

UCLA

UCLA Previously Published Works

Title

The path forward: 2015 International Children's Tumor Foundation conference on neurofibromatosis type 1, type 2, and schwannomatosis

Permalink

<https://escholarship.org/uc/item/32t721k9>

Journal

AMERICAN JOURNAL OF MEDICAL GENETICS PART A, 173(6)

ISSN

1552-4825

Authors

Blakeley, JO
Bakker, A
Barker, A
[et al.](#)

Publication Date

2017-06-01


DOI

10.1002/ajmg.a.38239

Peer reviewed

CONFERENCE REPORT

The path forward: 2015 International Children's Tumor Foundation conference on neurofibromatosis type 1, type 2, and schwannomatosis

Jaishri O. Blakeley¹  | Annette Bakker² | Anne Barker³ | Wade Clapp⁴ | Rosalie Ferner⁵ | Michael J. Fisher⁶ | Marco Giovannini⁷ | David H. Gutmann⁸ | Matthias A. Karajannis⁹ | Joseph L. Kissil¹⁰ | Eric Legius¹¹ | Alison C. Lloyd¹² | Roger J. Packer¹³ | Vijaya Ramesh¹⁴ | Vincent M. Riccardi¹⁵ | David A. Stevenson¹⁶ | Nicole J. Ullrich¹⁷ | Meena Upadhyaya¹⁸ | Anat Stemmer-Rachamimov¹⁹

¹ Johns Hopkins University School of Medicine, Baltimore, Maryland

² Children's Tumor Foundation, New York, New York

³ Arizona State University, Tempe, Arizona

⁴ Indiana University, Indianapolis, Indiana

⁵ Guy's Hospital and St. Thomas' Hospital, London, United Kingdom

⁶ Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

⁷ University of California Los Angeles, Los Angeles, California

⁸ Washington University School of Medicine, St. Louis, Missouri

⁹ New York University Medical Center, New York, New York

¹⁰ Scripps Research Institute, Jupiter, Florida

¹¹ Center for Human Genetics-University Hospital, Leuven, Belgium

¹² MRC Laboratory for Molecular Cell Biology, University College, London, United Kingdom

¹³ Children's National Medical Center, Washington, District of Columbia

¹⁴ Harvard Medical School, Boston, Massachusetts

¹⁵ The Neurofibromatosis Institute, La Crescenta, California

¹⁶ Lucille Packard Children's Hospital Stanford, Palo Alto, California

¹⁷ Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts

¹⁸ Institute of Cancer Genetics, Cardiff University, Wales, United Kingdom

¹⁹ Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Correspondence

Jaishri O. Blakeley, M.D., Johns Hopkins University School of Medicine, Neurofibromatosis Therapeutic Acceleration Program, 600 N. Wolfe Street, Meyer 100, Baltimore, MD 21287.
Email: jblakel3@jhmi.edu

Funding information

National Institute of Neurological Disorders and Stroke, Grant number: 2R13NS084619-03; The Children's Tumor Foundation

The Annual Children's Tumor Foundation International Neurofibromatosis Meeting is the premier venue for connecting discovery, translational and clinical scientists who are focused on neurofibromatosis types 1 and 2 (NF1 and NF2) and schwannomatosis (SWN). The meeting also features rare tumors such as glioma, meningioma, sarcoma, and neuroblastoma that occur both within these syndromes and spontaneously; associated with somatic mutations in NF1, NF2, and SWN. The meeting addresses both state of the field for current clinical care as well as emerging preclinical models fueling discovery of new therapeutic targets and discovery science initiatives investigating mechanisms of tumorigenesis. Importantly, this conference is a forum for presenting work in progress and bringing together all stakeholders in the scientific community. A highlight of the conference was the involvement of scientists from the pharmaceutical industry who presented growing efforts for rare disease therapeutic

development in general and specifically, in pediatric patients with rare tumor syndromes. Another highlight was the focus on new investigators who presented new data about biomarker discovery, tumor pathogenesis, and diagnostic tools for NF1, NF2, and SWN. This report summarizes the themes of the meeting and a synthesis of the scientific discoveries presented at the conference in order to make the larger research community aware of progress in the neurofibromatoses.

KEYWORDS

neurofibromatosis type 1, neurofibromatosis type 2, pediatric tumors, rare disease, schwannomatosis, therapeutic discovery

1 | INTRODUCTION

Neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), and schwannomatosis (SWN) are distinct neurogenetic syndromes caused by mutations in tumor suppressor genes that manifest with multiple tumors throughout the central and peripheral nervous system (CNS and PNS). There is a risk for malignancy in all three syndromes, but most pronounced in NF1 where there is risk for brain and optic pathway tumors, malignant peripheral nerve sheath tumor (MPNST), and juvenile myelomonocytic leukemia (JMML) (Evans et al., 2002; Fisher et al., 2012; Korf, 2000; Lauchle, Braun, Loh, & Shannon, 2006; Listerick, Charrow, Greenwald, & Mets, 1994; Pourtsidis et al., 2014). There are also multiple other manifestations, particularly in NF1, including vascular and bone malformations, altered neurocognitive development, and endocrine system tumors. All three syndromes are rare with NF2 and SWN estimated to effect between 1/25,000 and 1/40,000 births (Evans et al., 2005, 2010) and NF1 being the most common, with an estimated incidence of 1/2,500–1/3,000 births (Evans et al., 2010; Huson, Harper, & Compston, 1988).

