HHS Public Access

Author manuscript

Am J Med Genet A. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:

Am J Med Genet A. 2018 May ; 176(5): 1258–1269. doi:10.1002/ajmg.a.38675.

2016 CTF Conference on Neurofibromatosis Type 1, Neurofibromatosis Type 2 and Schwannomatosis

Michael J. Fisher[1], **Allan J. Belzberg**[2], **Peter de Blank**[3], **Thomas De Raedt**[4], **Florent Elefteriou**[5], **Rosalie E. Ferner**[6], **Marco Giovannini**[7], **Gordon J. Harris**[8], **Michel Kalamarides**[9], **Matthias A. Karajannis**[10], **Aerang Kim**[11], **Conxi Lázaro**[12], **Lu Q. Le**[13], **Wei Li**[14], **Robert Listernick**[15], **Staci Martin**[16], **Helen Morrison**[17], **Eric Pasmant**[18], **Nancy Ratner**[19], **Elisabeth Schorry**[20], **Nicole J. Ullrich**[21], **David Viskochil**[22], **Brian Weiss**[23], **Brigitte C. Widemann**[24], **Yuan Zhu**[25], **Annette Bakker**[26], and **Eduard Serra**[27]

[1]Division of Oncology, Children's Hospital of Philadelphia, and Department of Pediatrics, The Perelman School of Medicine at The University of Pennsylvania, Philadelphia, PA

[2]Department of Neurosurgery, The Johns Hopkins Hospital, Baltimore, MD

[3]Division of Oncology and Department of Pediatric, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

[4]Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[5]Center for Skeletal Medicine and Biology, Dept. of Molecular and Human Genetics and Orthopedic Surgery, Baylor College of Medicine, Houston, TX

[6]Neurofibromatosis Centre, Guy's and St. Thomas NHS Foundation Trust London UK

[7]Department of Head and Neck Surgery, David Geffen School of Medicine at University of California Los Angeles, Los Angeles, CA

[8]Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

[9]Department of Neurosurgery, Hospital Pitie-Salpetriere, AP-HP, Paris, France; Université Pierre et Marie Curie, Sorbonne Universités, Paris, France

[10]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY

[11]Division of Oncology, Children's National Medical Center, Washington DC

[12]Hereditary Cancer Program, Catalan Institute of Oncology (ICO-IDIBELL-CIBERONC), L'Hospitalet de Llobregat, Barcelona, Spain

[13]Department of Dermatology and Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX

[14]Department of Pediatrics, Department of Biochemistry & Molecular Biology, Penn State University College of Medicine, Hershey, PA

Correspondence to: Michael J. Fisher, The Children's Hospital of Philadelphia, Colket Translational Research Building - 10th Floor, 3501 Civic Center Blvd, Philadelphia, Pennsylvania 19104, Phone# (215)590-5188, Fax# (215)590-9956, fisherm@email.chop.edu.

[15]Division of Academic General Pediatrics, Ann & Robert H. Lurie Children's Hospital of Chicago, and Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago IL

[16]National Cancer Institute, National Institutes of Health, Bethesda, MD

[17]Leibniz Institute on Aging Research, Fritz Lipmann Institute, Jena, 18. Germany

[18]EA7331 and Cochin Hospital, Paris Descartes University, Faculty of Pharmacy of Paris, France

[19]Division of Experimental Hematology and Cancer Biology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH

[20]Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

[21]Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA

[22]Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT

[23]Division of Oncology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

[24]Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD

[25]The Gilbert Family Neurofibromatosis Institute, Centers for Cancer and Immunology Research and Neuroscience Research, Children's National Medical Center, Washington, DC

[26]Children's Tumor Foundation, New York, NY

[27]Hereditary Cancer Group, The Institute for Health Science Research Germans Trias i Pujol (IGTP) – PMPPC, Barcelona, Spain

Abstract

Organized and hosted by the Children's Tumor Foundation (CTF), the Neurofibromatosis (NF) Conference is the premier annual gathering for clinicians and researchers interested in neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2) and schwannomatosis (SWN). The 2016 edition constituted a blend of clinical and basic aspects of NF research that helped in clarifying different advances in the field. The incorporation of next generation sequencing is changing the way genetic diagnostics is performed for NF and related disorders, providing solutions to problems like genetic heterogeneity, overlapping clinical manifestations or the presence of mosaicism. The transformation from plexiform neurofibroma (PNF) to malignant peripheral nerve sheath tumor (MPNST) is being clarified, along with new management and treatments for benign and pre-malignant tumors. Promising new cellular and *in vivo* models for understanding the musculoskeletal abnormalities in NF1, the development of NF2 or SWN associated schwannomas, and clarifying the cells that give rise to NF1-associated optic pathway glioma were presented. The interaction of neurofibromin and S*PRED1* was described comprehensively, providing functional insight that will help in the interpretation of pathogenicity of certain missense variants identified in NF1 and Legius Syndrome patients. Novel promising imaging techniques are being developed, as well as new integrative and holistic management models for patients that take into account psychological, social and biological factors. Importantly, new therapeutic approaches for schwannomas, meningiomas, ependymomas, PNF, and MPNST

are being pursued. This report highlights the major advances that were presented at the 2016 CTF NF Conference.

Keywords

Neurofibromatosis; Schwannomatosis; Conference; Neurofibromin; Merlin; Neurofibroma; Malignant peripheral nerve sheath tumor; Schwannoma; Glioma; Ependymoma; Meningioma; Pseudoarthrosis; Autism

INTRODUCTION

The Neurofibromatosis (NF) Conference is the premier annual gathering of the NF professional community. This international meeting is organized and hosted by the Children's Tumor Foundation (CTF) and aims to bring the most up to date clinical and basic research findings to the NF community, foster critical debate, generate new ideas and promote collaboration and synergies among participants. There is also room to introduce experimental approaches, technology and ideas from outside NF via keynote addresses from experts in other fields and by attracting scientists from outside the NF community to expand collaborative opportunities. The promotion and consolidation of young scientists and clinicians engaged in a broad range of disciplines related to NF is also important to foster the development of emerging investigators in the field.

The 2016 edition took place in Austin, TX and constituted a blend of clinical and basic aspects of NF research, trying to maintain the difficult equilibrium between both worlds that need to interface to move the field forward. Most sessions were designed to be "bench to bedside" with both a basic and clinical research focus. Technical aspects such as the incorporation of next generation sequencing (NGS), cellular pathophysiology and the development of new preclinical model systems were mixed with the impact of novel imaging techniques and novel therapeutic approaches. NF1, NF2 and SWN were each addressed as were various complications including musculoskeletal abnormalities of NF1, central nervous system tumors in NF, and peripheral nerve sheath tumors. There was also an exploration of psychological, social and biological factors to build new models of NF management. In addition, updates from various NF consortia and two sessions focused on issues in clinical management were included. While the keynote speakers were once again primarily from investigators outside the field, a new initiative, "Perspectives Talks," was launched at the 2016 conference. The latter talks were from experts within the NF field, on topics related to and part of particular sessions, and included not just an update on the speaker's research but also their thoughts on new directions in the field.

KEYNOTE PRESENTATIONS

Dr. Stephen Friend, the president of Sage Bionetworks, kicked off the conference with a keynote on "big data." Dr. Friend stressed the necessity of building networks among the NF community. As an example, he highlighted "the Resilience Project," a retrospective study on 100 childhood diseases, as well as early examples in NF (CTF's Synodos projects) in which clinicians, bench scientists, and computational analysts are harnessing clinical, functional,

and “omics” data together to identify not just drivers of disease but modulators of disease outcome. He finished the keynote by noting the importance of empowering and partnering with patients in research, such as the development of phone apps to measure patient outcomes in clinical trials.

