

## What's New in Neurofibromatosis? Proceedings From the 2009 NF Conference: *New Frontiers*

Joseph L. Kissil,<sup>1</sup> Jaishri O. Blakeley,<sup>2</sup> Rosalie E. Ferner,<sup>3</sup> Susan M. Huson,<sup>4</sup> Michel Kalamarides,<sup>5</sup> Victor-Felix Mautner,<sup>6</sup> Frank McCormick,<sup>7</sup> Helen Morrison,<sup>8</sup> Roger Packer,<sup>9</sup> Vijaya Ramesh,<sup>10</sup> Nancy Ratner,<sup>11</sup> Katherine A. Rauen,<sup>7</sup> David A. Stevenson,<sup>12</sup> Kim Hunter-Schaedle,<sup>13\*</sup> and Kathryn North<sup>14</sup>

<sup>1</sup>The Wistar Institute, Philadelphia, Pennsylvania

<sup>2</sup>Johns Hopkins University, Baltimore, Maryland

<sup>3</sup>Guy's and St Thomas' NHS Trust, London, United Kingdom

<sup>4</sup>St. Mary's Hospital, University of Manchester, Manchester, United Kingdom

<sup>5</sup>Hopital Beaujon, APHP, Clichy, France and Inserm U674

<sup>6</sup>University Eppendorf, Hamburg, Germany

<sup>7</sup>University of California, San Francisco, California

<sup>8</sup>Leibniz Institute for Age Research, Jena, Germany

<sup>9</sup>Children's National Medical Center, Washington, District of Columbia

<sup>10</sup>Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

<sup>11</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

<sup>12</sup>University of Utah, Salt Lake City, Utah

<sup>13</sup>Children's Tumor Foundation, New York, New York

<sup>14</sup>University of Sydney and The Children's Hospital at Westmead, Sydney, Australia

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The NF Conference is the largest annual gathering of researchers and clinicians focused on neurofibromatosis and has been convened by the Children's Tumor Foundation for over 20 years. The 2009 NF Conference was held in Portland, Oregon from June 13 to June 16, 2009 and co-chaired by Kathryn North from the University of Sydney and The Children's Hospital at Westmead, Sydney, Australia; and Joseph Kissil from the Wistar Institute, Philadelphia. The Conference included 80 platform presentations in 9 sessions over 4 days; over 100 abstracts presented as posters; and three Keynote presentations. To date, there have been tremendous advances in basic research in the pathogenesis of neurofibromatosis, and more recently in progress toward identifying effective drug therapies and the commencement of neurofibromatosis clinical trials. The NF Conference attendees have significantly increased (doubling from 140 in 2005 to 280 attending in 2009) with a significant increase in attendance of physicians and clinical researchers. Correspondingly the NF Conference scope has expanded to include translational research, clinical trials and clinical management issues while retaining a core of basic research. These themes are reflected in the highlights from the 2009 NF Conference presented here. © 2010 Wiley-Liss, Inc.

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\*Correspondence to:

Dr. Kim Hunter-Schaedle, Children's Tumor Foundation, 95 Pine St, 16th Floor, New York, NY 10005. E-mail: [KHS@ctf.org](mailto:KHS@ctf.org)

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## INTRODUCTION

Neurofibromatosis affects 1:3,000 individuals, and characteristically causes largely benign but often debilitating tumors to grow in the nervous system. There are three main forms of NF: NF1, NF2 and schwannomatosis. NF1 and NF2 are autosomal dominant disorders caused by mutations in tumor-suppressor protein encoding genes. NF1 is typically diagnosed in childhood by appearance of dermal café au lait spots. Its course is unpredictable: it can cause a variety of benign nerve tumors including plexiform, dermal and optic glioma tumors; in some cases malignant peripheral nerve sheath tumors can develop from plexiforms. Around two-thirds of individuals with NF1 develop learning disabilities, and around a quarter may develop bone abnormalities such as tibial dysplasia [for recent NF1 review, see Boyd et al., 2009; Williams et al., 2009]. Recently, a milder NF1-like phenotype, termed Legius syndrome, was characterized, and is associated with café-au-lait spots, learning disabilities and minor bone abnormalities, but with absence of neural tumors [Brems et al., 2007; Spurlock et al., 2009]. NF2 is usually diagnosed in young adults by tinnitus or imbalance, and is characterized by bilateral vestibular schwannomas growing on the 8th cranial nerves. Those with NF2 will often lose their hearing due to auditory nerve damage during necessary surgery to remove these tumors. NF2 can also cause spinal schwannomas and meningiomas [for recent NF2 review, see Evans, 2009]. Overall the underlying genetics, biology and signaling pathways of NF1 and NF2 are fairly well understood and clinical trials are now underway. Schwannomatosis is the least understood form of neurofibromatosis. It is largely diagnosed in adulthood and characterized by peripheral schwannomas and pain that is very difficult to manage [MacCollin et al., 2005]. A candidate gene for schwannomatosis was recently identified [Hulsebos et al., 2007], and though not yet fully understood, efforts are well underway to better understand the genetics and clinical diagnosis of schwannomatosis as well to develop research tools such as mouse models for cellular and molecular studies.

All of these topics were addressed at the 2009 NF Conference: New Frontiers, and the highlights summarized here provide a snapshot of the current state of the neurofibromatosis research and clinical arena.

## WHAT IS THE STATUS OF NF CLINICAL TRIALS?

Roger Packer (Children's National Medical Center) and Jaishri Blakeley (Johns Hopkins University) chaired the opening session of the Conference, reviewing the status of therapeutic clinical trials for children and adults with NF1 and NF2, an area that has seen a dramatic increase in activity over the past few years. The earliest NF1 clinical trial efforts were funded by the Congressionally Directed Medical Research Program for Neurofibromatosis (CDMRP NFRP) and tested pirfenidone and farnesyl transferase inhibitors in prospective plexiform neurofibroma studies. Though neither drug emerged as a promising therapy, these trials paved the way for the design and implementation of NF1 biological agent trials. A major development came with the formation of the Phase II Neurofibromatosis Clinical Trials Consortium, established in 2005 and again, supported by CDMRP NFRP. Dr. Packer chairs this

program and he provided an update of progress. The Phase II NF Clinical Trials Consortium includes nine core sites in the United States. Additional sites, including international collaborators, may be included for selected studies. The Phase II NF Clinical Trials Consortium is focused on designing and implementing trials for NF1 manifestations; these include benign plexiform neurofibroma, optic pathway glioma or dermal neurofibroma tumors; malignant peripheral nerve sheath tumors; bone abnormalities such as tibial dysplasia; or learning disabilities.

The first trial of the Phase II NF Clinical Trials Consortium is evaluating the mTOR inhibitor Rapamycin in NF1 patients with progressive plexiform neurofibromas. The trial utilizes three-dimensional volumetric analysis imaging to monitor growth and shrinkage in these tumors which are often diffuse and otherwise difficult to measure. Prior natural history studies have shown that the time to progression for plexiform neurofibromas is 10.6 months on placebo, and these data will be used as the comparison arm for the Rapamycin Phase II clinical trials. This first trial of the Phase II NF Clinical Trials Consortium has completed accrual in patients with symptomatic disease, and is near completion of initial accrual goals for patients with radiographically progressive disease. The trial may be expanded to include patients with progressive lesions.

The second trial of the Phase II NF Clinical Trials Consortium is evaluating Lovastatin for the treatment of cognitive deficits, particularly visuospatial learning and attention, in a prospective, placebo-controlled randomized trial for children with NF1 between 10 and 17 years of age. This study opened in summer 2009. The third trial of the Phase II NF Clinical Trials Consortium was outlined by Nicole Ullrich (Children's Hospital Boston) and will assess a novel mTOR inhibitor RAD-001 in NF1 patients with progressive low-grade gliomas. Finally, a proposal has recently been submitted to the Phase II NF Clinical Trials Consortium to assess a new biologic agent in NF1 patients with recurrent malignant peripheral nerve sheath tumors (MPNSTs). This trial would be conducted in conjunction with the national Sarcoma Clinical Trials Consortium.

Ype Elgersma (Erasmus University, the Netherlands) described the results of a randomized, double-blind, placebo-controlled trial in 62 children evaluating whether the related statin drug simvastatin, might improve neuropsychological outcome in children with NF1. Though no clear-cut clinical improvement was seen with simvastatin treatment, there were a number of limitations in this study including marked practice effect in outcome measures in both controls and treated patients, inclusion of children with intellectual impairment, inclusion of patients on stimulant medication and very short duration of treatment on full dosage. These limitations have been taken into account in the design of the CDMRP Consortium's Lovastatin study described above; in addition only children with deficits in the primary outcome measures will be included in the current trial—since these demonstrated the greatest response to Simvastatin in the Netherlands study.

Brigitte Widemann (National Cancer Institute) reviewed data from recently completed and ongoing clinical trials for children and adults with symptomatic plexiform neurofibromas. These included: completed oral farnesyl transferase inhibitor trial and completed pirfenidone trials; an ongoing phase II study utilizing interferon and another utilizing Imatinib mesylate; and a phase I trial of

sorafenib which targets Raf and other kinases. The patient entry and evaluation criteria of these trials are slightly different from each other. Other studies are planned for treatment of recurrent MPNSTs, including the addition of biologic agents to standard chemotherapy.

Scott Plotkin (Harvard Medical School/Massachusetts General Hospital) described an ongoing trial assessing the effect of ranibizumab on the growth of dermal neurofibromas. Ranibizumab is a monoclonal antibody for VEGF-A, and could potentially inhibit vascularization of dermal neurofibromas and thereby halt growth. In addition to assessing the local response to Ranibizumab injection in dermal neurofibromas, the trial will assess circulating angiogenic proteins in blood and tumors of NF1 patients.

Madeleine Duvic (MD Anderson Cancer Center) discussed how trials are conducted for other, non-NF cutaneous syndromes including malignancies. From these trials, she suggested several therapeutic targets that may be worth investigating as NF1 dermal neurofibromas therapy approaches. These included mTOR, histone deacetylase inhibitors such as suberoylanilide hydroxamic acid or Ras pathway inhibitors such as farnesyl-protein transferase inhibitors.