Because of the inherent complexity of these syndromes, involvement of multiple specialties, including neuroscience, molecular genetics, chemistry, biology, pediatrics, genetics, oncology, neurology, psychology, pathology, surgery, radiology, pain management, radiation oncology, and internal medicine, is necessary for the optimal clinical care and therapeutic advancement for people with NF1, NF2, and SWN. A major tool for bringing these critical specialists together and advancing the discoveries that improve treatments for people with NF1, NF2, and SWN is the annual International Children's Tumor Foundation meeting. The 2015 annual meeting was held in Monterey, California with a novel agenda designed to address all of the major nodes in the therapeutic pathway from basic discovery, through key translational studies (including systems and computational biology), to emerging results of current clinical trials. In addition, the meeting provided education about the current standard of care for NF1, NF2, and SWN with an overall effort to synergize efforts between clinicians and basic scientists. This report provides highlights of the 2015 Children's Tumor Foundation Meeting inclusive of the major advances in NF1, NF2, and SWN.

2 | A STRONG FOUNDATION

Recognizing that NF1, NF2, and SWN have many shared challenges in all spheres of science and clinical management, the 2015 Children's Tumor Foundation Meeting was configured to focus on shared challenges and opportunities for breakthrough discoveries across all three syndromes. Exemplifying this theme, the first key note address was given by Anne Barker, PhD, Professor at Arizona State University and Director of the National Biomarker Development Alliance. Dr. Barker drew on her experiences as prior Deputy Director, National Cancer Institute (NCI) and her current work in developing a new systems-based clinical trial for glioblastoma to address the opportunities to radically change the approach to medical discovery in her talk, "The Myths and Realities of Transformative Healthcare." She challenged the NF research community to seize the opportunities to apply broad scientific principles and systematic discipline to enable development of individualized, molecularly based therapies for the various manifestations of NF1, NF2, and SWN. The first session following this inspirational opening talk was chaired by Drs. David Gutmann and Nicole Ullrich and focused on core concepts of diagnosis and management of NF1, NF2, and SWN. Dr. Bruce Korf reviewed the current status of clinically available genetic testing for NF1, NF2, and SWN. Presently, the sensitivity of testing differs for the three disorders and is typically performed to resolve a diagnostic uncertainty rather than to provide predictive or prognostic data. However, an argument was made that universal genetic testing would advance the discovery of predictive genotype–phenotype correlations moving forward. In addition, *NF1* is now included on many cancer molecular panels since it is a frequent somatic mutation in common cancers such as lung adenocarcinoma, breast cancer, and melanoma. This developing somatic *NF1* mutation dataset paired with germline testing of individuals who have the NF1 syndrome presents an exciting opportunity to explore the phenotypic spectrum of *NF1* genomic variants, ultimately increasing the prognostic value of *NF1* testing within and outside of the syndrome. Indeed, Dr. Gareth Evans presented a compelling example of the use of genotyping for molecular predictors of mortality in people with NF2. He and his team identified 1,192 people with NF2 through the United Kingdom National NF2 Registry and showed that early age at diagnosis and the

presence of intracranial meningiomas as well as germline truncating mutations have the worst prognosis. Interestingly, this study also showed that the mortality rate for patients with NF2 diagnosed in more recent decades was lower than for people diagnosed previously, suggesting that recent efforts to advance management of people with NF2 in specialist centers is having impact.