In another keynote address, Dr. Crystal Mackall (Stanford, CA) reviewed emerging immunotherapy strategies and how they might be harnessed for NF-related tumors. She cautioned that while checkpoint inhibitor therapy has induced durable responses in a wide range of cancers, at present, it is believed that tumors with low mutational burden are less likely to respond. Thus, while this approach may bear fruition in MPNST of high grade glioma, it is less likely to work with common NF-associated low grade tumors, such as low grade glioma, plexiform neurofibroma, or vestibular schwannoma. In addition, the toxicity of checkpoint inhibitors and chimeric antigen receptor T-cell strategies must be weighed when targeting low grade tumors. Perhaps if appropriate antigens were identified, vaccine approaches might be effective in preventing transformation of nerve sheath tumors from low to high grade.

Dr. David Solit’s (Memorial Sloan Kettering Cancer Center, NY) keynote address centered on the changing application of next generation sequencing (NGS) in the clinical oncology setting. Understanding which part of the genome is clinically actionable is helping to transform oncology trials. Traditionally, trials have been based on obtaining statistically significant benefits of genetically unselected populations, and now trials can identify unrecognized biomarkers of response to agents in a limited number of patients. Dr. Solit explained the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) approach to enable precision oncology in patients with solid tumors, and the basket study schema, in which treatments are not focused on histologically similar types of cancer but rather on heterogeneous tumors that have the same altered/mutated pathway(s).

Human pluripotent stem cells have great potential in many aspects of medicine, from tissue engineering to disease modeling. However, not many laboratories have expertise in using induced pluripotent stem cells (iPSCs) to generate neural crest stem cells (NCSC), from which many of the cell types affected in NF derive. One such laboratory is led by Dr. Stephen Dalton (University of Georgia, GA), who gave a keynote presentation on how to use iPSCs to understand NC development and disease. After reviewing the signaling pathways and epigenomics characterizing the pathway from pluripotent cells to NCs during embryo development, Dr. Dalton provided insight into different NC-associated diseases, such as Treacher-Collins Syndrome and DiGeorge Syndrome. He stressed the importance of studying the biology behind the formation of cells of the NC lineage to uncover key aspects of the development of tumors with a neural crest origin, like many of the ones related to NF.

Activation of RAS signaling is a hallmark of NF1 associated tumors. To date, attempts to target RAS have been ineffective. Dr. Frank McCormick (University of California San Francisco, CA) in a keynote speech suggested potential new approaches to target RAS signaling. He described the role of SPRED1 in recruiting neurofibromin to RAS.GTP, where the complex inactivates RAS by converting it to RAS.GDP. In the absence of neurofibromin,

RAS.GTP accumulates and drives downstream MAPK pathway signaling. Thus, a more complete understanding of this protein complex may help identify novel therapeutic targets. He also noted that RAS.GTP dimers activate the MAPK pathway, suggesting that disruption of dimerization may be an approach to prevent RAS signaling. In addition, he identified novel KRAS effector pathways. KRAS binds to calmodulin and inhibits calmodulin-dependent kinase leading to a stem cell-like phenotype, which is in part promoted by the secretion of LIF (leukemia inhibitory factor), which is expressed in high levels in KRAS mutant cells. Thus, targeting LIF may reduce stem-ness and be a novel way to target RAS.

NEXT GENERATION DIAGNOSTICS, GENEDISCOVERY AND GENOTYPE-PHENOTYPE CORRELATIONS FOR THE NFS

The first session of the 2016 NF Conference, chaired by Drs. Conxi Lázaro and Eric Pasmant, focused on recently discovered genes, the use of next generation sequencing (NGS) for the genetic diagnostics of NF, and the effect of mutations and modifier genes on phenotypes. Ludwine Messiaen (University of Alabama at Birmingham, AL) opened the session with a *Perspectives* talk reviewing the different ways molecular genetics have been applied to NF testing, including cDNA-analysis methods, multiplex ligation-dependent probe amplification (MLPA), and the recent application of NGS-based strategies. She provided detailed information on the *NF1* mutational spectrum: mutations affecting *NF1* splicing (and not detected by DNA-based methods), the different types of *NF1* microdeletions, over 500 different missense mutations (identified in ~20% of NF1 patients). She underscored the challenges of evaluating for mosaicism, often requiring biopsy-based testing of neural crest-derived cells and deep NGS. She reviewed the 3 clinically relevant NF1 genotype-phenotype correlations identified thus far (*NF1* microdeletions, p.Met991del and missense mutations affecting the p.Arg1809) and their relevance for genetic counseling and management. She highlighted the challenges of NF genetic diagnostics, such as genetic heterogeneity and overlapping clinical manifestations with other syndromes. She exemplified the latter by presenting three families with hypertrophic neuropathy, pigmentary skin lesions, enlarged nerves and massive burden of paraspinal tumors, originally diagnosed clinically as NF1; but all patients carried a mutation in the *PTPN11* protein tyrosine phosphatase catalytic domain, underscoring the clinical overlap between NF1 and other RASopathies.

Dr. Béatrice Parfait (Paris Descartes University, France) updated how her laboratory handles genetic heterogeneity and overlapping clinical manifestations by taking advantage of NGS capabilities and studying different gene combinations simultaneously, like *NF1* and *SPRED1*, or *NF2-SMARCB1-LZTR1-SMARCE1 and SUFU*. Dr. Parfait explained how NGS can be used to detect copy number alterations, identify mosaicism presentations of low frequency mutations by deep sequencing, and solving uncertain clinical presentations. In a complementary talk, Dr. Justin T. Jordan (Massachusetts General Hospital) presented novel methods for genotype-phenotype correlation in 32 patients with clinically diagnosed schwannomatosis. Targeted gene capture followed by NGS were performed to analyze the entire gene regions of *SMARCB1*, *LZTR1*, and *NF2* in the blood. Mutations were identified in *LZTR1* in 5 patients, *SMARCB1* in 9 patients, and no mutation was found in 18 patients.

SMARCB1 and *LZTR1* mutated schwannomatosis patients did not differ in the number of schwannomas developed, but in the latter group the pain level was higher.

Dr. Kristine Vogel (UT Health Science Center, San Antonio, TX) presented studies linking DNA damage repair genes and NF1 disease severity. Characterization of *NF1* loss-of-heterozygosity (LOH) and somatic mutation spectra in cutaneous neurofibromas raised the possibility that genetic differences in DNA damage repair capacity (influencing somatic mutation rates) might act as modifiers of disease severity. S100 positive Schwann cell cultures were established from cutaneous neurofibromas removed from four adults with NF1. Schwann cells were exposed to doxorubicin, and DNA damage sensitivity and repair efficiency was measured by p53 binding protein 1 immunostaining and comet assay, respectively. Significant differences in Schwann cell sensitivity to doxorubicin-induced DNA damage were found between all 4 subjects. Comet assay data also indicated different rates of DNA repair in Schwann cells from all 4 individuals. Dr. Vogel postulated that DNA damage and repair characteristics may act as modifiers of NF1 disease severity as potential contributors to neurofibroma burden in NF1 individuals.

The session ended with Dr. Akihiro Yoshimura (Keio University, Japan) presenting a detailed analysis of the interaction between the SPRED1 EVH1 domain and the N-terminal 16 amino acids and the C-terminal 20 amino acids surrounding the GAP-related domain (GRD) of neurofibromin. Dr. Yoshimura showed that these neurofibromin regions are dispensable for GAP activity and are not present in p120(GAP). His group evaluated several mutations in these N- and C-terminal regions of the GRD in NF1 patients and pathogenic missense mutations in the EVH1 domain of *SPRED1* in Legius syndrome individuals, showing a reduced binding affinity between the EVH1 domain and the GRD. EVH1 domain mutations with reduced binding to the GRD also disrupted the ERK suppression activity of SPRED1. These data point to SPRED1 inhibiting the Ras-ERK pathway by recruiting neurofibromin to Ras through the EVH1-GRD interaction, and also provide molecular basis for the pathogenic mutations of NF1 and Legius syndrome.