The recent development of consensus recommendations for conducting NF2 clinical trials [Evans et al., 2009] has resulted in commencement of clinical trials in this arena. NF2 patients may develop several tumor types in the brain and peripheral nerves including vestibular schwannoma (VS, the hallmark of the disease), cranial or spinal nerve schwannoma, peripheral nerve schwannoma, ependymoma or meningioma. Two trials are now open for NF2 patients, both focusing on VS endpoints. Jaishri Blakeley (Johns Hopkins University) described the first NF2 trial, a “Phase 0” study, assessing delivery of the ErbB2/EGFR inhibitor Lapatinib to tumor tissue and the effects of the drug on the targeted pathways within tissue. Patients may enroll if they have a scheduled VS surgery; drug is given prior to surgery, and the tumor may be examined after removal to see if the drug reached it. This study is designed to rapidly assess if a promising agent can reach the tumor site and, if successful, may rapidly progress to Phase II trials. The second NF2 study, presented by Harry Miao (PTC Therapeutics), is a Phase II trial of PTC299, a new drug entity that inhibits VEGF synthesis upstream via interruption of post-transcriptional processing, and thus decreases angiogenesis. The goal is to reduce blood perfusion and correspondingly VS volume, and potentially maintain or restore hearing for NF2 patients. This trial will enroll NF2 patients who have progressive growth of VS with hearing loss.

The session concluded with two presentations on Children’s Tumor Foundation initiatives to help accelerate NF clinical trials. Looking ahead to new drug therapies and drug targets, Mila McCurrach (Children’s Tumor Foundation) presented an update on the Children’s Tumor Foundation NF Preclinical Consortium (NFPC), which screens promising candidate drugs in parallel in multiple NF1 and NF2 tumor models. NFPC is making headway in engaging pharmaceutical companies: the first drug partnership tests Novartis drug BEZ-235, a dual PI3K/mTOR inhibitor. Mindell Seidlin (Children’s Tumor Foundation) spoke about a new initiative of the Foundation to develop an NF1 Biobank and Patient Registry. This resource would serve in part as the return pathway for translational research by facilitating investigation of patient-derived tissues to identify new therapeutic targets.

The clinical trials presentations indicated that there has been remarkable progress over the past few years in the development and testing of therapeutics in well-designed, statistically sound clinical trials for several of the disease processes that impact NF1 and NF2 patients.

## NEW NF THERAPEUTIC TARGETS AND PRECLINICAL DRUG SCREENING

Nancy Ratner (Cincinnati Children’s Hospital Medical Center) chaired a session that looked ahead to future drug targets and therapeutic approaches for NF. Ruihong Chen (NexGenix Pharmaceuticals) discussed a new drug therapy for dermal neurofibromas (DNFs) which develop in the large majority of adults with NF1. DNFs can range in number from a few to several thousand in one individual, and though benign, these tumors can cause disfigurement, pain and pruritus. Surgical removal of DNFs requires multiple injections of anesthetic or costly general anesthesia. Data from DNF cell-based assays, explants and xenografts, as well as from small proof-of-principle NF1 clinical trial conducted in Mexico City and Rio de Janeiro, employing a specially formulated drug doxycycline (NX101) were assessed in treatment of DNFs. This drug is an antibiotic in wide usage with known vascular disruption and possible anti-angiogenic capabilities. The NF1 trial conducted was a proof-of-principle open-label single dose clinical trial. From these trials NX101 was reported to be quite effective in clearing DNFs, causing vascular disruption, necrosis and clearing of lesions within days after one to two injections, with minimal side effects including minor pruritus and discomfort at the injection site. No complications or evidence of re-growth of lesions were observed in follow-up visits that extended to 12 months.

While activation of Ras and its down-stream effectors are observed in only some MPNST cell lines, Faris Farassati (University of Kansas) showed that RalA is activated in all studied mouse and human MPNST cell lines, and in MPNST tumor samples as compared to non-transformed Schwann cells. Although highly similar to Ras, Ral proteins (RalA and RalB) activate different effectors, influencing gene expression and translation through interaction with ZO-1 associated nucleic acid binding protein and RalA binding protein 1. Silencing Ral or using a dominant negative Ral reduced proliferation, invasiveness, and in vivo tumorigenicity of MPNST cells, and reduced the expression of epithelial-mesenchymal transition markers. Expression of NF1-GAP-related domain diminished the levels of Ral activation, implicating neurofibromin in regulating RalA activation. Exposure of MPNST cells to concentrations of geranyl-geranyl transferase inhibitors which reduced Ral activation but had no significant effects on Ras activation decreased cell proliferation, implicating the Ral pathway as a target for therapy of MPNST.

Geoffrey Kilili (Tufts University), a predoctoral fellow in the laboratory of John Kyriakis, is following leads from the *Drosophila* model where the orthologues of Merlin act upstream of and positively regulate the tumor suppressor Hippo (hpo), the *Drosophila* orthologue of the mammalian Ste20 like kinases 1&2 (Mst1&2). In mammals, the MAP3K protein Raf-1 interacts and negatively regulates Mst2. To determine if Merlin acts upstream of

Mst2 in human cells and to elucidate the role of the *hippo* pathway in mitogen mediated MAPK signaling they used RNAi knock down and adenoviral over expression systems. Importantly, these investigators found that Merlin did not act upstream of or positively regulate Mst2 in mammalian cells. Knockdown of Mst2 led to increased phosphorylation of Raf-1, decreased Raf-1 kinase activity, MEK1/2 phosphorylation and cell proliferation. Raf-1 hyperphosphorylation persisted after elimination of the PI3K/AKT signaling, a known Raf-1 targeting pathway. These data indicate that Mst2 has a positive role in mitogen mediated MAPK signaling via suppression of the inhibitory phosphorylation of Raf-1 on S259 through a mechanism that does not involve the PI3K/AKT pathway [O'Neill et al., 2004; Hamaratoglu et al., 2006].

C. Oliver Hanemann (Peninsula College for Medicine and Dentistry, United Kingdom) also described candidate drug therapies for NF2. Merlin loss causes benign schwannomas which show enhanced Rac1, Cdc42, and p21-activated kinase 2 (PAK2) activities. PAK regulates the activation of merlin; however, the role of PAK in the Rac pathway is insufficiently characterized. The group used a peptide inhibitor of the PixGEF interaction with Rac and a novel small molecule PAK inhibitor, IPA-3, to investigate the role of PAK activation on Rac1/Cdc42 activity, cell spreading and adhesion in human primary schwannoma and Schwann cells. In summary, IPA-3 blocks activation of PAK2 at Ser192/197, antagonizing interaction between PAK and Pix, resulting in decreased Pix-mediated Rac1 activation. They concluded that PAK acts upstream of Rac, and confirm studies showing that Rac activation in schwannoma cells is essential for cell spreading and adhesion. These studies support PAK as a potential therapeutic target in schwannomas [Flaiz et al., 2009a,b].

Ronen Marmorstein (The Wistar Institute) discussed several kinases downstream of Ras and Rac that are constitutively activated in many cancers, including schwannomas and neurofibromas, making them important drug targets. Three such kinases are BRAF, PI3K, and PAK1. Given that inhibitors to these kinases have not yet progressed beyond clinical trials, this team of investigators combined synthetic chemistry, small-molecule screening and structural biology to develop novel organometallic inhibitors that show high potency and selectivity for these kinases. In each case, he reported on inhibitors with IC<sub>50</sub> values in the mid-nanomolar range and with a high degree of kinase isoform selectivity and that function in cells. He also reported on the X-ray crystal structures of these inhibitors bound to their respective kinase targets revealing the molecular basis for inhibition and avenues for further inhibitor development. Together, these inhibitors provide lead compounds for the development of drugs to treat cancers of the nervous system.

Nancy Ratner described the progress of the NF1 microarray consortium. To identify novel biomarkers and therapeutic targets to fight NF1, this group have used transcriptional profiling to distinguish primary normal Schwann cells (n = 10) from NF1-derived primary Schwann cells, malignant peripheral nerve sheath tumor (MPNST) cell lines, and benign and malignant tumors (n = 67). Dr. Ratner reported on validation of differential expression of 82 genes including the neural crest transcription factor SOX9 and SOX9 predicted transcriptional targets. For example, SOX9 immunoreactivity was robust in neurofibroma and MPNST tissue sections. Using shRNAs targeting genes up-regulated in

neurofibromas and MPNST they are testing the role of SOX9 and its targets in cell growth and tumorigenesis. Targeting SOX9 caused MPNST cell death. SOX9 regulates EYA4 which is highly expressed in MPNST. EYA proteins interact with Dachsund (DACH) and/or SIX proteins to bind DNA and regulate gene transcription. Thus SOX9 is a biomarker of neurofibroma and MPNST, and SOX9 and its downstream targets may represent therapeutic targets in NF1 [Williams et al., 2008; Miller et al., 2009].

Karlyne Reilly (National Cancer Institute - Frederick) is investigating a very interesting novel natural product that is a therapeutic candidate for NF1 tumors. She presented data showing that it appears to target cells that have homozygous loss of Nf1, while wild-type and *Nf1*<sup>-/+</sup> cells are resistant to the effects of the natural product. The group determined that the natural product inhibits multiple tumor types associated with NF1. NF1-specific natural products may form the basis for NF1-specific therapy in the future.

## UNRAVELING THE SIGNALING PATHWAYS OF NF

A signaling-focused session chaired by Vijaya Ramesh (Harvard Medical School/Massachusetts General Hospital) and Frank McCormick (University of California, San Francisco) spanned talks on NF2/merlin functions, Schwann cell biology and NF1/Ras signaling. The session opened with Brendan Manning (Harvard School of Public Health) giving an overview of the complexity of mTOR signaling. There is significant convergence of many tumor suppressors on the mTORC1 pathway including *NF1*, *NF2*, *PTEN*, *LKB1*, *TSC1*, and *TSC2*. However, the downstream consequences of aberrant mTORC1 activation and therapeutic response of these different tumor suppressor syndromes remain poorly understood. The Manning laboratory focuses on the cellular and molecular effects of uncontrolled mTORC1 activity in TSC, via a combination of TSC gene disruption and rapamycin treatment to activate and inhibit mTORC1 respectively. From this system, his group has identified and characterized a set of transcripts that are strictly regulated by mTORC1 signaling. They have also shown that aberrant mTORC1 signaling can trigger adaptive stress response pathways that dampen the deleterious effects of metabolic changes and uncontrolled protein synthesis. The approaches taken by Dr. Manning's laboratory offer novel points of therapeutic intervention and may provide potential targets of cytotoxic agents to selectively kill tumor cells exhibiting elevated mTORC1 activity, a molecular defect common to NF1, NF2, and TSC.

Chunling Yi (The Wistar Institute), a postdoctoral fellow in the group of Joseph Kissil, has purified a novel protein complex composed of merlin and a number of tight junction associated proteins. A direct interaction between merlin and the tight junction proteins as well as co-localization of the proteins to both tight junctions and adherens junctions were presented. One of the interacting proteins was shown to function downstream of merlin as a positive regulator of Rac-Pak and MAPK signaling. This study elegantly demonstrated a novel merlin-mediated signaling route at the site of cell:cell junctions, which may contribute to the tumor suppressive function of merlin.