Dr. Christine Chiasson-MacKenzie presented data that the NF2 tumor suppressor protein, merlin, dynamically modulates mitogenic signaling upon the establishment of cell contact (Chiasson-MacKenzie et al., 2015) reinforcing therapeutic strategies that modulate this activity. Dr. Anat Stemmer Rachamimov discussed the importance of autopsies for the study of the evolution and progression of disease in NF1, NF2, and SWN and for enhancing the understanding of the clinical and radiologic observations in complex disease processes. Recent examples highlighting the power of autopsies for discovery are the finding that gastrointestinal stromal tumors (GISTs) are part of the spectrum of lesions encountered in NF1 and that NF1-associated GISTs have clinical and molecular characteristics that are distinct from their sporadic counterparts. Similarly, autopsy studies in people with NF2 revealed the presence of precursor schwannoma lesions, Schwann cell “tumorlets,” and showed that schwannomas are polyclonal and may in fact represent collision tumors composed of multiple tumorlets (Dewan et al., 2015; Stivaros et al., 2015). The study of surgical pathology specimens can also lead to the discovery of diagnostic and prognostic markers, for example the use of immunohistochemical markers (S100, glut1, claudin1, sox10, p16, and Ki67) to help differentiate the various types of nerve sheath tumors. Dr. Chetan Bettgowda presented exciting new data about the use of circulating tumor DNA (cDNA) as a “liquid biopsy” for NF1-associated tumors. He shared their early experiences using cDNA to monitor for cancer recurrence in the course of therapy for MPNST. As this is further developed and validated, this may serve as a new clinical tool for monitoring MPNST clinical status. Dr. Miriam Bredella then presented the state of the art imaging techniques used to assess tumor burden in patients across NF1, NF2, and SWN and the validation of imaging techniques for clinical trial endpoints for the tumors of these syndromes. Importantly, several studies have shown that volumetric tumor assessment is more accurate than 2D techniques such as RECIST for tumors such as plexiform neurofibroma (pNF) in NF1 and vestibular schwannoma (VS) in NF2 (Cai et al., 2009; Dombi et al., 2007; Harris et al., 2008; Solomon, Warren, Dombi, Patronas, & Widemann, 2004). Dr. Eva Dombi presented the preliminary results of a comparison of the MRI analysis methods used at the National Institutes of Health (NIH) and Massachusetts General Hospital to assess two volumetric analysis approaches for assessing tumor size for complex pNF. They used a dataset of 15 pNF at three different time points and showed similar accuracy of the assessment techniques for detecting tumor volume and changes over time. These results establish volumetric analysis as a sensitive and reliable method to detect small changes in pNF size; a critical step for applying this technology to both standard clinical care and as an endpoint in clinical trials. Dr. Karin Walsh then presented the approaches for evaluating cognitive function in individuals with NF1, both for clinical care and clinical trials, to allow assessment of therapies addressing cognitive

performance in people with NF1. The session closed with the much anticipated results of the Department of Defense sponsored multicenter, randomized, double-blind, placebo-controlled trial to assess the efficacy of lovastatin for the treatment of deficits in visuospatial learning and attention in children with NF1 presented by Dr. Kathryn North. Children with NF1 ($n = 146$) who demonstrated deficits in the primary cognitive outcome measures at baseline were randomly assigned to lovastatin ($n = 74$; 40 mg/day) or placebo ($n = 70$). Although lovastatin administered for 16 weeks was well tolerated, it did not improve cognitive or behavioral outcomes in children with NF1.

2.1 | Advancing discovery to improve therapeutics for NF1, NF2, and SWN

Dr. Barbara Slusher launched the second day of the meeting with the keynote “The Changing Ecosystem of Drug Discovery: Rise in Academic-Industry Partnerships.” She discussed the evolving landscape of therapeutic discovery and the associated opportunities for basic science discoveries to be translated to clinic via multi-disciplinary public-private partnerships. These opportunities for collaboration are particularly rich for rare diseases like NF and are increasingly valued in the pharmacology industry. Drs. Marco Giovannini and Wade Clapp then led the session: “Optimizing Tools for Discovery” addressing cell systems, inducible pluripotent stem cells (iPSCs), and new murine models. One clearly emerging opportunity to both understand disease pathogenesis and identify targeted therapies is the use of iPSCs. In his keynote presentation, Dr. Evan Snyder reviewed the basic biology of neuronal stem cells, emphasizing that these primordial cells have the capacity to differentiate into cells of many neuronal lineages, display a capacity for self-renewal and can migrate to target areas guided by microenvironmental cues. Utilizing these cells can both provide insights into disease pathogenesis and when induced to differentiate, create populations of neuronal cells that can potentially be used therapeutically. The two subsequent discussions focused on the use of iPSCs in the context of NF1 specific malignancies. Dr. David Gutmann utilized a panel of NF1 patient-derived iPSCs to identify genotype-phenotype differences relevant to improved modeling of NF1 optic gliomas (OPG) in mice, while Dr. Eduard Serra generated human iPSCs from pNF. Dr. Gutmann discussed approaches his laboratory is taking to define the individual contributions of the germline NF1 gene mutation and patient sex to NF1 clinical heterogeneity, focusing on optic glioma and hippocampal-based learning. Dr. Serra characterized the germline and somatic mutations from human pNF and then reprogrammed the schwann cells to iPSCs to create a renewable biologically relevant assay for discovery. These model systems allow investigation of key events in tumorigenesis and serve as drug therapeutic models with increased representation of the diversity of the genetic background of both OPG and pNF. Dr. Robert Kesterson then reviewed the current murine models for NF1. These are predominantly based on the absence of *Nf1*, which is of limited genetic diversity. He presented recent efforts to create murine models with splice site mutations to increase model diversity more closely modeling the human condition. Dr. Shuning followed with a

presentation about the pathways activated by loss of function in *Nf1* in a mutant zebrafish model. The *Nf1* zebrafish models provide novel systems for drug testing for *NF1* deficient high-grade gliomas, MPNSTs, and neuroblastoma. Notably, a tight genetic link between *Nf1* loss and MYC-N driven neuroblastoma tumorigenesis was demonstrated in this model. Dr. Peacock in the Steensma laboratory presented work using genetically engineered mice with disruption of *Nf1* and overexpression of c-met leading to development of MPNST, implicating c-met as a therapeutic target for MPNST. The session was closed by Drs. Anna Barker and Joshua Stewart presenting the current and developing bioinformatics infrastructure tools available to support computational analysis of complex datasets generated from both pre-clinical and clinical studies. These data approaches enhance the ability to predict patterns of tumor behavior and response to therapies as well as support biomarker discovery. Recognizing that we are in the midst of the era of "omics" and the need to make rational decisions about treatments based on a variety of complex data sources, these two talks were timely and instructive for the NF research community.