CLINICAL MYSTERY SESSION

Drs. Rosalie Ferner and Robert Listernick led a fun session on “Clinical Mysteries.” The aim of the education session was to promote understanding between clinicians and scientists in an informal atmosphere of a quiz. Portrayal of cases of NF in the Arts were discussed; and historical facts were presented that have a bearing on current practice, including von Recklinghausen’s methods of measuring neurofibromas and an early presentation of malignant peripheral nerve sheath tumor by Frederick Hale Thomson in 1840. Clinical manifestations of syndromes that overlap or could be confused with NF1 were presented and included Fanconi anemia, Jaffe-Campanaci syndrome, Russell-Silver syndrome, tuberous sclerosis, hypercorticolism in McCune-Albright syndrome and Legius syndrome. Unusual clinical problems were discussed ranging from superficial siderosis in NF2 to B-RAF positive gangliogloma in NF1 unresponsive to B-raf inhibitors. There was an active debate to discuss the advantages and pitfalls of using whole body MRI as a screening tool in young adults with NF1.

PLEXIFORM NEUROFIBROMA TO MALIGNANT PERIPHERAL NERVE SHEATH TUMOR

MPNST is a leading cause of mortality in patients with NF1, and outcomes have not improved over the last several decades. This session, chaired by Drs. AeRang Kim and Lu Le, focused on the substantial progress that has been made in understanding the pathogenesis of plexiform neurofibroma (PNF), atypical neurofibromas (ANF) and MPNST. In a *Perspectives* talk, Dr. Brigitte Widemann (National Institutes of Health, Bethesda, MD) presented an overview of the field and highlighted the use of longitudinal and whole body MRI with volumetric analysis to understand the natural history of PNF (Dombi et al., 2013; Gutmann, Blakeley, Korf, & Packer, 2013; Solomon, Warren, Dombi, Patronas, & Widemann, 2004). She also described that distinct nodular lesions within PNF may be precursor lesions to MPNST (Meany et al., 2013).

Identification of genomic alterations associated with PNF development and progression to MPNST may be useful in identifying treatment. David Largaespada (University of Minnesota, Minneapolis, MN) presented his work defining cancer driver and maintenance genes associated with PNF development and transformation to MPNST using Sleeping Beauty (SB) transposon-based forward primary genetic screens in mice and CRISPR/Cas9-based secondary genetic screens in human Schwann cells (Rahrmann et al., 2013). His laboratory has identified three genes, including *NF1*, *GOSR1*, and *PTCH1*, which when targeted, induced xenograft tumors. *GOSR1* encodes a SNARE complex member and is an entirely novel tumor suppressor gene candidate. PTCH1 loss activates sonic hedgehog (SHH) signaling and SHH pathway alterations may contribute to MPNST development or progression. Ping Chi (Memorial Sloan Kettering Cancer Center, NY, NY) described her work using comprehensive genomic approaches to identify loss of function somatic alterations of the Polycomb repressive complex 2 (PRC2) components (*EED* or *SUZ12*) in the majority of MPNST(Lee et al., 2014). In contrast, PRC2 was intact in all benign neurofibromas, indicating that PRC2 loss is likely involved in malignant transformation from benign neurofibroma to MPNST. MPNST with PRC2 loss showed complete loss of trimethylation at lysine 27 of histone H3 (H3K27me3) and aberrant transcriptional activation of multiple PRC2-repressed homeobox master regulators and their regulated developmental pathways. Introduction of the lost PRC2 component in a PRC2-deficient MPNST cell line restored H3K27me3 levels and decreased cell growth.

Alexander Pemov (National Institutes of Health, Bethesda, MD) presented his work on comparative genomic analysis of NF1-associated ANF and MPNST. ANF may represent an intermediate step in the malignant transformation from PNF to MPNST. He and others have identified deletion of the *CDKN2A/2B* locus as the most frequent genetic event distinctive from PNF in ANF (Beert et al., 2011; Nielsen et al., 1999). His group performed whole exome sequencing (WES) in 16 ANF and 4 MPNST matched with germline DNA obtained from 14 and 4 unrelated NF1 patients, respectively. It appears the PNF-to-ANF transition is predominantly driven by heterozygous deletion of the *CDKN2A/2B* locus, but no PRC2 mutations are identified in ANF. The somatic mutation burden in the ANF is relatively low and comparable to that in PNF, however the level of genomic instability is elevated in the

ANF. Two novel somatic mutations in PRC2 genes were found in two MPNST and also in a potential novel driver gene coding for a histone H4-specific acetyltransferase.

Christine Higham (NCI Pediatric Oncology Branch and Children's National Medical Center, Washington, DC) closed the session describing a collaborative retrospective analysis of 73 ANF in 63 patients. On MRI, these tumors were distinct nodular lesions and FDG avid on PET. More than half were associated with pain and were palpable or visible on exam. The majority were completely resected and did not recur with an average follow up time of 4 years. Approximately one third of patients had additional lesions suspicious for ANF, and 4 ANF transformed to MPNST. She concluded that growth of nodular lesions associated with pain and PET avidity should raise the concern for ANF, and suggested that complete resection of ANF may potentially prevent transformation to MPNST.

The growing knowledge presented in this session combined with the availability of relevant preclinical models and concerted clinical trial efforts can be used to accelerate the development of MPNST prevention and treatment strategies.

THE ROLE OF SURGERY IN THE NEUROFIBROMATOSES

In this session on surgical considerations in NF1-related tumors, meant to complement the preceding session, Drs. Allan Belzberg and David Viskochil assembled a panel of experts for a point-counterpoint discussion on the surgical management of peripheral nerve sheath tumors. Dr. Alexander Lazar (MD Anderson Cancer Center, Houston, TX), Dr. Lor Randall (Huntsman Cancer Institute, Salt Lake City, UT), and Dr. Carol Morris (Johns Hopkins University, Baltimore, MD) reviewed issues related to clinical care using two case scenarios. Case 1 was a young adult woman with a brachial plexus tumor, and discussion centered on PET/CT results, need for biopsy, management based on different hypothetical diagnoses of high grade MPNST versus atypical neurofibroma/low-grade MPNST, and surgical margins. The second case was a child with a family history of NF1-related MPNST and an extensive sciatic nerve plexiform neurofibroma with atypia. The question posed to the panel was whether prophylactically removal of atypical plexiform neurofibromas with resultant major neurological deficit is warranted.

In addition, Dr. Belzberg summarized the NIH/Johns Hopkins team approach to MPNST: surgical intervention with *en block* resection and negative surgical margins, similar to the approach used in sarcoma surgery. Pre- and post-surgical adjuvant therapy may include radiation and chemotherapy. For a low grade MPNST, an attempt at preserving vascular/neurological structures is made by accepting a "marginal margin." When the MPNST arises within a plexiform tumor, the plexiform tumor serves as the margin and radiation therapy is not used. Their team considers neurofibromas with atypia to be "pre-malignant" and thus recommends surgery in these cases using a nerve sparing technique.

The value of these discussions rested with the nuances of each case and the process by which surgeons elect to proceed with interventions. It highlighted the lack of consensus in clinical pathology assessment of atypical plexiform neurofibromas versus low-grade MPNSTs, the lack of long-term tumor control and functional outcome data for prophylactic

tumor resection, and the importance of a multidisciplinary approach to MPNST management.

