CONFERENCE REPORT

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Update from the 2013 International Neurofibromatosis Conference

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INTRODUCTION

The 2013 Neurofibromatosis (NF) Conference took place at the Portola Hotel and Spa, Monterey, CA, from June 8–11, 2013. This international meeting is sponsored annually by the Children's Tumor Foundation (CTF), with the goal of bringing together NF researchers and clinicians from disparate fields of expertise. The conference agenda included a range of preclinical topics with a focus on signaling pathways and mouse models and a number of clinical topics, including a symposium on the interaction of academics, government, and industry in NF clinical trials.

Neurofibromatosis is a group of inherited genetic disorders–NF1, NF2, and schwannomatosis–that together affect about 100,000 persons in the US. NF1 is the most common, with an estimated birth prevalence of 1:3,000 [Friedman, 1999; Lammert et al., 2005]; for NF2 and schwannomatosis, the estimated birth prevalence is 1:25,000 and 1:40,000, respectively [Antinheimo et al., 2000; Evans et al., 2005]. NF1, NF2, and schwannomatosis have in common that they predispose affected individuals to develop Schwann cell tumors such as neurofibromas and schwannomas. At a lower frequency, they also predispose to the development of a number of other benign and malignant tumor types, as well as some developmental abnormalities including learning disabilities (in NF1). The disorders arise from mutations in different genes, each of which plays a key role in regulating cellular function. The *NF1* gene on human chromosome 17 encodes an intracellular signaling molecule that functions as a GTPase activating protein for Ras proteins, whereas the *NF2* gene on human chromosome 22 encodes a cytoskeletal-membrane linking protein with reported roles in the suppression of several different growth-associated signaling pathways. The biology of schwannomatosis remains understudied despite the identification of germline *SMARCB1* mutations in schwannomatosis patients in 2007.

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NEUROFIBROMATOSIS 1

Basic Science

Neurobiology. Approximately 30–70% of individuals with neurofibromatosis type 1 (NF1) have learning disabilities, representing the most significant cause of lifetime morbidity associated with this disease. Moreover, recent findings indicate that signaling proteins within the Ras and the extracellular-signal regulated kinase (ERK) subfamily of mitogen-activated protein kinases (MAPK) pathway are mutated and hyperactivated in a number of human genetic syndromes (known as RASopathies) associated with varying levels of cognitive dysfunction substantiating the view that these pathways are important in regulating brain function [Rauen, 2013].

Yuan Zhu (University of Michigan) presented evidence that biallelic, but not monoallelic, inactivation of *Nf1* in developing neural stem cells increased the number of glial lineage cells but by distinct mechanisms depending on the region of the brain. In the dorsal forebrain *Nf1* loss in neural stem and progenitor cells altered their fate-specification resulting in increased numbers of glial cells in the corpus callosum at the expense of neurogenesis in the olfactory bulb. In contrast, in the ventral forebrain, overproduction of glial cells resulted from increased proliferation of glial-restricted progenitors. Importantly, both of these glial defects appeared to be caused by hyperactivation of Ras/ERK signaling, as a MEK inhibitor rescued the deficits. Yuan Wang (University of Michigan) presented data on the effects of biallelic loss of the *Nf1* gene in the cerebellum of mice and provided further evidence for the importance of the ERK pathway showing that hyperactivity of ERK underlies the defects seen in both neurons and glia of these mice, disrupting cerebellum structure and causing motor defects.

William Snider (University of North Carolina) performed both loss-of-function and gain-of-function studies on MEK1/2 in developing neural stem cells and post-mitotic neurons and showed that while loss of MEK1 and MEK2 impaired the generation of glial cells, hyperactive MEK/ERK signaling promoted gliogenesis during development. These results provide further genetic evidence that supports the observations of the Zhu group and others that accurate regulation of Ras signaling through the MEK/ERK pathway is critical for normal brain development.

Alcino Silva (University of California, Los Angeles) described the possible mechanisms by which loss of one allele of *Nf1* can cause deficits in long-term potentiation (LTP) and learning in adult mice. Dr. Silva also presented evidence that mutations in *SHP2* (identified in Noonan syndrome) cause similar deficits in LTP and learning. However, he emphasized that what appeared to be a similar phenotype occurred via distinct mechanisms, as the genetic mutations caused their effects in distinct cell types.

Freda Miller (University of Toronto, Canada) and her colleagues investigated signaling proteins within the Ras pathway and their

activating mutations in embryonic neural stem cells of the murine cortex, and arrived at two major conclusions. First, each protein, including SHP2, B-Raf, and Ras, plays an important role in regulating the genesis of neurons and astrocytes from developing neural precursors. Second, that when these proteins are hyperactivated, as in the Rasopathies, they deregulate cell genesis by distinct mechanisms.