Drs. Alison Lloyd and Vincent Riccardi led the session, "Biology and Discovery" in which novel work toward understanding the basic pathogenesis of the tumors associated with NF1, NF2, and SWN was presented. Mario Suva, MD, PhD reviewed the methodologies of chromatin analysis and the use of this technique for investigating tumor heterogeneity and identification of therapeutic targets through chromatin immunoprecipitation and high-throughput sequencing to map protein-DNA interactions (ChIP-seq). This approach allows identification of the epigenome of a given cell type. The signature chromatin structures can then be used to identify promoters, transcripts, enhancers, silencers, and repressive chromatin domains that can be manipulated for therapeutic gain (Suva, Riggi, & Bernstein, 2013). Dr. Karen Cichowski presented her lab's work in identifying ways to overcome therapeutic resistance in MPNSTs. Through focusing on cancer cell-specific metabolic and epigenetic vulnerabilities in combination with targets in the Ras pathway, they have developed new combination approaches for MPNST, some already currently evaluated in clinical trials (Malone et al., 2014) (www.clinicaltrials.gov, NCT01661283; NCT02008877). Dr. Helen Morrison discussed how microenvironment interactions contribute to tumorigenesis in *NF2* driven schwannomas. Her lab identified a severe re-myelination defect in combination with sustained macrophage infiltration as an underlying pathogenic process in the setting of a crush injury model in mice with *Nf2* deletions in Schwann cells and axons. These provocative results suggest that interactions between axons and their adjacent Schwann cells are important for schwannoma development beyond the biallelic *Nf2* loss in Schwann cells. Finally, Dr. Luis Parada presented data about the interaction between *Nf1* mutation, cerebral development, and the associated phenotypical intellectual deficits in murine models as well as the association between *Nf1*, *Trp53*, and *Pten* inactivation in adult progenitor cells and development of malignant gliomas. His lab has shown that neurofibromin is required for appropriate development of cerebellar folia and ERK inhibitors can reverse the effect, implying that *Nf1* is a key element of normal brain development.

Drs. Eric Legius and Meena Upadhyaya led the session "Along the Pathway" about the signaling transduction pathways and systems that influence NF1, NF2, and SWN pathogenesis. Dr. Jianqiang Wu presented data supporting the role of an *Nf1*-Egfr-Stat3-Arid1b/ β -catenin pathway in the initiation of *Nf1* neurofibroma, using unbiased insertional mutagenesis screening, mouse models, and molecular analyses. Genetic deletion of Stat3 in *Nf1* deficient Schwann cells progenitors (SCPs) and Schwann cells (SCs) prevents neurofibroma formation. Genetic gain- and loss-of-function mutations identify EGFR as a major upstream regulator of P-Stat3 in SCP; and Stat3 represses Arid1b through histone modification indicating that epigenetic modification plays a role in neurofibroma tumorigenesis. These data support testing JAK/STAT or Wnt/ β -catenin pathway inhibitors in neurofibroma therapeutic trials. Signaling pathways are usually presented as a linear cascade of protein interactions inducing post-transcriptional modifications and ending with the activation/inhibition of a transcription factor. However, the epigenetic state of a cell determines how these signals are received and translated into an actual change in transcriptional output. Further, transcription factors can only influence transcription if they physically bind to enhancers or promoters that are active or poised for activation and this status is determined by the histone/DNA modifications present. Hence, epigenetic factors are important considerations when therapeutically targeting signal transduction pathways. Dr. Thomas De Raedt outlined the role of PRC2 loss in MPNSTs. Loss of the PRC2 complex removes the repressive H3K27me3 mark and induces an epigenetic switch that allows for acetylation of H3K27. This H3K27Ac mark opens the chromatin structure allowing for transcriptional activation to occur. Moreover, the loss of PRC2 causes an upregulation of the RAS transcriptional output in murine MPNSTs (De Raedt et al., 2014).