Marlan Hansen (University of Iowa) presented data suggesting that ErbB2 and c-Jun N-terminal kinase (JNK) signaling contribute to vestibular schwannoma growth and radiosensitivity. ErbB2, a

receptor tyrosine kinase essential for Schwann cell development and proliferation resides in lipid rafts and is active in vestibular schwannoma (VS) cells. In addition, MEK/ERK, PI3K/AKT, and JNK signaling are active in VS cells, which is independent of ErbB2 signaling. Inhibition of JNK resulted in VS cell apoptosis and an increase in radiosensitivity.

Betty Chow (Fox Chase Cancer Center), a postdoctoral fellow in the group of Jonathan Chernoff, showed that a peptide inhibitor of Pak1, Pak2, and Pak3 restored normal morphology and slowed the growth of cells transformed with a dominant mutant allele of merlin. Xenografts of cells expressing the peptide inhibitor showed markedly reduced growth, when compared with cells expressing an inactive mutant form of this inhibitor. The growth of the xenograft tumors did not correlate with the level of ERK activation, indicating that the effects of Pak on cell proliferation and tumor growth are not mediated by ERK.

Pablo Hollstein (Harvard Medical School) discussed the molecular mechanisms by which neurofibromin is degraded during normal cell signaling. He reported identification of an E3 ubiquitin ligase that is specifically required for neurofibromin degradation. Depletion of this ubiquitin ligase specifically attenuates Ras signaling, resulting in impaired cellular proliferation. Dr. Hollstein suggested that understanding the mechanisms that regulate neurofibromin degradation may provide the opportunity for designing or applying effective therapies aimed at increasing neurofibromin protein stability, a strategy that may be most useful in manifestations related to haploinsufficiency at the *NF1* locus, which has been shown to contribute to some symptoms of the disease. Regulation of neurofibromin degradation might also be a useful strategy in other conditions driven by hyperactive Ras and cell proliferation.

Frank McCormick discussed therapeutic approaches to the Ras pathway, with special emphasis of the issue of “oncogene addiction.” According to this concept, cancer cells often become increasingly dependent on driver oncogenes for their survival, and therefore respond dramatically when these drivers are blocked through therapeutic intervention. In the case of NF1, Shannon, Lauchle and colleagues (submitted for publication) showed that early stages of myeloid disease associated with NF1 are not sensitive to intervention, using MEK inhibitors, but later stages that progress to AML, become sensitive. This suggests that drugs blocking hyperactive Ras signaling may have little effect on normal tissue in NF1 patients, but could selectively inhibit advanced disease. Dr McCormick also discussed intrinsic cellular pathways, involving ephrin signaling and sprouty-like proteins that regulate normal Ras and could, potentially, be a source of new drug targets to treat this disease.

Karen Cichowski (Harvard Medical School/Brigham and Women’s Hospital) discussed the use of mouse models to optimize treatment protocols for treating NF1. While mTOR inhibitors are currently being tested in the clinic, it is likely that sequential or combination therapies may ultimately be more effective. The Cichowski group has been delineating mechanisms of NF1 pathogenesis and investigating the biology of therapeutic response in these models. Responses *in vitro* have, so far, not been predictive of efficacy *in vivo*, stressing the importance of advanced mouse models to properly mimic disease and therefore predict response.

In glioblastoma, loss of neurofibromin occurs through deletion of the gene and through increased turnover and degradation, a process initiated by PKC. These insights may be useful in identifying new therapeutic approaches to NF1.

Jan Manent (INSERM, France) presented data evaluating the transcriptome of *Nf2*-deficient primary mouse Schwann cells (SCs) and tumors from mice and patients. In mouse SCs, loss of *Nf2* led to the activation of a molecular program reminiscent of myelination via the PI3K/Akt pathway. Mouse schwannoma transcription profiles share striking similarity to developing immature SCs and comparison of mouse and human schwannoma gene expression revealed common transcriptional signature. The data presented suggested that NF2 protein plays a role in axon-SC crosstalk and that loss of this specific function may be relevant to schwannoma formation.

Steven Scherer (University of Pennsylvania) provided an overview on junctional specialization of Schwann cells. Myelinating Schwann cells have gap, tight and adherens junctions that are composed of connexin32, claudin-19 and E-cadherin, respectively. In addition, they have specialized contacts with the axons at the nodes of Ranvier and the flanking paranodal region mediated by a different set of cell adhesion molecules. Cultured normal Schwann cells are joined by adherens junctions comprised of N-cadherin. Beta-catenin is associated with the adherens junctions of myelinating, non-myelinating and cultured normal Schwann cells. In contrast, NF2-deficient Schwannoma cells revealed poorly formed adherens junctions and an irregular distribution of N-cadherin and Beta-catenin, which has been implicated in tumorigenesis.

Andrea McClatchey (Harvard Medical School/Massachusetts General Hospital) presented on behalf of her predoctoral fellow Zachary Morris and showed how merlin controls EGFR distribution and signaling. Employing single particle tracking of individual EGFR molecules, it was shown that merlin immobilizes EGFR at the cell surface in confluent mouse-derived cells. Furthermore, merlin appears to govern the mobility and distribution of EGFR within the plasma membrane in a contact-independent manner. The efficacy of several EGFR inhibitors was tested in preclinical *in vitro* studies and pilot study results indicated that the compounds were effective in blocking proliferation and signaling in *Nf2*<sup>-/-</sup> mouse cells.

Ueli Suter (ETH Zürich, Switzerland) closed the session with a focus on Rho-GTPases in Schwann cells. The role of integrin-linked kinase (ILK) in Schwann cell migration, proliferation and morphological changes associated with the sorting, ensheathment and myelination of axons was reviewed as well as the roles of RhoGTPase and AKT signaling in signal transduction in Schwann cell development.

## CELLULAR ORIGIN OF NF TUMORS

Helen Morrison (Leibniz Institute for Age Research, Germany) chaired a session featuring talks that highlighted identifying the cell or origin or tumor initiating cells in NF tumors. The cancer stem cell (CSC) model of tumors has received tremendous attention in recent years, but the concept that tumor development is fundamentally a problem of stem cell biology remains somewhat controversial. The “CSC hypothesis” suggests that tumors derive from a subset of mutated stem cells that possess characteristics associated

with a normal stem cell such as self-renewal and the capacity to differentiate into actively proliferating tumor cells. Such cells are proposed to be resistant to conventional chemotherapy and considered to be a persistent and distinct sub population of cells that can give rise to new tumors. While the CSC hypothesis is a significant contribution, challenging questions remain. For example, do such cells really represent a minority population? What is the exact cell of origin of CSCs - stem cells that have lost the ability to regulate proliferation, or specialized cells from the adult tissue have acquired a stem-cell-like state? It is possible that several answers are correct, dependent on tumor type and phenotype. Identifying tumor initiating cells and whether they follow the CSC model or not is critical for a complete understanding of tumor behavior and has fundamental implications for therapeutic strategies. As an example, despite progress in understanding the molecular biology of malignant astrocytomas, a tumor with increased occurrence in NF1, the clinical prognosis of these brain tumors continues to be dismal. The development of therapeutic interventions, and ability to anticipate tumor behavior, are limited because the cell of origin of the tumor is unknown. Astrocytomas were originally thought to arise from differentiated glial cells that then undergo a process of dedifferentiation, but initiation of tumor formation in mature differentiated astrocytes *in vivo* has never been properly tested. In addition the recent identification of adult neural stem cells provokes the question whether these immature cells are in fact the targets of tumor causing mutations.

Sheila Alcantara (University of Texas Southwestern), a postdoctoral fellow in the group of Luis Parada, presented a study focused on identifying the tumor cell of origin in a mouse model of malignant astrocytoma. The Parada group has previously developed a tumor-developing mouse models containing a conditional inactivation of human astrocytoma-relevant tumor suppressors *p53*, *Nf1*, and *Pten* [Zhu et al., 2005; Kwon et al., 2008]. Tumor suppressor inactivation in neural stem cells or progenitor cells are both necessary and sufficient to induce astrocytoma formation as demonstrated via specific *in vivo* gene targeting to “neurogenic niches” such as the subventricular zone of the brain. Strikingly, the mice develop astrocytomas and the transformed cells undergo infiltration and multilineage differentiation. The tumor suppressor targeted neural stem/progenitor cultures from presymptomatic mice showed a growth advantage and altered differentiation, identifying a pre-tumorigenic cell population. This is the first report that normal neural stem/progenitor cells *in vivo* are cancer-initiating cells that can give rise to high-grade astrocytomas. Taken together these findings indicate the existence of CSC in malignant astrocytoma.

Johanna Buchstaller (University of Michigan), a postdoctoral fellow in the group of Sean Morrison, presented work identifying the tumor initiating cells in malignant peripheral nerve sheath tumors (MPNST). MPNSTs are very aggressive and invasive soft tissue sarcomas that appear in approximately 10% of patients with neurofibromatosis. Previous work from the Morrison group has addressed the question of whether cancers are driven by rare subpopulations of CSCs. While they appreciate that the cancer stem cell model is an important new idea they have suggested that the design of certain mouse transplantation experiments underestimates the frequency of human cancer cells that are capable of

contributing to disease progression. Using highly immunocompromised modified mice and transplanting human melanoma cells they could already demonstrate that a significant fraction of melanoma cells have common tumorigenic attributes, even when such cells appear rare in other less immunocompromised mice [Quintana et al., 2008]. They propose a model in which some human cancers follow a cancer stem cell model whereby disease progression is driven by small subpopulations of cancer stem cells, while other cancers do not follow this model. Assessing whether a cancer has rare or common tumorigenic potential is critical when designing therapeutic strategies. Cancers that follow a cancer stem cell model might be treated with therapies that target cancer stem cells. In contrast, for cancers in which tumorigenic potential is a more common attribute, therapies would have to target the majority of the cells in the tumor. Dr. Buchstaller presented work aimed at determining whether the progression of MPNSTs follows a cancer stem cell model. They employed mouse models for MPNSTs bearing mutations in *Nf1*, *Ink4Arf*, and *p53*, genes that are relevant for human patients, in which they have previously shown that MPNSTs appear to arise from differentiated glia and not neural crest stem cells [Joseph et al., 2008]. Performing limiting dilution transplantation assays, they found that a high number of cells isolated from primary MPNSTs of *Nf1+/- Ink4aARF-/-* mice were able to transfer disease when transplanted into syngenic mice. These results suggest that growth of MPNSTs in this mouse model does not follow a cancer stem cell model and that most tumor cells are capable of forming new tumors. These studies have fundamental implications for therapeutic strategies for MPNSTs, suggesting all cancer cells need to be targeted during tumor therapy bearing these mutations [Joseph et al., 2008].