Debra Mayes (Cincinnati Children's Hospital Medical Center) presented an analysis of the histological and behavioral changes of mice with hyperactive RAS signaling in oligodendrocytes. She hypothesized that upregulation of nitrous oxide plays a critical role in the phenotypes, which could be reversed with antioxidant treatment.

Tumor models. Thomas De Raedt (Brigham and Women's Hospital) presented work characterizing a more severe NF1 phenotype associated with microdeletions of the *NF1* region. He identified an additional gene within the deletion and, in a mouse model, recapitulated a more severe NF1 phenotype when this gene was lost in conjunction with the *NF1* gene. Nancy Ratner (Cincinnati Children's Hospital Medical Center) presented further work using the DhhCre; $NF1^{fl/fl}$ mouse model to test the requirement of specific signaling pathways for tumor growth. The studies verified the MEK/ERK pathway as a therapeutic target and identified STAT-3 as a potential new target. In studies of NF1-associated low grade gliomas, David Gutmann (Washington University) presented evidence that human tumors are monoclonal and have no other mutations other than loss of *NF1* gene expression [Gutmann et al., 2013]. However, in a genetically engineered mouse optic glioma model, loss of *Nf1* gene expression in glial cells alone is not sufficient to drive tumorigenesis but requires signals from $Nf1^{+/-}$ microglia in the surrounding microenvironment [Pong et al., 2013].

Luis Parada (UT Southwestern) presented work on a novel glioma mouse model in which stem cells are labeled and can be ablated following ganciclovir treatment. A stem cell population was found to be labeled in glioblastomas in these mice and was relatively quiescent. Following treatment of the tumor however, these stem-like cells differentially proliferated and were responsible for the regrowth of the tumor. These results reinforce the importance of targeting the stem-like cells found in many tumors. Dinorah Friedmann-Morvinski (The Salk Institute) presented a different model of glioblastoma in which tumors were induced from fully differentiated cortical neurons rather than from stem cells, demonstrating that the cell-of–origin may not always be obvious.

MPNST is the primary cause of early mortality in NF1 patients [Evans et al., 2011]. Using a Sleeping Beauty genetic screen, Adrienne Watson (University of Minnesota) identified Wnt signaling as a driver of Schwann cell tumorigenesis. Induction of Wnt signaling was sufficient to induce a transformed phenotype in human Schwann cells, while inhibition of both Wnt signaling and mTOR synergistically induced apoptosis of MPNST cell lines. Rebecca Dodd (Duke University) described a mouse model of soft tissue sarcoma in which *Nf1* and *lnk4a* were knocked out by the injection of a Cre-expressing adenovirus into either muscle or sciatic nerve. Treatment with a MEK inhibitor decreased sarcoma growth in these mice.

Clinical Research

Cognitive issues and clinical trials. Up to two-thirds of individuals with NF1 demonstrate signs of cognitive dysfunction, ranging from deficits in attention, visual-spatial memory and executive function, to language problems, learning disabilities, and academic underachievement. Nicole Ullrich (Boston Children's Hospital, Harvard Medical School) summarized the major challenges in clinical trial design for neurocognitive interventions, including trial design, selection of ideal outcome measures, selection of the appropriate drug and duration for intervention, dose-finding and appropriate inclusion criteria.

Maria Ribeiro (Coimbra University, Portugal) presented data using magnetic resonance spectroscopy to measure brain metabolites such as the inhibitory neurotransmitter GABA in the visual cortex of children and adolescents with NF1. Mouse models have implicated increased release of GABA in the pathophysiology of cognitive issues [Cui et al., 2008]. Children with NF1 have lower measured levels of unbound GABA, which might reflect depletion of GABA from synaptic vesicles [Violante et al., 2013]. Within the NF1-group, higher overall GABA-levels correlated with slower reaction times.

Thijs van der Vaart (Erasmus MC, The Netherlands) reported on the final results of the NF1-SIMCODA trial, a 12-month randomized placebo-controlled trial in 84 NF1-children aged 8–16 years. Earlier work had shown positive effects of lovastatin, a cholesterol-lowering drug, in a mouse model of NF1 [Li et al., 2005]. The NF1-SIMCODA trial aimed to detect beneficial effects of simvastatin on cognitive and behavioral problems in children with NF1. The current randomized controlled trial (RCT) was based on a previous 12-week pilot RCT with 61 NF1-children in the same age range, in which only one secondary outcome measure, the IQ-subtest "object assembly", demonstrated a positive effect of simvastatin over placebo [Krab et al., 2008]. The results of NF1-SIMCODA were disappointing, showing no benefit of simvastatin over placebo for treating cognitive and behavioral problems [van der Vaart et al., 2013].

Kristina Hardy (Children's National Medical Center) presented data on the use of a computerized training program, CogMed, in children with NF1. CogMed has been proposed as a neurocognitive intervention in an upcoming clinical trial through the NF Clinical Trials Consortium.