Dr. Nancy Ratner discussed the cell autonomous and non-cell autonomous effects of loss of *NF1* and H-RasG12V in oligodendrocytes, based on data from mouse models (Mayes et al., 2013). New findings confirm that the decompaction of oligodendrocyte myelin and diminished integrity of the blood brain barrier in these models are controlled, at least in part, by nitric oxide and MEK signaling. The findings suggest that oligodendrocytes, in addition to astrocytes and neurons, contribute to NF1 brain pathology. Shifting focus to NF2, Dr. Filippo Giancotti and coworkers shared evidence that Merlin's entry into the nucleus and inhibition of the E3 ubiquitin ligase CRL4-DCAF1 is necessary for tumor suppression (Li & Giancotti, 2010) and that CRL4-DCAF1 inhibits Lats thus activating the Hippo-YAP pathway (Li et al., 2014). There is now genetic and pharmacological evidence that CRL4-DCAF1 drives tumorigenesis in mouse models of schwannoma and mesothelioma. Dr. Matthew Karolak then presented new data about pseudoarthrosis pathophysiology in NF1. They found that FGFR1 signaling in hypertrophic growth plate chondrocytes is attenuated by neurofibromin to regulate growth plate catabolism and hypertrophic zone length. He emphasized that FGFR1 and FGFR3 signaling is likely to be attenuated by neurofibromin in the prehypertrophic zone to control proliferation zone length/organization and overall body length. It may be possible to translate these findings to treat NF1 associated pseudoarthrosis by inhibiting FGFR.

These pre-clinical discoveries and their critical role in developing clinically impactful interventions were further expounded upon in the session: "Therapeutics: From Target to Clinic" led by Drs. Vijaya Ramesh and Joe Kissil. Dr. Douglas Stewart discussed the use of genomic approaches to identify new therapeutic targets in NF1 and NF2. Although promising, as with many other solid tumors, whole-exome and whole-genome sequencing studies face challenges such as tissue procurement and annotation, the calling of insertion-deletion variants and complex arrangements, the assigning of significance to non-coding sequence, bioinformatics and issues with informed consent, patient confidentiality and sharing of large datasets. In addition, NF studies in particular are hampered by issues related to tumor heterogeneity, illustrated recently in NF2-associated VS, where different NF2 mutations were found in an apparently single lesion (Stivaros et al., 2015). Dr. Lei Xu discussed the use of anti-VEGF treatment to enhance responses to radiation therapy in NF2 schwannomas in xenograft mouse models. Their results suggest that anti-angiogenic treatment improves the effectiveness of radiation treatment of VS in NF2 patients. Dr. William Guerrant from the Scripps Research Institute then discussed efforts to characterize the role the transcriptional activator YAP plays in NF2 null schwannoma. They demonstrated that YAP is required for proliferation and survival of NF2-null Schwann cells in culture and for tumor formation in vivo again supporting the Hippo-YAP pathway as a therapeutic target in schwannomas. Dr. Dominique Lallemand discussed results using proteomic approaches, a combination of reverse phase protein array (RPPA), receptor tyrosine kinase (RTK) array, and immunohistochemistry to identify frequently activated signaling pathways in human schwannomas. His group identified a number of RTKs including Her2/3, PDGFR β , and Axl as promising targets. In addition, the RPPA analysis showed that Ki67 expression correlates to the levels of the Hippo pathway effector Yap and that in cell models tumor cell proliferation is linked to a signaling network driven by a small set of RTKs under the control of Yap. Dr. David Deyle from Mayo clinic presented the effects of measles virus Edmonston (MVE_{dm}) vaccine strain engineered to express the human sodium iodide symporter (MV-NIS) on MPNST. MPNST cell lines were found to highly express CD46, a cellular receptor required for measles viral entry on their cell surface. After in vitro MV-NIS infection, MPNST cell lines showed significant cytopathic effect and local administration of MV-NIS into MPNST-derived tumors resulted in significant regression of tumor and improved survival. Based on these results, they have launched a phase I clinical trial using oncolytic measles virus therapy to treat people with MPNST. Dr. Marc Ferrer from the National Center for Advancing Translational Science (NCATS) discussed the approach for a large scale high-throughput screening (HTS) to identify agents active against human immortalized NF1 deficient Schwann cells in collaboration with Neurofibromatosis Therapeutic Acceleration Program (NTAP) investigators. Six cell lines derived from human pNF created by Dr. Margaret Wallace were screened with roughly 2,000 compounds. The top 40 compounds were chosen after a single drug screen, which was followed by a combination screen to identify synergy among the drugs. Although

no clustering of pharmacological response by NF1 status was noted, this is a new tool that makes HTS feasible for single and combination agents.