MOLECULAR ASPECTS OF NF1 IN HEALTH AND DISEASE

Dr. Nancy Ratner chaired a session of selected platform presentations focused on clinical aspects and new therapeutic approaches for NF1-associated tumors, and molecular insights in the interaction between *NF1-SPRED1* gene products. Dr. Robert Avery (Children's Hospital of Philadelphia, PA) opened the session presenting recent data on the difficult task of discovering useful predictors of visual outcomes in children with NF1 and optic pathway glioma (OPG). He focused on how OPG size influences vision. Anterior visual pathway (AVP) tumor volume was measured in 38 individuals by high resolution MRI and retinal nerve fiber layer (RNFL) thickness was assessed by optical coherence tomography. An inverse correlation was identified between AVP tumor volume and RNFL thickness. In addition, all subjects with an optic chiasm volume greater than 1.3 mL had axonal damage, a biomarker of vision loss. Results presented by Theresia Dunzendorfer-Matt (Innsbruck Medical University, Austria) complemented and were consistent with those presented by Dr. Yoshimura in the session on next generation diagnostics on mapping the interaction domains of neurofibromin and Spred 1. She detailed the domains and key residues of both proteins in this interaction, and analyzed the interaction in a functional context. Collectively, the data provided by Drs. Dunzendorfer-Matt, Yoshimura, and McCormick provide a comprehensive view of how neurofibromin and Spred 1 interact and participate in RAS pathway down-regulation, and provide a framework for the interpretation of missense mutations in both genes. Clare F. Malone (Brigham and Women's Hospital, Boston, MA) and Verena Staedtke (Johns Hopkins University, Baltimore, MD) both presented data on novel MPNST preclinical therapeutics. Dr. Malone described the successful combined use of the histone deacetylase inhibitor vorinostat and the mTOR inhibitor rapamycin for MPNST regression, using both MPNST cell lines and the C56/BL6 NPcis mouse MPNST model. The mechanism of action of the combination is via activation of the unfolded protein response, cellular response to ER stress and induction of reactive oxygen species (ROS), and she identified some activators of apoptosis signaling as mediators of this combined effect, like the TXIP/thioredoxin pathway (Malone et al., 2017). Dr. Staedtke discussed the use of bacterial therapy for MPNST. This completely new therapeutic perspective consisted of intratumoral injections of *Clostridium novyi-NT* spores into MPNST tumors generated by injecting the NF90.8 human MPNST cell line subcutaneously in athymic mice. Eight dogs with spontaneous MPNSTs were also treated. The promising preclinical results provided a rational for an ongoing Phase 1 clinical trial in humans.

MUSCULOSKELETAL ABNORMALITIES IN NF1

A session on musculoskeletal abnormalities was chaired by Drs. Elizabeth Schorry and Florent Elefteriou. Studies by different groups have recently shown that young children with NF1 have decreased muscle strength compared to controls, including decreased handgrip strength (Stevenson et al., 2012). These motor findings in the past have been attributed to CNS involvement; however, it is becoming clear that direct muscle involvement is also a feature of NF1. Matthew Summers (University of Sydney) reported the presence of

myopathy associated with lipid storage in a mouse model in which *Nf1* is ablated in muscles. This observation was also made in muscle biopsies from 4 individuals with NF1. Mass spectrometry lipid analysis of the muscle tissues from the mouse model revealed large increases in esterified cholesterols and neutral lipids associated with long chain fatty acids. These observations thus suggest a role for Ras/MAPK signaling in muscle lipid metabolism and an underlying mitochondrial/lipid metabolism defect in NF1muscles, similar to other lipid-storage myopathies. The observation that these lipid abnormalities were MEK-dependent suggests that the muscle weakness could be reversible.

A subset of patients with NF1 can present with unilateral anterolateral bowing of the tibia with cortical thickening and medullary canal narrowing radiographically, which is often followed by fracture and pseudoarthrosis. Past studies have documented somatic NF1 “second hits” in pseudoarthrosis tissue samples, providing some evidence of the underlying etiology (Paria et al., 2014). However, little is known about the architecture and bone quality of the bowed tibia prior to fracture. Dr. David Stevenson (Stanford University, CA) presented micro-CT images of an NF1 bowed tibia prior to fracture, which showed decreased bone density and increased porosity. He also presented preliminary results revealing reduced speed of sound utilizing quantitative ultrasound (QUS) in non-fractured bowed tibia compared to the contralateral side in 23 NF1 patients. As preclinical studies suggest the possibility to prevent changes in bone quality via enzyme therapy against pyrophosphate accumulation in children with NF1 (de la Croix Ndong et al., 2014), QUS may be an appropriate outcome measure to predict bone quality and identify patients to recruit in future clinical trials.

Although the occurrence of somatic double hit mutations in the *NF1* gene has been demonstrated in cells from the diseased bony tissue of patients with NF1 pseudoarthrosis, the identity of the cell of origin responsible for tibia bowing, fracture and pseudoarthrosis is still unknown. Dr. Florent Elefteriou (Baylor College of Medicine, TX) summarized what was learned from mouse models of *Nf1* ablation in the bone mesenchymal lineage. These studies revealed that osteochondroprogenitors characterized by *Nf1* loss of function have a proliferative advantage over wild type cells, secrete increased amount of pyrophosphate (a strong inhibitor of bone mineralization) leading to the accumulation of non-mineralized tissue and poor bone quality, and fail to differentiate toward the osteoblast lineage. Based on the results of these studies, Dr. Elefteriou stressed the need for new methods to predict bone changes in NF1 and to identify children at risk of progressing toward bowing and fracture. He hypothesized the likely necessity of combination treatments to manage NF1 pseudoarthrosis. He also provided evidence suggesting that NF1 dystrophic scoliosis, like NF1 pseudoarthrosis, might be associated with a defect of mineralization originating from the bone or paraspinal tumors.

Carlijn Brekelmans (KU Leuven, Belgium) reported the detection of bi-allelic (germline and somatic) *NF1* inactivation in cultured cells of the primary pseudoarthrosis tissue in 8/8 NF1 patients who had an Ilizarov surgical procedure. In two patients sampled more extensively, a range of *NF1*+/− and *NF1*−/− cells was found in the pseudoarthrosis site. Unexpectedly, the periosteum outside the pseudoarthrosis region was reported to contain ±20% (proximal) to almost 100% (distal) NF1−/− cells. One patient had 30% *NF1* −/− periosteal cells in the

proximal 3rd of the tibia, far above the pseudoarthrosis tissue. These results confirmed the presence of cells with biallelic *NF1* inactivation in the pseudoarthrosis region and for the first time showed these cells in periosteum from both proximal and distal regions of the same tibia.

ASSESSING THERAPEUTICS IN NF2 AND SCHWANNOMATOSIS THROUGH TRANSLATIONAL RESEARCH

Drs. Marco Giovannini and Michel Kalamarides chaired a session focused on preclinical and clinical assessment of novel therapeutics approaches for the two major tumor types associated with NF2: schwannoma and meningioma. To identify potential drug targets in NF2-associated schwannomas, Dr. Joe Kissil (Scripps Research Institute, FL) assessed the consequences of inhibiting the tyrosine kinase receptor MET. Using activity-based protein profiling (ABPP), he identified FAK1 (PTK2) as the relevant target of crizotinib inhibition in *NF2*-null schwannoma cells. Inhibition of FAK1 was sufficient to suppress tumorigenesis in animal models of NF2, and crizotinib-resistant forms of FAK1 rescued the effects of treatment (Troutman et al., 2016). Dr. Alejandra Petrilli (University of Central Florida) tested the ability of ponatinib to inhibit proliferation/survival of merlin-null human Schwann cells (HSC). Ponatinib reduced the viability of merlin-null HSC in a dose dependent manner by inhibiting activity of Src kinase and the Platelet-Derived Growth Factor Receptors -alpha and -beta. Flow cytometry studies revealed that ponatinib caused a G1 cell cycle arrest of the merlin-null HSC. These studies identify two FDA approved drug as a potential treatment for NF2 and delineate the mechanism of action in *NF2*-null Schwann cells.