Recognizing that clinical trials for NF1 learning disability are currently underway, the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) group has been working to recommend a standardized set of outcome measures in clinical trials for NF. Karin Walsh (Children's National Medical Center) summarized the progress of the REiNS working group on neurocognitive outcome measurements. One concern of commonly utilized neuropsychological measures is that they are not designed to detect change over time. The ideal outcome measures for cognitive interventions should measure constructs of interest that are relevant to individuals with NF1, should detect change over time, and should be generalizable.

Optic pathway glioma. Peter de Blank (Rainbow Babies & Children's Hospital, Case Western Reserve University) retrospectively evaluated the ophthalmologic records and diffusion tensor imaging measurements of the optic pathway in children with NF1

and optic pathway gliomas. He found that a decrease in fractional anisotropy of the optic radiations was associated with abnormal visual acuity and could be a predictor of visual acuity loss in the following year [de Blank et al., 2013].

Autism spectrum disorder. Susan Huson (Oxford, UK) presented a study evaluating the prevalence of autistic features in a large population of children 4–16 years of age with NF1. In this population-based study, she found that approximately 30% of subjects met the diagnostic criteria for an autistic spectrum disorder [Garg et al., 2013]. These findings build on previous reports suggesting that autistic symptoms are common in NF1 patients but contrasts with a prior report in adult NF1 patients. [van Eeghen et al., 2013; Walsh et al., 2013].

Clinical efficiency. Amanda Bergner (Johns Hopkins School of Medicine) presented the results of a pilot project evaluating the use of a dedicated adult intake clinic at the Johns Hopkins Comprehensive NF Center in improving the efficiency of care. The intake clinic significantly reduced patient wait time, allowed for more effective time to be spent with NF providers, and generated new revenue for the clinic.

NF1 Clinical Trials Update

Brian Weiss (Cincinnati Children's Hospital Medical Center) presented the results from a 2-stratum phase II clinical trial of the mTOR inhibitor sirolimus for plexiform neurofibroma (PNF) in subjects with NF1. This trial specifically sought to determine whether sirolimus either: (a) increases time to progression (TTP) in progressive PNF (stratum 1) or; (b) results in objective radiographic responses in inoperable PNFs in the absence of documented radiographic progression at trial entry (stratum 2). Disease status was evaluated using volumetric MRI analysis at regular intervals; pain reduction and quality of life (QOL) outcomes were also assessed. No patients on stratum 2 experienced a radiographic response. [Weiss et al., 2013] In contrast, patients in stratum 1 had an estimated median TTP of 15.4 months, compared to 11.9 months in a control group from a completed NCI-sponsored study with near identical eligibility criteria.

Brigitte Widemann (National Cancer Institute) reported the preliminary results of an ongoing phase 1 trial of selumetinib, a MEK inhibitor, in children 3–18 years of age with NF1 and inoperable PNFs. Selumetinib is administered orally BID on a continuous dosing schedule. The maximally tolerated dose will be determined based on toxicities observed in the first three courses. Disease status is evaluated using volumetric MRI analysis at regular intervals. Nine subjects had enrolled to date (median age 15 years; range, 5–18 years). No dose limiting toxicities (DLTs) were observed at the 20 mg/m^2 dose, but one of three initial subjects developed a DLT at 30 mg/m^2 which was reversible and asymptomatic. This dose level has since been expanded to six subjects, with no additional DLTs to date. Decreases in PNF volume have been observed in all subjects who have undergone at least one restaging MRI, and partial response ($\geq$ 20% shrinkage in tumor volume) has been achieved in four of the six subjects. Chronic dosing of selumetinib has been tolerated by children with NF1 and PNF, and enrollment of this study is ongoing.

David Viskochil (University of Utah) presented the results of a phase II trial of chemotherapy in sporadic and NFI-associated high-grade malignant peripheral nerve sheath tumors (MPNSTs). Ifosfamide and doxorubicin followed by 2 cycles of ifosfamide and etoposide were used. The objective response rate was lower in NF1 than in sporadic MNST patients, which is consistent with retrospective literature reports.

NEUROFIBROMATOSIS 2

Basic Science

NF2 signaling. Merlin interacts with the E3 ubiquitin ligase CRL4-DCAF1 in the nucleus and inhibits its function [Li et al., 2010]. Filippo Giancotti (Memorial Sloan-Kettering Cancer Center) described a functional link between Merlin, CRL4-DCAF1, and LATS in human Schwann cells. These results provide a mechanism for Merlin's activation of Lats and the Hippo tumor suppressor pathway in the nucleus of Schwann cells. In addition, they indicate that YAP/TEAD inhibitors, which are undergoing preclinical testing, may be effective for the treatment of NF2.