The focus then shifted to "Clinical Therapeutics: Ongoing Efforts and Future Directions" led by Drs. Rosalie Ferner and Michael Fisher. Dr. Peter de Blank opened the session reviewing long-term vision, psychological and socio-economic outcomes from the Childhood Cancer Survivor Study in adult survivors of childhood glioma that included patients with NF1. Adults with bilateral severe sight impairment were more likely to be unemployed, unmarried, and live dependently. However, unilateral visual loss had very limited impact on adult socio-economic and psychological outcomes. Dr. Robert Avery then reported that in 46 participants (55 affected eyes) with OPG followed longitudinally; new vision loss was associated with a decline in retinal nerve fiber layer (RNFL) ($\geq 10\%$); however, there was no relationship between MRI progression and RNFL change indicating that RNFL thickness may be a valuable biomarker in children with OPG. Dr. Juha Peltonen then presented long-term outcome data using population databases from the Finnish Cancer Registry and the NF1 registry. The age of death and 5 year survival was significantly lower in NF1 patients (particularly in females) compared with matched controls. Cancer was the main cause of death in people with NF1 with the greatest risk of malignancy in childhood and women with most common cancers in NF1 being MPNST, gliomas, breast cancer, and GIST. Regarding clinical therapeutics, Dr. Scott Plotkin discussed emerging data on use of bevacizumab for patients with NF2. Results from a prospective multi-center phase 2 study (NCT01207687) revealed hearing improvement in 36% (5/14) of subjects and imaging responses in 6/14 (43%) of target VS that were durable for up to 3 months after stopping drug. However, some patients experienced hearing decline and tumor growth by 6 months showing that long-term treatment with bevacizumab is necessary to maintain clinical improvement. Imaging responses have also been reported in NF2 patients with symptomatic spinal schwannoma and ependymoma (Farschtschi et al., 2016). In contrast, response of NF2-related meningiomas to bevacizumab was less frequent and transient (Nunes et al., 2013). Finally, Dr. Brigitte Widemann presented the preliminary results of a phase I trial of the oral MEK inhibitor selumetinib for children with inoperable plexiform neurofibromas. The maximum tolerated dose was defined as 25 mg/m²/dose administered twice daily on a continuous schedule. Partial responses ($\geq 20\%$ reduction in tumor volume) were noted in 71% of patients. Dose limiting toxicities included infection, asymptomatic creatinine kinase elevation, and asymptomatic left ventricular function decline; all resolved (Dombi et al., 2016). A phase II study is ongoing (NCT01362803). The clinical session was closed by Dr. David Bennet who gave the final key note speech on the Neuropathic Pain and Peripheral Nerve Repair. He presented the state of the art in nerve pathophysiology and the opportunities for therapeutic strategies that enhance nerve repair. The applications to NF1, NF2, and SWN are broad ranging from managing the associated painful neuropathy to preventing new tumor formation.

Drs. Karajannis and Packer led the session "Pathway to Approval" focused on strategies on moving therapies into the clinical development space including business strategy, identification of biomarkers

that will allow therapeutic personalization and patient-focused outcomes. In this session, basic science researchers, clinical researchers, and industry partners discussed their various perspectives on opportunities and obstacles to drug approval for rare diseases like NF1, NF2, and SWN with special focus on bringing novel therapies to children. On the therapeutic front, Kairong Li, presented his research on drugs that suppress premature translation termination in treatment of NF1 patients with *NF1* nonsense mutations. One-fifth of NF1 patients carry nonsense mutations in the *NF1* gene. Dr. Li has created a novel NF1 mouse model (*Nf1st18-CKO*) carrying a recurrent nonsense mutation found in NF1 patients and showed preclinical in vivo data using *Nf1st18/st18* embryonic fibroblasts to screen candidate drugs for their ability to restore neurofibromin activity. Gentamicin, which enables reading through premature translation codons, coupled with amlexanox (an anti-inflammatory immunomodulator with nonsense-mediated mRNA inhibitor activity) partially restored neurofibromin function as measured by a significant reduction in downstream phospho-ERK activation. Further in vivo studies are planned to validate these findings. Dr. Stankovic presented gene expression analysis data pointing to overexpression of genes related to inflammation in human VS, as well as retrospective clinical data suggesting an association between aspirin intake and decreased growth rates of VS. She showed preclinical data indicating that cyclooxygenase 2 (COX2) and prostaglandins are overexpressed in VS, and that salicylates and a COX-2 inhibitor decreased the proliferation of cultured human VS cells with no effect on normal Schwann cells, setting a path for translation to clinical trials for NF2 associated VS. Dr. Mitchel Springer presented data on the identification of differentially expressed genes in responsive (shrinking) and non-responsive (growing) tumors from an NF1 plexiform neurofibroma mouse model treated with inhibitors of MEK, ErbB, PI3K, or Stat3. He identified a set of a candidate genes that may drive therapy resistance, including CXCL13. Based on these results, the Ratner laboratory is currently investigating the efficacy of a CXCL13 neutralizing antibody in a mouse model of NF1 plexiform neurofibromas to overcome this potential resistance mechanism.