There were two presentations focusing on meningioma, the second most frequent tumor type in NF2. Earlier work by Dr. Vijaya Ramesh's (Harvard University, MA) laboratory established aberrant activation of mTORC1 signaling in NF2-deficient meningiomas (James et al., 2009; James et al., 2012), which led to clinical trials with the rapamycin analog everolimus for NF2-associated tumors (Goutagny, Giovannini, & Kalamarides, 2017; Goutagny et al., 2015). Further, they showed that the dual mTORC1/mTORC2 inhibitor AZD2014 was more effective than rapamycin in blocking proliferation of meningioma cells. They have now undertaken studies to define the adaptive kinome response in isogenic NF2-expressing and NF2-null human arachnoidal cells treated with either rapamycin or AZD2014, and kinases preferentially induced by AZD2014 or rapamycin were identified (Beauchamp et al., 2015). Consistent activation of EPH receptor family members was found in both arachnoidal and meningioma cells with NF2 loss. Based on these observations, *in vitro* combination treatment with AZD2014 and dasatinib, a potent inhibitor of many of the EPH family members, was tested and synergy between these two drugs found to effectively inhibit meningioma cell growth. Defining adaptive kinome response has the potential to reveal effective combination therapies and will have direct impact on NF2 research as well as patient care. Dr. Matthias Karajannis presented a retrospective review of patients with NF2 and progressive vestibular schwannomas treated on a Phase II clinical trial with lapatinib, a dual EGFR/ErbB2 inhibitor (Karajannis et al., 2012). The data suggested that lapatinib has modest growth-inhibitory effects on meningiomas in NF2 patients, but

prospective studies of lapatinib for NF2 patients with progressive meningiomas may be warranted.

CENTRAL NERVOUS SYSTEM TUMORS IN THE NEUROFIBROMATOSES

Brain and spinal cord tumors are a common and challenging manifestation of NF. Drs. Yuan Zhu and Matthias Karajannis organized a session touching on central nervous system tumors in both NF1 and NF2. Dr. David Gutmann (Washington University, St. Louis, MO) gave a *Perspectives* talk dissecting the risk factors that predispose to optic glioma development and vision loss in mice. Using a series of novel *Nf1* genetically-engineered strains, he presented data that the germline *NF1* gene mutation, sex, glioma cell of origin, and timing of somatic *Nf1* loss all constitute critical factors that underlie optic glioma pathogenesis. He also underscored the importance of microglia in the optic pathway glioma "ecosystem" to both tumor cell growth and retinal ganglion cell health. Dr. Yuan Zhu (Children's National Medical Center, Washington, DC) presented a study investigating the critical cellular and molecular events during the development of optic nerves upon loss of *Nf1* in a genetically engineered mouse model. These studies suggest distinct roles of *Nf1*-deficient glial cells in the developing astrocytic and oligodendroglial lineages in the development of optic pathway gliomas, providing a proof-of-principle study to target NF1-associated optic pathway glioma at their earliest stages. Dr. Peter de Blank (Rainbow Babies & Children's Hospital, Cleveland, OH) presented initial experience of using magnetic resonance fingerprinting (MRF), a quantitative non-invasive tool to measure tissue characteristics and distinguish tissue type and tumor grade, in children with NF1.

Dr. Michel Kalamarides (Hopital Pitie-Salpietre, Paris, France) presented a retrospective cohort study of spinal ependymomas in NF2 from a series of patients at two large NF2 centers: Manchester (United Kingdom) and Paris/Lille (France). The management of spinal cord ependymomas in the context of NF2 has traditionally been conservative with the specific aim of avoiding surgical intervention to minimize morbidity. In Manchester, patients were almost invariably treated conservatively, whereas in France, surgery was an actively employed treatment option. Full clinical data was available in 24 patients from Manchester and 46 patients from Paris/Lille. In the Manchester cohort, 26% of patients deteriorated clinically during the course of the study. This effectively represents the natural history of ependymoma in NF2. In the French series, 0% of conservatively and 31% of surgically managed patients deteriorated, respectively. He concluded that spinal ependymomas can produce morbidity, and surgery can prevent or improve morbidity in select patients. Surgery should be considered for growing and symptomatic ependymomas, especially in patients who are not good candidates for treatment with bevacizumab.

Sarah Burns from Long-Sheng Chang's group at Nationwide Children's Hospital (Columbus, OH) presented early-phase clinical studies showing that treatment with AR-42 resulted in responses in NF2-associated meningiomas, while suppressing the growth rate of vestibular schwannoma. In genomic studies, they showed that NF2-associated vestibular schwannoma contained relatively few mutations in genes frequently altered in human cancers. Among these genes, NUP98 was identified as a potential genetic modifier for NF2. Vestibular schwannoma and meningiomas from the same patients harbored identical genetic

changes despite differences in response to AR-42 treatment, implying different epigenetic changes and/or microenvironment effects in these tumors. Vestibular schwannoma, but not meningiomas, expressed high levels of nuclear cMYC. Silencing cMYC reduced vestibular schwannoma, but not meningioma, cell proliferation. These results suggest that cMYC may be a target for therapeutic intervention in vestibular schwannoma.

PRESENT AND FUTURE IMPACT OF NOVEL IMAGING IN THE MANAGEMENT OF NF

In this session organized by Drs. Peter de Blank and Gordon Harris, four novel imaging approaches with potential impact on the assessment and management of subjects with neurofibromatosis were presented. These novel approaches go beyond the standard clinical assessments of brain structure to view parameters of neural microstructure and function that may be disrupted in subjects with NF.

Dr. Vikas Gulani (University Hospitals Case Medical Center, Cleveland, OH) discussed a number of advances in ultrafast MR acquisition which have resulted in the ability to quantitatively measure important tissue properties, including T1, T2, diffusion and perfusion properties. Multi-dimensional quantitative imaging allows for the possibility of non-invasively characterizing and following neoplasms, reducing the need for invasive biopsy. Importantly for children, the rapid imaging capabilities being developed can often be performed with significantly less scan time, allowing for free-breathing acquisitions and potentially decreasing the need for sedation in children. Recent advances in machine learning may make the analysis of complex data sets that include multi-parametric imaging as well as clinical data and circulating biomarker evaluations feasible in the near future.

Dr. Donald Mabbott (Hospital for Sick Children, Toronto, Canada) discussed white matter microstructure using diffusion imaging (tensor and kurtosis) and magnetization transfer imaging, and how this may relate to cognition. Among 32 children with low grade glioma treated without radiation, compromise of supratentorial white matter mediated the effect of treatment on intelligence quotient (IQ), suggesting that brain tumor therapies affect cognition by damaging white matter. Greater white matter damage was seen in five children with NF1 (Liu et al., 2015). However, exercise may help moderate the neurotoxic effect of cranial radiation. In an ongoing study, children exposed to cranial radiation participate in a 12-week exercise regimen to determine the effect of exercise on white matter integrity and cognitive impairment. Initial results demonstrate the feasibility of this approach.

Dr. Maria Acosta (Children’s National Medical Center, Washington, DC) examined the effects of cognitive training using a computerized visuospatial working memory tool and phone-based coaching (Cogmed®) on resting-state functional connectivity using functional MRI. Among 16 participants who underwent 6–10 weeks of training, changes in fractional amplitude of low frequency fluctuations and regional homogeneity pre- and post-training correlated with changes in performance on tasks of executive function and visuo-spatial working memory.

Dr. Timothy Roberts (Children's Hospital of Philadelphia, PA) discussed strategies to use magnetoencephalography (MEG) in the context of multimodal imaging to establish biomarkers for autistic spectrum disorder (ASD), which occurs with increased frequency in children with NF1. MEG captures the electrical activity of neurons by measuring the magnetic fields they produce over time. Latency prolongation in the auditory evoked response can be seen in children with ASD with or without language and speech deficits, and may be a useful predictive biomarker of ASD (Roberts et al., 2010). Evidence from diffusion tensor imaging and magnetic resonance spectroscopy experiments provide further evidence for a biological basis for latency prolongation in auditory processing.

