessed by linear measurements and qualitative analysis.*

Results: Nineteen patients (14 male), median age 15.5 years (range 6-60), had SNF. Eleven of these patients were enrolled on the pediatric, and eight patients on the adult phase II trial of selumetinib. Six patients had prior spinal decompression surgery. All patients had SNF extending into the central canal at one or more levels (14 cervical, 2 thoracic, 12 lumbosacral). Deformity of the spinal cord cross-section was observed in 17 patients. Sixteen patients completed at least 12 cycles of treatment, and 3 received 8 cycles. Selumetinib was safe and tolerable. No SNFs exhibited progression by linear measurements during treatment. SNF remained stable in 5 patients, and some improvement characterized by expansion of the CSF space (due to tumor shrinkage), with the CSF signal becoming circumferential, was observed in 14 patients (9 children, 5 adults). Correlations with functional and patient reported outcomes and database validation are ongoing.

Conclusions: This is the first study to assess the effect of selumetinib on SNF. In 14 of 19 patients with SNF, we observed improvements in one or more SNF attributes. Our findings indicate that selumetinib may prevent the worsening of cord compression, and in some patients reduce the need for surgical interventions. Prospective evaluation of selumetinib for the treatment of SNF should be considered.

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At Risk Phenotype and Mortality During Neurofibromatosis 1

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Background: Subcutaneous neurofibromas in patients with neurofibromatosis 1 (NF1) are correlated to internal neurofibromas which are responsible for major morbidity and mortality. We aimed to study mortality and its association with the phenotype at risk in a cohort of patients with NF1.

Methods: Retrospective cohort of patients with NF1 from a 2005 multicentric case-control study for whom clinical features had been standardly collected. The number of deaths and their causes when available was collected from medical files and civil registry. The mortality of patients with NF1 was compared with French general population death rates (continental France in 2010) by the estimation of the Standardized Mortality Ratio (SMR). SMR were then estimated for different age groups according to the different clinical features.

Results: Overall, 188 patients were analyzed in the study, median age was 40 years [extremes 20-77], 75 were men (40%). The median duration of follow-up was 8.5 years [0-11.2]. 98 patients (52%) had at least one subcutaneous neurofibroma (SC-NF), and 79 (42%) had 2 to 9 lesions. During the follow up, 10 patients died, including 1 due to malignant tumors of the nerve sheath and 1 due to stomach cancer. Among these patients, 7 (70%) had at least one SC-NF. Overall mortality was significantly increased in the NF1 cohort, SMR was 2.54 (95%CI, 1.21-4.66; P < 10-2). The excess mortality was significant among patients over 60 years (SMR, 10.80; CI, 2.90-27.64; P < 10-3). In patients with SC-NF significant excess mortality was found among the whole cohort (SMR, 3.23; CI, 1.29-6.66; P < 10-2) and patients over 50 (SMR, 10.32; CI, 2.78-26.43; P < 10-3). By contrast, no excess mortality was found among patients without SC-NF compared to general population.

Conclusions: NF1 was shown to be associated with a 15-year decrease in life expectancy due to higher risk of malignant tumors. This excess mortality compared to general population had been found in our cohort and especially among patients with at least one SC-NF. In these patients, the excess mortality is more important and occurs earlier than in the whole cohort.

Clinical follow-up should be focused on patients with SC-NF, which can be easily found by a routine clinical examination. Further monitoring of this cohort might lead to identify a threshold number of SC-NF associated with this increased mortality risk.

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Utility of Telehealth for Specialty Neurofibromatosis (NF) Care

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Background: Telehealth allows for clinical evaluation and management to be delivered over long distances, which may particularly benefit patients with rare neurological diseases such as neurofibromatosis (NF).

Methods: We performed a retrospective cohort study of patients receiving telehealth-based care in our NF clinic. We used our electronic medical record to collect demographics, reasons for visits, and visit outcomes. We used ArcGIS to calculate driving distance and time from patient’s home to clinic.

Results: 109 patients (70 female, median age: 37 years) had telehealth visits from May 2016-March 2018, eighteen (17%) of whom had multiple telehealth visits. Patient diagnosis was 33% NF1, 42% NF2, 8% Schwannomatosis, and 17% other, compared to our clinic population of 58% NF1, 31% NF2, 6% Schwannomatosis and 5% other. Only 7 patients (6%) were pediatric, compared to 15% pediatric population in our NF clinic. Telehealth visit indication was 62% routine follow up, 26% new test result follow up, 6% evaluation of a new problem, and 6% on-therapy follow up. The plan developed by telehealth visits included 24% new radiology ordered, 7% new medication ordered, 18% new specialty consultation ordered, and 51% no change in previous plan. Telehealth saved patients a median round trip drive of 108 miles (IQR 388 miles) and a median driving time of 170 minutes (IQR 292 minutes).

Conclusions: Telehealth improved NF specialty care through more accessible routine follow up, and urgent evaluation of new symptoms in 6% of patients. Telehealth visits led to new testing, referrals, or medications in 49% of cases, showing that telehealth fulfills a distinct need for services while saving patients time and travel. Adult patients were overrepresented among telehealth cases compared to our general clinic volume, suggesting that in-person examination of children may be preferred by NF providers or families. Given difficulties in access to specialty care, telehealth offers an opportunity to extend care for patients with rare neurological diseases who live at a distance from specialty centers.

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Mammography Screening in Neurofibromatosis Type 1

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Background: Women with neurofibromatosis type 1 (NF1) are at an increased risk of breast cancer. Characteristics of NF1, such as cutaneous neurofibromas, may interfere with mammography, yet no data on the utility of mammography screening in NF1 are available. We aim at elucidating the feasibility of mammography screening in patients with NF1 by a retrospective register-based analysis of mammography screening in Finland.

Methods: Finnish women aged 50–69 years are invited to mammography screening biannually. The invitations, attendance and diagnostic findings are recorded in the Finnish Mass Screening Registry. In addition, the Finnish Cancer Registry collects information on all cancers diagnosed in Finland. By linking these registries with the population-based Finnish NF1 registry, data on a cohort of 732 women with confirmed NF1 and 7262 matched non-NF1 controls were retrieved. The study adhered to the Declaration of Helsinki principles and was approved by the Ethical committee of the Hospital district of Southwestern Finland and the National Institute for Health and Welfare.

Results: Totals of 211 NF1 patients and 2239 controls were invited to mammography screening at least once in 1992–2014. Of the NF1 patients, 193 women attended the screening at least once, while the corresponding number was 2108 among controls, yielding 854 and 9500 visits by NF1 patients and controls, respectively. The participation activity did not differ between NF1 patients and controls ($P = .526$).

Each mammogram was scored by two radiologists on a scale from 1 to 5 for the possibility of malignancy. The scores of the radiologists differed by at least 1 point in 11.4% of the mammograms from NF1 patients, while the corresponding proportion was only 5.3% among controls ($P < .001$). NF1 patients were referred to further studies slightly more often than controls (4.0% vs 2.9%).

Thirteen female NF1 patients were diagnosed with breast cancer at ages 50–69 in 1992–2014. Nine of them had attended mammography screening within 3 years prior to their breast cancer diagnosis, and 5/9 (56%) of the cancers were diagnosed in screening mammography. Out of the 98 controls with breast cancer, 78 had attended mammography screening within 3 years, and in 33/78 cases (58%) the cancer was detected based on the screening.

Conclusions: NF1 may cause some uncertainty in the interpretation of mammograms, yet it does not affect the proportion of cancers detected in screening.

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Neurosurgical Contribution Within a Complex NF1 Service

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Background: The goal of this study was to review and present any neurosurgical related activity within a multidisciplinary nationally commissioned specialty NF-1 center.

Methods: We reviewed all NF-1 Neurosurgical MDTs, NF-1 Neurosurgical clinics and all neurosurgical procedures carried out in NF-1 patients over an 8-year period.

Results: Since the inception of the service in 2009 1505 cases were discussed at MDT, 171 clinic appointments in complex NF-1 patients with neurosurgical pathologies and 41 (cranial and spinal) operations were carried out.

Conclusions: The formation of a supraregional multidisciplinary team allows a better understanding of the disease, a comprehensive evaluation of radiological findings and a steep learning curve in the management of NF-1 surgical conditions.

We provide a holistic treatment of these patients via direct care, specialist advice and liaison with local units.

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Polish Standard of Coordinated Medical Care for Children with Neurofibromatosis Type 1 and Related RASopathies

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Background: Neurofibromatosis type 1 and its allelic forms together with Legius syndrome are currently classified as RASopathies. Historically they were considered as phacomatoses, what warranted neurological care in Poland, mostly resulted in unintentional malpractice. In opposition, the Polish system of pediatric oncology network appeared optimal to assure NF/RAS patients professional and multidisciplinary care. Yet, an outstanding phenotypes and wide spectrum of clinical complications significantly distinguish NF/RAS from remaining phacomatoses and RASopathies and warrants separate, patients’ oriented care system.

Methods: Among 5 centers currently provided consultations for NF/RAS in Poland, two ensure defined coordinated medical care (CMC) meant as a disease management process: the first settled in early 2000’ in Bydgoszcz and NF/RAS Coordinated Care Center in Warsaw, established in 2005.

Results: The essence of coordinated medical care, referred to as a yearly clinical consultation and ability to consult patient’s ailments throughout electronic media as well as differential diagnosis for patients presenting with multiple CALs and genetic counselling, is responsibility of an expert in disease management (“Nf-coordinator”). Routine annual visit consists of the precise examination of a patient with accurate anamnesis concerning period between the visits and results of external consultations, general and neurological examination, and detailed developmental assessment (growth, pubertal and behavioral problems). Considering disclosed problems and expected, age-related disturbances, the further prophylactic or diagnostic imaging, patient’s addressed treatment and rehabilitation with behavioral and cognitive therapies are planned. Finally, the present and expected problems, both behavioral and physical, and the precise plan of therapy or rehabilitation, are summarized and discussed. In the mean-time between visits, both parents and GP or other specialist engaged in the holistic care, have a permanent access to the Nf-coordinator consultation. Yet, the center is obliged to provide a forum for clinical audit and academic interaction through multidisciplinary meetings and telemedical conferencing.

Conclusions: The mainstay of NF/RAS patients’ CMC is age specific monitoring of disease manifestations and patient education, continuously supervised by NF coordinator responsible for patient’s oriented, holistic management provided a sense of medical security to the patient and ended his “diagnostic odyssey”.

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Rates and Nature of Hospitalization in Neurofibromatosis 1: A Danish Population-Based Cohort Study

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**Background:** The rates and nature of hospitalizations for individuals with neurofibromatosis 1 (NF1) are unknown. Therefore, we conducted a population-based cohort study of the risk for hospitalizations in 2,515 individuals with NF1 based on information from nationwide registers.

**Methods:** All 2,515 persons with NF1 in the Danish National Patient Register (1977–2013) and two national Centres for Rare Diseases (1995–2013) were matched to 24,629 general population comparisons from the Danish Civil Registration System. Based on discharge diagnoses from the Patient Register, hospitalization rates for 146 non-psychiatric diagnoses were used to calculate rate ratios (RRs) and absolute excess risks (AERs). The total number of bed days spent in hospital by sex and previous cancer diagnosis was expressed as bed day ratios (BDRs). In Cox proportional hazard models, a hazard ratio (HR) was estimated for 12 main diagnostics groups according to age and cancer status.

**Results:** The RR for a first hospital admission among NF1 persons in 146 selected diagnostic categories was 1.8 (95% confidence interval 1.7–1.9), with an AER of 304 per 10,000 person-years (95% CI, 274–334). A high AER was seen for all 12 main diagnostic groups, dominated by benign neoplasms (14%), malignant neoplasms (14%), disorders of the central nervous system and sense organs (13%), the respiratory (11%) and digestive systems (11%). Individuals with NF1 spent twice as many days in hospital as did the general population, with an overall mean of 9.5 and 4 hospitalizations, respectively, by age 60 years; children under 10 years averaged 3.5 hospitalizations compared to 1 in the population. For women with NF1, the overall BDRs were 5.2 (95% CI 5.1–5.3) and 1.7 (95% CI 1.7–1.7) for women with and without cancer, with similar BDRs in men being 4.6 (95% CI 4.4–4.7) and 1.9 (95% CI 1.8–1.9), respectively. An increased HR for any hospitalization was observed for all age groups, with or without an associated cancer.

**Conclusions:** NF1 persons have an overall greater likelihood of hospitalization, with more frequent and longer hospitalizations involving problems in every organ system. The results emphasize the need for lifelong follow-up of NF1 individuals in specialized NF1 clinics with the expertise to address the pleiotropic manifestations of the disease.

Disclosure of Interest: L. Kenborg has a conflict with: The study was supported by a grant from U.S. Army Medical Research and Material Command (The Neurofibromatosis Research Program). , A.-K. Duun-Henriksen: None Declared, V. Riccardi: None Declared, K. Rugbjerg: None Declared, C. Pedersen: None Declared, K. Doser: None Declared, S. Dalton: None Declared, C. Johansen: None Declared, K. Andersen: None Declared, J. Østergaard: None Declared, H. Hove: None Declared, S. A. Sørensen: None Declared, J. Mulvihill: None Declared, J. Winther: None Declared
Skin Manifestations in Children and Young Adults with Neurofibromatosis Type 1 (NF1)

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**Background:** Longitudinal changes in cutaneous manifestations of NF1 including neurofibromas (NFs), café-au-lait macules (CALM), and skin freckling have not been well characterized prospectively.

**Methods:** The NCI NF1 natural history study (NCT00314119) evaluates patients (pts) with NF1 including skin exam and tanner staging, yearly until age 18 and less frequently thereafter. Cutaneous neurofibromas (cNF) were categorized into three groups: cutaneous (exophytic), dermal (flat, violaceous color), and subcutaneous (deep). We documented, 1) number and location of NFs, 2) number, size, and location of CALM, and 3) presence / absence of freckling in 9 body sites at each exam. Using Spearman rank correlation, the relationship between clinical variables and skin manifestations were evaluated for patients with 3 year (yr) follow-up, stratified by age ≥ or < 13.

**Results:** This study enrolled 167 pts from February 2008 to August 2017, 130 of whom had detailed skin exams (age median 13.0 yrs, range 0.25-45.25). At baseline, the median number of cNF for pts ≥13 yrs or <13 yrs was 18.5 and 0, respectively. With 3 yr follow-up, the median change in cNF number was 6 for pts ≥13 yrs (n=23) and 0 for pts <13 yrs (n=37). Moderately strong correlations (r=0.48, 0.51) were found at baseline between pt age and the number of cutaneous and dermal NFs. Among pts who were postpubertal at baseline, there was a median increase of 14.5 cNFs over a 3 yr period (n=14, p=0.0005). Among those who were prepubertal at baseline and entered puberty during the 3 yr period, there was a median increase of 5.5 cNFs (n=16, p=0.002). The median number of CALM ≥0.5mm for pts ≥13 or <13 yrs was 14 and 16, respectively at baseline. The median change in CALM number, over a 3 yr period, was -0.5 (n=24) and 2.0 (n=41), respectively. There was a weak correlation between the number of body sites with freckling and the number of cutaneous/subcutaneous NFs at baseline in those ≥13 yrs or <13 yrs (r <0.3). This correlation became stronger (r=0.48, r=0.42) for cutaneous NFs at 3 yr follow up in both age groups.

**Conclusions:** This prospective study captured longitudinal changes in several NF1 skin manifestations. We saw an increase in cNFs over time, consistent with prior reports. In contrast, there was a decrease in number of CALM with age. These observations may aid in the development of future clinical trials for the NF1 skin manifestations. Data validation and longitudinal follow-up are ongoing.

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Adaptive Behavior in Children with Neurofibromatosis Type 1: A Longitudinal Investigation

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Background: Both young children and school-aged children with NF1 demonstrate poorer adaptive functioning in comparison to same-aged peers (Barton & North, 2004; Klein-Tasman et al., 2013). However, investigations of adaptive functioning of children with NF1 are limited in number and have not assessed the longitudinal pattern of adaptive behavior throughout childhood. The primary aim of this investigation was to examine parent reported adaptive behavior of children with NF1 longitudinally in the preschool and school-age years.

Methods: Participants included 26 children with NF1, along with their parent. Children were assessed at two time points: preschool years (ages 3-7; \( M=4.7, SD=1.33 \)) and school age years (age 9-13; \( M=10.57, SD=1.56 \)). The Scales of Independent Behavior – Revised (SIB-R) were used to assess adaptive behavior, yielding an overall adaptive behavior standard score (Broad Independence; BI; \( M=100, SD=15 \)) as well as standard scores for the following domains: Motor Skills (M), Social Interaction & Communication Skills (SIC), Personal Living Skills (PL), and Community Living Skills (CL). IRB approval was obtained for the conduct of this study.

Results: BI (\( \rho = .45, p = .011 \)), M (\( \rho = .346, p = .042 \)), PL (\( \rho = .37, p = .032 \)) and CL skills were significantly correlated from preschool to school age years (\( \rho = .42, p = .016 \)). In the preschool years, 15.4% of children with NF1 showed BI difficulties, 19.2% M, 11.5% SIC, 26.9% PL and 19.2% CL. In the school aged years, majority of children with NF1 showed difficulty on the BI (61.5%), M (73.1%), and CL (65.4%) domains. The frequency of difficulties in the school-age years was significantly higher than in the preschool years in all domains except PL (BI: \( p < .001 \); M: \( p = .001 \); SIC: \( p = .008 \); PL: \( p = .688 \); CL: \( p = .002 \)).

Conclusions: Adaptive behavior, motor skills, personal living skills, and community living skills of children with NF1 in the preschool years showed significant relations to these skills in the school-age years. The frequency of adaptive behavior difficulties shown by children with NF1 increased over time from preschool to school-age years. Hence, there is evidence for worsening of adaptive skills with age in children with NF1. These findings contribute to the limited adaptive behavior literature in children with NF1 which may be useful for the development of targeted interventions to support optimal functioning.

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Funding: NF Midwest, NF MidAtlantic, NF Northeast, UWM Research Growth Initiative, CTSA Grant UL1 RR024999
Executive Functioning in Children with NF1 with and without ADHD Using Lab-Based and Parent and Teacher Reported Functional Measures

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Background: Previous research suggests that executive function (EF) impairment occurs in children with neurofibromatosis type 1 (NF1), independent of an attention-deficit/hyperactivity disorder (ADHD) diagnosis, suggesting that the presence of EF impairments is not uniquely associated with ADHD in NF1. However, functional questionnaires from multiple informants have not been consistently utilized to assess the influence of ADHD diagnosis on EF. Therefore, examination of EF in children with NF1 and without ADHD using a multi-method approach is warranted.

Methods: Participants were 40 children with NF1 (n = 25 without ADHD; n = 15 with ADHD) ages 9-13 and their parent and teacher. Standardized lab-based measures (Differential Ability Scales-Second Edition: DAS-2, Recall of Sequential Order: RSO & Recall of Digits-Backward: RDB), NIH Toolbox List Sorting Working Memory: LSWM, Dimensional Change Card Sort Test: DCCS) were administered to children and standardized functional measures (Behavior Rating Inventory of Executive Function Global Executive Composite: BRIEF GEC; Conners-3 Short Executive Function scale: Conners) were administered to parents and teachers. The protocol had IRB approval.

Results: Children with and without ADHD did not differ significantly on lab-based measures of EF (RSO, t(39) = 1.45, p = 0.15; RDB, t(39) = 0.395, p = 0.69; LSWM, t(36) = 1.42, p = 0.16; DCCS, t(36) = 1.18, p = .24); however, children with ADHD tended to perform in the low average range, while children without ADHD performed in the average range. Significant differences in performance between children with and without ADHD were apparent on functional parent-reported EF measures (BRIEF GEC, t(39) = -3.69, p = .001; Conners, t(39) = -2.41, p = .02), as well as on functional teacher-reported measures (BRIEF GEC, t(29) = -3.34, p = .002; Conners, t(29) = -2.20, p = .03).

Conclusions: Results indicate that children with NF1 demonstrate similar EF profiles on lab-based measures, independent of comorbid ADHD diagnosis. However, children with ADHD demonstrated significantly elevated levels of executive dysfunction on a daily basis based on parent and teacher reported functional measures. Results highlight the utility of using functional measures when assessing EF in children with NF1, in conjunction with traditional lab-based performance measures of EF.

Rate and Frequency of Bully Victimization in School-Age Children with Neurofibromatosis Type 1 (NF1)

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Background: Bullying is a harmful behavior that involves a power imbalance between perpetrators and targets. Bullying has been reported to affect almost 25% of teenagers, with those with facial differences at increased risk. Children and adolescents with the genetic tumor predisposition syndrome Neurofibromatosis Type 1 (NF1) have varying degrees of physical stigmata characteristic of the disease and experience high rates of social difficulties which may increase their risk of being a target of bullying. We evaluated school-age children with NF1 to examine rates of bullying in this patient population. In order to assess the role of NF1-related physical differences associated with bullying risk, we developed a physical severity rating form to differentiate mildly and severely physically affected individuals.

Methods: Eighty-two school-age children with NF1, ages 8 to 18, completed established bullying questionnaires during a scheduled clinic visit. Two medical providers assessed each patient for physical stigmata of NF1, including any café au lait macules, freckling, neurofibromas, and bone changes which could be readily visible in a shorts/short-sleeved clothed child.

Results: About 62% of the children in the sample reported being bullied at least once in the past year, with 24.7% reporting being bullied daily. Males reported experiencing significantly higher rates of bullying than females. An interaction effect of gender and level of physical stigmata emerged, with males with high stigmata burden experiencing higher rates of bullying than females with similarly high stigmata burden. The physical severity rating form had minimal variability between raters.

Conclusions: The present study suggests that rates of bullying victimization in NF1 are quite high and are higher than previously reported for the general population. Physical NF1 stigmata did not clearly correlate with bullying risk; however, boys with more severe stigmata did experience higher rates of bullying victimization.
The Risk of Neurofibromatosis Type 2 in Young Patients Presenting with Unilateral Vestibular Schwannoma and the Role of Improved Diagnostics on Early Diagnosis

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Background: NF2 is usually characterized by bilateral vestibular schwannomas (BVS), however it can present with unilateral vestibular schwannoma (UVS). To avoid delay in diagnosis in patients that present with UVS before age 30, screening protocols have been proposed. While the risk of having this syndrome is small even for young UVS patients, avoiding a delay in diagnosis is of paramount importance, as this diagnosis has an impact in counseling and follow-up and may guide clinical decisions.

Methods: Medical records were reviewed from patients diagnosed with UVS under the age of 30. Patients with a family history of NF2 were excluded. In addition, clinical NF2 patients were retrospectively evaluated for age and tumor(s) at presentation.

Results: Results are shown in table 1. 70 patients diagnosed with UVS below the age of 30 were included; the first group (n=19) was followed to at least 35 years of age. In this group, 1 patient (5.3%) developed a BVS and therefore NF2. The second group (n=19) was followed to an age between 30 and 35; 1 of these patients was diagnosed with NF2 (5.3%). When reviewing the MR imaging of this patient, the second VS was in retrospect already present on first imaging, albeit very small. Finally, the third group (n=20) was followed to an age below 30; none so far developed NF2. No NF2 germline mutation was found in any of the tested patients (22/70, including those with clinical NF2).

For a second database including 66 NF2 patients (revised Manchester), information on the tumor(s) at presentation could be retrieved in 59, of which 19 (32%) initially were diagnosed with UVS. From the patients presenting with UVS, 12 (63%) had no additional NF2 stigmata (revised Manchester) or a family history of NF2 at the initial presentation. Reviewing MRI-scans at first presentation, showed that of these 19 patients, a very small contralateral BVS was present in 8 (MRI-scans were done at a mean age of 51 , range 27-75 y). The delay in diagnosis of NF2 diagnosis was 3 years on average for patients presenting with UVS.

Conclusions: This study raises the question whether NF2 screening in patients presenting with UVS under 30 years is still appropriate. It gives an indication that the risk is low (5.3%), for which more data is needed. Besides, it may have been caused by inferior imaging techniques and interpretations. An additional remarkable finding was that in cases where a second VS was initially missed, and therefore the NF2 diagnosis, the average age was comparatively advanced (51 years).


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The Heterogeneous Presentation of Neurofibromatosis Type 2

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Background: The initial presentation of Neurofibromatosis Type 2 (NF2) patients is characterized by hearing loss caused by (usually bilateral) vestibular schwannomas. However, the clinical symptoms at presentation may vary due to the diverse clinical manifestations of NF2. Recognition of de novo NF2 patients is especially challenging if the initial symptoms and signs differ from the classical presentation and do not include audiovestibular symptoms. As early identification and diagnosis is essential in the management of NF2 patients, a retrospective evaluation of the presenting symptoms of known NF2 patients is valuable.

Methods: NF2 patients treated by the skull base multidisciplinary team, were reviewed for age at onset, initial symptoms, and the delay in NF2 diagnosis.