Duojia Pan (Johns Hopkins University) discussed recent efforts in targeting the Hippo pathway as a potential strategy against NF2. He presented findings showing that loss of *NF2* mimics inactivation of Hippo signaling in the liver, the lens of the eye, and ependymal cells, suggesting that *NF2* is a physiological regulator of Hippo signaling in multiple mammalian tissues. Disruption of the YAP-TEAD complex, the nuclear effector of the Hippo pathway, potently suppresses hepatomegaly/tumorigenesis resulting from *Nf2* inactivation in the mouse liver. Screening of a library of drugs approved by the U.S. Food and Drug Administration (FDA) identified verteporfin as a small molecule that inhibits TEAD-YAP association and YAP-induced liver overgrowth, providing proof of principle for YAP inhibitors as molecular-targeted therapeutics for NF2 [Liu-Chittenden et al., 2012].

Joe Kissil (The Scripps Research Institute) detailed the identification of a novel Merlin-interacting protein–angiomotin (Amot)–associated with tight and adherens junctions and an interactor of several small G-protein modulators. Amot directly associates with Yap, a transcriptional activator regulated by the Hippo pathway. Moreover, Amot is required for hepatic "oval cell" response and tumorigenesis in response to toxin-induced injury or *Nf2* loss in a liver-specific Amot knockout mouse. In these studies, Amot appears to regulate Yap at multiple levels, both in the cytoplasm and in the nucleus. These studies implicate Amot as a critical downstream effector of Merlin and the Hippo/Yap pathway.

Dominique Lallemand (Curie Institute, France) reported the use of a combination of proteomic approaches to evaluate the expression, activity, and link to tumor cell proliferation of several of the signaling pathways regulated by Merlin in a large series of human schwannoma biopsies. They found that Her3, Her2, PDGFR-β, and Axl are the most frequently activated receptor tyrosine kinases (RTKs) in schwannomas and could represent key therapeutic targets. Using Reverse Phase Protein Array, they showed that the expression of the Ki67 proliferation marker is strongly associated with levels of Yap, activated Her3, and PDGFR-β. Also, the expression of PDGFR-β and Her3 can be stimulated by Yap in

schwannoma cells in culture suggesting that a signaling network under the control of Yap may be involved in tumor growth. Finally, they observed remarkable heterogeneity in the expression of all the proteins assessed by immunohistochemistry on tumor sections, indicating that the response to targeted treatments will likely be variable from patient to patient.

Alizee Boin (Curie Institute, Paris, France) presented data indicating that Merlin and YAP directly interact, with the possibility that Merlin sequesters YAP in the cytosol, preventing it from transactivating expression. Lastly, Alexander Schulz (Leibniz Institute, Germany) presented data showing how loss of Merlin in PNS axons leads to reduced expression of the myelin regulator, neuregulin 1 type III. They are currently studying the effects upon PNS myelination and the implications for the polyneuropathies seen in patients with NF2.

Preclinical Science

NF2 mouse models. Andrea McClatchey (Massachusetts General Hospital/Harvard Medical School) started off this session by presenting her studies of the origin of the *Alb-Cre;Nf2*$^{flox/flox}$ model of liver progenitor expansion and tumorigenesis. Surprisingly, this analysis uncovered a novel, proliferation-independent function for Merlin during liver development.

Michel Kalamarides (Hopital Pitié-Salpêtriére, Paris, France) presented an update on mouse models of human meningioma [Peyre et al., 2013] showing an important role of PDGF-β for the initiation and malignant progression of meningiomas associated with *NF2* gene inactivation. Two recent papers showed that recurrent mutations in SMO and AKT1 are mutually exclusive with *NF2* loss in meningioma initiation [Brastianos et al., 2013; Clark et al., 2013]. The lab's current strategy to reproduce these genetic events in mice was presented.

Charles Yates (Indiana University School of Medicine) described a genetically engineered NF2 mouse model with excision of the *Nf2* gene driven by Cre expression under control of a tissue-restricted 3.9 kb Periostin promoter element. By 10 months of age, 100% of these *Postn-Cre;Nf2*$^{flox/flox}$ mice develop spinal, peripheral, and cranial nerve tumors that are histologically identical to human schwannomas. In addition, the development of cranial nerve VIII tumors in these mice may correlate with functional impairments in hearing and balance, as measured by auditory brainstem response and vestibular testing. Embryonic analyses suggest an early Schwann cell progenitor tumor cell of origin.

Screening and Compound Testing

Cristina Fernandez-Valle (University of Central Florida) discussed the high-throughput screening of a 1280 compound library (LOPAC) using Merlin-null mouse Schwann cells in order to identify probes that reduced cell viability. Dr. Fernandez-Valle identified two compounds from an initial 40 "hits" that selectively reduced viability of Merlin-null mouse Schwann cells as compared to normal mouse Schwann cells. In a joint project, Cenix Bioscience validated a number of compounds from the drug screen as well as those in clinical trials for NF2. This demonstrated the validity of the cell line and high-throughput screening approach to identify candidate drugs for human studies.