BEYOND THE MEDICAL MODEL: EXPLORING PSYCHOLOGICAL, SOCIAL, AND BIOLOGICAL FACTORS IN NF

Jennifer Janusz (Children's Hospital Colorado, Denver, CO) laid the framework for this session, chaired by Drs. Staci Martin and Nicole Ullrich, by introducing the environmental and family variables that exist when caring for individuals with NF and the potential interventions that are available. The impact of illness on family and the impact of family on illness were defined, and a pediatric psychology health model was presented (Kazak et al., 2007). This model posits a tiered approach starting with broad-based and universal approaches for psycho-education, then moving to targeted interventions for specific symptoms, and lastly to intensified psychosocial services for specific issues using behavioral health specialists such as cognitive behavioral therapy and family problem solving interventions. The benefits of this approach include decreased behavioral problems and improved executive functioning skills. Interventions show more robust response in families from lower socioeconomic status and retention of benefits one year after treatment. Barriers include limited number of interventions that have been validated with the NF population, limited access to services, limited funding for services and limited insurance coverage for services.

Ana-Maria Vranceanu (Massachusetts General Hospital, Boston, MA) discussed her work related to a mind-body treatment that she adapted for patients with NF1, NF2 and schwannomatosis using qualitative information collected via focus groups. This intervention includes eight weekly sessions delivered via live video using Skype in small groups. Individuals are randomized to receive either "active training," which includes resiliency training, adaptive thinking and positive psychology, versus "inactive training," which includes educational information on stress, nutrition, exercise and medical care. The initial trial in adults was feasible with a high level of satisfaction among participants regardless of conditions (active versus inactive). In addition, "active training" resulted in improved quality of life and reduced anxiety and pain beyond that of "inactive training." Improvements were statistically significant, clinically meaningful and durable. Dr. Vranceanu also discussed her current projects testing this mind body treatment adapted for adolescents and adults with NF2 who are deaf.

Taylor Smith (California Polytechnic State University, San Luis Obispo, CA; Rhode Island Hospital) presented a collaboration with Children's National Medical Center (Washington,

DC) on coping in parents of children with NF1. Prior studies have demonstrated higher levels of parental stress among parents of children with NF1 (Esposito et al., 2014), and that the severity of disease in the child is associated with increased parental psychiatric symptoms, increased family conflict and less social support (Reiter-Purtill et al., 2008). Data were collected as part of a larger project which developed an online parenting resource/intervention. They found that learning difficulties and NF1 severity are associated with greater parenting stress and distress and that this relationship is partially mediated by parental coping strategy use. Limitations included the lack of control group and small numbers of familial NF1 cases. Results support the inclusion of mental health screening measures for parents, and parental cognitions and coping as targets for parental intervention.

Nicole Ullrich (Boston Children's Hospital, MA) presented a pilot study that uses social media (with restricted access as a community of shared experience) to improve quality of life and medical outcomes in adolescents with NF1 and their parents. Participants created a video intervention/prevention assessment about living with or parenting a child with NF1 and then quality of life and disease severity were assessed at key times. The goals were to learn about the experiences of adolescents and parents caring for adolescents with NF1 and to provide peer support for adolescents and parents of adolescents with NF1 through the social media platform. At baseline, adolescents were using social media more than their parents, and most adolescents and parents had not met/interacted with others with NF1 and felt isolated in their experience. They experienced improved awareness and quality of life after recording the visual narratives. Participants felt that the experience provided a forum to express their feelings and fears and to "put a face" on NF1. The majority considered the social media site a valuable 24/7 resource of peer to peer support and reliable medical information as well as a source for strategies for living with NF1.

CELLULAR PATHOPHYSIOLOGY IN NEUROFIBROMATOSIS TYPE 2 AND SCHWANNOMATOSIS

This session, chaired by Drs. Wei Li and Helen Morrison, focused on understanding the pathogenesis of schwannomatosis and NF2 at molecular and cellular levels, empathizing Schwann cell-related models. Dr. Lawrence Sherman (Oregon Health and Science University) presented his laboratory's work on the pain associated with schwannomatosis. They studied genetically engineered mice, in which the *SMARCB1* gene has been conditionally disrupted in Schwann cells. They found that such gene disruption does not lead to changes in peripheral nerve morphology, Schwann cell proliferation or alterations in cell cycle-related gene expression in peripheral nerves. However, the mice demonstrate behavioral phenotypes consistent with chronic pain. Interestingly, expression of several pain signaling related genes, such as *TRPV1*, are elevated in dorsal root ganglion (DRG) neurons in these mice. Sherman proposed that loss of SMARCB1 in Schwann cells leads to the secretion of factors that induce the expression of pain mediators in sensory neurons, therefore suggesting a mechanism for schwannomatosis pain.

Regeneration of the peripheral nerve system (PNS) following injury depends on the plasticity of Schwann cells. Dr. David Parkinson (Plymouth University, UK) presented work

on the roles of merlin and the Hippo pathway effector, Yes Associated Protein (YAP), in the control of PNS repair. They found loss of merlin in Schwann cells (SCs) caused a catastrophic failure of axonal regeneration and remyelination in the PNS. He showed that distal nerves exhibited increased SC proliferation as well as a persistent immune response after injury. Moreover, he showed activation of YAP expression in merlin null nerves after injury, and that loss of YAP in merlin null SCs restores axonal regrowth, functional repair and resolution of the immune response to injury. He concluded that merlin controls the proliferation and repair capacity of SCs following injury by regulating Hippo/YAP activity. The activity of this pathway is also critical to mediate contact inhibition, essential in tissue homeostasis. Taken together these studies give new insights into molecular pathways contributing to the regenerative potential of SCs and highlights the role of merlin deficient SCs generating an environment that is reminiscent of schwannoma tumors. Dr. Susana Moleirinho (Scripps Research Institute, FL) presented her work on the roles of Angiomotin in the regulation of this pathway. They found that merlin, Angiomotin and YAP form a complex, and Angiomotin is required for this association. In addition, she showed that Angiomotin phosphorylation status is not necessary for the complex formation but can impact the subcellular localization of the complex. Work to determine whether Angiomotin phosphorylation modulates cell proliferation and YAP transcriptional activities is ongoing.

Previous work has indicated that merlin plays its tumor suppressive function by antagonizing multiple oncogenic signaling. Dr. Robert Hennigan (Cincinnati Children's Hospital Medical Center, OH) presented work on the identification of merlin interacting protein partners by the proximity biotinylation technique. Through comparing the interactomes of wild type Merlin, a PIP2 binding mutant, merlin-6N, and two conformational mutants, merlin-FH and merlin AR, they identified 53 merlin-associated proteins. The proteins consisted predominately of cell junction associated proteins with actin binding activity, like afadin, an actin binding protein that is required for the formation of tight and adherent junctions. Dr. Hennigan showed that afadin directly interacts with merlin, and afadin loss mimics the effects of merlin loss on apoptosis, suggesting a role of afadin in mediating cell junction related signal transduction in Schwann cells.

NEW PRECLINICAL MODELS IN NF

Dr. Thomas De Raedt chaired a session on new preclinical models in NF. Dr. De Raedt (Harvard University, Boston, MA) kicked off the session with a report on the use of genetically engineered mouse models to develop novel therapies for MPNST. He showed that the combination of MEK (PD-0325901) and BRD4 inhibitors (JQ1) potently kills MPNST cells by synergistically inhibiting the RAS transcriptional output. Interestingly, the inhibition of MEK and BRD4 leads to a rapid influx of immune cells, suggesting that non-cell autonomous mechanism could contribute to the observed tumor shrinkage *in vivo.* This observation inspired De Raedt *et al.* to successfully combine MEK and BRD4 inhibitors with anti-PD1 checkpoint blockade. Additionally, they explored strategies to prevent MPNST formation, which is important especially for those patients with a high burden of plexiform neurofibromas or NF1 microdeletion, who have a high risk for MPNST development. De Raedt *et al.* reported that BRD4 inhibitors prevent the onset of MPNSTs in a mouse model for MPNST development in NF1 microdeletion patients (*NF1/P53/SUZ12*).