Results: In total, 67 NF2 patients (according to revised Manchester criteria) were identified of which the median age at diagnosis was 37 years (range 4-77). Only 4 patients were known to have a positive family history of NF2 (proband(s) excluded). The initial symptom(s) at presentation could be retrieved in 58 patients; a majority of the patients (n=34, 58%) presented with multiple symptoms. 37 (64%) patients presented with hearing loss at a median age of 40. Eight patients presented with other audiovestibular symptoms: vertigo, unsteadiness or tinnitus. 13 patients presented with non-audiovestibular symptoms at a median age of 28; these patients are shown in table 1. Two patients are not shown, of which one had no complaints and was diagnosed through cascade screening and the other had bilateral vestibular schwannomas that were coincidentally identified on imaging.

Conclusions: This retrospective study looks at the initial symptoms at presentation of NF2 and highlights the challenge for diagnosis because of its heterogeneous presentation. Non-classical presentations of NF2 are not rare, as 22% of our NF2 cases did not present with audiovestibular symptoms. The presented cases showed a delay in diagnosis ranging from 0-17 years. Awareness of NF2 and its potential initial symptoms among different specialties could potentially facilitate early diagnosis.

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Multidisciplinary Management of Neurofibromatosis (NF): Three Year Experience of a Pediatric Neurocutaneous Clinic in Greece

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Background: Neurocutaneous syndromes are a group of congenital disorders that include abnormalities of neuroectodermal and, sometimes, mesodermal development. The clinical spectrum is heterogeneous and often involves multiple organ systems. Additionally, patients may have a predisposition to develop tumors. A multidisciplinary approach to care has been advocated in order to provide optimum care for these complex disorders. Three years ago, the First Department of Pediatrics of the University of Athens at Children’s Hospital Agia Sofia started a pilot multidisciplinary clinic, in order to improve the clinical care of patients with neurocutaneous disorders. We are now presenting our experience with a special focus on the group of patients with neurofibromatosis (NF).

Methods: Patients were evaluated by a multidisciplinary team of specialists, including pediatric neurologist, dermatologist, oncologist, ophthalmologist, geneticist, orthopedist, neuroradiologist and psychologist. Patients underwent regular follow-up visits that included: physical examination, developmental assessment, growth and blood pressure monitoring, skin examination, ophthalmologic examination, MRI, spine X ray, hearing evaluation and other additional studies were performed based on clinically signs or symptoms.

Results: 98 children (age 5-months-17 years, 46 girls and 52 boys) were assessed. 58 patients had NF1, 4 segmental NF1, 4 NF2, 10 tuberous-sclerosis-complex, 5 incontinentia pigmenti, 2 Sturge Weber syndrome, 2 PHACE syndrome, 1 Delleman syndrome, 1 hemimegalencephaly and 8 without specific diagnosis. Molecular diagnosis was confirmed in 35 patients with NF1. One patient with segmental NF1 underwent molecular analysis of the *neurofibromin* gene in blood and in neurofibroma tissue. The main clinical findings in patients with NF1 were: learning disabilities (83%); optic-pathway-gliomas (50%); pilocytic astrocytomas (3%); sarcoma (1.7%); cutaneous neurofibromas (15%); plexiform neurofibromas (10%); scoliosis (28%); pseudoarthrosis (3%); hypertension due to vascular stenosis (10%) and short stature (21%).

Conclusions: The most frequent neurocutaneous syndrome seen in our clinic was NF1 (59%). The high proportion of optic-pathway-gliomas may reflect a referral bias. Establishment of a multidisciplinary center for neurocutaneous disorders can improve clinical care by providing multidimensional approach and contributing to early diagnosis and timely therapeutic intervention.

Emotional Functioning and Social Relationship Patients with NF1 According to Adaptive Behavior Concept

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Background: Besides specific learning difficulties the main complaints patients with NF1 are problems in social life and lack of participations in the open community activities. It happens even they demonstrate friendship seeking behavior. They have problems to maintain relationship with other non-family members. Often they experience social rejection (disregard or verbal abuse). Patients report this as situation that is incomprehensible to them. This situation is the reason of emotional disturbances, and the need for psychological support. The aim of this research is to discover the causes of failures in social contacts and its possible connections with behavioral phenotype of NF1.

Methods: Long-term psychological assessment and observation of whole group patients with NF1 were conducted at our center. Emotional functioning was assessed based on interview with parents, psychological investigation and observation of the patients. Data of 50 pts were completed and analyzed using some subscales of *Vineland Adaptive Behavior Scales* – second edition. Patients were 8-18 years old. 16 of them were treated due to the glioma of optic pathway and were low vision, attending regular schools. We assessed social competence of patients, like: cooperativeness, interpersonal appropriate, recognizing social cues, following social rules. We assessed also maladaptive behavior in this population as a risk of social rejection, especially ability to controlling anger, tendency to impulsive behavior; temper tantrums, lack of considerations, strange habits or ways, bizarre speech etc.

Results: In our group of patients we do not observe higher level of difficult behaviors (maladaptive behavior). It seems that other factors are the causes of social failures patients with NF1. Specific for these children are expressive emotionality and open communication of their needs. As additional factors we found physical appearance (café au lait spots, freckles, neurofibromas in visible parts of the body). No correlation between social life and low vision were found.

Conclusions: NF1 behavioral and physical phenotype is a risk of difficulties in interpersonal relationship and social rejection. Emotional support and social competences training are needed. The effective methods of psychological support and social competences development of patients with NF1 will be presented and discussed.
Utility of 18F-FDG PET/CT in Follow Up of Patients with Neurofibromatosis Type 1: A Clinical Registry Study of 70 Adult Patients

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Background: Some distinctive cancers are associated with NF1, such as malignant peripheral nerve sheath tumors (MPNST), gliomas and breast cancers. Few studies have actually aimed to establish the role of medical imaging in the management and follow-up of these patients. Positron emission tomography – computed tomography (PET/CT) is a nuclear medical imaging technique widely used in oncology that depicts the spatial distribution of metabolic activity in correlation with anatomic imaging obtained by CT scan. This diagnostic modality has been particularly effective in the detection of malignant transformation of plexiform neurofibromas in NF1 patients.

Objective: Our study aims to determine the overall risk of tumors in NF1 patients and to clarify the use of PET-CT in their follow-up.

Method: A retrospective observational study has been realized on 70 NF1 adult patients followed at the Neurogenetic Clinic of the CHU de Québec-Université Laval. The registry counts 45 women (64%) and 25 men (36%). The average of age is 45 years old. All diagnosed tumors and all medical imaging were compiled for each patient. Various clinical manifestations and complications, relevant personal characteristics, family history and results from genetic testing were also collected from their electronic records.

Results: All 70 patients presented cutaneous neurofibromas and café au lait spots. However, other disease manifestations were highly variable. Of these patients, 49 (70%) were diagnosed with NF1 before the age of 18 and 43 patients (61%) had an affected family member. A genetic testing was done on 57 patients (81%). Of them, 58% of mutations were substitutions; 32% deletions; 8% duplications; 2% insertions. In 13 of all patients followed, 17 tumors were diagnosed; 8 of them (47%) were thyroid cancers; 3 (18%) were gastrointestinal stromal tumors (GIST); 3 (18%) were breast cancers and 3 (18%) were MPNST. In all the cohort, 41 patients (59%) underwent a PET/CT, of which 12 (30%) yielded a positive result that in most cases revealed a previously undiagnosed tumor. So, 12 of the 17 diagnosed tumors (71%) were first detected by PET/CT.

Conclusion: A proportion of studied patients did benefit from the PET/CT as part of their routine NF1 follow up. This is the first study that attempt to establish the role of PET/CT in the follow-up of tumors in adult NF1 population. Studies on larger cohorts will have to be done to lead to significant conclusions.

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Lower Height Percentiles in Neurofibromatosis Type I (NF1) Patients on the NCI NF1 Natural History Study Inversely Correlate with Plexiform Neurofibroma (PN) Tumor Volume

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Background: Growth trends in the pediatric neurofibromatosis type I (NF1) population remain undefined. Moreover, the relationship between anthropometric measurements (e.g. height, weight) and plexiform neurofibroma (PN) tumor burden is unknown. The goals of this study were to define the anthropometric parameters for pediatric and young adult NF1 patients on the NCI NF1 Natural History Study and to investigate their relationship to PN burden. We hypothesized that NF1 patients would have lower height and weight percentiles compared to Center for Disease Control (CDC) reference populations, and that height and weight percentiles would correlate inversely with whole body PN burden.

Methods: We retrospectively investigated anthropometrics in 106 patients on the NCI NF1 Natural History Study (NCT00924196) who had volumetric MRI measurements of total body PN burden. We identified heights and weights measured within ~48 hours of whole-body MRI and determined CDC height and weight percentiles. We calculated the percentage of NF1 patients falling above or below clinically significant growth percentiles (height < 5th or > 75th percentile; weight < 5th or > 90th percentile). We examined growth velocity trends in NIH diagnostic criteria and genetic testing. Clinical features and whole body MRI findings were reviewed retrospectively.

Results: For NF1 patients over age 10 (n=67), 22% of boys (95% CI 11-37%) and 21% of girls (95% CI 8.0-40%) had height < 5th percentile. Only 2.2% of boys (95% CI 0.06-12%) and no girls (95% CI 0-12%) had height > 75th percentile. The trend towards high numbers of patients having height < 5th percentile was similar when patients with history of optic pathway glioma were excluded. We identified that among the patients with total tumor volume > 1000 ml (n=47), 38% (95% CI 25-54%) had height < 5th percentile. The association between tumor volume and height percentile was weakly negative (Somers' D: -0.25; 95% CI -0.37, -0.13).

Conclusions: The incidence of tumors detected on whole body MRI increased with age. However, malignant transformation was also noted in the pediatric patients. A serial follow-up study of whole body MRI in NF1 patients will help to understand long-term clinical course of their neurofibromas and to make a decision to intervene their progress by medical or surgical approach.

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Whole Body MRI Evaluation of Patients with NF-1

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Background: Neurofibromas develop in multiple organs in patients with neurofibromatosis type I (NF1). Deeper fusiform subcutaneous and plexiform tumors may undergo malignant change. Although these tumors are uncommon in childhood and asymptomatic in most cases, careful observation is required for them. This study was performed to investigate the efficacy of whole body MRI in evaluating tumor burden and cancer surveillance in Korean patients with NF1 and with variable age.

Methods: 71 Korean patients with NF-1 were enrolled in this study. The diagnosis was based on NIH diagnostic criteria and genetic testing. Clinical features and whole body MRI findings were reviewed retrospectively.

Results: 20 patients were below age 10 years (5.2 ± 2.3 years) and 8 patients (40%) showed peripheral or plexiform neurofibroma. One patient had a severe plexiform neurofibroma affecting the whole body and underwent debulking surgery. Another patient had pilocytic astrocytoma and malignant peripheral nerve sheath tumor in the mediastinum at age of 9 years. 12 patients were over age of 10 - 20 years (12.7 ± 2.8 years) and 7 patients (58%) had peripheral or plexiform neurofibroma. Pilocytic astrocytoma was suspicious in one patient. 39 patients were over age of 20 years (29 ± 12 years) and 29 patients (72%) had peripheral or plexiform neurofibroma. One patient was suspicious to have cranial nerve schwannoma at age of 28 years. One patient with an age of 32 years needed debulking surgery due to severe plexiform neurofibroma of the head and neck. Peripheral and plexiform neurofibroma were most commonly discovered in lower extremities and paravertebral spaces. Central nervous system was the least common region where tumor was identified. Bony dysplasia were found in 26 patients (37%). No specific relations were found between genotype and tumor burden or location.

Conclusions: The incidence of tumors detected on whole body MRI increased with age. However, malignant transformation was also noted in the pediatric patients. A serial follow-up study of whole body MRI in NF1 patients will help to understand long-term clinical course of their neurofibromas and to make a decision to intervene their progress by medical or surgical approach.

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Lower Height Percentiles in Neurofibromatosis Type I (NF1) Patients on the NCI NF1 Natural History Study Inversely Correlate with Plexiform Neurofibroma (PN) Tumor Volume
NF1-Like Optic Pathway Gliomas: Clinical and Molecular Characterization of a New Entity

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Background: NF1-associated or sporadic optic pathway gliomas (OPGs) are predominantly observed in children and they show different clinicoradiological features, treatment, and outcome. While NF1-associated OPGs are caused by complete loss-of-function of the NF1 gene, genetic alterations of the RAS-MAPK pathway have been described in their sporadic counterparts. In our clinical practice, we have identified patients with typical radiological presentation of NF1-associated OPG but without NF1 diagnosis criteria. For this reason, we have defined this presentation as NF1-like OPG. The aim of this study was to elucidate the molecular mechanism of paediatric NF1-like OPGs.

Methods: Selected patients with a diagnosis of NF1-like OPGs were followed at the pediatric neuro-oncology consultation at Gustave Roussy hospital (n=17). We centrally reviewed medical (age, signs at diagnosis, personal and family history, treatment, and evolution), radiological (MRI scans), and histological diagnoses. We performed next-generation sequencing of the NF1 gene and RAS-MAPK pathway major genes (constitutional DNAs, n=17 and paired tumor DNAs, n=11).

Results: In one patient, we identified a mosaic NF1, mainly confined to the brain: the c.7285C>T, p.(Arg2429*) NF1 mutation was observed with a ~70% VAF in tumor (suggesting LOH) and a ~8% VAF in leucocytes. This patient also presented signs of neurodevelopmental disorder associated with NF1 (macrocephaly, attention-deficit/hyperactivity disorder, and epilepsy) but no NF1 diagnosis criteria. Otherwise, we found somatic alterations of the RAS-MAPK pathway in seven tumors (four BRAF p.Val600Glu mutations and three BRAF fusions) and a somatic putative gain-of-function complex KRAS mutation in one tumor (c.197_203delins13), from eight different NF1-like OPGs patients.

Conclusions: Here, we described the largest cohort of NF1-like OPGs. We showed that NF1-like paediatric OPGs in patients with neurodevelopmental disorder can be caused by mosaic NF1. Although mosaicism is relatively frequent in NF1, no cases with manifestations mainly confined to the brain were previously reported. Our results also confirm the great implication of somatic mutations of the RAS-MAPK pathway in paediatric OPGs. For all other patients, further studies are warranted to explore unknown predisposition condition leading to the NF1-like presentation, in particular in patients with neurodevelopmental disorder.

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Cutaneous Mosaic Neurofibromatosis 1 (MNF1) at the McGill University Health Center (MUHC): Clinical and Molecular Findings in a Cohort of Pediatric and Adult NF1 Patients

Jessica Lu, Faculty of Medicine, McGill University

Background: Mosaic NF1 (MNF1) refers to a localized, under-recognized and usually mild form of NF1 due to somatic NF1 mutations. Most patients are suspected on skin exam by typical NF1 skin findings distributed in limited areas of the body. MNF1 individuals may have offspring with classical NF1. No diagnostic criteria exist for MNF1. Minimal percent of body surface area (BSA), required to identify patients at higher risk of gonadal mosaicism has not been determined. Here we describe the clinical characteristics of our MNF1 patients and our experience with molecular testing in this population.

Methods: Data of 500 NF1 patients followed at the MUHC NF1 Clinic were reviewed to identify patients with localized distribution of skin NF1 findings. Detailed clinical and molecular (blood and melanocyte) testing information was available, reviewed and recorded in a dedicated database. Statistical analysis was performed for trait distribution differences among groups. The protocol was approved by the MUHC-Research Ethics Board.

Results: We identified 43 patients with cutaneous MNF1. Mean age at diagnosis was 10.4 years (range: 7 months-47 years). All had café-au-lait spots (CALS) with or without freckling. 1 had pigmentary changes and a deep neurofibroma. 4/43 (9%) had learning/behavioral atypicalities, 6/43 (14%) had migraine. Development and physical examination was normal in all other patients. Brain MRI was done in 20/43. 1/20 had an NF1 related hyperintensity in the pulvinar. NF1 and SPRED1 testing on blood was done in 20 patients. 4/20 had next generation sequencing (NGS) and 16/20 had RNA based testing. NGS sequencing revealed non-mosaic Legius syndrome in 1 patient. RNA based testing confirmed MNF1 in 3/16. 5/16 with negative testing in blood subsequently underwent NF1 testing on melanocytes from CAL biopsy. MNF1 was confirmed in 4/5. In 1/5 the test was non-conclusive and biopsy sites on the anterior chest developed undesirable hypertrophic scars.

Conclusions: MNF1 is common in the setting of a large tertiary-care NF1 clinic. Given the risk for gonadal mosaicism, all MNF1 patients should be offered genetic testing preferably prior to child bearing. NGS based NF1 testing in blood may have a higher yield than RNA based testing. In MNF1 skin melanocyte testing is the gold standard. In patients with limited skin involvement it may be the preferred first line test. We recommend balancing the pros and cons of skin biopsy in locations with high risk of scarring.

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Clinical Guidelines for the Screening of Adult Patients with Neurofibromatosis Type One

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**Background:** In the literature there is a paucity of unified guidelines for the clinical management and surveillance of adult patients with Neurofibromatosis type One (NF1), in contrast to paediatrics where there are several guidelines published. Clear guidelines will help primary care and specialist physicians more confidently care for adult NF1 patients and prevent over investigation.

**Methods:** A literature search of UpToDate, National Comprehensive Cancer Network guidelines, Gene Reviews, American Academy of Pediatric Guidelines, International Guidelines and PubMed was conducted with the search term “neurofibromatosis type 1 OR neurofibromatosis 1 AND guideline OR screen OR management”. The search yielded nineteen relevant articles. This poster extrapolates paediatric NF1 guidelines that are relevant to young adults and consolidates the screening recommendations found for adults with NF1.

**Results:** In adults with NF1 the most consistent recommendations include yearly blood pressure monitoring, examination of the skin and historical inquiry regarding changing growth or pain associated with known plexiform neurofibromas (PN). Radiologic surveillance guidelines for early detection of malignant transformation of PN to malignant peripheral nerve sheath tumours have not been established. Investigations for pheochromocytoma are recommended prior to major surgery and in the setting of severe hypertension where work up for renal artery stenosis is also needed. Imaging of the brain and spine is not required in the absence of ophthalmological or neurologic symptoms. Screening ophthalmologic exams and visual fields are not required in asymptomatic adults. Anticipatory guidance in regard to the chronic issues such as orthopedic complications, cognitive impairment, and psychosocial evaluations has not been established.

**Conclusions:** This review of clinical recommendations can direct the management of adult patients with NF1 and identifies the need for a unified standard of care written by an expert NF1 panel.

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Topographic Correpondence of Choroidal Lesions Visualized with Structural Enface OCT and Near Infra-Red Reflectance

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**Background:** Near infra-red reflectance (NIR) images has been described as an useful non-invasive tool to detect choroidal nodules in neurofibromatosis type 1 (NF1). Recently, enface structural optical coherence tomography (eOCT) has been used to enhance visualization of retina and choroidal structures in several conditions. In this study, we report eOCT findings in NF1 along with NIR images.

**Methods:** Case series, including 5 NF1 patients from HCFMRP-USP (Ribeirão Preto - Brazil). All patients with NIH criteria for neurofibromatosis signed the inform-consent, and the study was approved by the local ethical board. Comprehensive ophthalmological examinations were performed in all patients, including measurement of best-corrected visual acuity (BCVA), slit-lamp and fundoscopy. Additionally, Heidelberg Engineering OCT-Angiography using NIR and eOCT focusing on choroidal layer, was used to record fundus images. One nodule detected with NIR was chosen to investigate correspondence with eOCT findings in each eye.

**Results:** All 5 cases presented choroidal nodules detectable with NIR. One patient showed optical glioma and reduced retinal fiber layer thickness. On eOCT, areas of higher infra-reflection were observed, and were topographically correspondent to the nodules found with NIR in 8 of 10 eyes.

**Conclusions:** These cases suggest that eOCT highlight nodules found with NIR in most of the NF1 eyes. But the lack of perfect correspondence between the two methods might indicate that eOCT can play a role on identifying choroidal nodules in NF1. Studies with larger samples and particularly with histopathological correlation are wanted to confirm these findings.

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Pre Implantation Genetic Diagnosis (PGD) for Neurofibromatosis Type 1 – Obstacles and Solutions

Mira Malcov, Wolfe IVF-PGD-Stem cell lab Lis maternity hospital

**Background:** NF1 is an autosomal dominant disorder with variable expression that affects approximately 1/2000 individuals. Carrier couples can choose Pre implantation genetic diagnosis (PGD) to prevent the birth of affected offspring. However this diagnosis is challenging due to the presence of several pseudogenes. Here we describe the molecular strategies used to overcome this obstacle and present our experience and outcomes in PGD for 26 carriers.

**Methods:** All cases referred to PGD following molecular diagnosis at our tertiary center. Molecular analyses were performed by direct mutation detection paralleled to a detailed haplotypes characterization based on 4 informative markers flanking both sides of the gene. A variety of molecular methods were used when variants exons had one or more pseudogenes, including unique primers, long-range PCR, differentially enzyme restriction, etc.

**Results:** Overall, 26 individuals underwent PGD due to NF1. Of these, 9 variants had pseudo-exons, including 1-8 copies of analogues carried the wild type sequences. Only in one case the mutation region was part of the pseudogenes. 92 cycles have been performed, 17 pregnancies were achieved and amniocentesis confirmed PGD results. 14 healthy babies were born and 3 pregnancies are ongoing, providing 58% (15/26) yield per patients.

**Conclusions:** Here we summarize one of the largest series of NF1 patients underwent PGD. Developing molecular solutions for the discrimination between pseudogene and the real coding NF1 gene, we are able to prevent misdiagnosis and to offer an accurate, feasible and reliable PGD analysis.

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De Novo Mutation Frequency of NF1 Gene in NF1 Families

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**Background:** Neurofibromatosis type 1 (NF1) is the most common autosomal dominant neurocutaneous disease that predisposes to develop benign and malignant tumours in the nervous system. NF1 is caused by mutations in the NF1 gene which has one of the highest mutation rates known for human genes (1-2x10^-4/gamete/generation). Therefore, this condition is very prevalent (1/3000) and sporadic cases account for more than 50% of all patients.

We report two independent NF1 mutations in first degree relatives from two families of our cohort of NF1 familial cases. We have estimated the de novo mutation frequency in NF1 patients resulting in a high value. This should be further confirmed in larger samples.

**Methods:** We developed a genetic protocol for molecular diagnosis of NF1 and we have characterised a mutation on 598 unrelated patients. This cohort was composed of 166 familial cases, 424 sporadic and 8 of unknown condition. The collection of familial cases consisted of 364 NF1 patients and 97 healthy individuals excluding healthy spouses. To estimate the frequency of the de novo mutation in NF1 patient’s offspring we have counted the number of mutant and wild type alleles transmitted from patients to their offspring.

**Results:** In two unrelated families we have identified two distinct NF1 mutations.

The first family (NF072) was constituted by three affected individuals, two brothers who harboured the c.1756_1759delACTA mutation and the daughter of one of them who did not carry the paternal mutation and had a de novo missense mutation (c.3587T>G; p.Leu1196Arg).

The second family (NF232) was a three-generation kindred. Six patients carried the c.7996_7997delAG, however one of the affected grandson did not carry the familial mutation but a de novo missense mutation (c.2543G>A, p.Gly848Glu).

Accordingly, we have found two de novo mutations in 252 total transmissions from 324 patients, so the estimated de novo mutation frequency in NF1 patients rises up to 7.9 x 10^-3.

**Conclusions:** These results remark that a comprehensive NF1 mutation screening should be performed in NF1 relatives with a discordant genotype-phenotype. Although we could not established the parental origin of the de novo mutation in our families, we hypothesise that the mutation rate in NF1 patients is higher than the estimated for healthy population.


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Correlation Between Depression and Functional Disability Due to Pain, and Assessment of their Prevalence in Children with Neurofibromatosis Type 1

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Background: Neurofibromatosis 1 (NF1) is an autosomal dominant, multi-system disorder associated with pain and psychological comorbidity. Majority (73%) of 6-18 years old NF-1 patients have reported pain interfering with their functioning and symptoms of depression and anxiety were significantly more common then in general population whether they were on medications or not. The study aims to find prevalence of depression and pain and functional impairment thereof, in children in NF1 clinic. Our hypothesis was that there is a positive correlation between depression and functional disability due to pain in NF1 patients, and that the prevalence of depression would be higher in NF1 patients then in the general pediatric population.

Methods: Patients were enrolled during their visits to NF1 clinic. Surveys evaluated the symptoms for 2 weeks prior to visit. Pain assessment was done using a set of 2 questions. Functional impairment was assessed with, both a parent and child Functional Disability Inventory (FDI) form (for children < 12 years parents only filled out the forms) Depression was assessed with the Moods and Feelings Questionnaire (MFQ). Kendall’s tau bivariate correlations with two tailed test for significance were used to examine the relationships between the primary variables.

Results: We have currently enrolled 35 patients in our study, with 21 of them aged 12-18 years. Depression was present in 5/35 children, prevalence of 8.5 %. Pain is frequently reported (54%) and functional limitations are experienced by even higher percentage of children (80%) even if they did not report any pain in the last 2 weeks. Adolescents (17/21 with mean score of 9.4) report much greater functional disability then their parents (17/21 vs 15/20, with mean score of 9.4 vs 5.7 respectively) perceive them to have.

Conclusions: The rate of depression in children with NF1 in our clinic is similar to that in the general population. Fear of precipitating/aggravating pain probably restricts the children’s normal activities. There does not appear to be a correlation between MFQ scores and frequent pain or functional disability.