Marco Giovannini (House Research Institute) reported on efficacy testing of HSP90 inhibitors for NF2. The antiproliferative activity of NXD30001 was tested in NF2-deficient cell lines and in human primary schwannoma and meningioma cultures in vitro, and in two allograft models and in one Nf2 transgenic model in vivo [Tanaka et al., 2013]. They found that NXD30001 induced degradation of client proteins and suppressed proliferation of NF2-deficient cells. In addition, differential expression analysis using a global transcriptome approach identified subsets of genes implicated in cell proliferation, cell survival, vascularization, and Schwann cell differentiation whose expression was altered by NXD30001 treatment. Overall, results showed that NXD30001 in NF2-deficient schwannoma suppressed multiple pathways necessary for tumorigenesis.

Dina Stepanova (Fox Chase Cancer Center) reported that *Nf2* null mouse cells exhibit higher levels of acetyl CoA and fatty acid synthase (Fasn) and that the Fasn inhibitor, cerulenin, selectively inhibits cell proliferation in xenografts. In humans, the treatment of *NF2* null vestibular tumors with anti-VEGF therapy (bevacizumab) alone has shown promise. Using a mouse xenograft model, Lei Xu (Massachusetts General Hospital, Harvard Medical School) showed promising results using a combination of radiotherapy and anti-VEGF therapy.

Clinical Research

Rosalie Ferner (Guy's and St. Thomas' NHS Foundation Trust, UK) presented the results of her multi-center longitudinal study of quality of life (QOL) in 288 patients with NF2 using the NF2 Impact on QOL (NFTI-QOL) questionnaire [Hornigold et al., 2012]. This measure was quick and easy to administer, and demonstrated good reliability and ability to detect significant QOL changes over time in this patient population.

Vanessa Merker (Massachusetts General Hospital) analyzed prospective data from the NF2 Natural History Consortium Study, using new endpoint measures recommended by the Response Evaluation in NF and Schwannomatosis (REiNS) committee. Vestibular schwannomas were evaluated for volumetric progression and hearing was assessed with word recognition scores. The median time to tumor progression was 14 months. A significant number of patients experienced tumor progression and hearing decline over 3 years [Plotkin et al., 2014].

NF2 Clinical Trials Update

Jaishri Blakely (Johns Hopkins University) presented the results of a multi-institutional study of bevacizumab, a VEGF-A antibody, in subjects >12 years of age with NF2 and symptomatic vestibular schwannomas (VS). Subjects were given bevacizumab every 3 weeks for 1 year, with hearing evaluations performed during treatment and twice after completion of treatment. The primary endpoint was a statistically-significant increase in word recognition score (WRS) compared with baseline. Brain MRI, whole body MRI, and QOL outcomes were also assessed. Fourteen subjects (median age 30 years; range, 14–79 years) were enrolled. No subjects had progressive hearing loss while on treatment. Thirty-six percent of (5/14) subjects had significant improvement in WRS maintained

for three consecutive months, and improvement was maintained off bevacizumab for those subjects. Four of five subjects with an improved WRS also had significant reduction in tumor volume on MRI. These prospective data confirm that bevacizumab is well tolerated in this patient population and that bevacizumab can reverse hearing loss due to NF2-related VS in select patients, independent of tumor volume.

Michel Kalamarides (Hopital Pitié-Salpêtriére, Paris, France) presented the intermediate results of a prospective trial of the mTOR inhibitor everolimus for progressive VS in NF2 patients. The rationale for the trial was based on preclinical results in a NF2 genetically engineered mouse schwannoma model and encouraging and consistent results in an index patient [Giovannini et al., 2014]. To date, no tumor shrinkage has been observed. However, preliminary analysis suggests that mTORC1 inhibition may delay time to tumor progression. In the second year of the trial, patients who experience tumor growth after discontinuation of everolimus will be allowed to restart the drug. This design is intended to determine whether stable disease on everolimus represents a true clinical response to treatment.

Matthias Karajannis (New York University) reported the results of a separate clinical trial using everolimus in ten patients with NF2 and progressive vestibular schwannomas. No objective improvement in hearing or imaging response was seen [Karajannis et al., 2014].

SCHWANNOMATOSIS

Preclinical Science

Xandra Breakefield (Massachusetts General Hospital/Harvard Medical School) described her collaboration with Gary Brenner and Giulia Fulci to develop an AAV1-PO-ICE vector that has a Schwann cell-specific promoter driving caspase-1. Intratumoral injection of the vector causes regression of schwannomas and a reduction in schwannoma-induced pain in NF2-derived human xenograft mice in sciatic nerve. This effect may occur via a process of pyroptosis and therefore has the potential to control growth of tumors that are not directly exposed to the vector.