Germline mutations of the *SMARCB1* gene are involved in two different tumor conditions: rhabdoid tumor predisposition syndrome and familial schwannomatosis. To study the mechanisms by which *SMARCB1* germline mutations predispose to rhabdoid tumors or schwannomas, Dr. Marco Giovannini (University of California Los Angeles, CA) and coworkers generated different tissue-specific conditional knockout mice with *Smarcb1* and/or *Nf2* deletion. They found that loss of *Smarcb1* in neural crest cells is sufficient to initiate tumorigenesis in cranial nerves and meninges with typical histological and molecular features of human rhabdoid tumors. By inducing *Smarcb1* loss at later developmental stage, in addition to biallelic *Nf2* gene inactivation, they generated the first mouse model to develop schwannomas with the same underlying gene mutations found in schwannomatosis patients (Vitte, Gao, Coppola, Judkins, & Giovannini, 2017). These mouse models represent invaluable pre-clinical drug-screening tools for *Smarcb1*- and *Smarcb1/Nf2*-deficient tumors.

A large number of children with NF1 have social and communication deficits similar to autism spectrum disorders (ASD). Dr. Anantha Shekhar (Indiana University School of Medicine, Indianapolis, IN) reported that NF1 heterozygous mice display deficits in social learning and have deficits in long-term potentiation in the basolateral amygdala, which is important for social learning. These social defects are rescued when PAK1 inhibitors are used or when these Nf1+/− mice are crossed to PAK1−/− mice. Surprisingly, mice that lack the *NF1* exon 23a have communication deficits but no social learning deficits. This suggests that the communication deficits in these mice are not caused by defects in the RAS signaling pathway and make this 23a mouse model an invaluable tool to discern the difference between communication and social defects in NF1.

The *NF1* gene is fully inactivated in the majority of sporadic (i.e. non-NF1 associated) MPNSTs. Patients with sporadic MPNST have a better outcome than those with NF1 associated MPNST. This suggests that the heterozygous state of *NF1* in the MPNST stroma plays an important role in outcome. To investigate the impact of stromal genetics on MPNST biology, Dr. Rebecca Dodd *et al.* (Duke University School of Medicine, Durham, NC) developed genetically engineered mouse models (adenoviral-Cre injection in $NF1^{Flox/flox}$; Ink4a/$Arf^{flox/flox}$ and $NF1^{Flox/-}$; Ink4a/$Arf^{flox/flox}$ mice) that model the different tumor microenvironments of the MPNST patient populations (NF1 wild type and NF1 +/− stroma respectively). They found that the presence of NF1 +/− stroma accelerates tumor onset and is accompanied by an increase in immune cells comprised of myeloid-derived suppressor cells (MDSC) and mast cells. However, the tumor microenvironment does not alter the response of MPNSTs to chemotherapy. Taken together, this helps to clarify the role of the NF1 haploinsufficient tumor microenvironment in MPNST development of NF1 patients.

CONSORTIA UPDATES

A recurring but important session of the annual meeting is the update on various NF consortia, which was chaired this year by Drs. Brian Weiss and Brigitte Widemann. A significant focus this year was on the multi-disciplinary and collaborative Synodos groups sponsored by Children's Tumor Foundation. Dr. Jaishri Blakeley (Johns Hopkins University, Baltimore, MD) presented updates on the Synodos for NF2 team (http://www.ctf.org/

research/synodos#nf2), which includes twelve laboratories/medical centers focused on creating new tumor models and novel therapies for NF2 associated tumors. Three new Synodos groups for NF1 (http://www.ctf.org/research/synodos#nf1) were presented, including one group focused on identifying the molecular risk factors and treatments targeted to the cellular and molecular properties unique to NF1-LGG (presented by Dr. David Gutmann, Washington University, St. Louis, MO), and two preclinical acceleration teams who are creating porcine models of NF1 (one presented by Dr. Christopher Moertel, University of Minnesota, Minneapolis, MN; the other by Dr. Jill Weimer, Children's Health Research Center at Sanford Research, Sioux Falls, SD).

Dr. Ophelia Maertens (Harvard Medical School, Boston, MA) presented the previous year's accomplishments of the NF Therapeutic Consortium (http://www.ctf.org/research/nf-therapeutic-consortium), which comprises four laboratories performing pre-clinical trials on murine models of plexiform neurofibroma, MPNST and juvenile myelomonocytic leukemia. Their work has informed the development of multiple clinical trials, including several of those being conducted by the Department of Defense NF Clinical Trials Consortium (http://cdmrp.army.mil/nfrp/consortium/nfrpctc). The progress of this consortium was presented by Dr. Michael Fisher (The Children's Hospital of Philadelphia, PA) highlighting the ongoing clinical trials for plexiform neurofibroma (NCT02101736, NCT02096471), low grade glioma (NCT02285439), and vestibular schwannoma (NCT01767792); the recently completed trials for MPNST (NCT01661283 and NCT02008877 performed in collaboration with the Sarcoma Alliance for Research through Collaboration) and low grade glioma (NCT01158651); and the upcoming tibial pseudoarthrosis trial (NCT02718131).

Dr. Scott Plotkin (Massachusetts General Hospital, Boston, MA) reported on the progress of the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) international collaboration (https://ccrod.cancer.gov/confluence/display/REINS/Home), an effort to develop standardized outcome measures for clinical trials in patients with NF1, NF2, and schwannomatosis. The session concluded with a presentation by Dr. Carolyn Compton (Arizona State University, Tempe, AZ) discussing the importance of biomarker development to the future of medicine including NF. She underscored some of the challenges in this development, particularly the need for high quality biospecimens, so that the analyses reflect disease biology and not artifacts of specimen handling. She advocated for the standardization of biospecimen collection, processing, transport and storage, in order to empower the specimens for biomarker development.

CONCLUSIONS

The 2016 CTF NF Conference blended basic, translational and clinical research to highlight important advances in the field. Collaborative multidisciplinary endeavors have grown exponentially, increasing the sense of community and group effort in NF. However, many important advances are still pending further research and development. It is time to bring precision medicine to the treatment of NF tumors. Better resources must be built for the community, such as robust antibodies, integrated databases with matching prospective clinical and molecular data, functional assays for pathogenicity interpretation, and biomarkers with predictive value. An improved understanding of the structure of the protein

products of NF genes may expand the ability to identify novel drugs and treatments with clinical utility. A balance and integration between basic and clinical science must be maintained, which will lead to a clear reward and advance for the NF field.

Acknowledgments

The Children's Tumor Foundation (CTF) sponsored the 2016 NF conference. The authors thank Patrice Pancza (Conference Director, Children's Tumor Foundation) for her invaluable effort in organizing and managing the conference and her help with manuscript development and submission. Additional acknowledgements to all of the CTF staff that contributed to the conference organization. The 2016 NF Conference was also supported in part by a grant from the National Institute of Health/National Institute of Neurological Disorders and Stroke (2R13NS084619-04).