Understanding Diagnostic Delay in Schwannomatosis: A Qualitative Study of Patients’ Experiences

Vanessa L. Merker, Massachusetts General Hospital; Boston University School of Public Health, Boston, United States

Background: Previous research has shown diagnostic delay is common in schwannomatosis (SWN), but the effects of diagnostic delay on patients is unknown.

Methods: Adults with SWN living in the United States were recruited from the International Schwannomatosis Database. Using IRB-approved procedures, we interviewed subjects over the phone about their diagnostic journey. Interview transcripts were analyzed using grounded thematic analysis, in which coding categories are emergent from the data. Transcripts were coded separately by two authors and themes were developed using the constant comparative method.

Results: 18 people (11 males, median age: 51 years) were interviewed, a median of 3.4 years after SWN diagnosis. Participants reported a median time to SWN diagnosis of 9.8 years from first symptom and 1.9 years from discovery of their second schwannoma. Eleven participants (61%) had schwannoma-related pain misdiagnosed, most often as a musculoskeletal or neuromuscular issue, but also in 3 cases as psychosomatic pain. Misdiagnoses led to provision of ineffective treatment (including invasive procedures in 3 subjects) and to delays in the receipt of effective treatment via schwanna removal. Negative consequences of diagnostic delay/misdiagnosis included stigmatization as drug-seeking or as having a psychological disorder; feelings of anxiety, loneliness, and depression; conflict with family members; and mistrust in healthcare providers. Distress during the diagnostic process was exacerbated by poor communication from providers who initially introduced the topic of neurofibromatosis (NF) or SWN, particularly regarding lack of differentiation between the types of NF and lack of referral for follow-up specialty care. Eventual SWN diagnosis led to mostly (but not exclusively) positive impacts, including feelings of reassurance and empowerment; improved ability to interpret and seek care for schwannoma symptoms; more holistic treatment planning; and diagnosis of other affected family members.

Conclusions: Diagnostic delay and misdiagnosis are common in SWN and can result in unnecessary surgery, delayed receipt of effective pain management, psychological distress and stigmatization. SWN diagnosis was often delayed even after discovery of a second schwannoma; the need for neurogenetic evaluation in these patients should be reinforced with non-specialists. To reduce patients’ psychological distress, clinicians should provide more information about NF/SWN and facilitate referral for specialty care.

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Characteristics of Spinal Pathology in Patients with Neurofibromatosis Type 1 (NF1): A Systematic Review

Midhun Mohan, Department of Neurosurgery, Salford Royal NHS Foundation Trust, Salford, United Kingdom

Background: Spinal pathology is common in patients with NF1. Existing studies have not yet comprehensively described the spectrum of spinal pathology that can arise. The aim of this systematic review was to describe the characteristics of reported spinal pathology in NF1 patients.

Methods: This systematic review was conducted as per PRISMA guidelines and registered on the PROSPERO database of systematic reviews. Systematic searches were performed on the following databases: PubMed, PsycINFO, Medline, HMIC, HBE, EMBASE, CINAHL, BNI, AMED and the Cochrane Database of Systematic Reviews. Studies with n > 1 describing any spinal abnormality in NF1 patients were included. Each pathology was described with a rate: number of positive cases/total number of cases evaluated for pathology.

Results: 23 studies were included from 1253 unique results. The evidence levels were as follows: I (n = 1), II (n = 2) and IV (n = 19). Data was available in 1809 patients with a mean age of 22.5 years and equal gender distribution (49.3% male). Reported spinal pathology included: dural ectasia – 93/290 (32.1%); scoliosis- 326/748 (43.6%); meningocoele – 5/51 (16.1%); syrinx – 10/97 (10%); cord signal abnormalities in the absence of tumour compression – 33/97 (34%); intramedullary tumour - 7/82 (8.5%); spinal nerve root tumour - 276/1350 (20.4%); and spinal plexiform tumour - 167/364 (45.9%). Degenerative spinal disease and Chiari malformation were not described to any meaningful extent.

Conclusions: Spinal pathology is common in patients with NF1 though existing literature is heterogeneous in how findings are presented. Most existing studies are of low quality. There is a need for more prospective studies on this theme to aid the establishment of a core outcome set for spinal disease in NF1 patients.

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C2 Neurofibromas in Neurofibromatosis Type 1 (NF1): Genetic and Imaging Characteristics

Mueez Waqar, Midhun Mohan, Neurosurgery, Salford Royal NHS Foundation Trust, Salford, United Kingdom

Background: C2 nerve root neurofibromas commonly arise in NF1 patients with spinal disease, though their genetic and imaging characteristics are unexplored. The aim of this study was to characterise genetic and spinal imaging findings in a large cohort of NF1 patients with C2 neurofibromas.

Methods: Review of referrals to a national NF1 referral centre in the UK between 2009-2016. Inclusion criteria: (1) at least one C2 root neurofibroma; (2) MRI cervical spine/whole spine available for analysis. Blinded imaging review by a neuroradiologist with interest in NF1. Multivariate logistic regression analysis was used to identify factors associated with need for surgery.

Results: 54 patients with 106 C2 neurofibromas were included. The median age was 32.5 years (range 15-61 years) and there was a slight male excess (M:F, 33:21). Splice-site (30%) and missense mutations (20%) were the commonest NF1 gene mutations found. Spinal neurofibromas were distributed in all spine regions (65%) or the cervical spine alone (22%). Most (93%) C2 neurofibromas were visible on MRI head scans. Intradural invasion and cord compression in the cervical spine included the C2 level in 95% and 80% of patients, respectively. Compared to all other cervical spine neurofibromas, C2 neurofibromas had higher rates of intraspinal extension (75% vs. 32%; OR=6.20, 95% CI 3.85-9.97; p<0.001), intradural invasion (53% vs. 26%; OR=3.20, 95% CI 2.08-4.92; p<0.001) and cord compression (25% vs. 13%; OR=2.26, 95% CI 1.35-3.79; p=0.002). However, C2 neurofibromas had lower rates of extraforaminal growth beyond the transverse process (12% vs. 62%; OR=0.09, 95% CI 0.05-0.16; p<0.001). 13% of patients underwent surgery to decompress the C2 level. Factors associated with surgery included myelopathy (p = 0.03) but not radiological cord compression (p > 0.99).

Conclusions: C2 neurofibromas are particularly aggressive due to preferential intraspinal growth. However, radiological findings alone are not an indication for surgery at our centre. A large proportion of patients with these tumours also paradoxically harbour a milder NF1 genotype. These observations require future study.

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A Regional Study of Aqueduct Stenosis in the Paediatric Complex Neurofibromatosis Type 1 Population

Christopher Murphy, University of Manchester, Manchester, United Kingdom

Background: Aqueduct stenosis (AS) is a rare association of neurofibromatosis type 1 (NF1), resulting in ventriculomegaly and hydrocephalus requiring surgical treatment. This study aims to identify the prevalence of AS and its patterns of clinical presentation, aetiology and treatment in the paediatric complex NF1 population.

Methods: Patients with NF1 aged 0-18 years were recruited from the Regional Genetic Family Register, following institutional review board approval. Magnetic resonance imaging data and clinical documents were reviewed with respect to clinical presentation, degree of ventriculomegaly, aetiologic factors and management of AS.

Results: 24 of the 233 paediatric patients identified with complex NF1 were found to have AS. This included 13 males and 11 females with a mean age of 9 years 5 months (range 8 months – 17 years). The majority of patients with AS presented with symptoms of raised intracranial pressure associated with ventriculomegaly and/or hydrocephalus (n=18). However, in 25% of patients, AS was an incidental finding on MRI and was observed both in the presence (n=2) and absence (n=4) of ventriculomegaly. In the majority of cases a single cause of AS was identified (n=16), of which tectal plate thickening (n=7) was most frequently observed. The remaining 8 patients had multiple causes of AS, in which tectal plate thickening (n=7) and aqueductal webs (n=5) were the most common observations. Surgery was performed on all patients with evidence of raised pressure (n=9) by performing endoscopic third ventriculostomy (ETV) (n=6) or ventriculopertoneal (VP)-shunting (n=3). Tectal plate thickening was most frequently observed in patients who underwent ETV (n=3), followed by aqueductual web (n=1) and T2-signal changes in the tectal plate (n=1). Patients treated with VP-shunt had 4th ventricle outflow obstruction (n=2) and a tectal plate tumour (n=1).

Conclusions: This study identifies that AS is more prevalent amongst the paediatric complex NF1 population than previously reported, occurring in 10% of cases. Our findings demonstrate that AS is most commonly symptomatic in presentation, but can be asymptomatic in 25% of paediatric complex NF1 patients. In this population, AS can occur both in the presence and absence of ventriculomegaly and therefore requires careful monitoring for development of hydrocephalus. In this study, over one third of patients with AS eventually required treatment.

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Health-Related Quality of Life for NF2 Patients in Clinical Trials with Everolimus

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Background: Several clinical trials have evaluated the efficacy of everolimus in the treatment of Neurofibromatosis type 2 (NF2) related Vestibular Schwannomas (VS) and found that this treatment can be effective in stopping tumor growth in some patients with progressive VS. However, these studies have not reported the impact of daily use of everolimus on the health-related quality of life (HRQOL) of NF2 patients and feasibility of testing HRQOL in a NF2 clinical trial.

Methods: HRQOL data from 2 single institution studies of everolimus are evaluated – 10 patients from France and 12 from the United States (US). All participants received everolimus daily up to 52 weeks. Subjects were withdrawn from the study for serious adverse events, and, in the US, also for tumor growth. Participants completed the Short Form 36 (SF-36) health survey at study enrollment, and at 24 weeks and 52 weeks (if applicable) on study. In the US, patients also completed the NF2-specific QOL questionnaire (NFTI-QOL) and the Tinnitus Handicap Inventory (THI) during these time points.

Results: In the US study, 8 patients completed the HRQOL surveys at all time-points, 2 completed the 24-weeks time-point, and 2 only at enrollment. At baseline, majority of patients reported deficits in physical functioning, fatigue, and pain on the SF-36. Patients also had a mean score of 7.7 (range 0-12) at baseline NFTI-QOL, and a mean of 182.6 (range 170-238) on the THI. At the end of the study, 4 out of 10 patients reported worsening health changes using the SF-36, and 2 with improved health change responses. 2 out of 10 had a decrease (of 2 or more) in NFTI-QOL score over the study, and 8 had stable baseline NFTI-QOL, and a mean of 182.6 (range 170-238) on the THI. At the end of the study, 4 out of 10 patients reported worsening health changes using the SF-36.

Conclusions: It is feasible to have HRQOL assessments in patients on a NF2 clinical trial. In general, few patients reported a worsening in HRQOL while on everolimus. Further data on comparison with results from patients enrolled in France and correlation of changes in QOL with tumor response will be presented.

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Health Complaints and Work Experiences among Adults with Neurofibromatosis 1

Livø K. Nyhus, The Norwegian National Advisory Unit on Rare Disorders, Frambu resource centre for rare disorders, Sandbakken

Background: Previous studies have provided valuable insights into the complexity of HQoL problems experienced by persons with NF1. However, a striking feature across previous studies is the lack of focus on employment status and work experiences among persons with NF1.

Methods: The primary aim was to examine the extent and nature of work involvement among persons with NF1. Health complaints were also examined in relation to work experiences.

A cross-sectional self-report survey of 142 adults with NF1 recruited from non-clinical settings (M age = 50.3 years, SD = 12.0, range 32 to 80; 62.0% females) was conducted. Population data from the Norwegian epidemiological survey HUNT3 was used as controls (N=46293). The participants completed questionnaires which comprised background information, health-related questions specific to the NF1 diagnosis and excerpts from HUNT3. Health complaints were measured with The Subjective Health Complaints Inventory (SHC; Eriksen, Ihlebæk & Ursin, 1999). Work experiences were measured with eight items drawn from the HUNT3-survey.

Results: In the NF1 sample 76.8% reported to be currently or previously working. The following main sources of income were reported: 45% regular salary, 35.2% disability pension, 9.8% benefits due to sick leave, unemployment or work assessment, 7.0% regular retirement pension. Compared to controls, significantly fewer in the NF1 sample experienced support from co-workers, and significantly more participants reported workplace bullying. Significantly fewer participants with NF1 reported autonomy in work tasks and decisions. More NF1 participants reported to feel exhausted after a working day compared to controls. More self-reported health complaints (SHC) were significantly associated with poorer social aspects of work experiences and more demanding physical aspects of work experiences.

Conclusions: To the best of the authors knowledge this is the first study examining the role of work experiences for HQoL domains among persons with NF1. This study showed less sense of belonging to work and colleagues in the NF sample compared to controls, and significant association between perceived work experiences and self-reported health complaints. Given that many persons with NF1 work, there are important findings showing improvement in work experiences for this population is needed.

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Social Information Processing, Peer Relationships and Cognitive Functioning of Preschool Children with Neurofibromatosis Type 1: A Retrospective Study

Neeltje Obergfell, Department of Paediatrics and Adolescent Medicine, Division of Neurooncology, Medical University of Vienna

Background: NF1 is known for its frequent cognitive and psychosocial complications. Studies indicate a relationship between cognitive variables and social functioning in children with NF1. Research has shown that NF1-patients have poorer results in social domains in comparison to their unaffected siblings, peers or normative samples. To our knowledge, no studies have examined this ability in preschool children with NF1. In this age, the acquirement of social skills plays an important role as this period is considered as the key time to develop social competencies in order to prepare children for school. Therefore the study aimed at analyzing aspects of the social-emotional competence and the ability to identify emotional facial expressions of preschool children with NF1. In addition, cognitive profiles for patients with and without peer-relationship-problems were examined.

Methods: Data of 25 preschool children with NF1 were analyzed retrospectively regarding social information processing and general cognitive abilities as well as parents’ reports on peer-relationship-problems, cognitive functioning and quality of life. Data were collected in the framework of the research project ‘Fit for School - Despite Neurofibromatosis Type I’. Results were compared to normative statistical data of the respective tests.

Results: Preschool children with NF1 scored significantly lower on processing social information (p=0.004) than the norm. In addition, 22.7% of parents reported abnormal peer-relationships. The investigation of cognitive profiles of children with peer-relationship-problems in comparison to NF1-children without these problems showed significant differences for full-scale IQ (p=0.038), verbal IQ (p=0.038) and performance IQ (p=0.032). Moreover, parents reported significantly lower cognitive abilities for NF1-children with peer-relationship-problems (p=0.001). Further, parents’ reports on peer-relationship-problems were associated with certain cognitive deficits of their children.

Conclusions: The results demonstrate for the first time that social information-processing-deficits in children with NF1 already exist in preschool age. Additionally, the finding of different cognitive profiles for children with and without peer-relationship-problems, and their relationship with social functioning, indicates that there is a high-risk group of NF1-preschool children that needs special attention regarding early interventions to prevent later social isolation.

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Disclosure of Interest: N. Obergfell has a conflict with: We have no conflicts of interest to declare. The project was fiscal and contentual supported by the Austrian patient organization “NF Kinder”...
Motor Problems and its Course in Young Children with NF1, Results from a Pilot Study

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Background: Motor problems are frequently observed in children with NF1. In addition to motor problems NF1 children may face problems with hypermobility, hypotonia, and limited exercise tolerance. We hypothesize that motor development and physical condition are related. Interventions may focus on either motor performance or condition or both. We present pilot data of a longitudinal project on motor development in children with NF1 and the effect of potential interventions.

Aim: To assess motor development and contributing factors to quality of motor performance in young children with NF1. In a subset the motor development in time will be longitudinally assessed.

Methods: Patients and setting: Children aged 4 to 6 years, Erasmus MC multidisciplinary NF1 expertise center, Rotterdam. Motor performance (Motor Assessment Battery for Children version 2, MABC-2), exercise tolerance (Bruce protocol), grip strength (hand-held dynamometer), joint hypermobility (Beighton scale) and fatigue (PedsQL), are compared to normative values. Effects of contributing factors on the motor performance were evaluated by generalized linear modelling.

Results: We evaluated 29 children, median age 5.3 years (IQR 4.5-5.7yrs), with 8 having repeated assessments (median interval 12.8 months (IQR 11.3-16.8). Twenty-three out of 29 children scored below the 16th percentile on MABC-2 (20 on balance, 19 on motor function and 11 on dexterity). Exercise tolerance was assessed in 24 children, with 12 below -2SD normative values. Grip strength was below -2SD normative values in 13 out of 25 children. Hypermobility was present in two out of 26 evaluated children. We observed worse parental reported scores for ‘General fatigue’ and ‘Cognitive fatigue’ (effect sizes -0.3). Independent determinants on motor performance were exercise tolerance and grip strength. In follow up, one out of eight had worse MABC-2 scores (decreasing motor function), and 2 improving (in on balance). Six children improved in exercise tolerance; 2 recovering to above -2SD. Grip strength improved in 4 out of 7; 2 children recovering to above -2SD. One additional child was classified as having hypermobility. Variable effects were observed on parental fatigue scores.

Conclusions: Limited exercise tolerance and (hand) grip strength seem the most important contributors to motor performance in young children with NF1. In a small subset with repeated assessments we observed improvement on exercise tolerance, but variable effects on MABC-2 scores.

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Inflammatory Infiltrates in Dysplastic Neurofibromas of NF1 Patients: A Rationale for the Use of Checkpoint Inhibitors?

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Background: Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas arising from deep neurofibromas (NF) in about 10% of patients with neurofibromatosis 1 (NF1). We have identified a particular aspect of the neurofibromas, termed “dysplastic” which is considered to represent the very initial step of malignant transformation of NF into MPNST. To date, there are only few published data regarding the potential benefit of checkpoint inhibitors to treat NF1 tumors, while these drugs have dramatically changed the prognosis of many cancers.

Methods: We have blindly quantified using the Image J software (5 high power fields at x400) the inflammatory infiltrates (mononuclear cells and CD3+ T-cells) in the micro-environment of 60 NF (28 cutaneous NF and 32 deep plexiform/diffuse NF), 14 dysplastic NF and 9 MPNST of NF1 patients. The phenotype of mononuclear cells (CD3, CD4, CD8, perforin, granzyme B, TIA1, FoxP3, CD20, CD163), MHC class I expression (MHC-I) in tumor cells (spindle Schwann or fibroblast cells) and PD1/PDL1 was evaluated semi-quantitatively (0 : negative staining, 1 : <10%, 2 : 10-20%,...) in a subset of samples using immunohistochemistry.

Results: Dysplastic NF showed a significantly higher density of mononuclear cells and CD3+ T-cells than non-transformed NF and MPNST (p<0.0001). The infiltrates comprised M2 (CD163+) macrophages (60-70%) and T-cells (30-40%), with mostly CD8+ non activated cytotoxic effectors (TIA1+, granzyme B-). The CD8+ T-cells were scattered or grouped in small clusters in dysplastic NF.

Around half of the tumors (15/26, 58%) expressed PDL1, but the staining was often heterogeneous (score 5 in 85% of cases) while the CMH I was weakly but always expressed. To note, most of the CD8+ T-cells expressed PD1.

Conclusions: The presence of immune cells, especially PD1+ CD8+ effector T-cells in NF and MPNST was already shown but no study focused on dysplastic NF, to the best of our knowledge. We hypothesize that NF1 associated tumors can promote an anti-tumor response, culminating at the very early stages of NF transformation, at the “dysplastic” stage, which is however ineffective and later controlled by immune escape mechanisms to decipher. The significant expression of PD1 by tumor infiltrating lymphocytes suggests that the PD1-PDL axis may play a role in such immune escape. Whether patient may benefit from the use of checkpoint inhibitor targeting CTLA-4 or PD1 to restore an efficient anti-tumor response remains to be studied.

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Safety and Feasibility of Serial Biopsies on a Phase II Trial of the MEK 1/2 Inhibitor Selumetinib (AZD6244, ARRY-142886 Hydrogen Sulfate) in Adults with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PN)

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Background: PN in NF1 are associated with significant morbidities, including pain and functional limitations. Currently, there are no approved medical treatments for PN. Selumetinib, an oral MEK inhibitor, demonstrated unprecedented activity in a phase I trial for children with NF1 and inoperable PN, with partial responses in 17 of 24 patients (pts) (71%), and similar activity in a follow up phase II study. A phase II single site study of selumetinib in adults with NF1 and inoperable PN is ongoing (NCT02407405), and includes a prospective evaluation of the feasibility and safety of serial PN and cutaneous neurofibroma (cNF) biopsies. To our knowledge, these have not been performed previously in trials targeting NF1 PN, and the safety of this approach has not been established.

Methods: Open label phase II study; selumetinib 50mg twice daily orally on a continuous dosing schedule (1 cycle = 28 days). The primary study objective is to determine the partial and complete response rate of PN using volumetric MRI analysis (PN volume decrease ≥ 20%). Secondary objectives include collection of mandatory pre- and on-treatment biopsies of PN and cNF to evaluate pharmacodynamic markers, including percent target inhibition using phospho-AKT/phospho-ERK levels. Simon 2-stage design; stage 2 defined by >5/20 partial responses; overall target response rate is 45%.

Results: Twenty-one pts enrolled as of June 1, 2018, and enrollment on stage 2 is ongoing. 19/21 pts had percutaneous PN and/or cNF biopsy; a safe biopsy site was not identified in 2pts. 14/21 pts had uncomplicated paired PN biopsies via 14-18 gauge needles. Procedurally, 2pts had general anesthesia; samples were otherwise obtained via deep sedation (8pts), conscious sedation (4pts) or local anesthesia (5pts). On treatment PN biopsies were not performed in 4pts due to unrelated event (hematoma, 1pt), drug hold for toxicity (1pt), and further therapy refusal (2pts). 5/14 pts had paired punch cNF biopsies. 35 samples have been collected in total to date, all with confirmed neurofibroma pathology; 5 atypical neurofibromas and 6 neurofibromas with mild atypia have been identified.

Conclusions: Serial biopsies of PN and cNF for evaluation of PD markers are feasible, safe and should be considered for future targeted therapy trials to elucidate mechanisms of response and resistance. Patient enrollment, PN volumetric response assessments, PD marker analysis, and functional/pt-reported outcomes are ongoing.


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Large Volume Destruction of Cutaneous Neurofibromata in NF1 Using Electrodesiccation

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Background: Patients with NF1 can have a variety of presentations. Individuals exhibiting cutaneous neurofibromata can have several hundred tumors over their entire body surface. They typically present in adulthood and can increase in size and number with time. Tumors can become disfiguring, painful, and a source of psychosocial stress. Currently, surgical excision or destruction remains the only option available for treatment. Electrodesiccation (ED) is a relatively new technique to address cutaneous neurofibromata and can be especially useful for those patients suffering from a large number of skin lesions.

Methods: A single-surgeon retrospective review was performed between 2016 and 2018, examining the effects of large volume ED of cutaneous neurofibromata. Patient demographics, diagnosis, operative time, areas of concern, complications, and patient satisfaction were recorded. Photographic documentation was obtained for all patients both pre- and postoperatively.

Results: A total of 36 patients underwent large volume ED between 2016 and 2018 using a Conmed Hyfrecator 2000 (Utica, NY). Hyfrecator settings ranged 15-31 watts using the high voltage terminal in all cases. All patients were treated on an outpatient basis under general anesthesia. Average operative time was 169 minutes (range 118-214 minutes). Thirty-one patients requested treatment of the face, neck, and arms as the primary areas of concern. Intraoperative treatment endpoints for ED were determined visually with change in appearance of tissues. Patient satisfaction was high at 97% for improvement of skin contour. Primary complaint postop was pruritus. Most patients required subsequent sessions of ED to treat additional anatomic sections and to perform light re-treatment of poorly responsive areas.

Conclusions: ED is an effective way to treat large areas of cutaneous neurofibromata in NF1 patients. Operative time is limited in order to minimize wounding and lengthy postoperative healing period. An estimated 200-700 cutaneous neurofibromata can be treated in one session of ED. Limitations, however, do exist. Lesions larger than 5mm in diameter either respond poorly or produce unacceptable scarring in our experience. These larger lesions are usually excised simultaneously with ED. Another limitation is that ED is very technique-dependent. Modifications with this technique are constantly required to improve tumor destruction while minimizing scarring. Finally, longer follow-up periods are required to determine long-term efficacy.
Usefulness of an Individualized Neuropsychological Intervention in Children with Neurofibromatosis Type 1

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Background: Cognitive deficits, learning disabilities and social and behavioural problems which can negatively affect quality of life are detected in a large number of Neurofibromatosis type 1 patients. Studies using cognitive interventions are limited and some of them are based on computerized programs. The aim of this study is to assess the efficacy of a multimodal individualized neuropsychological intervention (child – parents – school) targeted to improve behavior and cognitive function in children with Neurofibromatosis type 1 and learning disabilities.

Methods: 1 year randomized controlled clinical trial, comparing neuropsychological intervention vs. no intervention. Total sample of 38 children with Neurofibromatosis type 1 and learning disabilities aged 6-16 years recruited from the pediatric national referral centre in Spain. Those with epilepsy and brain tumors – except asymptomatic gliomas – were excluded. All participants receive assessment (baseline and post-treatment) with an extensive battery of neuropsychological test. Subsequently subjects were randomly assigned to a control group (not receiving treatment) and the rest to the intervention group. The multimodal neuropsychological intervention – during 6 months – included 20 individual sessions conducted to the child / adolescent, 6 parents’ group sessions where behavior management techniques were trained and follow-up/counseling to schools once at each scholar trimester. In the intervention group, results of the evaluations pre and post intervention will be compared by means of a paired t test.