Larry Sherman (Oregon Health and Science University) discussed the underlying mechanisms of pain in schwannomatosis. He described a SMARCB1 conditional knockout mouse with nerves that showed a similar expression profile and morphology to wild type cells, but which were quicker to respond to painful stimuli. An expression array from cultured dorsal root ganglia of these mice showed elevated VR1 capsaicin receptors (TRPV1) suggesting that this pathway could be considered for therapeutic intervention.

Clinical research. Miriam Smith (University of Manchester, England) presented exome sequencing data showing that multiple spinal meningiomas can be inherited as a distinct genetic condition, caused by mutations in the chromatin remodeling complex subunit *SMARCE1*. Theo Hulsebos (Academic Medical Center, Netherlands) presented an analysis of mutant SMARCB1 protein expression in schwannomas from schwannomatosis patients, showing that mutant forms of SMARCB1 can be found in these tumors and that the type of mutation influences the amount of protein present. However, the type of mutation does not influence the presence of the typical mosaic pattern of staining in tissue preparations. Alvaro Pinto (Massachusetts General Hospital) presented his research correlating *SMARCB1* mutation analysis and SMARCB1 immunohistochemical expression patterns in syndromic and sporadic schwannomas. He found a discrepancy between the high rate of altered, mosaic SMARCB1 expression in syndromic schwannomas (familial schwannomatosis, sporadic schwannomatosis, NF2) and low rates of mutations in the *SMARCB1* gene, suggesting other mechanisms may play a role in tumor development. In addition, despite a high rate of mosaic pattern of SMARCB1 expression in NF2 tumors, *SMARCB1* mutations were not present in any of the NF2-associated tumors.

Judith Eelloo (Central Manchester University Hospitals Foundation Trust, UK) presented a case report of a patient with SMARCB1-associated schwannomatosis with three independent malignancies (lymphoma, neuroendocrine tumor, and sarcoma). This further highlighted the clinical overlap between syndromes and stressed the importance of seeking care in an expert center as well as the need for on-going genetic and phenotypic characterization of schwannomatosis. Amanda Bergner presented the progress of the International Schwannomatosis Database (ISD). The ISD is a medically curated, anonymous database of schwannomatosis patients, and was designed as a resource to identify people who would be suitable candidates for clinical trials or research studies. The ISD is being contributed to by 10 academic centers around the globe and recently, a remote patient entry mechanism was added to allow patients not affiliated with an enrolling center to participate (www.schwannomatosis.com).

NOVEL SESSIONS AT THE 2013 CONFERENCE

The 2013 conference incorporated novel sessions including educational sessions, small group sessions, and an industry/academia/government symposium.

Clinical Phenotypes: What do They Tell Us?

NF1, NF2, and schwannomatosis are characterized by wide clinical variability. Making a clinical diagnosis can be challenging due to the overlap in tumor types encountered in the neurofibromatoses and in the clinical presentation with a variety of other conditions.

Ludwine Messiaen (University of Alabama, Birmingham) discussed clinical and molecular characteristics of skin hyper pigmentation in NF1 and in Legius syndrome. In addition, an array of other conditions associated with multiple cafe-au-lait macules (CALM) were reviewed, including NF2, McCune-Albright syndrome, ring chromosomes (ch. 7, 11, 12, 15, 17, 22), LEOPARD syndrome, constitutive mismatch repair deficiency, Cowden, Fanconi anemia, tuberous sclerosis complex, Silver-Russell, and piebaldism. She noted that the NIH diagnostic criteria for NF1 are not specific, as some patients with pigmentary signs only (CALM and skinfold freckling) have Legius syndrome, due to mutations in *SPRED1*, instead of *NF1*.

Gareth Evans (Saint Mary's Hospital, United Kingdom) discussed the lack of sensitivity of the current NIH diagnostic consensus criteria for NF1 patients with "spinal neurofibromatosis". These patients present with multiple spinal tumors but few, if any, CALMs, skinfold freckling, or cutaneous neurofibromas [Burkitt

Wright et al., 2013]. Dr. Evans recommended testing of both *NF1* and *SMARCB1* genes in blood and, in selected cases, in tumor biopsies in order to distinguish between NF1, mosaic NF2, and schwannomatosis.

Anat Stemmer-Rachamimov (Massachusetts General Hospital, Harvard Medical School) discussed that hybrid tumors are peripheral nerve sheath tumors that have mixed pathological features of two or more types of peripheral nerve sheath tumors and are therefore difficult to classify. However, their presence in a patient is highly suggestive of one of the syndromic forms of neurofibromatosis [Harder et al., 2012].

Dusica Babovic-Vuksanovic (Mayo Clinic) presented a recently published series of patients with multiple orbital neurofibromas, painful peripheral nerve tumors, distinctive face, and marfanoid habitus [Babovic-Vuksanovic et al., 2012]. Four unrelated patients have been described with this condition, and this is thought to be a new syndrome, as none of the known genes associated with predisposition to develop neurofibromas or schwannomas were mutated in any of these patients.