Dr. Aileen Healy, Vice President, Preclinical Development of Cydan Development Inc. provided an overview of drug development for rare diseases from the perspective of a company with a mission of de-risking studies to bridge the preclinical to clinical development process, and enable human proof-of-concept studies to promote later stage investment and development. Dr. Stephen Simko, Associate Medical Director, Innovative Pediatric Oncology Drug Development for Genentech Inc. then described the evolving regulatory requirements and incentives for increasing the involvement of pharmaceutical companies in pediatric oncology drug development. He discussed factors internal to the pharmaceutical industry that influence investigation of new agents in children, including the prioritization of molecule expertise over pediatric disease-specific expertise, inconsistency in pediatric development strategy across different molecule programs, and regulatory requirements. In order to maximize therapeutic opportunities for children with cancer, a mechanism-of-action based approach has been adopted within Genentech, including robust preclinical assessments, on-target

molecule sensitivity in vitro and in vivo, biomarker validation, resistance mechanisms, and potential therapeutic combinations early in the development process. The information shared in this session demonstrates the tremendous opportunities that are now available to move agents from the preclinical state to clinical trials in the hopes of developing a menu of approved drugs for pediatric tumors. The pharmaceutical industry is clearly committed to developing drugs for rare diseases and Drs. Healy and Simko presented the case for strong business models that should be established to support such development.

3 | THE PATH FORWARD

The meeting concluded with sessions focused on the active programs in NF1, NF2, and SWN. During the session "Report Card" leaders of influential programs focused on specific aspects of NF1, NF2, and SWN research presented the current activities of each group. Dr. Roger Packer presented the activities of the Department of Defense Neurofibromatosis Clinical Trials Consortium (NFCTC) including the ongoing clinical trials for pNF (NCT02101736, NCT02096471) and MPNST (NCT01661283, NCT02008877) and upcoming studies for pseudarthroses and low grade gliomas (<http://cdmrp.army.mil/nfrp/consortium/nfrpctc.shtml>). The accomplishments of the Response Evaluation in Neurofibromatosis and Schwannomatosis (REINS) Consortium was presented by Drs. Scott Plotkin and Brigitte Widemann. This consortium is dedicated to identifying or developing validated endpoints to be incorporated into clinical trials for NF1, NF2, and SWN (<http://www.reinscollaboration.org/>). The Neurofibromatosis Therapeutic Consortium is a collaborative of for veteran NF research laboratories (Drs. Cichowski, Clapp, Ratner, and Shannon) focused on pre-clinical efficacy studies for NF1 associated JMML, pNF, and MPNST. The most current iteration of the consortium (initiated in 2012) has completed >50 pre-clinical studies and several of these have informed subsequent clinical trials. Dr. Ophelia Maertens presented the work by this consortium over the last year and the efforts they have made to collaborate with the clinical trials consortium to advance promising pre-clinical findings to clinical trials. Dr. Oliver Hanneman and Pamela Knight presented the progress through the NF biobank and the bioregistry initiative. Through these remarkable efforts, hundreds of cutaneous neurofibromas have been collated and are in the process of being fully analyzed with the data to be made public and minimal clinical datasets have been created for each tumor type. CTF has also collaborated with NDRI to allow people with NF1, NF2, and SWN who wish to donate their body to expand scientific understating of NF.

Dr. Michael Fisher presented updates on the newly formed Optic Pathway Glioma Consortium. This consortium is working to define the treatment indications and factors predictive of visual decline and tumor progression for OPGs via a prospective natural history study of NF1-OPG with standardized visual assessment methods and clear definitions of visual outcomes and data acquisition time points. Dr. Wade Clapp presented the updates about the work of Synodos for NF2 (<http://www.ctf.org/Research/Synodos.html>) a team-science initiative sponsored by CTF to create better models and find new therapies for NF2 associated schwannomas and meningiomas.

Dr. Allan Belzberg provided updates about the progress of the International Schwannomatosis Database which connects people living with schwannomatosis and researchers with one another to enhance collaborations focused on this rarest of the neurofibromatoses (<http://sid2011.squarespace.com/>). Finally, Dr. Jaishri Blakeley presented the activities of the Neurofibromatosis Therapeutic Acceleration Program (NTAP), a program focused on therapeutics for plexiform neurofibromas (<http://www.n-tap.org/>).

The "Path Forward Panel Discussion – A Reflection and Synthesis of Successes and Limitations in Neurofibromatosis Research" with Drs. Allan Belzberg and Larry Sherman representing SWN; Drs. Elizabeth Schorry and David Gutmann representing NF1; and Drs. James Gusella and Gareth Evans representing NF2 followed. The session was moderated by Drs. Jaishri Blakeley and David Stevenson who opened the session with data from a survey completed by people enrolled in the CTF NF registry in advance of the meeting asking what their greatest clinical and research priorities were. These data were presented relative to the published literature for each syndrome, the CDMRP NF areas of emphasis and the abstracts and presentations from the 2015 conference in order to provide a synthesis of the successes and limitations in the field to date. The review of the 2015 NF Conference poster abstracts showed that roughly 79% focused on NF1, 20% on NF2, and 1% on SWN. Poster abstracts covered basic research topics 43% of the time versus 57% addressing clinical research topics. Within the NF1 posters the most common topics were MPNST, development/cognition, and neurofibromas. Areas of success identified included the number of clinical trials now available, the growing collaborations across basic science and clinical scientists specializing in NF, and the creation of new resources for research including the CTF biobank and registry. Areas with opportunities for growth include enhanced sharing of data and open access resources, the need to invest in all forms of biomarker discovery to improve the precision of therapeutics across heterogeneous tumors, the need for investment for hearing restoration strategies that may help people with NF2 and the need to advance research in all forms for SWN.