Results: 20 participants were in the treatment group and 18 in the control group.

All the participants on the treatment group presented difficulties on academic performance. Deficits in attention and executive function were present on all of them but only 55% met ADHD criteria. 40% showed reading disabilities, 40% difficulties in mathematics and 90% presented behavior difficulties.

Most of the families referred an improvement on academic performance and on executive function on daily life, meaningly in planning, organizing, supervision... this fact was verified by the scales. Those parents who applied behavior management techniques reported an amelioration of behavioral difficulties.

Conclusions: Multimodal individualized neuropsychological intervention can be a useful tool to improve learning disabilities and behavior difficulties in daily life of patients with NF1.

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Retinal Nerve Fiber Layer Thickness as a Surrogate of Visual Acuity in Pediatric Patients Affected by Optic Pathway Glioma Secondary to Neurofibromatosis Type 1

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Background: The use of optical coherence tomography (OCT) to detect peripapillary retinal nerve fiber layer (RNFL) changes secondary to the presence of optic pathway glioma (OPG) has recently been proposed as a new clinical diagnostic test to detect OPG in patients affected by neurofibromatosis type-1 (NF1). The aim of the present study is to evaluate the use of RNFL thickness measured by OCT as a surrogate of visual acuity (VA) in a population of pediatric patients affected by OPG associated with NF1, and to identify a cut-off value allowing to discriminate between eyes with normal and pathological VA.

Methods: 38 pediatric patients (66 eyes) affected by MRI-proven optic pathway gliomas were included. Each patient underwent complete ophthalmological evaluation, including age-appropriate visual function assessment and RNFL analysis by OCT (Spectralis, Heidelberg, Germany). VA was classified as normal or pathologic using age-based normative data.

Results: VA was classified as normal in forty-three eyes (65%) and pathologic in twenty-three (35%) eyes. The mean RNFL thickness of each analyzed sector was significantly lower in eyes with abnormal VA (p<0.05). The best balance cut-off value of global RNFL thickness allowing to discriminate between eyes with normal and pathological VA resulted 76.25 µm (91%, 76% 67% and 94% of sensitivity, specificity, positive and negative predicting value, respectively) (Fig.1). Considering best balanced cut-off values of other analyzed RNFL sectors, the superior (p = 0.0029) and the inferior (p = 0.0024) sectors reached the higher sensitivity (87% and 87%, respectively) and specificity (81% and 79%, respectively)(Fig 1).

Conclusions: RNFL thickness is directly related to VA in children affected by NF1-related OPG, and should be considered as a potential surrogate marker of visual acuity. RNFL thickness cut-off values can be used in pediatric patients unable to complete age-appropriate visual function tests or to discriminate false-positive results obtained by VA measurement.

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<table>
<thead>
<tr>
<th>RNFL thickness</th>
<th>Cut-off value*</th>
<th>SE</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>76  µm</td>
<td>91.3%</td>
<td>76.7%</td>
</tr>
<tr>
<td>Temporal</td>
<td>49  µm</td>
<td>87.0%</td>
<td>76.7%</td>
</tr>
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<td>Superior</td>
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<td>81.4%</td>
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<tr>
<td>Nasal</td>
<td>54  µm</td>
<td>78.3%</td>
<td>72.1%</td>
</tr>
<tr>
<td>Inferior</td>
<td>99  µm</td>
<td>87.0%</td>
<td>79.1%</td>
</tr>
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RNFL = retinal nerve fibre layer; SE: specificity; SP: sensitivity; * Best balance.
Reproducibility of Cognitive and Behavioral Outcomes for Clinical Trials in Children with Neurofibromatosis Type 1: Problems and Solutions

Jonathan M. Payne, Department of Paediatrics, University of Melbourne; Murdoch Children's Research Institute, Melbourne

Background: Despite significant promise of preclinical trials demonstrating reversal of the murine behavioral phenotype, early attempts at translating these findings to patients in randomized controlled trials have been challenging. Poor reproducibility of commonly used cognitive and behavioral endpoints may provide one explanation for this difficulty. In this study, we examined the suitability of various neuropsychological measures as cognitive outcomes in clinical trials by reanalysing real-life trial data in children with neurofibromatosis type 1 (NF1). We report on the severity of cognitive deficits at baseline, test-retest reliability of the measures and the application of statistical methods to improve reproducibility.

Methods: Data were analyzed from the STARS clinical trial (n=146), a multi-center double-blind placebo-controlled trial of lovastatin, conducted by the NF Clinical Trials Consortium. Cognitive outcomes were compared to normative data to determine severity of deficits at baseline. Intra-class correlations were generated between pre- and post-performances on efficacy endpoints in the placebo group to determine test-retest reliabilities. Confirmatory factor analysis (CFA) was used to reduce data into cognitive domains and account for measurement error.

Results: Largest differences between mean scores at baseline and normative reference data were seen on cognitive measures of attention and inattentive symptoms. Test retest reliabilities of outcome measures were variable, ranging from unacceptable to good. In general, endpoints utilizing observer report were more reliable than those directly assessing the child. Data reduction using CFA confirmed four distinct neuropsychological domains: executive functioning/attention, visuospatial, memory and behaviour. Reliability of these domain scores improved to acceptable levels for clinical trials. Applicability and utility of our model was demonstrated by homogenous effect sizes in the reanalyzed efficacy data.

Conclusions: Poor reliability of cognitive outcomes is a critical issue for clinical trials in NF1 and these data demonstrate that single observed endpoints are not appropriate to determine efficacy. Recommendations to improve reproducibility are outlined and should guide future trial design.

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Social Function and Autism Spectrum Disorder in Neurofibromatosis Type 1: A Systematic Review and Meta-Analysis

Jonathan M. Payne, Department of Paediatrics, University of Melbourne; Murdoch Children's Research Institute, Melbourne

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic condition associated with cognitive deficits, attention deficit/hyperactivity disorder (ADHD), and learning disabilities. There is a growing body of literature describing a range of social difficulties, with several research groups reporting elevated autism spectrum disorder (ASD) trait burden. The aims of the present study were to perform a systematic review and meta-analysis of the existing data in children and adults with NF1, and to place findings within the context of a theoretical framework, the Socio-Cognitive Integration Abilities Model (SOCIAL).

Methods: Medline, PsycINFO, Embase, and PubMed databases were searched in August 2017 for references back to 1980. A total of 35 original articles fulfilled the selection criteria and 22 manuscripts provided sufficient data for meta-analysis. Separate meta-analyses were carried out for the three social domains, comprising social function (12 studies, 575 NF1, 518 controls), ASD symptomatology (8 studies, 620 NF1, 657 controls), and social cognition (6 studies, 201 NF1, 189 controls).

Results: The overall mean effect (Hedges' g = 0.785) for the social function domain was medium-to-large in magnitude, demonstrating that individuals with NF1 displayed significantly lower social function than comparison samples. A large mean effect (Hedges' g = 0.905) was obtained for the ASD symptoms domain, indicating significantly greater prevalence of ASD symptoms in NF1 cohorts compared to controls. Poorer social cognition was also detected in NF1 cohorts relative to controls, with an overall mean effect size that was medium in magnitude (Hedges' g = 0.651). Social outcomes in NF1 were found to be heterogeneous, and meta-regression and qualitative synthesis provided support for age, sex, and comorbid ADHD symptoms as moderating factors.

Conclusions: Our data demonstrate significant social deficits in NF1 and provide robust evidence for the presence of ASD traits in a subset of individuals with NF1. Limited evidence also indicates the presence of social cognitive deficits. Further research is required to better understand predictors of poor social outcomes and elevated ASD traits in NF1. The use of a framework such as the SOCIAL model is warranted to guide future investigations and to provide theory-based targets for intervention.

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Neurofibromatosis Type 1 of the Child Increases Birth Weight

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**Background:** The average height of patients with neurofibromatosis type 1 (NF1) is slightly smaller than the height of the general population. NF1 has been reported to lead to intrauterine growth restriction. Using total population based NF1 cohort, we have recently reported that NF1 of the fetus is associated with slightly shortened pregnancy. However, no cohort data has been published on birth size of the newborns with NF1.

**Methods:** This retrospective cohort study includes a cohort of 1,410 persons with a confirmed diagnosis of NF1, and a matched (10:1) comparison cohort from the general population. Figures for weight, length and head circumference at birth were retrieved from the Medical Birth Register for those born since 1987. Data were converted to standard deviation scores (SDS), defined as standard deviation difference to the reference population. Analyses were adjusted for gestational age, maternal age, maternal weight, maternal height, parity, smoking during pregnancy, gestational diabetes and year of the delivery.

**Results:** The birth weight among infants with NF1 was higher than among infants in the comparison cohort [adjusted mean difference (95% confidence interval): 0.53 (0.19 to 0.87)]. The difference was even more pronounced in the subgroup of NF1 infants with non-NF1 mothers [0.90 (0.46 to 1.34)]. Also the head circumference at birth was longer among NF1 infants [0.58 (0.26 to 0.90)]. The average length of the NF1 infants at birth did not differ significantly from the infants in the comparison cohort. The birth weight among infants born to mothers with NF1 was decreased compared to infants of mothers in the comparison cohort [-0.28 (-0.51 to -0.06)], as was the birth length [-0.22 (-0.45 to 0.00)].

**Conclusions:** The results show that NF1 of the mother is associated with reduced birth weight and length of the infant while NF1 of the child increases birth weight and head circumference.

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Cutaneous Adverse Events in SPRINT: A Phase 2 Trial of the MEK Inhibitor Selumetinib for Pediatric Patients with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PN)

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**Background:** MEK inhibitors have been associated with cutaneous adverse events (cAE) in patients (pts) with cancers and NF1. Patients with NF1 frequently remain on MEK inhibitor therapy for prolonged time periods. Characterization of cAE in this population may allow for earlier recognition of cAE and the development of effective treatment strategies. We characterize the cAE observed in the phase II trial (NCT01362803) of selumetinib (AZD6244, ARRY-142886) for pediatric pts with NF1 and inoperable PN.

**Methods:** The records of all pts enrolled on study between August 2015 and November 2017 were reviewed. The time of cAE development relative to start of study drug, the duration and the management of the cAE, and any consequence to the study drug as a result of the cAE (e.g. drug interruption, dose modification) were evaluated.

**Results:** Sixty-nine pts (age range 3.5 to 18.1 years old), received selumetinib (25 mg/m2/dose) BID for 1 to 29 cycles (median: 20, 1 cycle=28 days). Over the treatment duration, 372 individual cAE were documented in 65 pts. Ninety-seven percent (360/372) of the cAE were grade 1 or grade 2, and resulted in no changes to selumetinib dose or course. Three percent (11/372) of cAE were grade 3 and 1 cAE was grade 4. The most common cAE, acneiform eruption, accounted for 22% (80/372) of all cAE, was seen in 52% of pts, and first occurred early in treatment (median cycle: 3, range: 1-20). Management included topical or oral antimicrobial drugs and topical corticosteroid. Paronychia accounted for 20% (76/372) of all cAE, was seen in 35% of pts, and first occurred later in treatment (median cycle: 13, range: 1-25). Paronychia resulted in the highest number of drug interruption or dose reduction (n=7). Paronychia was initially managed conservatively with soaks, topical antimicrobials, oral antibiotics if necessary, and surgically when refractory. Less frequent cAE included eczema, xerosis, folliculitis, pigmentary dilution, hair thinning, and mucositis.

**Conclusions:** Cutaneous adverse events in children with NF1 and PN receiving selumetinib are frequent, but mostly low grade, and infrequently required dose modifications when addressed early. Characterization of the cAE will allow us to better understand, anticipate, and manage these events. Recommendations for standardized treatment approaches to the most frequent selumetinib related cAE, acneiform eruption and paronychia, are in development. This data is preliminary and data verification is ongoing.

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Brain Function During Emotion Processing in Children with Neurofibromatosis Type 1

Natalie Pride, Institute for Neuroscience and Muscle Research, The Children’s Hospital at Westmead; Sydney Medical School, The University of Sydney

Background: Emotion processing deficits are notable in children with Neurofibromatosis Type 1 (NF1) and are associated with poorer social skills and autism spectrum disorder traits. The underlying neural mechanisms associated with the social and emotional deficits in NF1 remains unclear. To further understand the neural substrates underlying emotion processing deficits in NF1 we evaluated cerebral blood flow response in the "social brain" network during a facial emotion processing task. We hypothesised that children with NF1 will have diminished activation in regions associated with facial perception.

Methods: Seventeen children with NF1 and nineteen typically developing (TD) children viewed facial displays of happiness, anger, fear and disgust as well as neutral faces. Functional magnetic resonance imaging (fMRI) was used to measure blood-oxygen-level dependent signal changes in response to 1) explicit processing of negative emotional expressions relative to neutral expressions and 2) explicit processing of individual types of emotions relative to neutral expressions.

Results: When processing negative emotional expressions, children with NF1 demonstrated hypoactivation in the right inferior occipital gyrus (rIOG) when compared to TD controls (FWE corrected, \( p \leq 0.05 \)). Group comparisons of individual emotions also showed significantly less activation of the rIOG and left fusiform gyrus during processing of happy expressions, the rIOG during disgusted expressions and the right superior temporal gyrus during angry expressions in children with NF1 (FWE corrected, \( p \leq 0.05 \)).

Conclusions: When processing facial expressions of emotions, children with NF1 show atypical activation of brain regions that are part of the core face perception network. These findings partially support the hypothesis that abnormal facial processing may contribute to emotion processing deficits in patients with NF1.

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Pallister-Killian Syndrome Variant Diagnosed in Workup for Segmental Neurofibromatosis Type 1

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**Background:** A 3-year-old boy was evaluated for neurofibromatosis type 1 (NF1) He had 6 café au lait macules (CALMs) present at 1 year of age, with more over time, and a history of developmental delay (DD). Ht/wt/HC were 75th-94th%iles. He had about 12 CALMs measuring more than 0.5 cm on his left upper body. No freckling or neurofibroma was noted. His father and brother, not present, were described as having several CALMs.

**Methods:** Two of the CALMs were biopsied for NF1/SPRED1 analysis including sequencing and copy number analysis on the cultured melanocytes as previously described (Maertens O. et al, AJHG, 82:243,2007). Due to his DD, we also obtained fragile X DNA and chromosome microarray (CMA) testing.

**Results:** Blood and CALM melanocyte testing for NF1 and SPRED1 and fragile X testing showed negative results. The CMA showed arr[GRCh37] 12p13.33p13.31(173786_6201932)x3, a 6.03 Mb copy number gain of the p13.33p13.31 region of chromosome 12. When he returned, he had more CALMs on his left side with a linear appearance, per lines of Blaschko. Chromosomes showed an abnormal male karyotype 46,XY,der(21)(t(12;21)(p13.3;q22.3), with a derivative chromosome 21 from an unbalanced translocation between chromosome regions 12p13.3 and 21q22.3; this results in a copy number gain (trisomy) for the region 12p13.3a12p13.33pter. Subtelomeric probes for chromosome 12 showed a copy of the 12p subtelomere signal at the end of the long arm of chromosome 21, confirming the karyotype findings. Parental studies and examination of father and brother were underway to determine the inheritance of the chromosome abnormality and potential association with the pigmented findings.

**Conclusions:** Numerous disorders are in the differential diagnosis (DDx) of segmental NF1, also known as localized mosaic NF1 (Wimmer K et al, Clin Gen, 2016). Pallister-Killian syndrome (PKS) has not been included. Typical PKS is mosaic tetrasomy 12p, with the extra chromosome 12 material present as a marker. Recently patients have been reported with nonmosaic trisomy 12p, introducing the concept of a critical region for the PKS phenotype. We know of no other trisomy 12p individual described with café au lait macules in lines of Blaschko associated with the mosaic tetrasomy 12p phenotype. Based on this, we suggest PKS be considered in the DDx of patients with segmental CALMs and that patients with the appearance of segmental NF1 be fully investigated for segmental NF1 and other disorders.

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Evaluation of Behavioral Outcome Measures of a Trial for Adolescents with Neurofibromatosis Type 1

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**Background:** Although Neurofibromatosis type 1 (NF1) is defined by somatic symptoms, cognitive and behavioral disorders are among the most significant manifestations for patients and their families. Advances in understanding the underlying mechanisms of deficits in NF1 have led to a running registered randomized double-blind controlled trial aiming at determining the effect of lamotrigine on cognition in adolescents (aged 12-17.5) with NF1. The current study uses the baseline data from this study to evaluate the relevance, interconnection, and independence of the selected outcome measures.

**Methods:** Selected outcome measures were intelligence, visual perception, motor skills, attention, parent-rated symptoms of attention-deficit/hyperactivity disorder (ADHD), parent-rated executive function, and level of education. Spearman’s correlations were computed and a principal component analysis was performed to explore the relationships between the outcome measures. An ordinal logistic regression analysis was used to determine the predictive value of the outcome measures for the level of education of the adolescents.

**Results:** Performance IQ, verbal IQ, visual-motor skills, and visual perception skills were strongly associated. In a second factor, parent-rated executive functions, parent-rated ADHD symptoms, and motor functions were strongly associated. Furthermore, intelligence (p < .001) and parent-rated executive function (p < .01) were found to be significant predictors of level of education, together explaining 66% of the variance. None of the other outcome measures had a significant additional predictive value for level of education.

**Conclusions:** Next to well-known criteria for the selection of outcome measures, new criteria can be derived from studies focusing on delineating the phenotype of NF1, factors impacting on daily life, and the association of outcome measures within trials. These criteria can supplement recommendations from the ‘Response Evaluation in Neurofibromatosis and Schwannomatosis’ (REINS), an international collaboration aiming for standardization in clinical trials.

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Could Musical Training Improve NF1 Social and Cognitive Deficits? An Ongoing Study

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Background: Neurofibromatosis type 1 (NF1) affected individuals present pleomorphic clinical features including cognitive deficits. Previous studies have showed auditory processing disorder (APD) and amusia in NF1 (Cota, et al., 2018). We have hypothesized if musical training could improve the DPA, amusia and other cognitive deficits.

Methods: We have just initiated the first phase of the experimental study with 30 NF1 (at least three diagnosis criteria) male and female (50%) teenagers (12-16 y). The volunteers will be evaluated before, during and after 6 month of weekly-supervised musical training by clinical and psychological experts and submitted to phono audiological, electrophysiological and musical validated tests. The tests used are Montreal Battery of Evaluation of Amusia (MBEA) for musical perception; Gaps In Noise (GIN) for APD measurements; Multiplex Ligand-dependent Probe Amplification (MLPA) to detect whole NF1 gene deletion; Magnetic Resonance Image for T2/FLAIR hyper intensities measurement; Mismatch Negativity (MMN) for auditory electrophysiological function measurement. The cognitive evaluation will take in account the intelligence quotient, inhibitory control and selective attention, executive functioning and teenager’s social abilities. After initial evaluation, all volunteers will be randomly divided in two groups, one of them will receive the musical training and the other will wait as a control group. After six month, there will be a new global reevaluation and cross treatment change. After 12 months, all volunteers will be reevaluated and the data processed statistically (see Figure 1).

Results: We have just started the initial phase in May 2018. We expect to contribute to a better understanding of NF1 cognitive deficits and, perhaps, to offer a new therapeutic intervention to improve life quality of NF1 affected individuals.

Conclusions: The study is ongoing and the present presentation is a chance to discuss it with NF1 experts during NF meeting.

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A New Surgical Approach to Multiple Compressive Cervical Spinal Neurofibromas

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Background: Neurofibromatosis type 1 (NF1) is a genetic autosomal dominant disease with full penetrance and some affected individuals present cervical spinal neurofibromas (CSN) with progressive limb weakness and neuropathic pain due to compressive myelopathy. Traditional neurosurgical treatment has been just one tumor excision via opened dura mater, which increases fistulae risk and requires further surgeries.

Purpose: To report two cases of NF1 patients with neuropathic pain and tetraparesis caused by myelopathy secondary to multiple level CSN. They were treated using a new approach with multiple CSN excisions in just one-time surgery, without opening dura mater and avoiding subarachnoid invasion.

Methods: Two NF1 teenagers (ELL, 18 y, female and PIB, male, 15 y) with inability to walk (MF2/2) were treated with median cervical large posterior incision with total laminectomy (C1-C5), bilateral subtotal facetectomy performed with high speed drill (C3-C6) without vertebral artery visualization to exposed all lesions spots. Using microscopy, cross open incisions were made in the posterior center of neurofibromas, with sharp dissection and fine separation of the tumor from the dura and avoiding arachnoid damage. We did not use traditional tumor forceps or scalpel and the excisions were done using ultrasound aspiration device to break tumors and avoid traction. The preservation of the anterior motor root was the main objective, achieved with multiple neurophysiological stimuli that guided the limit of excision. The mean surgery time was 4 hours to do 5 (ELL) and 7 (PIB) tumor resections (total or partial).

Results: Both patients achieved total pain suppression, recovering locomotion and a near normal quality of life. They did not present fistulae, pseudo meningoceles or infection. The control imaging studies showed absence of compressive myelopathy. The main handicaps of this new surgical approach were longer surgical time with higher blood losses, lasting intensive treatment therapy for recovery and transient orthopedic cervical collar until arthrodesis to prevent pseudo arthrosis. Further cases could be submitted to a small number of spinal neurofibromas excision to reduce these undesirable side effects.

Conclusions: The present new surgical technique of exclusive extra-arachnoid approach seems to innovate the surgical treatment of compressive neurofibroma myelopathy and deserve further studies.

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New Correlation Between NF1 Whole Gene Deletion and Toe/Foot Phenotype

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Background: Neurofibromatosis type 1 (NF1) is a genetic autosomal dominant disease with full penetrance. NF1 is characterized by neurocutaneous and osseous dysplasia, cognitive deficits and behavioral disorders and multiple benign and malign tumors (Rodrigues, et al., 2014). NF1 is usually caused by mutations in the NF1 gene, but 5-10% of NF1 probands present with NF1 whole gene deletions (WDGs) (Huson, 2011). The NF1 deletion (or microdeletions) is associated with more severe forms of the disease (Mautner, Kluwe, & Friedrich, 2010). A novel toe/foot phenotype in nearly 10% of NF1 persons was identified: an “upright” second toe, micronychia, large feet and increased soft tissue, which was called Second Toe Signal (STS) (Faria, Rodrigues, Diniz, Rezende, & Rodrigues, 2012) (Figure 1). A further study showed association between STS and WGD (Rodrigues, et al., 2017).

Methods: In the present study, new blood genomic analyses were done by Multiplex Ligand-dependent Probe Amplification (MPLA) and correlated with 41 NF1 persons and their foot phenotypes and clinical presentation.

Results: The results showed that STS in a NF1 person indicates a 5 times greater chance of NF1 WGD [(STS with 52% chance of NF1 WGD versus a 9.0% chance of NF1 WGD in the absence of STS (p<0.01)] (Figure 2). The clinical presentations of 12 NF1 people with NF1 WGD were consistent with previous reports on emerging genotype-phenotype relationships in patients with large NF1 deletions, the most frequent recurring mutation in NF1 disease (Kehrer-Sawatzki, Mautner, & Cooper, 2017).

Conclusions: We conclude that the STS phenotype is a clinical marker to instigate further genetic analysis (MPLA) to afford better management of NF1 persons.

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Figure 1 – Typical Second Toe Signal (STS) in an NF1 patient with an NF1 whole gene deletion, showing upright second toe, micronychia, large feet and increased soft tissue.

Figure 2 – Association between Second Toe Signal and NF1 whole gene deletion (WGD). The NF1 WGD was found in 52% of positive STS phenotype and in 9.5% of negative STS phenotype (p<0.01).
Association Between NF1 Toe/Foot Phenotype and Congenital Tibia Dysplasia

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disease with full penetrance. NF1 is characterized by neurocutaneous and osseous dysplasia, cognitive deficits, behavioral disorders and multiple benign and malignant tumors (Rodrigues, et al., 2014). Nearly 10% of NF1 individuals present the more severe disease forms caused by NF1 whole gene deletion (WGD) (Kehrer-Sawatzki, Mautner, & Cooper, 2017). A novel toe/foot phenotype was identified in nearly 10% of individuals with NF1 described as: bilateral “upright” second toe, micronichya, large foot and increased soft tissue, which was called Second Toe Signal (STS) (Faria, Rodrigues, Diniz, Rezende, & Rodrigues, 2012) (Figure 1). An ensuing study showed association between bilateral STS and WGD (Rodrigues, et al., 2017)

Methods: To describe STS phenotype in NF1 individuals with tibial dysplasia (TD)

Results: We have observed 11 NF1 individuals (10 with unilateral TD e 1 with bilateral TD) and 9 of them presented simultaneously with STS phenotype (6 of them with ipsilateral STS and 3 with bilateral STS). Only one of these NF1 individuals with unilateral STS and TD underwent DNA analysis (MLPA) and it was negative for germline whole gene deletion.