Small Group Sessions

NF-related neuropathy. Neuropathies are a significant problem in many patients with NF1, NF2, and schwannomatosis. Susan Huson (NF Centre, Manchester, UK) reviewed the clinical aspects of neuropathy in NF2, including length dependant peripheral neuropathy and focal amyotrophy/mononeuropathy [Trivedi et al., 2000; Sperfeld et al., 2002]. In the latter group, the diagnosis can be made only after exclusion of a causative tumor. Gareth Evans (personal communication) identified 77/396 (19%) NF2 patients in the UK registry with amyotrophy/mononeuropathy; the most common symptom is sudden unilateral facial weakness (40/77) followed by foot drop (23/77); 8.6% (34/396) of patients have a peripheral neuropathy.

In another study of NF2 peripheral neuropathy [Sperfeld et al., 2002], 7/15 patients were found to have a clinically detectable neuropathy and a further three had nerve conduction abnormalities. Sural nerve biopsies in NF2 neuropathy cases have shown diffuse Schwann cell proliferation and small endoneurial tumorlets of schwannomas and perineuriomas associated with a reduction in nerve fiber density. Electron microscopy demonstrated de-differentiated Schwann cells isolated, or in complexes, and multiple interdigitating cell processes compatible with merlin malfunction [Sperfeld et al., 2002]. Three hypotheses were suggested as to the cause of the neuropathy: nerve compression by multiple tumorlets, an unknown local toxic effect of abnormal endoneurial cells, or the inability of NF2 Schwann cells to adhere to nerve sheath axons. Support for the compression hypothesis came from a MRI study of peripheral nerves in NF2 patients that demonstrated accumulation of non- compressive fascicular lesions with the clinical severity being proportional to the number of lesions [Baumer et al., 2013].

Helen Morrison (Leibniz Institute for Age Research, Germany) highlighted that NF2-neuropathy is likely of multifactorial origin. She reported a novel merlin-specific function in maintaining axonal integrity and proposed that reduced axonal *nf2* gene dosage influences NF2-associated polyneuropathy. Genetically engineered mice that specifically lack neuronal merlin display abnormal axons and these mice suffer from polyneuropathy-like symptoms of axonal origin. This finding is of clinical interest because, in addition to the observed Schwann cell tumorlets, reduced merlin expression in axons may contribute to NF2-polyneuropathy [Schulz et al., 2013].

The clinical and biological aspects of NF1 associated neuropathies were reviewed by Rosalie Ferner (Guy′s and St. Thomas′ NHS Foundation Trust, UK). NF1 neuropathy is a length-dependent, predominantly axonal sensorimotor neuropathy. It occurs less frequently than the NF2 neuropathy and the symptoms and neurological signs are usually mild and non–progressive. The pathogenesis of NF1 neuropathy has not been determined but there is a putative abnormality in signaling between Schwann cells, fibroblasts, and perineurial cells.

Neuropathy associated with schwannomatosis was reviewed by Gareth Evans (University of Manchester, UK). Typically, schwannomatosis is characterized by painful schwannomas with minimal deficit in motor or sensory function. Tumor morphology is discrete rather than invasive and therefore peripheral neuropathy and amyotrophy are rare. The main clinical challenge is treating neuropathic pain.

Musculoskeletal biology and metabolism in NF. While the skeletal complications associated with NF1 are well described, several research groups have begun to explore the prevalence and impact of muscle weakness. Reductions in the muscle compartment in NF1 individuals have been documented by peripheral quantitative computed tomography, which initiated a number of studies examining muscle performance.

David Stevenson (University of Utah) described studies in children with NF1 showing significant reductions in total motor composite scores and strength. In addition, preliminary data from 16 individuals with NF1 suggest that energy expenditure/metabolism may also be abnormal in NF1 muscles. He theorized that impaired muscle function may be a feature common to RASopathies, suggesting an underlying importance of RAS signaling in muscle function [Stevenson et al., 2012]. These clinical findings were supported by hand-held dynamometry results from the lab of Joshua Burns (Children's Hospital, Westmead, Sydney, Australia) showing mean reductions of 30–45% in strength in a range of muscle movements. Juliana de Souza (Federal University of Minas Gerais, Belo Horizonte, Brazil) presented data showing that a range of muscle outcomes in NF1 patients were affected (aerobic capacity [de Souza et al., 2013], handgrip strength, 6 minute walk, respiratory muscle force) and were not affected (cardiac function, myocardial mass and pulmonary function). As researchers and clinicians increasingly recognize that weakness and reduced muscle tone are features of NF1, research has turned to exploring the benefits of physical therapy and discovering the underlying molecular mechanism.