## NF2 AND SCHWANNOMATOSIS—WHATS NEW?

This session, chaired by Joseph Kissil (The Wistar Institute) offered an update on the progress and major open questions in NF2 and schwannomatosis. Allan Belzberg (Johns Hopkins University) opened with several case studies that demonstrated the current and urgent need for a study to delineate the natural history of schwannomatosis. While diagnostic criteria have been established, there is a paucity of information regarding the underlying genetic mechanisms and the natural course of the disease that significantly hampers the development of therapeutic approaches. Belzberg described a new initiative to address this, that is, assembly of a schwannomatosis natural history database. A panel of international clinical experts will develop the questions to form a new schwannomatosis-centered database that will include a case-reporting interface. These studies will provide preliminary information regarding the natural history of the disease and assess the utility of developing and expanding the database worldwide.

Anat Stemmer-Rachamimov (Harvard Medical School/Massachusetts General Hospital) went on to present exciting data regarding the expression status of the in sporadic and familial cases of schwannomatosis, NF2 and sporadic, solitary schwannomas. The findings indicate that loss of expression of *SMARCB1/INI1* is seen not only in schwannomatosis associated schwannomas, but also in NF2 associated schwannomas. This contrasts with solitary, sporadic schwannomas in which most tumors retain INI1 expression

suggestive that the pathogenesis of solitary sporadic tumors is different than that of hereditary and multiple schwannoma syndromes [Patil et al., 2008]. Furthermore, INI1 loss of expression is much more common in schwannomatosis associated schwannomas than the reported rates of INI1/SMARCB1 mutations found in other series implying that other, epigenetic mechanisms may be responsible for silencing the INI1 gene in some cases [Boyd et al., 2008; Hadfield et al., 2008].

Emmanuelle di Tomaso (Harvard Medical School/Massachusetts General Hospital) presented some exciting new data on an NF2 clinical trial. First, she and her colleagues examined vascular patterning, vessel morphology and expression of angiogenic molecules such as VEGF, VEGFR2, NRP1, and NRP2 in vestibular schwannoma specimens, and the results suggested a role for VEGF in schwannoma pathophysiology. Next, 10 patients with progressive vestibular schwannomas and chronic progressive hearing loss in their only hearing ear were treated by an anti-VEGF monoclonal antibody. Overall results were encouraging with the majority of tumors shrinking and a hearing response was seen in 4 of 6 patients (66%) who were capable of hearing improvement. The results suggest that the pathophysiology of hearing loss from neurofibromatosis-associated vestibular schwannomas is related, in part, to the action of VEGF and provide the first evidence that a medical treatment can restore functional hearing in a subset of NF2 patients [Plotkin et al., 2009].

Gareth Evans (St. Mary's Hospital, University of Manchester) described the significant diagnostic and prognostic dilemma associated with diagnosing patients with schwannomas. The approach taken in attempt to resolve this is to establish if schwannomas could be differentiated on the basis of gene expression profiling. By comparing array profiles from different schwannomas, normal nerve and fibromas, a set of transcripts was identified that exhibit significant differential expression between the schwannoma subtypes and between controls and schwannomas. Hierarchical clustering analysis indicated that the gene expression pattern in NF2 and unilateral vestibular schwannomas (UVS) is very similar as reflected by the small number of genes that are differentially expressed between these two groups. In addition, there was no apparent clustering correlating to the size of tumor, location (right/left) or gender of the patient. A few differentially expressed genes were identified in NF2 schwannomas relative to schwannomatosis and in both NF2 and UVS schwannomas relative to schwannomatosis. These studies provide an initial molecular phenotype to aid differentiation of schwannomas subtypes and predict clinical outcome and may provide targets for therapeutic intervention.

Rachael Hornigold (Guy's and St Thomas' NHS Trust) discussed the appropriateness and timing of surgical intervention in NF2. The majority of patients will undergo multiple surgical procedures within their lifetime, many resulting in significant postoperative deficits. A retrospective case note analysis examined the number and timing of surgical procedures and post-operative complications. These were correlated to a number of parameters including tumor type and burden, associated clinical features, age at presentation and genotype. Of the cohort examined, over 75% of patients have undergone some form of surgical intervention, with a mean of three procedures. 39.5% of patients had surgery prior to diagnosis with NF2, and 74.4% had surgery prior to specialist review.

Management within a multi-disciplinary clinic and correct timing of surgical procedures is essential to avoid unnecessary complications and to avoid the need for emergency intervention.

Vijaya Ramesh (Harvard Medical School/Massachusetts General Hospital) presented exciting new data implicating merlin as a novel regulator of TSC/mTORC1 signaling and described the identification of the mammalian target of rapamycin complex 1 (mTORC1) as a novel mediator of merlin's tumor suppressor activity. Merlin-deficient human meningioma and merlin knockdown arachnoidal cells exhibit rapamycin sensitive constitutive mTORC1 activation and increased growth. Also, NF2 patient tumors and *Nf2*-deficient MEFs, demonstrate elevated mTORC1 signaling and, conversely, the exogenous expression of wild-type merlin isoforms, but not a patient-derived mutant, L64P, suppresses mTORC1 signaling. It appears Merlin does not regulate mTORC1 via the established PI3K-Akt or MAPK/ERK-mediated TSC2 inactivation, and may instead regulate TSC/mTOR signaling in a novel fashion. These findings suggest that rapamycin or rapamycin analogues in combination with PI3K inhibitors, may provide promise as new therapeutics in the treatment of NF2-associated meningiomas and schwannomas.

## NOVEL CELLULAR AND ANIMAL MODELS OF NF

Michel Kalamarides (Hopital Beaujon, Paris) chaired a session focused on novel cellular and animal models that are a vital resource for NF research. Dr. Kalamarides first reported a new mouse model of NF2 meningioma which account for one-third of all primary central nervous system tumors. The frequent *NF2* gene inactivation event in human meningiomas has been utilized to generate this meningioma mouse model and while it represents a significant advance in meningioma modeling, questions persist as to the meningioma cell of origin [Kalamarides et al., 2002]. To address this, the Kalamarides group has identified a specific marker of mouse and human arachnoid cells, the prostaglandin D2 synthase (PGDS). This arachnoid-specific promoter has been used to generate conditional *Nf2* knockout mice with restricted biallelic *Nf2* inactivation in the primordial meninges, before formation of the meningeal layers. Using genetic and injection methods (*adCre*) the group found that the timing of *Nf2* biallelic inactivation in a PGDS+ meningeal precursor cell is responsible for the developmental heterogeneity of meningioma subtypes. Pre-natal and early postnatal biallelic *Nf2* inactivation in PGDS+ progenitor cells can give rise to meningiomas, whereas more mature cells cannot. Together, this study not only has established a novel mouse model for meningioma, but also provided important insights into the cell of origin of the different histological subtypes of meningiomas.

Larry Sherman (Oregon Health & Science University) is exploring the role of SWI/SNF factors in Schwann cells as potential regulators of pain and tumor growth in schwannomatosis. Mutations in the *SNF5* gene (also called *SMARCB1* and *INI1*) have been observed in schwannomas from schwannomatosis patients. SNF5 is a subunit of SWI/SNF chromatin remodeling complexes that can regulate both cell cycle progression and the transcriptional activation of a wide range of genes. They observed Schwann cell hyperproliferation along peripheral nerves of mice with conditional mutations in brahma-related gene-1 (*Brg1*), an ATPase subunit of

SWI/SNF complexes. This effect was cell autonomous as loss of Brg1 in primary cultures of Schwann cells was sufficient to induce a two- to threefold increase in bromodeoxyuridine (BrdU) uptake in vitro. In contrast, mice with conditional mutations in the *Snf5* gene and *Snf5*-null Schwann cells grown in vitro did not display significant changes in Schwann cell proliferation. Interestingly, they found that rhabdoid tumors with *SNF5* mutations have elevated levels of transcripts of a member of the neurotrophin family of growth factors brain-derived-neurotrophic factor (BDNF), and that Schwann cells lacking *Snf5* express twice the amount of a BDNF as wild type Schwann cells. Indeed, growing evidence has implicated BDNF in neuropathic pain and increased pain sensitivity. They are currently testing if these effects on sensory neurons can be reversed by BDNF-neutralizing antibodies, and whether mice with conditional *Snf5* mutations have increased pain sensitivity.

Andrew B. Gladden (Massachusetts General Hospital/Harvard Medical School) a postdoctoral fellow from the group of Andrea McClatchey presented work on the role of the NF2 protein merlin on the regulation of the epithelial cell polarity and proliferation. The group has previously shown that Merlin function is critical for the establishment of adherens junctions (AJ) and contact-dependent inhibition of proliferation in cells. Given the requirement for merlin in AJ formation in vitro and the importance of cell junction and polarity components in the basal cells of the skin, they investigated the function of Merlin during skin development in vivo. They found that *K14-Cre;Nf2lox/lox* mice display a dramatic epidermal phenotype with little initial hair growth and severe dehydration leading to death during early postnatal development. Histological analysis of *K14-Cre;Nf2lox/lox* skin reveals a pronounced expansion of the epidermal progenitor cell population in the basal layer. This expansion can be ascribed to altered cell:cell communication and defective basal cell polarity during skin development. In fact, molecular studies suggest that merlin is a vital bridge between AJ and polarity components. These studies introduce new evidence for a role for merlin in epithelial progenitor cell adhesion, proliferation and polarity and provide a platform for further studies of merlin function in these processes.

Lou Chang (University of Michigan) presented two novel murine models that generate NF1-related MPNST with high penetrance. The two models differ in their timing of *Nf1* inactivation, where one model losses *Nf1* in conjunction with *p53*, while the other model inactivates *Nf1* first with subsequent loss of *p53*. While both models produce MPNSTs, when *Nf1* was inactivated prior to *p53* loss, neurofibromas also developed, mimicking NF1 patients. Comparison of these two mouse models enables elucidation of the role of neurofibromas in MPNST development. From a pre-clinical view, these novel murine models develop large numbers of MPNSTs, allowing testing of diagnostic markers to differentiate benign from malignant peripheral nerve sheath tumors, and offer improved NF1 models for pre-clinical therapeutic trials.

Yuan Zhu (University of Michigan) provided important insights into the role of the *Nf1* heterozygous microenvironment in the development of benign and malignant peripheral nerve sheath tumors. In their study, by using a conditional *Nf1* allele, this group established a mouse model that develops plexiform neurofibromas throughout the PNS (extending along the length of peripheral nerves and involving multiple branches of large nerves in humans)

as well as discrete neurofibromas in the skin (typically small, localized and often arising in the dermis or epidermis in humans). Furthermore, they demonstrated that the *Nf1* heterozygous microenvironment is critical for neurofibromas progression, but not for initiation or malignant transformation. This study has added a novel mouse model for neurofibromas and MPNST, and also provided important insights into the role of the *Nf1* heterozygous microenvironment in the development of benign and malignant peripheral nerve sheath tumor.