Conclusions: STS was previously associated with large NF1 deletions and its coexistence with unilateral tibial dysplasia lead us to hypothesize that ipsilateral STS and TD could result from a mosaic somatic post zygotic whole gene deletion (type 1, 2 or 3) in the affected member. These results deserve further investigation, including DNA analysis both in the blood as well as in some affected member tissue sample.

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What Do People with Neurofibromatoses Look For? - Analysis of More Than 250 Thousand Visitors to a Blog of Medical Information

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Background: Search for internet medical information is a common behavior, especially for people with rare diseases, which are looking for health professionals and treatment. Neurofibromatoses (NF) are rare diseases, with great diversity of signs, symptoms and clinical complexity and they have few health professionals with adequate knowledge for its diagnosis and follow-up. To provide NF information based on scientific evidence we have created a blog (http://amaf.org.br/blog/) in Portuguese. The blog has been active since 2015 and has received about 15 thousand visitors per month.

Purpose: to evaluate 262,679 blog visitation sessions (8/5/2015 to 11/17/2017) during 30 months.

Methods: The visitors were studied through Blogger's analysis system and Google Analytics to identify the most sought topics among the 405 published, as well as some user’s characteristics.

Results: Most of the visits were made by people located in Brazil (83%), using Portuguese Language (92%) in smartphones (54%), accessed from São Paulo (10.8%), Belo Horizonte (9.7%) and Rio de Janeiro (5.8%). The most sought topics were information on Type 1 Neurofibromatosis (NF1 – OMIM # 162200), especially on the diagnosis of the disease (50.9%) and life expectancy (20.3%). Other topics have aroused interest like life expectancy (20.3%), psychiatric medicines (13.5%), effects of nutrition (5.2%) and treatment (neurofibromas) (3.9%), doctor/patient relationship (2.9%) and disease prognostic (2.3%). The mean of all other access was 1.1%. (Figure 1). Most of the access occurred using Android (44%) and IOS (10%), indicating that half of the people have used cell phones to access the site. The average duration of visits was 3 minutes due to a) low interest aroused by most of the published topics; or b) shallow reading of most subjects; or c) inadequacy of the language of the blog to the level of formal instruction of internet users, or all these causes together.

Conclusions: The blog with scientific medical information on neurofibromatoses attracted thousands of visitors, who were especially interested in the diagnosis of the disease. Short on reading suggests the need to review and simplify selection of topics of greatest interest.

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Evaluation of a Neuropsychological Training Programme for Children with NF1 Focussing on Attention and Behavioural Problems

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Background: Many studies have shown that children with Neurofibromatosis Type 1 (NF1) suffer from a higher risk of developing learning difficulties and behavioural problems. Neurocognitive impairments are common and deficits in attention are often associated with the disease. Impacts on daily life seem to be a sequel as the children are often lacking appropriate strategies to regulate their behaviour. Neuropsychological training of cognitive abilities may help reducing attention problems, but there is little research on such treatment options. Therefore the present study aimed at identifying benefits of a neuropsychological training programme with respect to different aspects of attention and behaviour.

Methods: We developed a neuropsychological training programme specialized for groups of children with NF1, targeting distinct neurocognitive and psychosocial domains often found to be impaired in these patients. We compared and evaluated the data of 12 children (ages 5 through 18), who are treated at the Department of Paediatrics and Adolescent Medicine (Medical University of Vienna), and their parents. The children were split into three different age groups and received an age-dependent neuropsychological training. We used qualitative and quantitative measurements to detect early changes in quality of life, self-awareness, school participation and the children’s abilities in solving problems. Furthermore, we assessed stress levels and if the diagnosis affected everyday life. Questionnaires were completed by the children and their parents before and after the training and three months later to identify long-term effects.

Results: The questionnaires returned with a rate of 83.3%. The results confirmed behavioural changes in the treated children. Parents reported distinct improvements in the children’s behaviour and self-awareness. The children developed strategies regarding attention maintenance and behavioural regulation.

Conclusions: The findings indicate that there is a high need for neuropsychological training programmes in children with NF1. The implementation of such a programme appears to be a fundamental step towards a standard-of-care for patients with NF1. Nevertheless, current results must be interpreted with caution due to a small sample size and a lack of controls.

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Disclosure of Interest: V. Rosenmayr has a conflict with: We have no conflicts of interest to declare. The project was realized by the fiscal support of the "Gemeinsame Gesundheitsziele aus dem Rahmen-Pharmavertrag", a cooperation of the Austrian pharmaceutical industry and social insurance fund., N. Obergfell: None Declared, V. Fohn: None Declared, U. Leiss: None Declared, A. Azizi: None Declared, I. Slavc: None Declared, T. Pletschko: None Declared
Substantial Pain and Reduced Quality of Life (QOL) in Adolescents and Young Adults (AYA) with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PNs) Enrolled in NF Consortium PN Clinical Trials

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Background: PNs can cause pain, disfigurement, and neurologic deficits. To understand the impact of PNs on daily functioning and QOL in AYA with NF1, we examined patient-reported outcomes collected prior to treatment in PN clinical trials.

Methods: All patients >16 years with NF1 and symptomatic, inoperable PNs, who enrolled on NF Clinical Trials Consortium PN treatment trials (NCT02101736, NCT02096471), completed a background information form, the Numeric Rating Scale (NRS-11), Brief Pain Inventory (BPI) Pain Interference Scale, and NF1 PedsQL Scale at baseline.

Results: Thirty-eight subjects (20 M, 18 F; median age=23 years; 16-39) participated. Sixty-eight percent completed high school or some college, but only 32% were employed; 42% took pain medication regularly with 23% taking prescription medication. Subjects rated tumor visibility as mild (40%), moderate (47%), or severe (13%) and NF1 symptoms as mild (26%), moderate (50%), or severe (24%). On the NRS-11 (0-10 scale), mean overall tumor pain was 5.3 (+3.0) with 90% rating some level of tumor pain (21% mild, 29% moderate, 40% severe). On the BPI (0-10 scale), general pain interference in daily life was 3.0 (+2.9). On the NF PedsQL, the mean Total Functioning Score (0-100 scale; higher scores=better QOL) was 68.1 (+19.6). Baseline PN volume correlated with total functioning (p<0.05) but not pain scores; total functioning correlated with pain intensity and pain interference (each p<0.01). Most affected QOL domains were physical functioning, worry, pain/hurt, and fatigue while least affected were skin irritation, daily activities, and treatment anxiety. Self-selected tumor pain, overall tumor pain, pain interference, total functioning, physical functioning, communication, pain/hurt, movement/balance, daily activities, fatigue and treatment anxiety were significantly worse in those with more severe ratings of NF1 symptoms (each p<0.01). Participants not regularly using pain medication had significantly worse tumor pain, pain interference, total functioning, worry, pain/hurt, and paresthesias, and tumor pain was significantly worse in women compared to men (each p<0.01). There were no significant differences in any domain between employed and unemployed participants.

Conclusions: PNs in AYA with NF1 are associated with substantial pain and reduced QOL, highlighting the need for effective drug therapies as well as interventions to help cope with symptoms, engage in daily activities, and improve QOL.

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Clinical and Radiological Efficacy of Trametinib in Plexiform Neurofibromas in Patients with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is a neurocutaneous genetic disorder with an incidence of approximately 1 in 3,000 live births. 50% of these patients may develop plexiform neurofibromas (PNF).

PNF, although considered as benign lesion, can cause substantial morbidity and has potential malignant transformation to MPNST.

The only effective treatment for PNF is surgical resection, which is not possible in most cases due to its infiltrative condition and the potential post-surgery neurological sequels.

Based on animal model results about the utility of MEK inhibitors, the NCI group preliminary results in a pediatric group with risk PNF, and the low efficacy with other biological therapies; our group started treatment on a compassionate use with Trametinib in patients with symptomatic unresectable PNF followed up by the Neurocutaneous Disorders Unit in HSJD.

Methods: From September 2015 to the present, 24 patients with symptomatic unresectable NF1 and PNF have started Trametinib (MEK inhibitor) and were on treatment for more than 6 month. We evaluated clinical and radiological response of targeted lesions, in obedience to the REINS criteria,

Radiological response was assessed by MRI volumetric analysis contrasting images at diagnosis and after 6 months treatment. To evaluate the clinical response we made a survey assessing pain intensity, pain interference with daily life and perceived physical and functional appearance. Both of them according to REINS recommendations

Results: Tumor progression is not demonstrated in any of the patients but 2 (one of them with mixed response). In a significant percentage of them (80%) clear tumor volume reduction was demonstrated and almost all of them experiment a clinical and emotional benefit.

Conclusions: Trametinib is a useful drug in patients with symptomatic unresectable plexiform neurofibromas.

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Safety and Acceptability of Trametinib in Pediatric Patients with Neurofibromatosis Type 1: Experience Based in a Case Series

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**Background:** Describe our experience about the safety and acceptability using trametinib in pediatric patients with neurofibromatosis type 1.

**Methods:** Description of the side effects, safety, and treatment acceptability in a case series of 25 pediatric age patients with neurofibromatosis type 1.

These patients were included in the trametinib compassionate use program for pediatric patients of Novartis.

Novartis provided the trametinib powder for the preparation of oral suspension (0.05 mg/ml for 90 ml suspension) in ground-glass bottling.

Treatment indications were unresectable plexiform neurofibromas in 24 patients (6 of them also with an optic pathway glioma) and 7 patients with only optic pathway gliomas (3/7 with also a symptomatic brainstem glioma).

The side effects and safety data were collected by a retrospective study reviewing thoroughly the clinical histories of each patient, checking up on this information through an interview with the caregivers. The information about the acceptability (including syrup reconstitution, palatability, flavor and administration) was also appraised during the interviews.

The side effects were assessed and reported in accordance to the National Cancer Institute Common Terminology Criteria of Adverse Events (CTCAE v4.03, 2010) definitions.

**Results:** 31 patients (ages: 21 months-17 years old, average age 8 years old, 15 boys) were treated from September 2015 to nowadays, with at least 3 months of treatment duration.

The most frequent side effects reported were cutaneous toxicity, gastrointestinal disease and epistaxis. It should be pointed out 5 bone fractures.

The vast majority of the reported side effects were grade 1 and 2 of the CTCAE scale and they were sorted out and well managed with support treatments.

In 2 patients it was necessary to permanent discontinue the treatment (both skin toxicities grade 3).

The treatment was satisfactorily accepted in almost all the patients.

**Conclusions:** Trametinib powder for oral suspension was satisfactorily accepted and tolerated. The majority of the side effects reported were mild and responded to supportive treatments.

Security and efficacy should be validated by clinical trials.

We consider important and necessary to register the side effects of targeted therapies because the lack of awareness of the potential long-term side effects.

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Management of Itch in Neurofibromatosis Type 1 (NF1): A Single-Centre Experience

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**Background:** Previous studies have reported a high frequency of itch amongst people with Neurofibromatosis Type 1 (NF1). Most authors concur it is most consistent with neuropathic itch, but there remains a paucity of literature regarding the management for this symptom. Here we report on the characteristics and management of itch in NF1 from a single-centre experience.

**Methods:** As part of routine clinical care since 2017, patients attending an NF1 clinical genetics dermatological service (skin clinic) in Sydney, Australia, were questioned regarding their experience of itch and previous treatments. Patients who reported itch were offered treatment based on accepted intervention for neuropathic itch, and outcomes were recorded.

**Results:** 72% (21 of 29) of patients described itchiness of the skin, with ranging severities (29% mild, 43% moderate, 29% severe). Frequency of symptoms varied greatly across individuals and distribution was reported to be generalised or localised at particular regions of the body or to cutaneous neurofibromas. Heat was identified as a strong exacerbator of itch. Most patients reported previous treatments with antihistamines, topical steroids and emollients, with little to no effect. Seven patients commenced treatment with a low dose tricyclic antidepressant, and five reported improvements. Side effects included weight gain, sedation and a wearing-off effect. Mild symptoms, unacceptable side effects and medication stigma formed barriers for treatment uptake.

**Conclusions:** Treatment of NF1-associated itch with low dose tricyclic antidepressant was effective treatment in most patients. Findings of this study will enhance our understanding of itch in NF1 and inform clinical management.

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Third Case of Genetically Confirmed Paternal NF1 Germ Cell Mosaicism

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**Background:** The neurofibromatosis type 1 (NF1) gene exhibits one of the highest mutation rates of human disease genes indicated by the sporadic occurrence of NF1 in ca. 50%. Mosaicism is suspected in at least 10% of sporadic NF1 patients. Somatic and gonado-somatic NF1 mosaicism may clinically present as (mild) generalized, segmental or oligo-symptomatic NF1. Germline mosaicism however only comes to attention in families with more than one affected child born to unaffected parents. Very few of such families have been described so far and only in two of them germline mosaicism was molecularly confirmed.

**Methods:** A third case of germline mosaicism is presented here. The first child of unaffected parents had been diagnosed with NF1 caused by a recurrent NF1 mutation (p.Arg304*). Having been made aware of the rare possibility of germline mosaicism, the couple opted for prenatal testing in the second pregnancy, despite the absence of the mutation in their blood lymphocytes.

**Results:** The NF1 mutation p.Arg304* was also present in chorionic villi sampling of the second pregnancy. Sanger sequencing confirmed presence of the mutation in the father’s sperm cells. Subsequently performed amplicon-based deep sequencing confirmed that approximately 9% of sperm cells carried the mutation that was undetectable in other tissues of the father, representing the three germ layers (blood lymphocytes, buccal mucosa and urothelium cells).

**Conclusions:** The majority of intragenic NF1 point mutations are expected to arise in paternal germ cells. Hence, offering analysis of the father’s spermatozoa should improve recurrence risk assessment in future cases and uncover the frequency of NF1 germline mosaicism.

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Pain in Children and Adults with Neurofibromatosis 1

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**Background:** Pain is a common feature in patients with Neurofibromatosis 1 (NF1). However, little information is available about prevalence, etiologies, effects, location, and interference in the broad NF1 population. To address this lack of knowledge, surveys addressing pain types, chronicity, and interference were administered to pediatric and adult NF1 patients and controls.

**Methods:** Patients (90) and Controls (112) were recruited at several locations, including a local NF Clinic, a local NF Family Symposium, and an NF Walk. Measures included the Numeric Rating Scale 11 (NRS11) for pain intensity; NIH PROMIS (pediatric and adult forms) for pain interference; and a supplemental questionnaire for pain characteristics, frequency, and demographic features.

**Results:** Data were collected from 51 children with NF1 (mean age 12.1 years); 20 pediatric controls (mean age 11.4 years); 39 adults with NF1 (mean age 32.6 years) and 92 unaffected adult controls (mean age 41.5 years). Regularly-occurring pain was reported in 54.9% of children with NF1 versus 20% of Controls (p=0.009); and in 74.4% of NF1 adults versus 52.8% of adult controls (p=0.022). Chronic pain, defined as pain lasting longer than 3 months in the past year, was seen in 27.5% of NF1 children and 74.4% of NF1 adults. Migraine headaches were reported in 25.5% of NF1 children and 38.5% of NF1 adults, both significantly higher than control groups. Tumor-related pain occurred in both NF1 children and adults, but was more frequent in adults; it was not reported in controls. Frequent itching was seen in 33.3% of NF1 children and 59.0% of NF1 adults. Over 40% of children and adults had used some type of pain medication. Children and adults with NF1 reported greater pain-related interference with daily activities compared to controls. Of importance, anxiety/depression was seen at an elevated rate in children and adults with NF1 (29.4% children, 53.9% of adults), and was associated with chronic pain (p<0.026).

**Conclusions:** Pain occurs at a high rate in a general NF1 population, with frequent chronic pain and interference with daily life in both children and adults. Migraine headaches and tumor-related pain are common sources of pain. Chronic pain is strongly correlated with anxiety and depression in both children and adults with NF1. Further research is needed on best management modalities for pain in the NF1 population.

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Features of Dural Ectasia in Neurofibromatosis Type I

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**Background:** This study aims to establish the prevalence of dural ectasia in patients with complex NF-1 and the possible aetiological link with key spinal deformities that manifest alongside. It also aim to establish which deformities progress over time and compare the burden of these deformities on the different sections of the spine. The lack of available research surrounding this key complication of NF-1 and its link to abnormal curvatures of the spine has prompted this specific study.

**Methods:** The neuroradiological notes of 378 complex NF-1 patients were reviewed from 24 months of multidisciplinary team meetings (from one of two specialist centres for NF-1 in the UK). Data relating to patients with dural ectasia and spinal deformity was obtained and statistically interpreted. The MRI films of all patients dural ectasia were reviewed and graded according to the severity. An attempt to establish a link between severity and concurrent deformity was made.

**Results:** Of these 378 patients, 38 (10.05%) were identified as having spinal dural ectasia and 103 (27.25%) of these patients were identified as having a spinal deformity of some degree. 90.91% of the patients with a major form of dural ectasia had a concurrent deformity whereas only 18.18% of patients with a minor form of dural ectasia had a concurrent deformity.

**Conclusions:** In conclusion, the more severe the dural ectasia, the greater the likelihood a concurrent spinal deformity will be present in patients with NF-1. The vertebral bodies and pedicles are more commonly involved than the posterior elements.

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Complex Coordination of Care Across a Spectrum of Neurofibromatosis in One Family: Utilization of Resources in a Neurology Neurofibromatosis Type I Clinic Complementing a Multidisciplinary Program at a Larger Tertiary Care Center

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**Background:** Our institution has a Neurofibromatosis Type 1 (NF1) Multidisciplinary Clinic (MDC) and Neurology NF Clinic to manage patients with NF related Neurology concerns. The MDC combines providers from neurology, oncology, neuropsychology, genetics, rehabilitation, and ophthalmology and is designed for patients with moderate to severe symptoms from NF1. Patients in the MDC require at least three of the specialties present. The Neurology NF Clinic sees patients who need coordinated care but less than three specialties as well as medically complex patient unable to wait the length of time to be seen in MDC and requiring triage to specialties more rapidly.

In the Neurology NF1 Clinic, there is a specially trained Neurology Advanced Practice Provider (APP) and the nurse coordinator. The APP provides a medical assessment, recommends testing, and is able to make referrals to appropriate specialties with the nurse coordinator facilitating education and ensuring recommendations are followed through.

Neurofibromatosis is a spectrum disorder with a variety of phenotypes seen within the population. As a result, affected members in the same family may have variable needs of care. We present a family requiring complex coordination of care in three siblings with NF1.

**Methods:** In this family, there are 11 year old male, 14 year old female, and 18 year old male siblings who travel from a significant distance (over 3 hours away from clinic) with a range of concerns including: migraine, non-epileptic spells, intellectual disability, ADHD, learning concerns, anxiety, depression, insomnia, urinary incontinence, and abnormal imaging monitoring. These needs were addressed with monitoring, referrals, testing coordination, and discussion at their Neurology NF appointment.

**Results:** For this complex family with varying needs, the infrastructure in place in this clinic has provided enhanced and coordinated care for these NF1 patients.

**Conclusions:** Improving access to care with a rapid paced clinic for complex needs patients is effective and beneficial to families. This clinic role is crucial in improving and developing a NF1 multidisciplinary program with in a large tertiary care center. The nurse coordinator role is essential to ensuring success of the clinic and follow through with various recommendations.

Headaches in Neurofibromatosis Patients: What is Different about Headache Management in the Pediatric Neurofibromatosis Population

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**Background:** Our institution has a Neurofibromatosis Type 1 (NF1) Multidisciplinary Clinic (MDC) and Neurology NF Clinic to manage patients with NF related Neurology concerns including headaches. Headaches are a common complaint in the general and NF populations with a higher incidence of hydrocephalus or aqueduct stenosis noted in the NF population. In addition, there are other concerns including sphenoid wing dysplasia and cerebrovascular diseases in NF which require a thorough evaluation in the NF patient.

**Methods:** Treating headaches in NF1 patients is similar to those in the general population. They typically respond well to standard preventive and abortive care. Genetics and family history are contributing factors as well as lifestyle modifications. Due to the increased risk of complications related to NF, a lower threshold for MRI and vascular imaging should be utilized in this patient population. Monitoring for signs of ICP including papilledema and changes on scans is also necessary to fully characterize the correct diagnosis.

**Results:** Headaches in NF patients require a lower threshold for imaging of the brain and vascular structures than the general population. Special attention should be paid to increased intracranial symptoms and pressure.

**Conclusions:** Headaches in NF patients have similarities to the general population but require increased vigilance and a higher level of attention and evaluation to rule out secondary causes which are less frequent in the general population.
Clinical Characteristics of Patients with Neurofibromatosis Type 1 and Phaeochromocytoma

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Background: Phaeochromocytoma are rare tumours of the adrenal medulla and extra-adrenal chromaffin tissue which secrete catecholamines. They occur in less than 0.2% of patients with hypertension. The frequency of phaeochromocytoma in patients with Neurofibromatosis type 1 (NF1) varies widely and is quoted to occur in ~0.1% - 5.7% of patients.

Methods: We performed a retrospective review of adult NF1 patient records who were diagnosed with phaeochromocytoma in a nationally commissioned NF1 service from 2009 - 2017. We reviewed patient demographics, male to female ratio, age at diagnosis, percentage of patients with cardinal symptoms, duration of symptoms and presence of other symptoms.

Results: Our preliminary review of 1400 adult NF1 patients has shown 11 patients (0.79%) were diagnosed with a phaeochromocytoma. All patients clinically had generalised NF1. Average age at diagnosis was 36 years (range 21 – 61 years) with males and females equally affected. Average duration of symptoms preceding diagnosis was 19 months. All phaeochromocytomas were adrenal and unilateral. 3 (27%) were malignant. One case was diagnosed incidentally on MRI scan. All other cases were diagnosed as the patient was hypertensive and symptomatic. One patient reported all 3 cardinal features of phaeochromocytoma, namely: headache, palpitations and sweating. 3 patients had 2 cardinal symptoms, 2 patient had 1 cardinal symptom, and 2 patients did not have any cardinal symptoms. Other symptoms reported were weight loss (3 patients), panic attacks (2 patients) and visual symptoms (3 patients). 10 (91%) patients had sustained hypertension. One patient had an isolated mildly elevated blood pressure but a subsequent 24-hour ambulatory blood pressure study was normal.

Conclusions: The frequency of NF1 related phaeochromocytoma in our cohort of patients is 0.79% and is lower than the frequency reported in some of the medical literature. Phaeochromocytomas in patients with NF1 present with a wide range of symptoms and none of the cardinal features may be present. We advise clinicians to have a low threshold for screening for phaeochromocytoma in patients with NF1 and hypertension with any of the cardinal symptoms (headache, palpitations and sweating) or other reported symptoms in our cohort (weight loss, panic attacks and visual symptoms). Where there is a clinical suspicion of phaeochromocytoma but 24-hour ambulatory blood pressure monitoring is normal, screening should still be considered as hypertension maybe paroxysmal.

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Change in Tumor Enhancement on Magnetic Resonance Imaging is Not a Predictor of Optic Pathway Glioma Disease Progression in Children with Neurofibromatosis Type 1 (NF1)

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Background: Optic pathway gliomas (OPGs) arise in 15-20% of children with NF1, but less than half will require therapy. Treatment is usually reserved for patients with documented progression; however, what constitutes meaningful progression warranting treatment is controversial. Common indications for treatment include decline in visual acuity and/or tumor progression by MRI (usually defined by increase in size). However, changes in tumor enhancement on post-gadolinium MRI sequences have also been used by some as a marker of tumor progression. No prior studies have specifically determined the clinical significance of change in tumor enhancement for OPG.

Methods: We conducted a single center retrospective analysis of children with NF1 managed at the Children’s Hospital of Philadelphia who had an OPG diagnosed between 01/01/2005 and 06/30/2016. Eligibility included enhancement of the OPG during the study period and a sufficient number of scans after enhancement was noted. Subjects were divided based on whether there was an increase in enhancement (defined as an increase in area of enhancement) or not. Change in overall tumor size (by T2 sequences) concurrent with change in enhancement was recorded. Primary endpoint was whether the subject required OPG-directed treatment.

Results: 82 subjects were enrolled (28 M, 54 F); median age at OPG diagnosis 32.5 months (range 5 – 147 months). 33 subjects had an increase in enhancement during the study period; 49 subjects were in the stable enhancement group. Subjects with an increase in enhancement were not more likely to require therapy in the year following the enhancement increase than those whose enhancement pattern remained stable (p=0.55). Of note, all of the children who had an increase in enhancement who required subsequent treatment also had a concurrent increase in tumor size at the time of increased enhancement. We are presently analyzing whether increase in enhancement predicts a decline in visual acuity in the year following the change in enhancement.

Conclusions: In the absence of a change in tumor size, an increase in OPG enhancement does not predict the need for initiation of tumor treatment in the following year. Thus, management decisions for NF1 OPG should not be made on the basis of tumor enhancement.

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Long-Term Outcomes of Radiation Therapy (RT) in the Management of Malignant Peripheral Nerve Sheath Tumors (MPNST) in Patients with Neurofibromatosis Type 1 (NF1)

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Background: There is limited information regarding outcomes of patients with NF1-associated MPNST, and specifically after radiation therapy (RT). Our primary objective was to determine the local control (LC) rate in patients with NF1-associated MPNST treated with RT. We also assessed overall survival (OS) and frequency of toxicities following RT. We hypothesize that RT is an effective and safe treatment modality in patients with NF1 with MPNST.