References

Beauchamp RL, James MF, DeSouza PA, Wagh V, Zhao WN, Jordan JT, … Ramesh V. A high-throughput kinome screen reveals serum/glucocorticoid-regulated kinase 1 as a therapeutic target for NF2-deficient meningiomas. Oncotarget. 2015; 6(19):16981–16997. DOI: 10.18632/oncotarget.4858 [PubMed: 26219339]

Beert E, Brems H, Daniels B, De Wever I, Van Calenbergh F, Schoenaers J, … Legius E. Atypical neurofibromas in neurofibromatosis type 1 are premalignant tumors. Genes Chromosomes Cancer. 2011; 50(12):1021–1032. DOI: 10.1002/gcc.20921 [PubMed: 21987445]

de la Croix Ndong J, Makowski AJ, Uppuganti S, Vignaux G, Ono K, Perrien DS, … Elefteriou F. Asfotase-alpha improves bone growth, mineralization and strength in mouse models of neurofibromatosis type-1. Nat Med. 2014; 20(8):904–910. DOI: 10.1038/nm.3583 [PubMed: 24997609]

Dombi E, Ardern-Holmes SL, Babovic-Vuksanovic D, Barker FG, Connor S, Evans DG, … Collaboration REI. Recommendations for imaging tumor response in neurofibromatosis clinical trials. Neurology. 2013; 81(21 Suppl 1):S33–40. DOI: 10.1212/01.wnl.0000435744.57038.af [PubMed: 24249804]

Esposito M, Marotta R, Roccella M, Gallai B, Parisi L, Lavano SM, Carotenuto M. Pediatric neurofibromatosis 1 and parental stress: a multicenter study. Neuropsychiatr Dis Treat. 2014; 10:141–146. DOI: 10.2147/NDT.S55518 [PubMed: 24489471]

Goutagny S, Giovannini M, Kalamarides M. A 4-year phase II study of everolimus in NF2 patients with growing vestibular schwannomas. J Neurooncol. 2017; 133(2):443–445. DOI: 10.1007/s11060-017-2447-3 [PubMed: 28434114]

Goutagny S, Raymond E, Esposito-Farese M, Trunet S, Mawrin C, Bernardeschi D, … Kalamarides M. Phase II study of mTORC1 inhibition by everolimus in neurofibromatosis type 2 patients with growing vestibular schwannomas. J Neurooncol. 2015; 122(2):313–320. DOI: 10.1007/s11060-014-1710-0 [PubMed: 25567352]

Gutmann DH, Blakeley JO, Korf BR, Packer RJ. Optimizing biologically targeted clinical trials for neurofibromatosis. Expert Opin Investig Drugs. 2013; 22(4):443–462. DOI: 10.1517/13543784.2013.772979

James MF, Han S, Polizzano C, Plotkin SR, Manning BD, Stemmer-Rachamimov AO, … Ramesh V. NF2/merlin is a novel negative regulator of mTOR complex 1, and activation of mTORC1 is associated with meningioma and schwannoma growth. Mol Cell Biol. 2009; 29(15):4250–4261. DOI: 10.1128/MCB.01581-08 [PubMed: 19451225]

James MF, Stivison E, Beauchamp R, Han S, Li H, Wallace MR, … Ramesh V. Regulation of mTOR complex 2 signaling in neurofibromatosis 2-deficient target cell types. Mol Cancer Res. 2012; 10(5):649–659. DOI: 10.1158/1541-7786.MCR-11-0425-T [PubMed: 22426462]

Karajannis MA, Legault G, Hagiwara M, Ballas MS, Brown K, Nusbaum AO, … Allen JC. Phase II trial of lapatinib in adult and pediatric patients with neurofibromatosis type 2 and progressive vestibular schwannomas. Neuro Oncol. 2012; 14(9):1163–1170. DOI: 10.1093/neuonc/nos146 [PubMed: 22844108]

Kazak AE, Rourke MT, Alderfer MA, Pai A, Reilly AF, Meadows AT. Evidence-based assessment, intervention and psychosocial care in pediatric oncology: a blueprint for comprehensive services across treatment. J Pediatr Psychol. 2007; 32(9):1099–1110. DOI: 10.1093/jpepsy/jsm031 [PubMed: 17626069]

Lee W, Teckie S, Wiesner T, Ran L, Prieto Granada CN, Lin M, … Chi P. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat Genet. 2014; 46(11):1227–1232. DOI: 10.1038/ng.3095 [PubMed: 25240281]

Liu F, Scantlebury N, Tabori U, Bouffet E, Laughlin S, Strother D, … Mabbott DJ. White matter compromise predicts poor intellectual outcome in survivors of pediatric low-grade glioma. Neuro Oncol. 2015; 17(4):604–613. DOI: 10.1093/neuonc/nou306 [PubMed: 25395463]

Malone CF, Emerson C, Ingraham R, Barbosa W, Guerra S, Yoon H, … Cichowski K. mTOR and HDAC Inhibitors Converge on the TXNIP/Thioredoxin Pathway to Cause Catastrophic Oxidative Stress and Regression of RAS-Driven Tumors. Cancer Discov. 2017; 7(12):1450–1463. DOI: 10.1158/2159-8290.CD-17-0177 [PubMed: 28963352]

Meany H, Dombi E, Reynolds J, Whatley M, Kurwa A, Tsokos M, … Widemann B. 18-fluorodeoxyglucose-positron emission tomography (FDG-PET) evaluation of nodular lesions in patients with Neurofibromatosis type 1 and plexiform neurofibromas (PN) or malignant peripheral nerve sheath tumors (MPNST). Pediatr Blood Cancer. 2013; 60(1):59–64. DOI: 10.1002/pbc.24212 [PubMed: 22645095]

Nielsen GP, Stemmer-Rachamimov AO, Ino Y, Moller MB, Rosenberg AE, Louis DN. Malignant transformation of neurofibromas in neurofibromatosis 1 is associated with CDKN2A/p16 inactivation. Am J Pathol. 1999; 155(6):1879–1884. DOI: 10.1016/S0002-9440(10)65507-1 [PubMed: 10595918]

Paria N, Cho TJ, Choi IH, Kamiya N, Kayembe K, Mao R, … Rios JJ. Neurofibromin deficiency-associated transcriptional dysregulation suggests a novel therapy for tibial pseudoarthrosis in NF1. J Bone Miner Res. 2014; 29(12):2636–2642. DOI: 10.1002/jbmr.2298 [PubMed: 24932921]

Rahrmann EP, Watson AL, Keng VW, Choi K, Moriarity BS, Beckmann DA, … Largaespada DA. Forward genetic screen for malignant peripheral nerve sheath tumor formation identifies new genes and pathways driving tumorigenesis. Nat Genet. 2013; 45(7):756–766. DOI: 10.1038/ng.2641 [PubMed: 23685747]

Reiter-Purtill J, Schorry EK, Lovell AM, Vannatta K, Gerhardt CA, Noll RB. Parental distress, family functioning, and social support in families with and without a child with neurofibromatosis 1. J Pediatr Psychol. 2008; 33(4):422–434. DOI: 10.1093/jpepsy/jsm077 [PubMed: 17905803]

Roberts TP, Khan SY, Rey M, Monroe JF, Cannon K, Blaskey L, … Edgar JC. MEG detection of delayed auditory evoked responses in autism spectrum disorders: towards an imaging biomarker for autism. Autism Res. 2010; 3(1):8–18. DOI: 10.1002/aur.111 [PubMed: 20063319]

Solomon J, Warren K, Dombi E, Patronas N, Widemann B. Automated detection and volume measurement of plexiform neurofibromas in neurofibromatosis 1 using magnetic resonance imaging. Comput Med Imaging Graph. 2004; 28(5):257–265. DOI: 10.1016/j.compmedimag.2004.03.002 [PubMed: 15249071]

Stevenson DA, Allen S, Tidyman WE, Carey JC, Viskochil DH, Stevens A, … Rauen KA. Peripheral muscle weakness in RASopathies. Muscle Nerve. 2012; 46(3):394–399. DOI: 10.1002/mus.23324 [PubMed: 22907230]

Troutman S, Moleirinho S, Kota S, Nettles K, Fallahi M, Johnson GL, Kissil JL. Crizotinib inhibits NF2-associated schwannoma through inhibition of focal adhesion kinase 1. Oncotarget. 2016; 7(34):54515–54525. DOI: 10.18632/oncotarget.10248 [PubMed: 27363027]

Vitte J, Gao F, Coppola G, Judkins AR, Giovannini M. Timing of Smarcb1 and Nf2 inactivation determines schwannoma versus rhabdoid tumor development. Nat Commun. 2017; 8(1):300.doi: 10.1038/s41467-017-00346-5 [PubMed: 28824165]