Walter J. Jessen (Cincinnati Children's Hospital Medical Center) a postdoctoral fellow in the group of Nancy Ratner presented work on the nerve gene expression patterning in NF1 mouse models. To elucidate the molecular changes that occur, they compared the transcriptome of adult nerves from five mouse models CNPase-hEGFR/+, CNPase-hEGFR/EGFR, CNP-HRas12V/+, *DhhCre;Nf1<sup>lox/lox</sup>* and NPcis and evaluated changes in gene expression relative to *Nf1<sup>lox/-</sup>* and *Nf1<sup>lox/lox</sup>* controls. The group identified 1,783 transcripts statistically different ( $P = 0.01$ ) and performed unsupervised two-way hierarchical cluster analysis to distinguish specific subtypes. They identified different principle patterns of gene expression and assessed each cluster to explore the potential biological significance. Transcripts up- or down-regulated across all mouse nerve samples were enriched for genes associated with apoptotic regulation, unfolded protein response, nervous system development, angiogenesis and cell cycle progression. Transcripts up- or down-regulated across NPcis, *DhhCre;Nf1<sup>lox/lox</sup>*, CNPase-hEGFR/+ and a subset of CNP-HRas12V/+ nerve samples were enriched for genes associated with complement and coagulation cascades, wound healing, cell adhesion and migration, and the intermediate filament cytoskeleton. Lastly, transcripts up- or down-regulated across CNPase-hEGFR/+, CNPase-hEGFR/EGFR and *DhhCre;Nf1<sup>lox/lox</sup>* nerve samples were enriched for genes associated with intermediate filament bundle assembly, nervous system development, angiogenesis, Wnt signaling, cell adhesion, response to wounding, cholesterol biosynthesis, and the endoplasmic reticulum. These preliminary results demonstrate that transcriptional changes in NF1 mouse models are diverse and represent different molecular aspects of the disease.

## CLINICAL FEATURES AND MANAGEMENT OF NF

Victor-Felix Mautner (Eppendorf University, Hamburg) chaired a session reviewing updates on some of the clinical features in NF and how these can be managed. Plexiform neurofibromas occur in about 60% of NF1 patients and can lead to severe morbidity by disfigurement or compression of vital structures, in addition these tumors can undergo or malignant transformation to MPNSTs. However complete surgical resection of plexiform tumors is rarely possible due to local infiltration of normal tissue. As an alternative to standard drug therapies, Michael Fisher (Children's Hospital of Philadelphia) is conducting a Phase I trial of photodynamic therapy (PDT) for treatment of regional plexiform tumors in children. The trial will determine the maximal tolerated dose of light combined with a systemically administered photosensitizer (talaporfin sodium).

Ian McCutcheon (MD Anderson Cancer Center) reviewed some of the features of NF 1 related MPNSTs. NF1 patients have a lifelong

risk of 8–13% to develop MPNST. These tumors arise from pre-existing plexiform neurofibromas and in NF1 carry a high risk of mortality: the 5-year disease survival in 72 NF1 patients with MPNST was 35% compared to 50% in non-NF1 patients who had developed sporadic MPNSTs. Negative predictors of disease survival in these patients were MPNSTs of greater than 10 cm at diagnosis, partial resection, and metastasis development. Improved clinical, pathological and molecular staging for MPNST are needed to optimize the prognostication and management of these tumors [Zou et al., 2009].

Determination of NF tumor growth patterns is essential to allow appropriate treatment decisions and conventional MRI scans without volumetric measurements show an error range up to 30% dependent on slice thickness. Gordon Harris (Harvard Medical School/Massachusetts General Hospital) has established a centralized service that can receive MRI scans electronically, perform volumetric analyses on these and report resulting images, measurements and graphs.

Beside MPNST, vasculopathy is one of the most serious life threatening events that can occur in NF1. However its pathogenesis is not well understood. Kimberly Jett (University of British Columbia), a predoctoral student in the group of Jan Friedman, described a study in which flow-mediated vasodilation (FMD) and glyceryl-trinitrate-mediated dilatation (NMD) were carried out to assess vascular endothelial and smooth muscle cell function. In addition, poly-chromatic flow cytometry was used to test for evidence of vascular inflammatory cells from the peripheral blood of NF1 patients. The FMD and NMD data demonstrate that vascular endothelial function is altered in NF1 patients. The study also showed for the first time that NF1 patients have evidence of chronic inflammation that could lead to subsequent vascular disease.

Yemima Berman (Children's Hospital, University Sydney) reported a study of 22 children with velopharyngeal insufficiency (VPI) and NF1. VPI is a speech disorder resulting in nasal sounding speech and reduced speech intelligibility. Amongst those patients with VPI, 10/22 (45.5%) had brainstem tumors and five (22.7%) had *NF1* microdeletion. These frequencies are much higher than usually expected within the NF1 population; 5–11% and 4.4%, respectively. The frequency of brainstem tumors appears to increase with severity of abnormal nasal airflow during speech. In five patients, VPI was detected prior to identification of brainstem tumors, suggesting this may be a useful clinical sign for early detection of brainstem tumors. This study suggests that VPI is an important and under-recognized cause of morbidity amongst patients with NF1. VPI may also be a useful clinical feature for identifying patients at increased risk of brainstem pathology and appears to be more common amongst patients with *NF1* microdeletion.

Anat Stemmer-Rachamimov (Harvard Medical School/Massachusetts General Hospital) described the problem of the multiplicity of terminologies used to describe neurofibromas in the clinical practice of NF1. The use of multiple classification schemes by different specialties is an impediment for transfer of knowledge between specialties and also between mouse models and human pathology. The formulation of a unifying classification scheme for neurofibromas has been developed by a panel of specialists representing multiple disciplines.

## CORRELATING THE NF1 PHENOTYPE AND GENOTYPE

Chaired by Susan Huson (St. Mary's Hospital, University of Manchester), this session comprised a range of topics ranging from new quantitative methods for defining the NF1 phenotype, through work on identification of modifying genes and then an area about which little work has been done—the natural history of NF1 in old age.

Bruce Korf (University of Alabama at Birmingham) highlighted the renewed interest in NF1 genotype–phenotype correlation that has resulted from the emergence of several related recognizable phenotypes. Furthermore, the identification of SPRED1 as a non-allelic cause of a dominantly inherited mild NF1-like phenotype (Legius syndrome) raises the possibility that there may be other genes in the Ras/MAPK pathway which share the pigmented phenotype. The other well recognized clinically significant genotypes are the NF1 microdeletions (usually associated with more severe neurofibroma burden and developmental/learning issues) and the three base deletion in exon 17 (associated with lack of neurofibroma development in adults). Another distinct phenotype associated with paucity of dermal tumors but an extreme burden of internal tumors (spinal neurofibromatosis) is also emerging; and this appears to be associated with an excess of missense and splice site mutations. These emerging phenotypes and the overlap of Legius syndrome and NF1 mean that consideration needs to be given to revision of the NF1 diagnostic criteria. Dr. Korf felt more data were needed before this can be done. Dr. Korf also shared his groups' work on quantitative recording of clinical features to assist in accurate phenotyping and in preparation for clinical trials. They are using a combination of serial photography and laser scanning to quantify the number of dermal tumors and follow their growth rate.

The emerging genotype–phenotype correlations probably still account for no more than 10% of an NF1 clinic population. For the majority of NF1 families there is significant intra-familial variability and evidence for the role of modifying genes. Technological developments mean that we should now be able to identify these. Andre Bernards (Harvard Medical School/Massachusetts General Hospital) provided an overview of his groups' work in *Drosophila* and humans. *Drosophila* NF1 null mutants exhibit a learning defect and a 20% reduction in post-embryonic growth. They have identified a neuronal tyrosine kinase which contributes to the phenotype. From human studies, patients with an NF1 microdeletion have early onset of large numbers of neurofibromas. The Bernard's group has performed preliminary analysis of two conserved microRNA genes, 200 kb downstream of NF1, which target the relevant pathway. The final approach that the group is about to embark on involves a genome association study on samples from a large cohort of NF1 patients selected for lower/higher numbers than average of dermal neurofibromas for their age.

Recent advances in MRI scanning offer new ways of measuring the internal tumor burden in NF1, NF2 and schwannomatosis. Scott Plotkin (Harvard Medical School/Massachusetts General Hospital) presented some preliminary data from an international prospective observational study. The technique clearly gives excellent images, in a relatively short time, without the use of ionizing radiation. There is the option for doing PET scans when lesions are

>5 cm. The combined data from the first 143 subjects was presented. Sixty percent of patients had at least one internal tumor. It will be interesting to see whether the study shows evidence of benefit in patient management as this cohort of 250 patients is followed prospectively.

John Mulvihill (University of Oklahoma) gave a progress report on their studies of an aspect of NF1 about which little is known: the natural history and health needs in old age. Working with NF1 research groups in Vancouver and Denmark, they are analyzing existing databases, reviewing previously identified NF1 cohorts and running focus groups to obtain a multifaceted view of ageing in NF1, key issues raised at the focus groups.

## RECENT ADVANCES IN UNDERSTANDING NF1 COGNITIVE DEFICITS

Kathryn North chaired this session and also provided the opening presentation to set the stage on issues to consider in developing effective therapies and informative clinical trials. NF1 is associated with a lowering IQ with median full scale IQ usually in the low 90s. Fifty to 60% of children with NF1 have academic difficulties. Thirty-eight percent have attention deficit disorders and there is a high frequency of specific deficits in visuospatial function, executive function, working memory and receptive and expressive language. Natural history studies have suggested that cognitive function in NF1 is static with age compared to controls.

The NF1 cognitive phenotype is a composite of two types of deficit. (1) Cognitive deficits that are developmental in origin and are likely to be irreversible. For example, increased gray matter volume and increased cross-section of the corpus callosum are associated with lower IQ; macrocephaly per se is not associated with lowered cognitive function. (2) Cognitive deficits that are associated with the biochemical effects of loss of neurofibromin in the Ras/MAPK pathway and are likely to be reversible. The aspects of cognitive phenotype in NF1 that are likely to be reversible are attention, working memory and learning (this correlates with the hippocampal defects seen in the mouse model of NF1). It must be noted that IQ—as a measure of aptitude—is a composite of reversible and irreversible deficits.