Methods: This is a retrospective review of patients treated at our institution with the diagnosis of NF1 and pathologically-confirmed MPNST who underwent definitive RT from 2009-2017. Patients without follow-up or who had a history of previous RT to the affected area were excluded. Kaplan–Meier curves were used to estimate LC and OS rates.

Results: 15 patients (F=9, M=6) were identified with median age of 25 (range 10-38) years. The median follow up was 29.1 (9.9-84.5) months. 47% of patients had tumors located in an extremity and 53% within the trunk. Four MPNSTs were <5 cm (27%), five 5-10 cm (33%) and six >10 cm (40%). All patients received initial therapy for a new diagnosis of MPNST, except one treated for recurrent disease after resection only. Patients were treated with several paradigms: 13% surgery + adjuvant RT, 33% surgery + adjuvant systemic therapy (ST) and RT, 40% neoadjuvant ST and RT + surgery, 7% interdigitated chemotherapy and neoadjuvant RT + surgery, 7% ST and RT. The median RT dose delivered was 50 Gy (44-63 Gy) via IMRT (87%), SBRT (7%), or 3D (7%). LC was estimated to be 100%, 91.7%, and 91.7% at 1, 3, and 5 years, and OS was identified as 100%, 79.2%, and 53.2% at 1, 3, and 5 years, respectively. Three patients progressed distantly (brain=2, lungs=1) and two developed second primaries (second MPNST, gliosarcoma). The most common toxicities at the end of RT were grade 1 fatigue and grade 1 pain. No secondary malignancies within the radiation field were reported.

Conclusions: In our study, therapeutic strategies that include RT resulted in excellent LC rates and were well tolerated for NF1-associated MPNST. Given the paucity of data about safety and efficacy of RT for NF1-associated MPNST, this dataset is encouraging, as outcomes were comparable to those reported for other types of soft tissue sarcomas. However, longer duration follow-up and larger cohorts are required to characterize the risk of second tumors and develop optimal approaches regarding timing and RT delivery.

Schwannomatosis: A Genetic and Epidemiological Study

Miriam J. Smith, The University of Manchester, Manchester, United Kingdom

Background: Schwannomatosis is a dominantly inherited neurogenetic condition predisposing to schwannomas that occur predominantly on the spinal and peripheral nerves. Schwannomatosis has some diagnostic overlap with neurofibromatosis type 2 (NF2), but the underlying epidemiology is poorly understood. Here, we present regional (population=4.8 million) birth incidence and prevalence for schwannomatosis and NF2.

Methods: Cases of schwannomatosis and NF2 were ascertained. Point prevalence and birth incidence were calculated from regional birth statistics. Genetic analysis of NF2, LZTR1 and SMARCB1 was also performed on blood and tumour DNA where samples were available.

Results: Regional prevalence for schwannomatosis and NF2 were 1 in 126,315 and 1 in 50,000, respectively. Calculated birth incidences were 1 in 68,956 and 1 in 27,956, respectively. Mosaic NF2 causes a substantial overlap with schwannomatosis, resulting in the misdiagnosis of at least 9% of schwannomatosis cases. LZTR1-associated schwannomatosis also causes a small number of cases that are misdiagnosed as NF2 (1-2%), due to the occurrence of a unilateral vestibular schwannoma. Schwannomatosis patients had lower numbers of non-vestibular cranial schwannomas, but higher numbers of peripheral and spinal nerve schwannomas and higher incidence of pain as a predominant presenting symptom. Life expectancy was significantly higher in schwannomatosis cases. LC was estimated to be 100%, 91.7%, and 91.7% at 1, 3, and 5 years, and OS was identified as 100%, 79.2%, and 53.2% at 1, 3, and 5 years, respectively.

Conclusions: In our study, therapeutic strategies that include RT resulted in excellent LC rates and were well tolerated for NF1-associated MPNST. Given the paucity of data about safety and efficacy of RT for NF1-associated MPNST, this dataset is encouraging, as outcomes were comparable to those reported for other types of soft tissue sarcomas. However, longer duration follow-up and larger cohorts are required to characterize the risk of second tumors and develop optimal approaches regarding timing and RT delivery.

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Disclosure of Interest: L. Sloan: None Declared, S. A. Terezakis has a conflict with: Elekta grant- funds Johns Hopkins pediatric research consortium, B. Slobogean: None Declared, L. R. Kleinberg has a conflict with: Accuray. Inc.-research support, honorarium, J. O. Blakeley: None Declared

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Resting Metabolic Rate and Its Association with Body Composition and Muscle Strength in Neurofibromatosis Type 1

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**Background:** Resting metabolic rate (RMR) is a measurement of the energy body expends to maintain its vital functions. Indirect calorimetry (IC) is the gold standard method to assess RMR, by analyzing oxygen consumption and carbon dioxide production from the metabolism of nutrients. This study aimed to assess RMR in individuals with NF1 using IC and evaluate its correlation with body composition and muscle strength.

**Methods:** Twenty-six adults with NF1 (14 male), ranging from 18 to 45 years of age, underwent IC to assess RMR, respiratory quotient (RQ) and substrate utilization. Body composition was assessed by dual energy X-ray absorptiometry (DXA). Weight, height and waist circumference (WC) were also measured. Muscular strength was measured by handgrip test using a dynamometer. NF1 group was compared to 26 unaffected controls, matched by sex, age, body mass index (BMI), and physical activity level.

**Results:** There were no differences in weight (69.1 ± 14.1; 62.5 ± 17.0 kg; \(P=0.18\)), WC (82.4 ± 11.4; 81.4 ± 14.6 cm; \(P=0.81\)), fat mass (FM) (22.2 ± 7.6; 20.0 ± 8.7 kg; \(P=0.42\)) and body fat percentage (BFP) (32.0 ± 8.4; 31.6 ± 9.0 %; \(P=0.88\)). Appendicular lean mass (ALM) adjusted by BMI (ALMBMI) was lower in NF1 group (0.828 ± 0.161; 0.743 ± 0.190; \(P=0.048\)), as well as maximum muscle strength (Fmax) (37.5 ± 10.6; 31.1 ± 12.2 kg; \(P=0.04\)). RMR adjusted by weight, lean mass or ALM was higher in NF1 individuals (median 21.9, 26.3, \(P=0.046\); 36.0 ± 5.5, 40.6 ± 6.3, \(P=0.02\); and 60.1 ± 12.7, 94.5 ± 17.9, \(P=0.01\), respectively). RQ was lower in NF1 (0.9 ± 0.1; 0.8 ± 0.1; \(P=0.01\), showing that individuals with NF1 oxidized more lipids and fewer carbohydrates than controls (0.04 ± 0.04, 0.06 ± 0.03 g/min, \(P=0.01\); and 0.20 ± 0.09, 0.15 ± 0.09 g/min, \(P=0.03\), respectively). RMR correlated negatively with BFP (-0.30, \(P=0.03\)), and positively with weight, height, BMI, WC, body surface, lean mass, ALM, ALMBMI, bone mineral content, Fmax and Farea (0.75, 0.6, 0.62, 0.7, 0.74, 0.85, 0.81, 0.56, 0.72, 0.59, 0.5, respectively; \(P<0.05\)). Regression analysis showed that lean mass was the variable that most influenced RMR in the NF1 group.

**Conclusions:** Individuals with NF1 presented with increased RMR (adjusted by weight, lean mass or ALM), and lower RQ when compared to controls, in association with lower ALMBMI and Fmax, which may indicate premature sarcopenia in this population. Further investigation, concerning energy metabolism in NF1, may be helpful to explain the mechanisms involved in such profile.

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Bone Phenotype and Its Association with Nutrient Intake in Adults with Neurofibromatosis Type 1

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**Background:** Poor nutrition and insufficient intake of nutrients related to bone metabolism are risk factors for low bone mass. More than 20 nutrients are required for adequate bone health, and insufficient nutritional intake was previously observed among adults with NF1. This study aimed to assess bone phenotype of adults with NF1, using dual energy X-ray absorptiometry (DXA), and verify its association with their nutrient intake.

**Methods:** Twenty-six adults with NF1 (14 male), ranging from 18 to 45 years of age, underwent bone phenotype assessment using DXA and food intake evaluation (three non-consecutive food records), and were compared to 26 unaffected controls, matched by sex, age, body mass index (BMI) and physical activity level. Weight, height and waist circumference (WC) were also measured. DXA provided bone mineral density (BMD) and bone mineral content (BMC) for total body, spine and hip (total and femoral neck). Food intake was evaluated for macro and micronutrients.

**Results:** There were no differences in weight (69.1 ± 14.1; 62.5 ± 17.0; \(P=0.18\)), and WC (82.4 ± 11.4; 81.4 ± 14.6; \(P=0.81\)). Stature was lower in NF1 group (1.68 ± 0.1; 1.61 ± 0.1; \(P=0.01\)). BMC was lower in NF1 group (2.3 ± 0.4; 2.0 ± 0.5; \(P=0.046\)). Individuals with NF1 also presented with lower total body and spine BMD in g/cm² (1.1 ± 0.1, 1.0 ± 0.1, \(P=0.04\); 1.0 ± 0.1, 0.9 ± 0.1; \(P=0.02\), respectively) and in Z-escore (\(P=0.049\) and \(P=0.03\), respectively). Prevalence of total body bone mass below the expected level for age was higher in NF1 (7.7%, 34.6%, \(P=0.02\)). No differences were observed on total hip and femoral neck BMD (0.9 ± 0.1, 0.9 ± 0.1, \(P=0.31\); 0.9 ± 0.2, 0.8 ± 0.1, \(P=0.11\), respectively). There were no differences on energy and macronutrients intake. The NF1 group consumed lower amounts of calcium, iron, vitamin A, and higher of sodium and polyunsaturated fatty acids, especially omega 6, compared to controls. BMC and total body BMD correlated negatively with protein (g/kg/day), total fat (g/kg/day) and omega 3 intake (\(P<0.05\)), and positively with weight, height, BMI and WC (\(P<0.05\)). BMC also correlated positively with iron intake (\(P<0.05\)).

**Conclusions:** Adults with NF1 presented with lower BMC and lower BMD in spine and total body evaluated by DXA. Lower consumption of calcium, iron and vitamin A, and higher intake of sodium and omega 6, nutrients related to bone health, have also been observed in the NF1 group. However, with no strong association between bone phenotype and nutrient intake.


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A Unique Play Therapy, Auditory Habituation and Social Story Patient Preparation Paradigm for Awake Multi-Parametric Magnetic Resonance Imaging in NF1 Children With Autism

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Background: Multi-Parametric Magnetic Resonance Imaging (MP-MRI) is a non-invasive methodology for measuring anatomical, neurochemical, physiological and functional parameters in the developing brain. It plays a vital role in the assessment and follow-up of children with NF1 and is an increasingly important tool for in-vivo research assessment. The SANTA trial (a phase one pharmaco intervention study) in autistic NF1 children relied upon such MP-MRI. We present a novel childhood imaging preparation paradigm developed as part of this study involving play therapy, scanner noise acclimatisation and a social story. We hypothesised that such a patient preparation approach would allow MP-MRI to be performed on awake NF1 children with autism negating the need for general anaesthesia or sedation.

Methods: A blinded, randomised control trial of oral simvastatin vs placebo treatment in NF1 autistic children was undertaken following ethical approval. The MP-MRI scanning included both anatomical, physiological and functional MRI acquisition with a total awake scan time of one hour. Scanning occurred at baseline and week twelve. In cases of incomplete imaging assessment there was an optional week four scan which could be used to acquire any missing data. Play therapy support was provided at initial assessment and during MRI scanning. Auditory preparation included earplugs, a set of “dummy headphones” and a two week habituation procedure using mp3 sound recordings of the scan sequences to be used in the study. A social story booklet was also introduced to the children and reinforced by their carers over the same two week period.

Results: In total 26 patients were imaged during the study (mean age 7.9, range 4.6-10.4). Only 4 required a week four scan (to re-acquire 1 motion degraded sequence in each of the 4 children). This equated to an imaging success rate of over 99% over the 3120 minutes total scan time in the study. Comparison with age matched controls indicates that two thirds would have been referred for general anaesthesia equating to a cost saving of £12,800 in anesthetic costs alone.

Conclusions: In the challenging setting of NF1 childhood autism, successful awake MP-MRI assessment can be undertaken following our preparation paradigm. This negates the potential confounding effects of anaesthesia or sedation on the MP-MRI findings. This study demonstrates this novel approach facilitates obtaining MRI scans in awake children which is acceptable to parents and results in significant cost saving.
Validating Advanced Volumetric Techniques for Cutaneous Neurofibromas in Clinical Trials

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Background: Cutaneous neurofibromas (cNF) affect up to 99% of all patients with neurofibromatosis 1 (NF1), and can be a major source of emotional and social distress. Treatment options for cNF are limited and drug development has been hampered by the inability to accurately detect small changes in tumor size. Caliper measurements, the current standard for cNF measurement, can only assess the superficial portion of tumors above the skin and are subject to inter-rater variability. Thus, the need for a reliable measurement technique with adequate sensitivity to change over timeframes relevant to clinical trials is a critical gap in the field.

Methods: We are currently recruiting participants with NF1 to a study assessing the intra- and inter-rater reliability of various measurement techniques for cNF. Six cNF on each subject are assessed independently by three different examiners using calipers, 3D photography, and high frequency ultrasound (HFUS). Photographs and ultrasound images are processed using manufacturer provided software, and tumors are measured via manual outlining of tumors by up to three separate assessors. Linear and volumetric assessments are compared using intraclass correlation coefficient (ICC) to determine the intra- and inter-rater reliability of each measurement technique. Patients will be followed every 4 months over the course of one year to assess the ability of each technique to detect changes in tumor size.

Results: Preliminary experience shows that cutaneous neurofibromas appear as well demarcated, hypoechoic masses on HFUS. Image acquisition is reproducible across examiners, and linear and volumetric measurement of cNF are feasible (figure A and B, respectively). Acquisition of 3D photographs and HFUS images is rapid (less than 30 seconds) and examiners found these devices easy to use. Both systems require no action from the patient, other than sitting still for brief periods of time, and are easily tolerated.

Conclusions: HFUS and 3D photography are feasible, non-invasive methods for imaging and measurement of cNF. These methods are easily utilized across examiners, and are currently being evaluated as potential methods to assess changes in cNF size over time. This study is currently enrolling patients, and we will present preliminary baseline data on the inter-rater and intra-rater reliability of each technique. This information will ultimately inform clinical trial design and facilitate the development of interventional treatments for cNF.

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Disclosure of Interest: R. D. Thalheimer: None Declared, V. L. Merker: None Declared, S. K. Verma: None Declared, S. S. Saluja: None Declared, A. J. Wuklan: None Declared, J. T. Jordan: None Declared, I. Ly: None Declared, A. S. Nam: None Declared, O. Thanigaivelan: None Declared, A. Muzikansky: None Declared, B. Vakoc: None Declared, F. Sakamoto: None Declared, J. O. Blakeley: None Declared, R. R. Anderson: None Declared, S. R. Plotkin has a conflict with: Funding Statement: This work was supported by an agreement from The Johns Hopkins University School of Medicine and the Neurofibromatosis Therapeutic Acceleration Program (NTAP). Its contents are solely the responsibilities of the authors and do not necessarily represent the official views of The Johns Hopkins University School of Medicine.
The Relationship Between Physical Manifestations of NF1 and NF2 and Communication Disorders

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Background: Individuals with neurofibromatosis 1 (NF1) and 2 (NF2) can exhibit communication concerns that negatively impact quality of life and are related to the physical manifestations of these disorders. As healthcare professionals do not routinely screen for communication concerns in these populations, they may be under-recognized and under-treated, leading to a negative impact on quality of life and a lack of comprehensive care. This project was undertaken to assist clinicians in understanding the relationship between physical manifestations of NF1 and NF2 and communication concerns, highlighting the need for routine screening in this population in order to facilitate referrals to appropriate rehabilitative services.

Methods: A self-selected group of experts from the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) International Collaboration, Patient Reported Outcomes subgroup, collaborated to review common physical manifestations of NF1 and NF2 and identify those that could impact communication. A literature review further refined the selected manifestations. The list was reviewed by four expert clinicians in neurofibromatosis who were not otherwise associated with the project and their feedback was incorporated. Potential related communication concerns and appropriate rehabilitative referrals were identified for each manifestation through group discussion.

Results: A table was developed summarizing the physical manifestations commonly observed in individuals with NF1 and NF2, resultant communication concerns, and appropriate referrals for rehabilitation. The table presents information separately for NF1 and NF2, and classifies physical manifestations according to body region.

Conclusions: This table is a tool that can support comprehensive care of individuals with NF1 or NF2 by increasing awareness of the nature of communication concerns resulting from common physical manifestations of these disorders. This tool can aid in clinical decision-making and lead to more appropriate referrals for people with NF1 or NF2 and communication concerns, thereby positively impacting quality of life. Gaps identified through creation of this tool highlight the need for further research examining the communication concerns of people with NF1 and NF2, with a particular need for interventions targeting causative physical manifestations of these disorders.

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NF1, NF2, Schwannomatosis, and Dysphagia: A Systematic Review of the Literature

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Background: Dysphagia (swallowing disorder), is defined as difficulty in moving food from the mouth to the stomach without spillage or residue in the oral cavity, pharynx, or esophagus. Tumors and surgery associated with neurofibromatosis type 1 (NF1) or 2 (NF2) and schwannomatosis may affect neuronal control of the muscles needed to coordinate swallowing, leading to dysphagia and potentially fatal aspiration pneumonia or infection. The purpose of this systematic review was to delineate current assessment and intervention approaches for dysphagia in NF1, NF2, and schwannomatosis with the goal of establishing pathways of care typically delivered by speech-language pathologists.

Methods: Electronic database searches were conducted utilizing the following search terms (“Neurofibromatosis 1” OR NF1 OR “Neurofibromatosis 2” OR NF2 OR neurofibromatosis OR schwannomatosis OR “Von Recklinghausen”) AND (swallowing OR dysphagia OR throat OR deglutition). Databases searched included The Cochrane Library, PubMed, CINAHL, PsycINFO, Web of Science, and ClinicalTrials.gov. Inclusion criteria were all article types published in English after 1988 that described any assessment, intervention, or path of care for dysphagia in individuals with a clinical or genetic diagnosis of NF1, NF2, or schwannomatosis. Hand-searching ensured inclusion of all relevant literature. To reduce inclusion/exclusion bias, three investigators individually ranked each publication using the Newcastle-Ottawa Scale modified to include line and page numbers.

Results: One hundred and thirty-four papers were retrieved. Twenty-nine papers met inclusion criteria. Eleven papers included a direct evaluation of swallowing (FEES [Fiber-optic Endoscopic Evaluation of Swallowing] or barium swallow study) and of those, only three had an evaluation of dysphagia post-treatment.

Conclusions: There is a paucity of literature and no established clinical practice for the assessment and treatment of dysphagia in patients with NF1, NF2, or schwannomatosis. Evidence from this review suggests that dysphagia associated with vagal dysfunction, due to lower cranial nerve lesions or compression, is a risk-factor for aspiration pneumonia and subsequent mortality. Additional research is needed to evaluate the frequency and severity of dysphagia in populations with NF1, NF2, and schwannomatosis.

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Interprofessional Education for Improving Care of Patients with Neurofibromatosis Type 1

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Background: Patients with neurofibromatosis (NF) type 1 require care from a variety of health professionals, from various disciplines, and typically attend many separate appointments. Research suggests that interprofessional practice leads to improved health outcomes. Students in healthcare fields do not routinely receive training on how to work collaboratively as part of an NF team. Interprofessional Education (IPE) can be provided to allow students from different disciplines to learn from and with each other, leading to a better prepared workforce and improved health outcomes. There is a paucity of research documenting IPE in the care of individuals with NF. This project was set to examine outcomes of an IPE training experience for students/professionals from multiple disciplines. It was hypothesized that participants would show improvements with interprofessional interactions, values, and knowledge of roles of collaborative practice in NF care as a result of the training received.

Methods: This pre-post IPE training design was approved by the University’s IRB Institute. Procedures took place on the University campus. Participants were a convenience sample of students and professionals from a variety of healthcare disciplines (N=10). Participants completed an investigator-developed readiness assurance quiz prior to a 4-hour IPE training experience (IPENF) to assess knowledge of NF. Two reliable and valid pre- to post-training IPE questionnaires were administered [The IPEC competency self-assessment tool-3 (IPEC) and The SPICE-R2]. To determine participants’ perception of the utility of the IPENF, a post-IPENF questionnaire was administered. Results of the questionnaires were analyzed using paired-samples t-tests.

Results: Results of the t-tests were significantly different for the IPEC domains of interaction (p < 0.001) and values (p = 0.04) from pre- to post. The domain roles of collaborative practice as measured by the SPICE-R2 was also significantly different from pre- to post (p = 0.008). Participants exhibited a mean score of 22/25 on the post-IPENF questionnaire, indicating that learning occurred as a result of the IPENF.

Conclusions: Participants exhibited increased knowledge in NF as a result of the IPENF. Pre- to post increases in interaction and values, and knowledge of roles of collaborative practice suggest that participants were increasingly collaborative and practice-ready at the end of the IPENF. These results support the need for IPE in NF care.

Long Term Follow Up of the Relationship Between Clinical, Cognitive Manifestations and Imaging Findings in Children with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is a relatively common inherited disorder with wide variety of manifestations including skin and nervous system involvement, ophthalmic, renal and bone involvement as well as high incidence of learning difficulties and Attention Deficit and Hyperactivity Disorder (ADHD) and Autistic Spectrum Disorder( ASD). The relationship between the neurocognitive profile and findings in imaging studies has been previously studied.

Methods: A retrospective data of NF-1 children with both follow up imaging and psychological evaluations was collected, comparing the changes over time.

Results: 29 NF-1 children completed at least two different Magnetic Resonance Imaging (MRI) exams and two different formal psychological assessment within three years of follow up period, 16 (55.2%) were males. Full scale IQ (FIQ) and verbal IQ (VIQ) values did not change while a mild decrease in Performance IQ (PIQ) (although not statistically significant, p=0.06) was noted in follow up visits. In the youngest age group (mean age between 37.5- 52 month) a significant change was noted in all measures over time: FIQ, VIQ and PIQ. The T2 hyperintensities (T2H) findings in MRI were set as a score of the sum of areas of T2H changes, and showed significant change over time (p=0.05).

Conclusions: No significant change was noted in IQ over time except in the youngest age group of children with NF1, although T2H findings in MRI consecutive studies did show significant change.

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The R1038G Missense Variant in NF1 is Associated with a Mild Phenotype Characterized by Café-Au-Lait Spots Without Neurofibromatosis 1-Associated Complications

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant condition caused by inactivating mutations of the NF1 gene. The wide allelic heterogeneity of this condition, with more than 3000 different pathogenic variants reported so far, is paralleled by its high clinical variability, which is observed even within the same family.

The definition of genotype-phenotype correlations has been hampered by the complexity of the NF1 gene, the absence of mutational hotspots and the variable timing and nature of the somatic mutation leading to the loss of heterozygosity. Although few exceptions have been recognized, the clinical course remains unpredictable in most patients.

Methods: We sequenced the NF1 gene in a large series of NF1 patients using different techniques since 2002. When no mutation could be detected or in the presence of variants of unknown significance, MLPA was carried out to search for whole gene or intragenic deletions/duplications.

Results: The novel missense variant c.3112A>G p.(Arg1038Gly) was identified in a three generation family segregating a CALs-only phenotype. Another unrelated patient of our cohort, who displays a mild phenotype, shows the same substitution. MLPA excluded the presence of deletions/duplications.

Both cDNA analysis from patient's RNA and a hybrid minigene assay did not detect any splicing defect.

The Arg1038Gly substitution has not been reported so far in any of the available databases, segregates in affected patients in both families and involves a highly conserved residue. Although our data confirm that it does not affect splicing, the molecular mechanism of the mutation remains unknown, given the lack of information about the function of this region of the protein.

We observed that all the seven patients examined display a mild phenotype characterized by the presence of café-au-lait spots (CALs) with freckling in some cases, but without neurofibromas or other NF1-associated complications.

Conclusions: Our data strongly support a novel correlation between the Arg1038Gly missense mutation and a mild phenotype of NF1, which may have relevant implications for patients and genetic counseling, but also to get insights into the function of neurofibromin. The identification of this rare variant in further families will help to confirm this finding.

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Pediatric Neurofibromatosis 1 (NF1) and Vasculopathy - Congenital Dysplasia or Progressive Occlusion?

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Background: Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders. While neurofibromas and café-au-lait macules are well-known traits of the disease, peripheral or cerebral vasculopathies are only rarely observed in children with NF1. However, recent studies demonstrated that vascular abnormalities in NF1 can affect the internal carotid arteries, the aorta, the renal and mesenterial arteries and may also be found in the venous circulation system. The clinical spectrum includes occlusion, stroke, aneurysm, ectasia, stenosis, fistula and rupture. It has been proposed that NF1-associated vasculopathy results from pathologic neurofibromin function in cells that form the vessel wall. Possible pathogenetic mechanisms include smooth muscle cell hyperproliferation, neo-intima formation and an altered inflammatory response to vascular injury.