Kate Quinlan (University of Sydney, Australia) described how mouse models were used to understand metabolic changes associated with α-actinin deficiency, a common inherited polymorphism [Quinlan et al., 2010] and then documented an underlying metabolic deficiency in NF1 mouse models. [Sullivan et al., 2014] in the *MyoD:Nf1*$^{-/-}$ mice lacking *Nf1* expression in muscles, a neonatal lethal phenotype with intramyocellular fat inclusions was observed. Examination of the *Prx1:Nf1*$^{-/-}$ mice, which lack Nf1 expression in

the developing limbs, showed reduced muscle mass and strength [Kossler et al., 2011], and increased muscle triglycerides.

Integrative Management of Children With NF1

The Washington University NF Center described a multidisciplinary approach to the management of children with NF1. A patient coordinator assists in scheduling same day appointments, especially for families who travel long distances. A physical therapist completes standardized testing of general development in children younger than eight years, and gross and fine motor skills for older children. Based on these and other assessments performed during the clinic visit, individualized recommendations for home activities are provided. In addition, an occupational therapist integrates haptic technology into daily activities and school routines to improve achievement in children with NF1. A pediatric neuropsychologist performs early screening of academic skills in children with NF1.

Multiple Phases of Neurofibroma Progression

In this session, the group led by Vincent Riccardi (Neurofibromatosis Institute, La Crescenta, CA) discussed the initiation and progression of neurofibromas and characterized the stages of progression. The main discussion centered around whether loss of the second *NF1* allele in Schwann cells was always the initiating event in neurofibroma formation and the role of macrophages, pericytes and CD34+ cells in tumor progression.

Using Genetically Engineered Mouse Models for Preclinical Drug Screening

Chaired by David Gutmann (Washington University), the discussion leveraged the expertise of investigators versed in clinical trial design and execution as well as those leading preclinical small-animal model discovery and evaluation efforts. The panel emphasized the successes that have directly resulted from generating and intelligently using genetically-engineered mouse (GEM) strains. Important concerns raised included the timely access of drugs in the clinical pipeline to preclinical GEM researchers and the need to collect biospecimens from individuals participating in human clinical trials. In addition, members of the panel emphasized the importance of matching GEM models with specific subtypes of NF-associated clinical features to best reflect the inherent heterogeneity of these conditions as well as the need to obtain detailed pharmacokinetic, pharmacodynamic, and long-term toxicity data from GEM preclinical studies relevant to the treatment of both adults and children with NF1 and NF2.

Industry/Academia/Government Symposium

In this session, leaders from industry, governmental agencies, and academia discussed the pathways to accelerate the development of effective therapies for NF1, NF2, and schwannomatosis. Salvatore LaRosa (Children's Tumor Foundation) described three CTF-sponsored preclinical initiatives: (1) NF Therapeutics Consortium (formerly the NF Preclinical Consortium, also funded by the Neurofibromatosis Therapeutic Accelerations Program), a collaborative effort amongst leading NF mouse model laboratories focusing on the evaluation of new or repurposed drugs for NF tumors; (2) Synodos, a new pre-clinical effort to fund new approaches to the diagnosis and treatment of NF2-related vestibular schwannomas and meningiomas; and (3) Drug Discovery Initiative, which since its launch in 2006 has provided >$1.4 million in funding for rapid screening of new drugs for manifestations of NF.

Roger Packer (Children's National Medical Center) reviewed the current and future clinical trials of the DOD NF Clinical Trials Consortium, which has been expanded to include 13 sites. He also discussed some of the clinical and therapeutic challenges the consortium has faced, including selection of appropriate outcome measures and study design, and the limitations imposed based on the geographic location of the consortium members.

Helen Chen (CTEP, National Cancer Institute) reviewed the CTEP program, which sponsors clinical trials to evaluate new anticancer agents. CTEP has a broad list of agents in clinical development including agents targeting specific signaling pathways, apoptosis, and the cell cycle. In addition to supplying drug, CTEP provides data infrastructure and regulatory support for clinical trials. In the past, CTEP has sponsored several clinical trials directed at NF1-related plexiform neurofibromas and NF2-related vestibular schwannomas. Dr. Chen commented on the challenges in selecting drug combinations including the potential for increased toxicity and the challenge in defining the optimal dose and schedule of the agents.

Gregory Reaman (Center for Drug Evaluation and Research, FDA) reviewed the Orphan Drug Act, which promotes the development of products that demonstrate promise for the diagnosis and/or treatment of rare diseases. He commented that while orphan products are held to the same approval standards as products for common diseases by requiring substantial evidence of safety and effectiveness for approval, regulations allow for "flexibility" and "scientific judgment" in how this is achieved. He also discussed the FDA Safety and Innovation Act and the Prescription Drug User Fee Act, both of which aim to bring critical new medicines to the market for patients and to support industry in their pursuit of innovative treatments for rare diseases.

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