In design of pharmacological clinical trials for NF1, it is important to target aspects of learning that are related to modulation of Ras/MAPK levels. For the Phase 2 Lovastatin trial that has commenced mid 2009, the study protocol has been designed to target deficits demonstrated to be reversible in Nf1 mouse model (e.g., hippocampal based visual memory and learning presented by Alcino Silva and Jonathan Payne and described below) as well as targeting deficits known to be common in NF1 (e.g., attention). Patients enrolled must have a deficit in one of the primary outcome measures and should not be on other medications such as stimulant medication that may influence reversible deficits.

Sheryl Rimrodt (Kennedy Krieger Institute) presented data from two ongoing studies comparing children with NF1 associated with reading disability (NF+RD) and children with idiopathic RD (IRD) to control participants. The first study suggests that the visual spatial deficits in NF + RD appear to be more extensive than

those required on the commonly used JLO measure; NF + RD performance was impaired, relative to controls and IRD, on tasks requiring visual closure and visualization of position in space. This result is distinct from findings on more complex visual spatial tasks with increased working memory demands, on which both RD groups (NF + RD and IRD) performed more similarly to each other than to controls. The second is a clinical trial comparing two approaches to intensive instructional intervention in both NF + RD and IRD; namely, it compares a strongly phonological approach to a more visual spatially based fluency approach. These preliminary data suggest that children with NF + RD appear to respond to reading interventions.

Jonathan Payne (Children's Hospital, University of Sydney) presented data concerning extrapolation from the *NF1* mouse behavioral phenotype to human clinical trials. When considering the applicability of treatment effects in animals to humans, it is important to understand the nature of the impairment across both species. NF1+/- mice display reversible impairments on the Morris water maze, a measure of visual learning that has been shown to rely on the integrity of the hippocampus. The Cambridge Neuropsychological Test Automated Battery (CANTAB) has been developed based on animal behavior paradigms to facilitate cross-species studies of cognition. The Paired Associate Learning (PAL) subtest of CANTAB measures changes in human hippocampal function, and this function is impaired in over 40% of children with NF1, independent of IQ and attention. In addition the PAL has multiple versions of the test to minimize practice effects as part of clinical trials. This instrument is thus an ideal primary outcome measure to use in studies of therapy for reversible cognitive deficits in NF1.

Ype Elgersma (Erasmus University, The Netherlands) presented data suggesting that exon 9a of the *NF1* gene is important for regulating neuronal RAS activity. Although *NF1* is expressed in all cells of the central nervous system, expression of *NF1* containing alternatively spliced exon 9a is restricted to the CNS neurons with high expression in the forebrain. Mice with mutant exon 9a have no change in total neurofibromin, but have deficits in synaptic plasticity and learning with impaired function on the Morris water maze and impaired LTP in the hippocampus.

Linnea Vose (New York Medical College), a predoctoral fellow in the laboratory of Frances Hannan, described studies in the *Drosophila NF1* model showing that Lovastatin placed in food rescues adult learning after 24 hr of treatment. Adult flies were tested for learning using an established associative learning protocol that measures the ability of the flies to distinguish between two odors, one of which was paired with an electric shock. Drug testing in larvae was also performed to replicate young patients with NF1. Larvae were tested using a similar shock-odor protocol recently developed in their laboratory. Treatment with lovastatin and rolipram rescues learning defects in both adult and larval NF1 mutants. Rapamycin, which targets the downstream Rheb/mTOR pathway had no effects on learning. The larval learning system pairing shock and odor provides a rapid (1 min) and robust protocol that will be useful for screening drugs and novel compounds that can advance to be tested in mouse models and eventual clinical trials in humans.

## PROGRESS IN MANAGEMENT OF NF1 BONE ABNORMALITIES

Both focal and localized bone abnormalities are part of the phenotypic expression of NF1, but the natural history and pathogenesis of the various skeletal abnormalities are not well delineated. However, over the past decade significant progress has been made in understanding the effects of aberrant Ras signaling on bone. In 2008, the Children's Tumor Foundation (CTF) convened an International NF1 Bone Abnormalities Consortium to develop consensus on the clinical management of NF1 skeletal manifestations and forge collaborations for preclinical and clinical bone studies [Elefteriou et al., 2009]. A session of the 2009 NF Conference chaired by David Stevenson (University of Utah) highlighted progress made since that meeting detailing salient clinical and molecular findings which will be important in developing better management guidelines and future therapeutic development.

David Feldman (New York University Langone Medical Center) reviewed dural ectasia in NF1, which is otherwise seen in relatively few other disorders and whose etiology is still unknown. It was hypothesized that in the context of NF1, dural ectasia is a mesodermal dysplasia with progressive osseous erosion rather than a focal expansion. The hypothesis of fluid pressure leading to dural ectasia in NF1 was thought to be unlikely.

Janet Hock (Maine Institute of Genetics and Health) reported on osteoporosis in NF1. The definition of osteoporosis in the general population, particularly in children, is evolving. Low bone density, a surrogate measure of bone fragility, appears to be detected at a much earlier age in NF1, and long before the more common postmenopausal osteoporosis and elderly male osteoporosis. Although decreased bone mineral density has been reported in NF1, bone quality and fracture risk are still not well understood in NF1 patients particularly when accounting for different age ranges. Dr. Hock stated that currently until clinical trial data become available on osteoporosis in NF1, standard of care guidelines and the use of regulatory-approved drugs for low bone density and fractures should typically follow the guidelines approved for osteoporosis in the general population.

David Viskochil (University of Utah) reported on a multicenter study investigating the spinal abnormalities of prepubertal NF1 children without scoliosis clinically. Approximately half of the individuals had at least one spine abnormality (31% had vertebral abnormalities and 17% had dural ectasia). This emphasizes the need for close follow up and detailed clinical evaluation of the back in a bending position in order to screen for individuals with potential spinal abnormalities.

Florent Elefteriou (Vanderbilt University) described a conditional mouse model that recapitulates many of the skeletal findings of NF1 including scoliosis, pectus deformities, and long bone bowing. The bone quality was poor with dramatic cortical porosity.

Juha Peltonen (University of Turku) reported on the biology of NF1 osteoclasts in vivo and in vitro. The osteoclast progenitors had an increased survival rate compared to controls with resistance to apoptosis induced by growth factor deprivation. Dr. Peltonen concluded that even when isolated from the factors mediated by other cell types of the NF1 microenvironment, NF1 osteoclasts display an altered phenotype.

Weixi Wang (Vanderbilt University), a postdoctoral fellow in the laboratory of Dr. Elefteriou, described results of the use of local delivery of low dose lovastatin via microparticles in a fracture model using the wild type and *Nf1<sup>ob-/-</sup>* mouse. There was an improvement in the biomechanical properties of the calluses in the treated *Nf1<sup>ob-/-</sup>* mouse compared to the untreated mice, suggesting that local delivery of low dose Lovastatin may be a consideration for use in future clinical trials.

Aaron Schindeler (Children's Hospital, University of Sydney) has modeled double inactivation of *NF1* in mice in hopes of creating regionalized double inactivation of *NF1* analogous to what has been described in some pseudarthrosis tissue in humans. This would potentially generate a mouse phenotype more consistent with the clinical human phenotype.

In summary, skeletal abnormalities are a significant part of the NF1 phenotype. This session demonstrated that though the pathophysiology of the skeletal abnormalities is still not well defined there has been rapid progress in understanding the biology, developing preclinical models, and in elucidating the clinical phenotype and natural history of NF1 skeletal manifestations. An important reference on this area is provided by the publication of the recent consensus report [Elefteriou et al., 2009] from the NF1 Bone Consortium, a working group assembled following the Children's Tumor Foundation Bone Workshop held in early 2008.

## NF CLINICAL CASES THAT TAUGHT ME SOMETHING NEW

In the field of neurofibromatosis there exists a symbiotic relationship between scientist clinician and patient. In recent years clinical sessions have gained a prominent educational role in the NF Conference demonstrating how scientific findings relate to clinical practice and impact on patient care. Rosalie Ferner (Guys and St. Thomas's NHS Trust) and Kathryn North led a session where expert clinicians presented epidemiological data and case histories of individuals with NF1 and NF2 to illustrate lessons they had learnt from patients, research and from clinical experience. An interactive "quiz" with teams from Europe and the United States as well as audience participation demonstrated unusual clinical presentations and disease complications of NF1 and NF2.

Gareth Evans (St. Mary's Hospital, University of Manchester) highlighted the importance of knowing the frequency of NF1 and NF2 in order to plan for allocation of resources. The recent results from a Genetic Register Study in North West England are in line with previous studies: a birth incidence of 1 in 2,699 for NF1 and 1 in 33,000 for NF2; disease prevalence of 1 in 4,560 (NF1) and 1 in 56,162 (NF2); new mutations in 42% of NF1 and 56% of NF2 individuals. Eric Legius (University of Leuven) emphasized that patients presenting with ring chromosome 22 (severe cognitive and behavioral impairment, café-au-lait patches and facial dysmorphism) can develop NF2-related tumors and should be monitored for vestibular schwannomas from adolescence onwards. Several experts stressed the importance of meticulous attention to the patient's clinical history, examination and investigations. Pitfalls await those who rely solely on clinical protocols and pattern recognition to diagnose neurocutaneous disease. Misinterpretation

of symptoms can lead to over- or under- diagnosis [Brems et al., 2007; Denayer et al., 2008].

Rosalie Ferner (Guy's and St Thomas' NHS Trust) explained that a rapidly growing, painful swelling behind the knee in an NF1 patient with a large plexiform neurofibroma was thought to arise from a malignant peripheral nerve sheath tumor but subsequent investigation revealed a benign Baker's cyst. Robert Listernick (Northwestern University) described an adolescent male with NF1 with nocturnal calf pain, and mild weakness of hip flexion and extension. These problems raised the suspicion of malignant peripheral nerve sheath tumor but no relevant palpable mass was detected and the neurofibromas on neuroimaging were not relevant to his symptoms. The patient's mother worked for a vascular surgeon and recognized the signs as claudication. The patient was diagnosed with an occlusion of the superficial femoral artery due to NF1 vasculopathy. Susan Huson pointed out that NF2 in children is a severe disease and frequently under-recognized and misdiagnosed as NF1. She reported a labial plexiform tumor as an unusual early NF2 manifestation and with Kathryn North she noted that proptosis and visual impairment in young children with café-au-lait patches can be due to NF2 rather than NF1. Neuroimaging was the key in Kathryn North's patient and revealed bilateral vestibular schwannomas as well as a meningioma surrounding the normal sized nerve, unlike the optic nerve enlargement in NF1 gliomas.