Methods: Our department provides care for 800 children with NF1. Vasculopathy was identified in 4 children (2 male, 2 female), all of them with cerebrovascular abnormalities. All children were asymptomatic, despite progressive vascular occlusion on serial MRIs. Vasculopathy was diagnosed by accident when MRI scans were performed for other reasons, e.g. optic pathway glioma or chronic headache.

Results: We diagnosed moyamoya syndrome, hypoplastic internal carotid artery, stenosis and progressive occlusion of the main trunk of the artery cerebri media. During follow-up all of our patients showed progressive occlusion with absent flow void. The internal carotid artery was most commonly affected. One patient was treated with aspirine, surgery was no option in any case.

Conclusions: Vasculopathy is a rare manifestation in pediatric NF1 and occurs in 2-5% of NF1 patients. Mostly it is an incidental finding during MR imaging and does not cause clinical symptoms - even in cases with a progressive and almost complete vascular obstruction over time. Therefore we do not recommend routine MR imaging in asymptomatic patients. However, MRI and MRA are helpful tools for follow-up of known and diagnosis or presumed vascular lesions and should be performed when symptoms occur that are indicative of a vascular etiology.

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Phenotypic Characterization and Functional Analysis of Cases Suspect for Neurofibromatosis Type 1

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Background: A molecular diagnosis of Neurofibromatosis type 1 (NF1) can sometimes be challenging, especially in cases without an identifiable mutation, or a variant of unknown clinical significance (VUS). We aimed to compare the clinical characteristics of patients with NF1-VUS or no NF1 mutation identified (NF1-NMI) to those with a pathogenic NF1 mutation. Secondly, we performed in vitro functional assessment to try and classify NF1 VUS.

Methods: Clinical characterization
Clinical and genetic information of clinically suspected NF1 patients was extracted from the departmental NF1 patient database (1993-2016). NF1 variants were classified as pathogenic (class 5), VUS (class 2-4) or benign (class 1). Clinical characteristics of the NF1-VUS and NF1-NMI groups were compared to the NF1 pathogenic mutation group using descriptive statistics.

Functional characterization of NF1-VUS
Expression constructs encoding NF1 VUS were derived by site-directed mutagenesis. We performed in vitro assays. First we estimated the RAS GAP activity of the neurofibromin variant proteins using a RAS-GTP pull-down assay. Second, we used coimmunoprecipitation to investigate whether the interaction between neurofibromin and SPRED1 was affected.

Results: Clinical and genetic information was available for 617 individuals clinically suspect for NF1. We identified 417 (68%) pathogenic NF1 variants, 33 (5%) VUS and 167 (27%) NF1-NMI individuals. The percentage of patients in each group who met the clinical NF1-NIH criteria was 64%, 67% and 34% respectively. The most frequently reported features in all groups were café-au-lait spots and freckling, whereas neurofibromas were most prevalent in the pathogenic variant group (50% vs 24% vs 23%; p<0.01). In the NF1-NMI group 45% of the individuals only had one NF1-NIH criterion identified. So far we have performed functional assessment of 13 NF1-VUS, of which five were reclassified as pathogenic.

Conclusions: An NF1 variant was identified in almost 75% of individuals suspect for NF1. Clinically, the NF1-VUS group was largely comparable to the pathogenic mutation group, and functional analysis was a useful tool to further classify NF1 VUS. Due to the clinical and familial consequences of an NF1 diagnosis, future studies should focus on optimizing assays of neurofibromin function to facilitate establishing an accurate molecular diagnosis. In the NF1-NMI group only one third of patients met NIH criteria and mosaicism or other diagnoses should be considered.

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Implications of Focal Cortical Dysplasia in Paediatric NF2

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Background: Focal cortical dysplasia is sometimes noted in children and young adults with NF2. The implication of this finding is uncertain. The true prevalence of FCD in the general population is unknown. Data from published epilepsy surgery series indicates that FCD is present in up to 25% of drug resistant epilepsies. Moreover the occurrence of FCD in any patient can only be confirmed by histological examination where it is divided into Type I, Type II and Type III. Brain MRI imaging is more likely to show abnormalities in Type I FCD than type II. Type 1 is usually implicated in temporal lobe seizures while in type II the location is more often extratemporal and mainly in the frontal lobe.

Methods: Records of all children and young adults who attended the Manchester Centre for Genomic Medicine NF2 clinic over the past two years were identified. The clinical records and the MR scans of the brain for these patients were reviewed and the presence or otherwise of focal cortical dysplasia and/or epilepsy noted.

Results: 27 children and young adults from 3-18 years with NF2 were reviewed in clinic over the past two years. 11 were female and 16 were male. Median age was 12 years. 24 out of the 27 children and young adults had had MR scans of the Brain.

None of the 27 children and young adults had epilepsy. Three out of the 24 (12.5%) who had neuroimaging had focal cortical dysplasia. Two affecting the right frontal lobe and one affecting the right parietal area. All were males.

Conclusions: In our series the location of FCD on MR brain imaging was extratemporal making it more likely to be Type II. The male preponderance of FCD in published literature is also confirmed in our Paediatric NF2 series with all our cases being male.

Focal cortical dysplasia is not uncommon in children with NF2 (12.5% in this series) but does not seem to be associated with Epilepsy. This is important when discussing the implications of FCD on MR brain imaging with families of children and young adults with NF2.

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The Prevalence of Perceived Fatigue in Children and Young Adults with Neurofibromatosis Type 1

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**Background:** Perceived fatigue is a common parental reported symptom in children and young adults with neurofibromatosis type I (NF1) however its severity and prevalence is unknown. This preliminary study aimed to describe the prevalence of reported perceived fatigue in children and young adults with NF1.

**Methods:** Ethical committee approval was granted to identify individuals aged 2-18 years with NF1 who had attended Manchester Centre for Genomic Medicine’s NF1 clinic within the last two years from clinical records.

The presence of fatigue was assessed using the PedsQL Multidimensional Fatigue Scale Parental and child report for children and young adults with age ranges of 2-18 years (2-4 years, parent only). This is a validated measure of fatigue comprising three domains: cognitive, physical and sleep/rest on a 0-100 scale. Higher scores indicate less severe fatigue.

The primary outcome measure was the aggregated fatigue score with the sub scales being secondary outcomes.

The Index of Multiple Deprivation (IMD) was determined from patient postcodes. Scores are summarised as mean±SD. NF1 patients were compared to sibling controls using multiple linear regression adjusting for age, sex, and IMD. Scores were compared to the published standards in Varni et al using z-scores.

**Results:** 286 were invited, 156 patients/parents expressed an interest and 91 participated, 75 with NF1 and 16 unaffected siblings. The numbers in the different age and IMD groups were similarly distributed between NF1 patients and sibling controls. There were no significant differences noted in the fatigue scores with age, sex or IMD.

There were statistically significant differences between NF1 and controls in the aggregated fatigue core (child report 55±19 v 75 (14), p<0.001; parent 54±20 v 73±18, p=0.001) and the individual score of the three sub domains: cognitive (child 48±27 v 75±23, p<0.001), physical (child 59±19 v 82±14, p<0.001) and sleep/rest (child 59±19 v 71±15, p=0.018) suggesting more significant fatigue in the NF1 group.

Similarly the NF1 group had much more fatigue than the published controls (aggregated child z-score -1.9±1.4, p<0.001; parent -3.2±1.8, p<0.001).

**Conclusions:** This study suggests that children with NF1 are significantly affected by perceived fatigue when compared to healthy children who do not have NF1. Further studies exploring interventions to reduce fatigue are indicated.

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Targeting of Cutaneous Neurofibromas: Uniform Datasets for Tissue Based Studies

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Background: Cutaneous neurofibromas (cNF) are one of the hallmark findings of neurofibromatosis type 1 (NF1). As there is no known way to prevent these tumors from developing and current treatments are limited to various procedural based approaches, the Neurofibromatosis Therapeutic Acceleration Program (NTAP) is targeting cNF therapy as part of its strategic mission. Understanding the molecular mechanisms underlying the formation of cNF, with a focus on translational approaches that have a high likelihood of having clinical impact in the near term, are a key focus area. As the studies aim to establish associations between clinical and molecular data for cNF, high quality tissue samples representing the diversity of human cutaneous tumors is required. However, there are no standardized sample collection or analysis protocols. Non-universal terminology for cNF appearance, multiple methods for the removal of tumors, and varied patient privacy considerations across countries represent additional challenges.

Methods: Investigators participating in the NTAP sponsored cNF initiative convened at a cNF summit in October 2017 and in a series of follow up discussions to establish criteria for prospective tissue collection with paired clinical datasets applicable to future cNF translational studies. The goals were to: (1) produce a systematically collected tumor dataset across translational scientists, (2) provide clinical context to support translational hypotheses, and (3) be in concordance with international patient privacy requirements.

Results: Tumors resected for translational studies are classified as: nascent, flat, sessile, globular, or pedunculated. An atlas representing these cNF subclassifications is being created to allow pictorial representation of cNF type in open share databases without requiring individual patient images that might be identifiable. A limited clinical dataset accompanying tumor samples includes sex, tumor body region, growth status at the time of sampling (if possible), and decade of birth. It was agreed that samples would be removed by blade based surgical excision with accompanying uninvolved skin when feasible. The derivative data will be shared in accordance with the guidelines of the Health Insurance Portability and Accountability Act (HIPAA) of 1996 and EU General Data Protection Regulation (GDPR) of 2018.

Conclusions: The recommendations described provide clinical and translational scientists with a common set of guidelines to collect and study cNF biospecimens.
Non-Ossifying Fibromas in NF1 (Neurofibromatosis Type 1)

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Background: Well-recognized orthopedic manifestations of neurofibromatosis type 1 (NF1) include non-ossifying fibromas (NOFs; aka fibrous cortical defects FCDs); however, the prevalence of NOFs in NF1 has not been ascertained and relative risk in NF1 versus the unaffected population has not been determined. In general, individuals with NF1 and NOFs tend to have multiple lesions that may have a higher association with pathogenic fracture. The pathogenesis of NOFs in NF1 has yet to be elucidated, although a key hypothesis is the "tug" of tendons at insertion sites of the distal femur and proximal tibia. Regardless of etiology, orthopedic management guidelines have not been established for the NF1 population, and it is not clear that pathogenic fractures are more likely in NF1-NOFs versus NOFs in the non-NF1 population.

Methods: The medical literature was reviewed as a component of care for 2 individuals with NF1 and NOFs who required surgical resection. Both cases presented to the orthopedic service with knee pain and radiographs were compatible with NOFs of the proximal tibiae without fracture. Surgical resection provided tissue for histologic assessment. Genotype analyses of blood and cultured cells derived from resected tissue is underway for case 2.

Results: Both symptomatic individuals with NF1 obtained relief from surgical resection. Resected tissue samples from case 1 comprised of an aggregate of irregular tan-to-pink soft tissue and bony fragments were provided for pathologic preparation and review. Microscopic examination showed moderately hypercellular spindle cell proliferation with irregularly distributed multinucleated giant cells within the proliferation. Focal hemosiderin deposition was noted. Resected tissue from case 2 comprised of tan to yellow soft tissue fragments and microscopic evaluation showed bland spindle cells arranged in a storiform pattern with scattered multinucleated osteoclast-like giant cells with hemosiderin deposition. Genotype analyses on DNA from cultured cells derived from tissue from case 2 has not yet been completed.

Conclusions: Non-ossifying fibromas (NOFs), also known as fibrous cortical defects (FCDs), in the context of NF1 are benign cell collections of unspecified cellular origin and pathogenesis. Even though NF1-NOFs appear more severe than in the non-NF1 population, there is no evidence to suggest they should be managed differently than NOFs’s in those without NF1.

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The Elephant in the Room; Suicidal Ideations Among Internationally Diverse Patients with NF

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Background: Neurofibromatoses are incurable diseases associated with impaired quality of life, high symptom burden and high emotional distress. Prior published and anecdotal case reports have raised awareness of suicidality and suicidal ideations in this population. However, this issue has never been properly explored within quantitative studies. The aim of this study was to provide the first report of rates of suicidal ideations in 3 geographically diverse international populations of patients with NF: 1) adults with NF1, NF2 and schwannomatosis (N = 118); 2) adolescents with NF1 and NF2 (N = 51); and 3) adults with NF2 who are deaf (N=45). Secondarily, we compared these rates with those in the general population and explored demographic and clinical factors associated with suicidal ideations.

Methods: Patients were recruited through an international NF registry as part of 4 separate clinical trials aimed at improving quality of life. Baseline suicidal ideation was determined based on participants’ answers to question 9 (“Did you have thoughts that you would be better off dead”) on the Patient Health Questionnaire-9 for adults (PHQ-9), and adolescents (PHQ-A).

Results: Among the combined sample (N=219), almost half (48.1%) endorsed suicidal ideations at least “several days”. Among the 118 adults, 63 (53%) endorsed suicidal ideations. The highest rates were observed in adults with schwannomatosis (10/14; 71%), followed by NF2 (41/72; 57%) and NF1 (41/72; 53%). Among the 51 adolescents, 32 (63%) endorsed suicidal ideations. Adolescents with NF1 reported higher rates (16/24; 67% versus 3/8; 37.5% in NF2). Finally, among the 45 adults with NF2 who are deaf 17 (38%) endorsed suicidal ideations. These rates are significantly higher than general population suicidal ideations rates for adults (3.9%; p<.001) and adolescents (11.4%; p<.001). Neither demographic factors (e.g., age, gender, race, learning disability) nor clinical variables (e.g., self-reported learning disability) were significantly associated with suicidal ideations (p>.05).

Conclusions: The high rates of suicidal ideations in NF are concerning. Clinicians are urged to assess and address suicidal ideations in all their NF patients. Interventions to directly address the emotional toll that NF places on adults and adolescents should also be developed.

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Prevalence of Neurofibromatosis Type 1 in a Pediatric Cohort of Café-Au-Lait Macules

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Background: Café-au-lait macules (CAL) are common among the general population but can also be an early indicator of Neurofibromatosis Type 1 (NF1). Legius syndrome (LS) and Noonan syndrome (NS). As a result, CAL are a common indication for referral to a genetics clinic. Young children without a family history often do not meet NF1 NIH criteria until later in childhood.

Methods: To evaluate the clinical outcomes of these referrals, a retrospective chart review was conducted for all patients with CAL referred to the RASopathy clinic between August 2016 and May 2018. This study was approved by the Institutional Review Board at Cincinnati Children’s Hospital Medical Center.

Results: In the study period, the RASopathy clinic received 37 CAL referrals. The average age was 5.4 years old, ranging from 2 months to 18 years. The majority of referrals (34/37) were placed by a pediatrician. The majority of CAL observed were typical (29/37), and 52% had fewer than 6 CAL. Of the 37 patients referred, 10 patients (27%) were diagnosed with NF1 and 2 patients with segmental NF1 (5%). Of those diagnosed with NF1, 4 were diagnosed after a physical exam, 3 were diagnosed after positive genetic testing, and 3 were diagnosed after an optic pathway glioma or Lisch nodules were identified. Three patients (8%) with atypical CAL were diagnosed with cutaneous mastocytosis. NF1 genetic testing was ordered for 22 patients (60%), ophthalmology evaluations in 20 patients (54%) and brain MRI in 13 patients (35%). Additional genetic testing was ordered for patients with atypical features including chromosomes (2), GNAS (1), TSC1/2 (1), intellectual disability panel (1), and Fanconi anemia breakage studies (1). All of those studies were normal. Six patients (16%) were discharged from clinic after a genetic etiology for their symptoms was ruled out due to their age and absence of manifestations. The remaining 16 patients were likely isolated café au lait macules but parents opted to return to the genetics clinic for additional follow up.

Conclusions: The majority of evaluated patients had isolated CAL without signs of a genetic disorder. A third of the referred CAL patients received a clinical diagnosis of NF1 after evaluation in the RASopathy clinic and half met clinical NIH criteria on first evaluation. Surprisingly, no other RASopathy diagnosis was made in this population. The most common diagnosis in patients with atypical café au lait macules was cutaneous mastocytosis which typically has a more benign clinical course.

A Prospective Study of the Impact of MEK1/2 Inhibition on Neurocognitive Functioning in Children and Adults with NF1

Karin Walsh, Children's National Health System

Background: In NF1, Ras is overactive and associated with cognitive deficits. Manipulation of the Ras/ERK pathway has rescued cognitive impairments in the NF1 mouse. In trials directed at NF1 tumors, MEK inhibitors have shown activity. A NF1 mouse study suggested that inhibition of MEK1/2 may improve cognitive function. This prospective, multi-center, single-arm, ancillary study aims to examine the potential benefit of MEK inhibitors on cognition in children and adults with NF1.

Methods: Sixty-three patients with NF1, 5-33 years (median = 12), enrolled on MEK inhibitor trials for NF1 tumor completed computerized cognitive assessment and parent-reported executive functioning at baseline (T0), 3, 6 (T2), and 12 months. The primary outcome is change in metacognition (CogState ONB+OCL Composite, BRIEF Metacognitive Index [MCI]) from T0 to T2. One-sample t-tests analyzed group mean differences from T0 to T2, and reliable change analysis evaluated individual clinically significant change.

Results: At T0, 21.5% of patients were rated to have clinically elevated deficits in metacognition (BRIEF, MCI), which was significantly higher than at T2 (15.7%, p = .002). Behavior regulation impairments were reported in 11.5% at T0, also significantly higher than at T2 (7.7%, p = .01). Mean scores on the CogState composite were significantly lower at T0 (M=-0.24, SD=1.08) and T2 (M=0.10, SD=0.87; p = .02, d = 0.32). Reliable change analyses indicated that more patients receiving selumetinib showed clinically significant improvement in metacognition (BRIEF MCI) at T2 compared to that expected by chance (25.5% improved v. 10% expected at 80% CI; p = .09). The number of participants improved in executive function efficiency on CogState was not significantly greater than chance (16.4% improved, 10% expected) but working memory accuracy approached significance (18.9% improved, 10% expected, p = .07).

Conclusions: Our study demonstrates significant group improvement in metacognitive functions within the first 6 months of treatment. Further, parents/ informants report significant clinical improvement in daily metacognitive functions. Findings are limited without a comparison group but support further research on the potential therapeutic effect of MEK inhibition on cognitive morbidities in NF1.
Exploring Brain Morphology in Adults with NF1 Using Magnetic Resonance Imaging

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Background: Although multiple studies have described alterations of brain morphology in children with neurofibromatosis 1 (NF1), similar data on adults with NF1 are limited. We used head MRI exams to measure the corpus collosum (CC), ocular globes, and brain stem in adult NF1 patients.

Methods: Adults with NF1 and sex- and age-matched (± 24 months) unaffected controls underwent head MRI exams in a 1.5 or 3.0 Tesla scanner. Multiple coronal, axial and/or sagittal T1, T2 and FLAIR images were obtained. OsirIX Lite was used for measurements of the CC (area, length, height, genu width, anterior body width, mid-body width, posterior body width, and splenium width), eyes (anterior-posterior length, diameter, anterior-to-diameter length, anterior-to-interzygomatic line length, posterior-to-interzygomatic line length, optic nerve shortest path and optic nerve tortuosity), and brain stem (midbrain width, midbrain anterior-posterior length, pons anterior-posterior length, middle cerebellar peduncle length, medulla width, and medulla anterior-posterior length). We report an initial exploratory analysis of CC measurements in 68 NF1 patients and 19 controls, and measurements of the ocular globes, optic nerve, and brainstem in 104 NF1 patients and 25 controls. Statistical analysis was performed with Shapiro-Wilk, Fisher’s F, Student-T, Welch’s T, or Mann-Whitney U tests, as appropriate. False Discovery Rates were estimated to take multiple comparisons into account.

Results: After adjustment for multiple comparisons using the False Discovery Rate (q = 0.05), five of the 29 measurements (CC genu width, CC height, CC mid-body width, medulla width, and left middle cerebellar peduncle length) were found to differ significantly between adults with NF1 and controls.

Conclusions: The findings from this exploratory study indicate that brain morphology differs between adults with NF1 and unaffected individuals.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>NF1 (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>p (adjusted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC genu width</td>
<td>1.0 ± 0.17 cm</td>
<td>1.2 ± 0.15 cm</td>
<td>0.0013</td>
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<tr>
<td>CC height</td>
<td>2.8 ± 0.37 cm</td>
<td>2.4 ± 0.30 cm</td>
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<tr>
<td>CC mid-body width</td>
<td>0.68 ± 0.10 cm</td>
<td>0.63 ± 0.10 cm</td>
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<tr>
<td>Medulla width</td>
<td>1.9 ± 0.12 cm</td>
<td>1.8 ± 0.10 cm</td>
<td>0.00063</td>
</tr>
<tr>
<td>Left middle cerebellar peduncle length</td>
<td>1.8 ± 0.17 cm</td>
<td>1.7 ± 0.14 cm</td>
<td>0.0490</td>
</tr>
</tbody>
</table>

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Dramatic Response of Recurrent and Refractory Diffuse Astrocytoma to Trametinib in a 12 Year Old Boy with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is defined by a mutation in the NF1 tumor suppressor gene, impairing production of neurofibromin, which negatively regulates the RAS/RAF/MEK/ERK/MAPK pathway. Activation of MAPK leads to the tumorigenic manifestations of NF1. Although most CNS tumors associated with NF1 are low grade astrocytoma (LGA), these may induce significant morbidity when complete resection cannot be achieved due to location. Radiotherapy is often omitted if possible due to the risk of complications including secondary malignancy. When these patients fail conventional chemotherapy, typically with carboplatin and/or vinca alkaloids, further treatment options are limited.

Trametinib is a third generation inhibitor of MEK1/MEK2. MEK inhibition has been shown to be a promising treatment modality for malignancies associated with MAPK activation and NF1 associated plexiform neurofibroma, however, its use in NF1-associated LGA has not been reported. We present a patient with refractory diffuse astrocytoma who, having failed multiple chemotherapeutic regimens, experienced a dramatic response to trametinib monotherapy.

Methods: Retrospective case review.

Results: Our patient was diagnosed with NF1 in infancy and received vincristine, carboplatin and temozolomide for progressive optic pathway glioma, with decrease in size and stable vision. Two years after completion of therapy, a WHO grade II diffuse astrocytoma was identified in the mesial right temporal lobe and underwent a gross total resection. MRI one year later showed an additional enhancing lesion in the left mesial temporal lobe. As the tumor was in close proximity to the optic nerve, he underwent biopsy only, and pathology was consistent with a diffuse astrocytoma as well. Despite an initial response to vinorelbine, 15 months after treatment completion, he experienced seizures associated with tumor re-occurrence. He progressed on vinorelbine retreatment, vinblastine, combined sirolimus/erlotinib, and temozolomide. Finally, he received trametinib monotherapy with a dramatic radiologic response by 3 months; he has now been receiving trametinib for 7 months and is tolerating it well, with further tumor shrinkage. Genomic profiling of the tumor is in process.

Conclusions: MEK inhibition such as trametinib monotherapy or in combination with other agents should be evaluated in a clinical trial for patients with NF1-associated LGA who have failed conventional therapies.

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Factor VII Deficiency Associated with Massive Intraoperative Hemorrhage in Two Patients with Neurofibromatosis Type 1

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Background: Plexiform neurofibromatosis (PN) is a typical manifestation of Neurofibromatosis type 1 (NF1) and may cause functional deficits and disfiguration. As highly vascular tumors, resection is often difficult and may be complicated by hemorrhage. Surgery is therefore deferred where possible, but debulking may become necessary.

FVII deficiency is the commonest of the rare bleeding disorders (incidence 1/500 000), and is due to mutations in one or both copies of the F7 gene (13q34) resulting in low FVII activity and an increased bleeding risk. Heterozygotes have mildly decreased levels, but remain at risk of severe hemorrhage as FVII activity does not correlate with bleeding phenotype.

We present two patients with NF1 and FVII deficiency – an association not previously reported – both of whom experienced massive intraoperative hemorrhages.

Methods: Retrospective case series.

Results: Patient 1 required calvarial reshaping for craniosynostosis at 8 months of age. She experienced significant blood loss, requiring 4 units of fresh frozen plasma (FFP), 3 units of platelets, and 6 units of packed red blood cells (PRBC) intraoperatively. Subsequent investigations demonstrated mildly prolonged PT (13.0 seconds), normal aPTT (30.0 seconds) and low FVII activity of 30-51%; exome sequencing confirmed heterozygous FVII mutation [c.1061C>T (p.Ala354Val)]. She received recombinant FVIIa for subsequent surgeries without need for further transfusion.

Patient 2 developed a left thigh PN at 2 years of age, which enlarged into the pelvis causing significant morbidity. Investigations at the time of a spontaneous intratumoral hemorrhage showed prolonged PT of 12.8 seconds, normal aPTT of 22.8 seconds, and FVII activity of 34-62% (normal 80-120%). On account of severe, progressive deficits due to left thigh/pelvic PN, surgical resection was attempted but aborted due to intraoperative hemorrhage necessitating massive transfusion(16 units of PRBC); a subsequent attempt was made using pre-operative recombinant FVIIa, but he unfortunately succumbed to massive hemorrhage intraoperatively.