Bruce Korf (University of Alabama at Birmingham) drew attention to indolent optic pathway gliomas as potential manifestation of mosaic NF1 and showed the importance of detailed molecular analysis to confirm the clinical findings. He presented a young man with an optic pathway glioma and reduced vision detected as an incidental finding when neuroimaging was performed following head trauma. Careful clinical assessment revealed a few skin papules, a patch of hyperpigmentation and freckling on one limb. Mutation testing confirmed NF1 mosaicism and skin biopsy showed a compound melanocytic naevus. David Viskochil (University of Utah) focused on the dignity, joie de vivre and emotional fortitude of a young woman in the presence of multiple disease complications from NF1. She had to deal with learning problems, anorexia nervosa, gastrointestinal symptoms due to a gastro-intestinal stromal tumor and an inoperable cervical plexiform tumor that was treated with radiotherapy. He illustrated the devastating consequences of inappropriate administration of radiotherapy to individuals with NF1 as benign plexiform neurofibroma underwent untreatable malignant transformation following radiotherapy.

Vincent Riccardi (The Neurofibromatosis Institute) underlined the importance of divergent thinking in NF1 by showing an adult male with a large plexiform neurofibroma on the back associated with hypopigmentation. His job as a truck mechanic contributed to the growth of the plexiform neurofibroma due to repeated trauma from lying on his back. David Gutmann (Washington University) focused on a common complaint as the manifestation of an uncommon diagnosis – facial itching was indicative of an underlying brainstem tumor in a young male with NF1. Rosalie Ferner pointed out that although malignant transformation is usually associated with plexiform neurofibromas and is rare in schwannomas, it should be suspected in patients with rapidly enlarging, hard painful lesions.

The session highlighted that advances in molecular biology and neuroimaging have proven beneficial in aiding diagnosis and management of NF patients. However, the role of clinical experience, clinical acumen, lateral thinking and above all listening to patients and their families cannot be underestimated.

## NF1 IN CONTEXT—A VIEW FROM RAS/MAPK DISORDERS

Katherine Rauen (University of California, San Francisco) chaired this session which brought NF1 into context of other genetic syndromes related to NF1. NF1 belongs to a class of genetic syndromes caused by germline mutations in genes which encode components of the Ras/mitogen activated protein kinase (MAPK) pathway. This pathway plays an essential role in the control of the cell cycle and differentiation; therefore its dysregulation has profound developmental consequences. These “RASopathies” each exhibit unique phenotypic features; however, many share characteristic overlapping features including craniofacial dysmorphism, cardiac malformations and cutaneous, musculoskeletal and ocular abnormalities, varying degrees of neurocognitive impairment and, in some syndromes, an increased risk of developing cancer [Tidyman and Rauen, 2009]. In this session, several RASopathies were discussed which demonstrated that there exists similar developmental phenotypes among this group of genetic syndromes.

Ludwine Messiaen (University of Alabama at Birmingham), Meena Upadhyaya (Cardiff University) and Eric Legius (University of Leuven) discussed Legius syndrome, a recently identified autosomal dominant disorder which shares many phenotypic features with NF1. Individuals with Legius syndrome may present with café-au-lait maculae, freckling, mild neurocognitive impairment and macrocephaly with some having a Noonan-like facies. However, in Legius syndrome, features such as neurofibromas, iris Lisch nodules and central nervous system tumors—commonly seen in NF1—are lacking. Legius syndrome is caused by mutations in *SPRED1* resulting in haploinsufficiency of the protein SPRED1, a member of the SPROUTY/SPRED proteins family. SPRED1 is a negative regulator of Ras and acts by inhibiting Raf phosphorylation. Heterogenous *SPRED1* mutations associated with Legius syndrome cause truncation of the protein resulting in a loss of SPRED1 function thereby increasing signaling down the Ras/MAPK pathway. Dr. Messiaen and colleagues performed *SPRED1* mutation analysis in 1,318 unrelated patients with the clinical diagnosis of NF1 and in whom no NF1 mutations had been identified. In this cohort, they identified 34 different *SPRED1* mutations in 43 probands: 27 were pathogenic (including 2 missense mutations) and 7 missense mutations were classified as probably benign. They estimated that 1.2–2.9% of individuals with the clinical diagnosis of NF1 have Legius syndrome.

Dr. Upadhyaya and colleagues set out to ascertain the frequency of *SPRED1* mutations in individuals with an NF1 phenotype by examining 85 unrelated mildly affected NF1 patients known to be negative for NF1. A subset of these patients negative for NF1 and *SPRED1* were subsequently screened for mutations of the related *SPRED2-3* and *SPRY1-4* genes to determine whether mutations in other *SPRED/SPRY* genes were causal in these patients. *SPRED1*

mutations were identified in 7 unrelated patients. A potential sequence alteration of the *SPRY1* gene was identified in one patient but further analysis is required to evaluate its pathogenicity.

Dr. Legius and colleagues examined the cognitive abilities in Legius syndrome. They investigated a group of nine children with a *SPRED1* mutation and compared IQ test scores with seven siblings. The mean performal IQ score in the *SPRED1* group was 15 IQ points lower compared to the sibling group with lower scores than sibs also identified in additional developmental testing. In addition, analysis of cognition in *Spre1* knockout mice showed a significantly slower learning in hippocampus dependent tests such as the hidden Morris Water Maze and the T-maze. These findings correlated with a decreased long-term potentiation in hippocampal slices and an increased long term depression in *Spre1* knockout mice. Biochemical analysis corroborated this finding by increased ERK phosphorylation. They are further investigating if the cognitive defects in *Spre1* mice can be rescued by treatment with statins. Cognitive defects are present in humans and mice with a *SPRED1* mutation but are generally milder than in the NF1 group.

Miikka Vikkula (Université catholique de Louvain) discussed capillary malformation-arteriovenous malformation syndrome (CM-AVM), which is an autosomal dominant inherited disorder characterized by multifocal capillary malformations which may be associated with arteriovenous malformations and fistulas [Eerola et al., 2003; Revencu et al., 2008]. CM-AVM syndrome is caused by heterozygous inactivating mutations in the gene *RASA1*, which like *NF1*, encodes a RasGAP. The hallmark of this syndrome is the multifocality of the malformations. AVMs can occur in many tissues including skin, muscle, bone, and in various internal organs including the brain. In addition, *RASA1* mutations have been associated with individuals diagnosed with Parkes-Weber syndrome and vein of Galen malformations. Haploinsufficiency of p120-RasGAP, the protein product of *RASA1*, causes a reduction in the hydrolysis of Ras-GTP and, therefore, increases Ras/MAPK pathway signaling. Interestingly, CM-AVM patients may be at increased risk of developing tumors.

Noonan syndrome (NS) is an autosomal dominant disorder with prevalence in the population similar in frequency to NF1. NS, which was discussed by Amy Roberts (Children's Hospital Boston), is characterized by distinctive craniofacial features, short stature, congenital cardiac anomalies, bleeding disorders and a variable degree neurocognitive delay. Individuals with NS have an increased risk of cancer. At present four genes, *PTPN11*, *KRAS*, *SOS1*, and *RAF1* harboring heterozygous germline mutations cause NS with all genes encoding various components of the Ras/MAPK pathway. However, there are still more genes to be identified. The most common gene associated with NS is *PTPN11* which accounts for approximately half of all cases.

Katherine Rauen discussed Costello syndrome (CS) and cardiofacio-cutaneous syndrome (CFC), two other syndromes that are part of the Ras/MAPK pathway. CS is a rare developmental disorder with multiple anomalies, including characteristic dysmorphic craniofacial features, failure to thrive, cardiac, musculoskeletal and ectodermal abnormalities and neurocognitive delay. Individuals with CS are at increased risk of developing neoplasms, both benign and malignant. Heterozygous germline mutations in *HRAS* cause CS. The distribution frequency of mutations reveals that more than

80% of individuals have a G12S substitution, followed by the second most common, G12A. CFC, like CS, is rare, and shares many overlapping phenotypic features with NS and CS, and to some extent with NF1. CFC individuals have a Noonan-like facies, cardiac malformations, ectodermal, gastrointestinal, ocular and musculoskeletal abnormalities, with most having short stature. Neurologic abnormalities are universally present to varying degrees and include hypotonia, motor delay, speech delay and/or learning disability. Three genes that encode proteins in the Ras/MAPK pathway downstream of Ras, have been associated with CFC syndrome: *BRAF*, *MAP2K1* (*MEK1*), and *MAP2K2* (*MEK2*).

Michelle Strecker (University of California, San Francisco) described a new and novel clinic at UCSF where she is the clinic coordinator: the NF/Ras Pathway Clinic. This was adapted from the Children's Tumor Foundation's structure for the national NF Clinic Network. This pathway-based clinic encompasses the entire group of Ras syndromes with have a similar underlying pathogenic mechanisms, and thus, overlapping phenotypes.

## KEYNOTE PRESENTATIONS: NEW PERSPECTIVES

Conference highlights included three keynote presentations from David Kwiatkowski (Harvard Medical School) on tuberous sclerosis; Allan Balmain (University of California, San Francisco) on the 'multiple faces of Ras'; and Luis Parada (University of Texas, South Western) who also honored as the 2009 recipient of the Friedrich Von Recklinghausen Award, which is given annually by the Children's Tumor Foundation to recognize individuals making outstanding contributions to NF research and clinical care.

Allan Balmain discussed the tissue-specific expression and mutation of Ras family members, the effect of germline mouse strain polymorphisms on preferential expression and mutation of specific parental Ras alleles. He also discussed amplification of mutant or loss of wild type Ras alleles late in tumorigenesis, and identification of other genes involved in tumor resistance of certain mouse strains. Data from his laboratory over the past 3 years implicate K-Ras4A oncogenic mutation in regulating cell fate decisions initiating, but not necessary for maintaining, lung tumorigenesis. A requirement for the 4A isoform of K-Ras may be linked to the farnesylation motif at its carboxy terminus, specifying subcellular localization. Using mouse strains susceptible or resistant to lung tumorigenesis, his group found that increased expression of K-Ras2, controlled by a *cis*-acting quantitative trait locus, itself controls tumor susceptibility. The balance between expression levels of wild type and mutant KRas alleles controls progression of lung cancer, consistent with wild type Ras blocking effects of oncogenic Ras proteins, and with selection for loss of wild type Ras alleles in tumorigenesis. New work using bioinformatic methods is revealing pathways modulating regulation of the Ras pathway at defined stages of tumorigenesis that may be useful in categorizing human tumors.

David Kwiatkowski presented on the tuberous sclerosis signaling hub and its broader role in neural development and cancer. Tuberous sclerosis (TSC) is an autosomal dominant tumor syndrome, with many similarities to but also many differences from NF1. The protein products of the genes which cause TSC are TSC1 and TSC2, and these are at the central hub of a signaling pathway that extends through the PI3K and ERK signaling pathways to

TSC1/TSC2 to regulate activation of mTORC1. The TSC1/TSC2 proteins play a particularly important role in brain development, reflected by the neuropathology and neurological manifestations. Although in general these genes are not common targets for mutation/inactivation in human cancer, there are some tumors in which they seem to be an important target.

The 2009 recipient of the Friedrich von Recklinghausen Award, Luis Parada presented on “a decade of modeling NF1 in the mouse.” Plexiform neurofibromas are embryonic in origin, are at least 15–25% penetrant and can progress to malignancy suggesting they have a different cell of origin than do dermal neurofibromas which are 100% penetrant, have later onset and are not predisposed to malignancy. Studies in human tumors suggest that only the Schwann cell lineage needs to undergo loss of heterozygosity for tumors to develop but do not provide a timeline for the progression of tumors. Mice lacking NF1 in Schwann cell precursors develop neurofibromas but do not develop neurofibromas in a wild type cell environment—that is, neurofibromas only develop on an *NF1*<sup>+/-</sup> background. In 2008, Parada and colleagues uncovered a genetic contribution of the environment to tumor formation, likely attributable to the mast cell. In mouse, mast cells degranulate prior to the development of tumors suggesting an inflammatory response precedes tumor development. In the *Nf1*<sup>+/-</sup> mouse with a selective knock-out of NF1 in Schwann cells (*-/-*) neurofibromas develop. However, following a bone marrow transplant to ensure development of *+/+* mast cells, no tumors develop. Thus, *+/-* mast cells interact with Schwann cells that have loss of heterozygosity to lead to the development of neurofibromas. Mast cells require the cKIT receptor for development and function. This is inhibited by Imatinib. Treatment with Imatinib has been demonstrated to lead to shrinkage of plexiform neurofibroma in one case. A Phase II trial currently underway suggests further efficacy. In 2009, Parada and colleagues propose that dermal neurofibromas arise from dermal stem cell progenitors. Dermal tumors have the same cellular composition as plexiform neurofibromas but have limited growth potential. The dermal stem cell progenitors (SKPs) have neural crest like properties and development of neurofibromas appears to depend on the microenvironment. The Parada group is now studying the origin of these cells in quail/chick chimeras. They have also noted the high frequency of mutations in *NF1* in sporadic malignant gliomas.

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## REFERENCES

- Boyd C, Smith MJ, Kluwe L, Balogh A, MacCollin M, Plotkin SR. 2008. Alterations in the SMARCB1 (INI1) tumor suppressor gene in familial schwannomatosis. *Clin Genet* 74:358–366.
- Boyd KP, Korf BR, Theos A. 2009. Neurofibromatosis type 1. *J Am Acad Dermatol* 61:1–14.
- Brems H, Chmara M, Sahbatou M, Denayer E, Taniguchi K, Kato R, Somers R, Messiaen L, De Schepper S, Fryns JP, Cools J, Marynen P, Thomas G, Yoshimura A, Legius E. 2007. Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype. *Nat Genet* 39:1120–1126.
- Denayer E, Ahmed T, Brems H, Van Woerden G, Borgesisu NZ, Callaerts-Vegh Z, Yoshimura A, Hartmann D, Elgersma Y, D’Hooge R, Legius E, Balschun D. 2008. Spred1 is required for synaptic plasticity and hippocampus-dependent learning. *J Neurosci* 28:14443–14449.
- Eerola I, Boon LM, Mulliken JB, Burrows PE, Domp Martin A, Watanabe S, Vanwijck R, Vikkula M. 2003. Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. *Am J Hum Genet* 73:1240–1249.
- Eleftheriou F, Kolanczyk M, Schindeler A, Viskochil DH, Hock JM, Schorry EK, Crawford AH, Friedman JM, Little D, Peltonen J, Carey JC, Feldman D, Yu X, Armstrong L, Birch P, Kandler DL, Mundlos S, Yang F-C, Agiostratidou G, Hunter-Schaedle K, Stevenson DA. 2009. Skeletal abnormalities in neurofibromatosis type 1: Approaches to therapeutic options. *Am J Med Genet Part A* 149A:2327–2338.
- Evans DG. 2009. Neurofibromatosis type 2 (NF2): A clinical and molecular review. *Orphanet J Rare Dis* 4:16.
- Evans DG, Kalamarides M, Hunter-Schaedle K, Blakeley J, Allen J, Babovic-Vuskanovic D, Belzberg A, Bollag G, Chen R, DiTomaso E, Golfinos J, Harris G, Jacob A, Kalpana G, Karajannis M, Korf B, Kurzrock R, Law M, McClatchey A, Packer R, Roehm P, Rubenstein P, Slattery W III, Tonsgard JH, Welling DB, Widemann B, Yohay K, Giovannini M. 2009. Consensus recommendations to accelerate clinical trials for neurofibromatosis type 2. *Clin Cancer Res* 15:5032–5039.
- Flaiz C, Ammoun S, Biebl A, Hanemann CO. 2009a. Altered adhesive structures and their relation to RhoGTPase activation in merlin-deficient Schwannoma. *Brain Pathol* 19:27–38.
- Flaiz C, Chernoff J, Ammoun S, Peterson JR, Hanemann CO. 2009b. PAK kinase regulates Rac GTPase and is a potential target in human schwannomas. *Exp Neurol* 218:137–144.
- Hadfield KD, Newman WG, Bowers NL, Wallace A, Bolger C, Colley A, McCann E, Trump D, Prescott T, Evans DG. 2008. Molecular characterisation of SMARCB1 and NF2 in familial and sporadic schwannomatosis. *J Med Genet* 45:332–339.
- Hamaratoglu F, Willecke M, Kango-Singh M, Nolo R, Hyun E, Tao C, Jafar-Nejad H, Halder G. 2006. The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. *Nat Cell Biol* 8:27–36.
- Hulsebos TJ, Plomp AS, Wolterman RA, Robanus-Maandag E, Baas F, Wesseling P. 2007. Germline mutation of INI1/SMARCB1 in familial schwannomatosis. *Am J Hum Genet* 80:805–810.
- Joseph NM, Mosher JT, Buchstaller J, Snider P, McKeever P, Lim M, Conway SJ, Parada LF, Zhu Y, Morrison SJ. 2008. The loss of NF1 transiently promotes self-renewal but not tumorigenesis by neural crest stem cells. *Cancer Cell* 13:129–140.
- Kalamarides M, Niwa-Kawakita M, Leblais H, Abramowski V, Perricaudet M, Janin A, Thomas G, Gutmann DH, Giovannini M. 2002. Nf2 gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse. *Genes Dev* 16:1060–1065.
- Kwon CH, Zhao D, Chen J, Alcantara S, Li Y, Burns DK, Mason RP, Lee EY, Wu H, Parada LF. 2008. Pten haploinsufficiency accelerates formation of high-grade astrocytomas. *Cancer Res* 68:3286–3294.
- MacCollin M, Chiocca EA, Evans DG, Friedman JM, Horvitz R, Jaramillo D, Lev M, Mautner VF, Niimura M, Plotkin SR, Sang CN, Stemmer-

- Rachamimov A, Roach ES. 2005. Diagnostic criteria for schwannomatosis. *Neurology* 64:1838–1845.
- Miller SJ, Jessen WJ, Mehta T, Hardiman A, Sites E, Kaiser S, Jegga AG, Li H, Upadhyaya M, Giovannini M, Muir D, Wallace MR, Lopez E, Serra E, Nielsen GP, Lazaro C, Stemmer-Rachamimov A, Page G, Aronow BJ, Ratner N. 2009. Integrative genomic analyses show altered Schwann cell development in Neurofibromatosis tumors and implicate SOX9 as an addicting oncogene. *EMBO Mol Med* 1:236–248.
- O'Neill E, Rushworth L, Baccharini M, Kolch W. 2004. Role of the kinase MST2 in suppression of apoptosis by the proto-oncogene product Raf-1. *Science* 306:2267–2270.
- Patil S, Perry A, MacCollin M, Dong S, Betebsky RA, Yeh TH, Gutmann DH, Stemmer-Rachamimov AO. 2008. Immunohistochemical analysis supports a role for INI1/SMARCB1 in hereditary forms of schwannomas, but not in solitary, sporadic schwannomas. *Brain Pathol* 18:517–519.
- Plotkin SR, Stemmer-Rachamimov AO, Barker FG II, Halpin C, Padera TP, Tyrrell A, Sorensen AG, Jain RK, di Tomaso E. 2009. Hearing improvement after bevacizumab in patients with neurofibromatosis type 2. *N Engl J Med* 361:358–367.
- Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. 2008. Efficient tumour formation by single human melanoma cells. *Nature* 456:593–598.
- Revenu N, Boon LM, Mulliken JB, Enjolras O, Cordisco MR, Burrows PE, Clapuyt P, Hammer F, Dubois J, Baselga E, Brancati F, Carder R, Quintal JM, Dallapiccola P, Fischer G, Frieden IJ, Garzon M, Harper J, Johnson-Patel J, Labreze C, Martorell L, Paltiel HJ, Pohl A, Prendiville J, Quere I, Siegel DH, Valente EM, Van Haagen A, Van Hest L, Vaux KK, Vicente A, Weibel L, Chitayat D, Vikkula M. 2008. Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. *Hum Mutat* 29:959–965.
- Spurlock G, Bennett E, Chuzhanova N, Thomas N, Jim HP, Side L, Davies S, Haan E, Kerr B, Huson SM, Upadhyaya M. 2009. SPRED1 mutations (Legius syndrome): Another clinically useful genotype for dissecting the neurofibromatosis type 1 phenotype. *J Med Genet* 46:431–437.
- Tidyman WE, Rauen KA. 2009. The RASopathies: Developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev* 19:230–236.
- Williams JP, Wu J, Johansson G, Rizvi TA, Miller SC, Geiger H, Malik P, Li W, Mukoyama YS, Cancelas JA, Ratner N. 2008. Nf1 mutation expands an EGFR-dependent peripheral nerve progenitor that confers neurofibroma tumorigenic potential. *Cell Stem Cell* 3:658–669.
- Williams VC, Lucas J, Babcock MA, Gutmann DH, Korf B, Maria BL. 2009. Neurofibromatosis type 1 revisited. *Pediatrics* 123:124–133.
- Zhu Y, Guignard F, Zhao D, Liu L, Burns DK, Mason RP, Messing A, Parada LF. 2005. Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer Cell* 8:119–130.
- Zou C, Smith KD, Liu J, Lahat G, Myers S, Wang WL, Zhang W, McCutcheon IE, Slopis JM, Lazar AJ, Pollock RE, Lev D. 2009. Clinical, pathological, and molecular variables predictive of malignant peripheral nerve sheath tumor outcome. *Ann Surg* 249:1014–1022.