Conclusions: NF1 patients, particularly those undergoing surgeries with a high risk of blood loss (e.g. PN resection) should undergo pre-operative screening for bleeding disorders even in the absence of a bleeding history. If such a disorder is identified, appropriate preoperative management may effectively reduce surgical risk and minimize the need for transfusion.

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Characterisation of Retinal Abnormalities Using Optical Coherence Tomography in NF2: A Potential New Diagnostic Criterion?

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Background: Neurofibromatosis Type 2 (NF2) is an autosomal dominant genetic disorder characterised by the presence of nervous system tumours, of which bilateral vestibular schwannomas are the hallmark of the disease. It also comprises the presence of ocular features such as cataracts, which form part of the diagnostic criteria of NF2, and two forms of retinal abnormality: hamartomas of the retina with or without retinal pigment epithelial involvement, and epiretinal membranes. Spectral domain optical coherence tomography (SD-OCT) is a non-invasive diagnostic technique that is able to visualise the retinal anatomy. In NF2 patients, this has allowed these retinal abnormalities to be characterised, and has identified other features such as retinal tufts. We sought to study these features and to measure their potential as a diagnostic tool in NF2.

Methods: A retrospective case-control study was performed. 54 NF2 patients underwent OCT imaging as part of their examination in the eye clinic. Stored images of OCTs from 55 healthy volunteers served as a control group. Mutation testing had been performed on all NF2 patients. 2 masked ophthalmologists analysed and graded OCT images according to the presence of retinal abnormalities.

Results: Retinal abnormalities were found in 29 NF2 patients (53.7%) and 2 control patients (3.6%). Retinal tufts were the most common abnormality, and were seen in 26 NF2 patients (48.1%); there were no retinal tufts in the control group. The specificity and sensitivity of an abnormal scan in NF2 was 96% and 54% respectively, with a positive predictive value of 94%. Retinal tufts had a specificity of 100%, a sensitivity of 48% and a positive predictive value of 100%.

Conclusions: We present the largest study of retinal abnormalities in an NF2 population as seen on SD-OCT imaging. Our results show a high frequency of retinal abnormalities that are readily detected by OCT imaging. The presence of retinal tufts may be a novel marker of NF2 with both high specificity and positive predictive value for NF2, and may have a place in the diagnostic for NF2.

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Patients’ Preferences for Psychological Support Within the Neurofibromatosis Type 1 Service

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Background: Children, young people and adults living with Neurofibromatosis Type 1 are at an increased risk for the development of behavioural, social, emotional, psychological and cognitive impairments, and a decreased quality of life. There is consistent evidence that psychological assessment and intervention should be routine for all patients. This scoping exercise aimed to obtain patients’ and parents’/carers’ preferences for the most accessible psychological support

Methods: A brief (fewer than 5 minutes to complete) questionnaire was developed to ask people living with NF1, or their parents / carers, what common difficulties are experienced for people living with NF1, whether support is needed for those difficulties, what interventions were preferred and when would be most convenient to access these interventions. Questionnaires were given to all patients attending their NF1 clinic appointment

Results: Only 30 completed and non-spoiled questionnaires were returned. Adults living with NF1 (n=22), young people living with NF1 (n=6) and parents / carers of someone with NF1 (n=3) reported fatigue (81%), pain (60%), sleep (57%), anxiety and worry (56%), memory difficulties (54%) and low mood (50%) to be the biggest difficulties. In contrast, fewer individuals felt anger and behavioural difficulties (19%), friendship or isolation problems (24%) and treatment difficulties (25%) were problems. Surprisingly, of the individuals who regarded fatigue to be problematic, only 18% felt support would be helpful. Of the few individuals who felt that anger or behavioural problems were problematic, 50% felt that support would be helpful. Individuals preferred 1:1 appointments with an NF1 clinical psychologist (71%) to groups, run by carers of people with NF1 (55%), NF1 professionals (52%) or people living with NF1 (50%). There was a preference for evening and weekend appointments.

Conclusions: Living with NF1 can lead to a wide range of psychosocial impairments. Individuals may report problems but not want to access support. Perhaps patients’ most severe difficulties are considered to be life-long, and support may be disregarded, whereas behavioural concerns or emotional difficulties are considered to be amenable to change, and support is more widely welcomed. Further data are essential, but the preliminary findings highlight that psychological assessment is recommended for all patients living with NF1 and individualised psychological intervention would be welcomed

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Phase 1 and Phase 0 Studies of AR-42, a Pan Histone Deacetylase Inhibitor, in Subjects with Neurofibromatosis Type 2 (NF2)-Associated Vestibular Schwannomas and Meningiomas

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**Background:** Patients with NF2 frequently develop bilateral vestibular schwannomas (VS) and multiple meningiomas. Presently, an FDA-approved medical therapy is not available. The histone deacetylase inhibitor AR-42, suppresses schwannoma growth and causes tumor shrinkage in meningiomas in preclinical models.

**Methods:** As part of a phase I trial of AR-42 in patients with advanced or recurrent solid tumors for which no standard therapy is available or patients who decline available standard treatment options, patients with NF2-associated VS and meningiomas were enrolled and received AR-42 treatment 3 times weekly for 3 weeks followed by 1-week break. Subsequently, a phase 0 exploratory evaluation of AR-42 for intratumoral pharmacodynamics and pharmacokinetics in VS and meningioma was performed.

**Results:** In the phase I trial, 5 NF2 patients were recruited. AR-42 was overall well-tolerated, and the maximum tolerated dose (MTD) was defined at 60 mg. AR-42 demonstrated anti-tumor activity mostly in the form of tumor stability. In one NF2 patient treated for 10 months, AR-42 significantly reduced tumor size in meningiomas and slowed VS growth rates. After cessation of treatment, meningiomas remained small, but VS quickly resumed growth. Tissue samples including a bilateral VS, a skull base meningioma, and an optic meningioma were collected from this patient. A VS from a second person with NF2 and these 4 tumors underwent comprehensive genomic profiling via FoundationOne. Mutations in NF2 and NUP98 were found. A phase 0 study with the primary objective to estimate p-AKT and p16INK4A levels after 3 weeks of oral AR-42 at 40 mg every other day, 3 times a week for 3 weeks preceding surgery, in NF2-related and sporadic VS and meningiomas, as well as control tumor samples from a tissue bank is ongoing. The secondary objectives include assessment of audiometric changes and volumetric tumor reduction and determination of plasma and intratumoral AR-42 concentrations. So far, 5 patients were studied. AR-42 concentrations in the plasma and VS of treated patients reached levels around the IC50 value determined *in vitro* and was more preferentially concentrated in the tumors. AR-42 decreased the levels of p-AKT, pERK1/2, p-PRAS40, and p-S6 in treated VS.

**Conclusions:** AR-42 achieves therapeutic concentrations in the tumors and hits its targets. Further investigation of AR-42 as an NF2 treatment is ongoing.

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(Funding: The US Department of Defense, The Galloway Family, and Advocure NF2)
Changes in Plexiform Neurofibroma (PN) Volume, Appearance and Patient Reported and Functional Outcomes in Patients Enrolled on SPRINT: A Phase 2 Trial of the MEK Inhibitor Selumetinib (AZD6244, ARRY-142886) for Children with NF1 and PN: A Case Series

Trish Whitcomb, NCI

Background: PN in NF1 can cause substantial morbidity. Our ongoing phase II study (NCT01362803) of selumetinib in children with NF1 and inoperable PN causing morbidities showed a partial response rate (PR, ≥20% PN volume decrease) of 72%. Key secondary endpoints of this trial include the evaluation of changes in PN related function, patient reported outcomes (PRO), and appearance using standardized evaluations longitudinally at the same time as response evaluation with MRI. A major challenge has been the wide range of morbidities that can be related to PN, which can be located anywhere in the body. In addition to assessing changes in PN morbidity across all patients, we are performing detailed analyses in each individual patient to most meaningfully describe selumetinib related changes in morbidity.

Methods: PN related present and potential morbidities are determined at enrollment based on medical records, clinical evaluation and location of the PN by MRI. Patients then undergo PRO measures, standardized photography of visible PN, and functional evaluations depending on the PN location. We provide here three detailed examples of patients, with a variety of PN related morbidities, to illustrate how longitudinal and standardized evaluations allow us to assess changes in appearance, function and PRO over time.

Results: In the patient examples, standardized photography demonstrates visible decrease in PN appearance. Pain and quality of life assessments demonstrate measurable and clinically meaningful changes on treatment with selumetinib. The global impression of change scale meaningfully reflects patient experience. Functional evaluations, such as strength and range of motion, allow us to capture changes over time.

Conclusions: Given the wide range of PN morbidities, detailed analyses of each patient provide added value to the assessment of the effect of selumetinib in children with NF1 and inoperable PN.

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Plexiform Neurofibromas in 150 Complex NF1 Patients 2017-2018

Victoria Williams, Neurofibromatosis Centre, Department of Neurology, Guy’s Hospital, London, United Kingdom

Background: Plexiform Neurofibromas (PN) in NF1 are a cause of significant morbidity with risk of malignant transformation. The aim of this study was to review the clinical manifestations of PN in a large cohort of patients at a national NF1 clinic.

Methods: We conducted a retrospective review of patients with complex NF1 and PN under the care of our national NF clinic in the year 2017-2018. Complex NF1 was defined according to criteria agreed between our national NF1 centres, to identify patients with significant morbidity due to NF. This definition includes all patients with symptomatic PN. Demographics, site, symptoms, signs and surgical intervention were assessed.

Results: Of the 1596 patients seen in 2017-18, 442 were defined as having complex NF1. 150 (34%) of those patients were known to have a PN, 78 males, 72 females, 159 PN in total. The age range was 1-64 years, median 24. The site of the PN was: head and neck 38%, abdomen and pelvis 19%, paraspinal 12%, lower limb 12%, upper limb 9%, thorax 8%, brachial plexus 4%. Patients presented with: pain 35%, disfigurement 30%, growth 13%, neurological deficit 13%, haemorrhage 6%, hard texture 1%. 9% of patients were asymptomatic. 26% of patients with symptomatic PN were referred for debulking surgery, 9% had biopsy only. There were 4 malignant peripheral nerve sheath tumours and 2 atypical neurofibromas.

Conclusions: We have presented a 1 year review of our cohort of 150 patients with complex NF1, with 159 plexiform neurofibromas. These arose most commonly in the head & neck and abdomen & pelvis. The most common symptoms were pain and disfigurement. We identified 4 new MPNSTs and 2 atypical neurofibromas amongst these tumours. PN are a significant cause of morbidity amongst people with NF1 and benefit from multidisciplinary management.

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Neurofibromatosis, Stress, and the Family: Examining the Efficacy of the NF Family Wellness Retreat Model

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Background: The social-emotional and cognitive difficulties related to Neurofibromatosis (NF) can severely impact quality of life and family functioning. Recent studies have reported anxiety and depression rates above 40% and high rates of attention and cognitive difficulties in NF populations. These psychosocial challenges can generate stress, lower quality of life, and impede family functioning, making them critical health intervention targets. Using Family Systems Theory to understand the family as a reactive network and a Mindfulness Model as a framework for stress reduction, the NF Family Wellness Retreat addresses the psychosocial challenges of NF through wellness-based programming.

Methods: To evaluate the retreat, researchers conducted quantitative surveys and qualitative interviews with retreat participants. Participants affirmed their informed consent, and parents gave additional consent to survey respondents under 18. The surveys, designed by the researchers, elicited information about the frequency of wellness behaviors and stress, the strength of family and community functioning, and knowledge about NF through 29 Likert-scale questions. We distributed pre-surveys at the retreat’s beginning and mailed post-surveys one month later. Sixteen participants completed both surveys. We analyzed these data using paired t-tests, a logit model to test correlations between responses, and average response trends. We conducted nine interviews by phone and a screen-sharing application. Participants responded to eight open ended questions, designed by the researchers, about stress, their families, and the retreat. Two independent coders recorded and coded the responses.

Results: The analysis of the average survey responses showed a slight decrease in reported stress, but none of the quantitative measures were statistically significant. The interview data found that all participant families experience NF-related stress, and indicated that the community support, relaxation techniques, and information provided at the retreat contributed to improved coping.

Conclusions: Supported by the quantitative trend in stress reduction, the qualitative data suggest that the retreat model improves coping with the psychosocial challenges of NF through robust social support structures, stress reduction practices, and information sharing.

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Experiences and Attitudes of Adults with Neurofibromatosis Type 1 Attending a Specialist Skin Clinic

Claire Wong, Clinical Genetics, Royal North Shore Hospital, St Leonards NSW; University of Sydney, Sydney NSW

Background: The appearance of Neurofibromatosis Type 1 (NF1) skin lesions can result in emotional distress and social isolation. In 2015, a novel clinical genetics dermatological service (skin clinic) dedicated to the management of NF1 skin manifestations was initiated in a tertiary genetics centre in Sydney, Australia. This exploratory qualitative study aimed to evaluate the usefulness and limitations of this service from a patient’s perspective.

Methods: Nine adult patients with NF1 (6 female, 3 male, age range 28-68 years) participated in a semi-structured interview about their views and experience of attending the skin clinic. Interviews were transcribed and analysed using inductive thematic methodology.

Results: Adults highly valued the availability of the skin clinic and NF1 expertise of the health professionals involved, even where cosmetic improvement was minimal. Key themes included high perceived value of the skin clinic for self and others, satisfaction with and preference for LASER therapy, and request for increased service provision.

Conclusions: Addressing skin concerns may be a previously unmet and important need of adults with NF1. This study highlights important roles of a specialised multidisciplinary approach and the potential of LASER therapy in the design of a dedicated clinical service for managing NF1 skin concerns. Furthermore, findings of this study demonstrate the complex disease burden associated with NF1 and illustrate the benefits of a specialist skin service to address patient needs.

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Application of Research Electronic Data Capture (REDCap) and Patient-Reported Outcome Measures (PROMs) in Neurofibromatosis Type 1 (NF1)

Claire Wong, Clinical Genetics, Royal North Shore Hospital, St Leonards NSW

Background: There is growing interest internationally in the use of patient-reported outcome measures (PROMs) to incorporate patient perspectives in healthcare and to promote patient-centred care. We utilised Research Electronic Data Capture (REDCap), a secure web application developed by the Vanderbilt University, as a reporting tool to evaluate under-investigated health domains in Neurofibromatosis Type 1 (NF1).

Methods: REDCap was used to design a PROM survey to document the prevalence of prominent NF1 manifestations and to assess individuals’ experience with itch and treatment. The survey includes targeted health questions and the validated 5-D itch scale, with readability appropriately modified for the NF1 population. Individuals affected by NF1 will be invited via the Australian CTF registry to participate through an online link. Validations and branching logic are used to improve quality of data, and data collected will be exported to common statistical packages for analysis.

Results: The process and utility of REDCap for collecting and managing PROMs will be described.

Conclusions: REDCap is a freely-accessible and powerful tool that can enable large-scale collection and management of PROMs, allowing individuals affected by NF1 to report on aspects of their health and wellbeing. Together, REDCap and PROMs represent key tools for guiding quality improvements that target healthcare to the needs of patients.

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NF2 Management in China

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Background: NF2 is a heritable syndrome that leads to the development of multiple intracranial tumors, including schwannomas, meningiomas and gliomas. Bilateral VSs are identified in 90–95% of NF2 patients. There is about 35000 NF2 patients in China. The treatment options for NF2-associated VSs mainly include observation, stereotactic radiosurgery, microsurgery and drug therapy.

Methods: Retrospective analysis of 63 patients with NF2 visited our center from January 2011 to May 2017. Family history, previous surgical or medicine treatment, patients' and immediate relatives' peripheral blood and tumor specimens (if surgical resection), NF2 gene testing, ophthalmological tests, neurological examination, audiological assessment, radiological data and individualized management for patients were collected. Postoperative follow-up included MRI every six months, cranial nerve function assessment, and audiological assessment. The average follow-up time was 3.6 ± 1.3 years.

Results: The median age was 22 years old (11 to 62 years old). There were 6 cases with family history and 57 cases without family history. The cases with family history showed only bilateral VSs and those without family history showed different clinical phenotypes. NF2 gene mutation could be detected in peripheral blood in all cases, but no obvious hotspot mutations was found. The mutations in cases with family history were consistent. No NF2 mutation was found in immediate relatives of cases without family history. 15 cases showed only bilateral VSs, and 48 cases showed multiple intracranial tumors (3-12 tumors, average at 4.7 tumors). 36 patients underwent at least one surgery, and 15 patients received medicine treatment. 10 tumors grew again of poor tumor control or after discontinuation of medicine. Of the 63 patients, 9 were lost, 22 underwent wait and scan, 6 were assisted by hearing aids, 12 underwent surgical resection of cerebospinal tumors, 9 underwent surgical resection of acoustic neuroma, and 5 underwent cochlear implantation.

Conclusions: NF2 is a multidisciplinary systemic disease involving the central nervous system, which needs multimodality therapy. Further studies are needed in order to find out the best treatment for NF2 patients with VSs in China. In our center, MDT is routinely conducted in treating NF2 patients and several studies, involving 500 NF2 patients, about drug therapy and treatment strategy of NF2 are carried out.
Epidemiologic Analysis of Major Complications Requiring Medical Interventions in Patients with Neurofibromatosis 1

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**Background:** Neurofibromatosis 1 (NF1) is an autosomal genetic disease that has various complications including dermatological, neurological and bone manifestations. Although about one-third of patients have major health impairments, a nationwide survey on the frequencies of complications requiring medical interventions has not been performed. We conducted a retrospective study for 3530 NF1 patients to elucidate the frequencies of NF1-related major complications in need of medical interventions.

**Methods:** We investigated clinical information for patients who met the diagnostic criteria of NF1 by the National Institutes of Health registered at the Ministry of Health, Labour and Welfare from 2001 to 2014 in Japan. The Ethics Committee approved the study protocol.

**Results:** Finally, data for 3505 patients (1595 males and 1910 females) were analyzed in this study. The prevalence peak was between 30 and 39 years of age (mean age, 38.3 years; age range, 0-93 years). The ratio of certified patients requiring medical interventions was 83%. The patients classified in the most severe grade suffered from dermatological complications (71.8% of the patients), neurological complications (38.1%) and bone complications (33.3%). In patients with dermatological manifestations, medical treatment was needed for cutaneous neurofibromas (58%), diffuse plexiform neurofibromas (31%) and malignant peripheral nerve sheath tumors (10%). Patients with neurological manifestations needed medical treatment mainly for intellectual disability (26%) and brain tumors (53%), and patients with bone manifestations needed medical treatment for pseudoarthrosis (9%), scoliosis (55%) and bone defect (16%). The frequencies of other complications including cardiovascular diseases and endocrinological diseases were low.

**Conclusions:** Our study has provided important information for the management of NF1. It is necessary for physicians to be aware of the priority of NF1-related complications requiring medical interventions in order to provide appropriate care for patients with NF1.

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A Soft Boot Brace for Patients with Drooping Giant Neurofibroma of the Leg, for Overall Improvement in the Quality of Life

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Background: Giant neurofibroma (GN) often degrades the quality of life of patients with neurofibromatosis type 1 (NF1). Though surgery is the standard therapeutic option, total resection of GN located at anatomically complicated sites is still challenging. In such cases, supportive treatment is an option. Here, we discuss the usefulness of a soft boot brace designed for a NF1 patient with drooping GN in the lower leg.

Methods: Case report: A 64-year-old man had GN in his lower right leg. He was first diagnosed with NF1 at the age of 59 though he recognized masses in the leg from early childhood. He did not have any history of surgery associated with NF1. The leg and ankle were diffusely swelling and drooping and the masses were frequently injured during walking. The circumference of the lower right leg was up to 61.5 cm, while that of the lower left leg, the left thigh and the right thigh was 33 cm, 36 cm and 33 cm, respectively. Magnetic resonance imaging demonstrated that large, diffuse and plexiform neurofibromas surrounded the neurovascular bundles so resection of the tumors was not done by plastic surgeons. As he suffered from severe cellulitis and abscess formation, we decided to apply a customized boot-type brace in order to protect the leg from injuries and avoid further infection. The brace had a bell bottom-like configuration that fits the shape of the leg. The upper edge of the cover was set higher than the site of the tumors and there was a soft pad on the upper edge of the cover to prevent the brace from rubbing against the skin. The fit is adjustable with hooks and loop fasteners to control edema in the leg. The sole was wide and tough so that the suspended part of the tumors never touch the floor. Due to the heaviness of the affected leg, we employed a double door-opening system, which allowed taking off the boot both through the frontal and back side. To correct length discrepancy between the legs, we also made a boot for the healthy leg.

Results: In the three years following the application of the brace, the patient never had any recurrence of cellulitis or any other skin infection. Moreover, the skin on the leg became softer and well-hydrated. The stability of the leg in the brace contributed to safe and easy walking.

Conclusions: Literature is lacking on brace therapies for NF1. This report can become a guide for clinicians treating patients with GN in the leg.

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Program Development and Evaluation: Establishing Needs in Pediatric Neurofibromatosis Patients

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Background: Research evidence has consistently demonstrated that children diagnosed with neurofibromatosis (NF), as a group, tend to struggle academically, socially, and developmentally. Issues concerning learning and various behavior problems (e.g., difficulties with attention, peer relationships, and challenging behaviors) are among the most commonly cited concerns by parents of children with NF. Gaining a better understanding of the effects of NF within the emotional, cognitive and social domains, as well as the direct impact they have on overall quality of life will potentially lead to better clinical interventions and programmatic supports within pediatric multi-disciplinary NF clinics. Thus, the purpose of the current study was to identify and evaluate the following within a pediatric multi-disciplinary NF clinic: (1) Psychosocial needs before, during, and after being diagnosed and receiving medical treatment for NF, (2) Educational status, social interactions, peer relationships, and psychosocial functioning, and (3) Patient psychosocial and educational support initiatives embedded within the NF multi-disciplinary program.

Methods: Pediatric patients with a diagnosis NF between birth and 21 years of age receiving treatment at a multi-disciplinary clinic in the Northeastern United States were eligible to participate in the present study. Participants were recruited during their new patient or follow-up appointment during the monthly pediatric NF multi-disciplinary clinic. Participants who consented to participate in the study were given a self-report assessment battery, which included the following: (1) Child Behavior Checklist or Adult Self-Report, (2) Pediatric Quality of Life Inventory, (3) Pediatric Quality of Life Inventory Neurofibromatosis Module, and (4) Survey of Well Being of Young Children, to complete during their clinic appointment.

Results: Preliminary data suggests that pediatric patients diagnosed with NF experience a multitude of psychosocial and educational challenges that impact their overall quality of life.

Conclusions: Data from the current study demonstrates that it is imperative for the psychosocial and educational challenges of pediatric patients' diagnosed with NF be identified and addressed within pediatric NF multi-disciplinary clinics, which should include medical specialists, as well as a licensed psychologist and a social worker.

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Proactive Early Hearing Rehabilitation in Type 2 Neurofibromatosis: A Shift of Paradigm to Contrast the Bilateral Deafness

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Background: Bilateral vestibular schwannomas (VS) are the hallmark lesion in patients affected by neurofibromatosis type 2 (NF2). Raised awareness of this condition led to early diagnosis and management of small VS, thus allowing the surgeon to evaluate the best treatment strategy for the patient. Decision making in VS management is determined by several patients’- and tumor factors, but in the last years the need of hearing preservation has become the probably most relevant aim to be pursued.

Methods: We retrospectively reviewed the medical charts of 22 NF2 patients that referred to our Institution between 2011 and 2017. Different treatment options have been proposed according to the patients’ diagnostic features, namely observation, hearing preservation surgery (HPS) through retrosigmoid approach, hearing rehabilitation surgery through translabyrinthine approach and consecutive cochlear implant (CI) or auditory brainstem implant positioning. In case of HPS failure, CI was indicated as option of hearing rehabilitation.

Results: Twelve patients (54.5%) with median age of 14 years (IQR: 11.15.5 years) underwent surgical intervention for VS removal with indications to hearing preservation surgery (50%), tumor growth (25%), tumor dimension in the pontocerebellar angle > 16 mm (17%) and neurological symptoms (8%). Seven patients were submitted to retrosigmoid approach with retrolabyrinthine meatotomy and 5 received translabyrinthine approach (in one case extended to the petrous apex). Cochlear nerve was intraoperatively preserved in 92% of cases. Cochlear implant was positioned in 8 patients. Five patients received CI after HPS failure, while 3 as part of the hearing rehabilitation program. Two major complications occurred, cerebellar edema, that required DVP and cerebellopontine angle hemorrhage in postoperative day 1, that required surgical revision. Hearing outcomes from our experience are discussed and presented.

Conclusions: Although hearing preservation in NF2 patients is still a surgical challenge, early proactive surgery - when feasible - allows hearing rehabilitation as an attempt to contrast the ineludible loss of hearing, which is the fate of bilateral VS in NF2 patients, especially when the severe type "Wishart" is diagnosed in young patients. HPS and cochlear nerve preservation or hearing rehabilitation with CI redefined the treatment strategies in NF2 patients.

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SEPT. 20-22

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The Children’s Tumor Foundation is grateful for the generous grant support from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health of the 2018 Joint Global Neurofibromatosis Conference.*

*Research reported in this publication was supported by NINDS of the NIH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.