An exciting development in the 2015 CTF meeting was a focus on the high number of exceptional posters. These were highlighted each day with a "posters of the day" session in which the authors of the top ranked posters were invited to give a "micro-talk" highlighting their work. The winners of the poster of the day (Drs. Robert Avery, Kwangmin Choi, Peter deBlank, Alexander Schultz, Brian Stansfield, Elina Uusitalo, and Adrienne L. Watson) presented topics ranging from microRNA-mediated gene regulatory network in MPNST to the development of a swine model of NF1 to the effect of carboplatin-based chemotherapy on NF1 white matter tracts associated with cognition. These exciting and scientifically rigorous reports were presented by young investigators from around the world invested in neurofibromatosis research.

4 | CONCLUSIONS

The Path Forward: 2015 International Children's Tumor Foundation Conference on Neurofibromatosis Type 1, Type 2 and Schwannomatosis

successfully integrated basic, translational, and clinical science in a single track that addressed shared scientific questions across NF1, NF2, and SWN. In addition, a major focus was placed on integration of academic and pharmaceutical scientific efforts, systems based and computational biology approaches to leverage, and expand the multiple discovery efforts ongoing for NF1, NF2, and SWN and there was a focus on the excellent science presented in posters. New, critical clinical data about lovastatin for NF1 cognitive function, selumetinib for plexiform neurofibromas, and natural history studies for NF1 and NF2 were presented. Finally, there were many compelling new discovery programs in both NF1 and NF2 presented as described above. The conference succeeded in highlighting the ways in which investigators within and outside of the NF field can work together to speed and expand discovery to benefit people living with these syndromes.

ACKNOWLEDGMENTS

The conference is sponsored by The Children's Tumor Foundation and specifically, the authors thank Patrice Pancza for her extraordinary efforts in coordinating the conference and to Rhonda Jackson for her administrative assistance. Web Resources include: www.ctf.org; www.clinicaltrials.gov; <http://cdmrp.army.mil/nfrp/>; <http://sid2011.squarespace.com>; www.n-tap.org

CONFLICTS OF INTEREST

None.

REFERENCES

- Cai, W., Kassarijan, A., Bredella, M. A., Harris, G. J., Yoshida, H., Mautner, V. F., ... Plotkin, S. R. (2009). Tumor burden in patients with neurofibromatosis types 1 and 2 and schwannomatosis: Determination on whole-body MR images. *Radiology*, 250, 665–673.
- Chiasson-MacKenzie, C., Morris, Z. S., Baca, Q., Morris, B., Coker, J. K., Mirchev, R., ... McClatchey, A. I. (2015). NF2/Merlin mediates contact-dependent inhibition of EGFR mobility and internalization via cortical actomyosin. *The Journal of Cell Biology*, 211, 391–405.
- De Raedt, T., Beert, E., Pasmant, E., Luscan, A., Brems, H., Ortonne, N., ... Cichowski, K. (2014). PRC2 loss amplifies Ras-driven transcription and confers sensitivity to BRD4-based therapies. *Nature*, 514, 247–251.
- Dewan, R., Pemov, A., Kim, H. J., Morgan, K. L., Vasquez, R. A., Chittiboina, P., ... Asthagiri, A. R. (2015). Evidence of polyclonality in neurofibromatosis type 2-associated multilobulated vestibular schwannomas. *Neuro-Oncology*, 17, 566–573.
- Dombi, E., Baldwin, A., Marcus, L. J., Fisher, M. J., Weiss, B., Kim, A., ... Widemann, B. C. (2016). Activity of selumetinib in neurofibromatosis type 1-related plexiform neurofibromas. *N Engl J Med*, 375, 2550–2560.
- Dombi, E., Solomon, J., Gillespie, A. J., Fox, E., Balis, F. M., Patronas, N., ... Widemann, B. C. (2007). NF1 plexiform neurofibroma growth rate by volumetric MRI: Relationship to age and body weight. *Neurology*, 68, 643–647.
- Evans, D. G., Baser, M. E., McGaughan, J., Sharif, S., Howard, E., & Moran, A. (2002). Malignant peripheral nerve sheath tumours in neurofibromatosis 1. *Journal of Medical Genetics*, 39, 311–314.
- Evans, D. G., Baser, M. E., O'Reilly, B., Rowe, J., Gleeson, M., Saeed, S., ... Ramsden, R. (2005). Management of the patient and family with neurofibromatosis 2: A consensus conference statement. *British Journal of Neurosurgery*, 19, 5–12.

- Evans, D. G., Howard, E., Giblin, C., Clancy, T., Spencer, H., Huson, S. M., & Laloo, F. (2010). Birth incidence and prevalence of tumor-prone syndromes: Estimates from a UK family genetic register service. *American Journal of Medical Genetics Part A*, 152A, 327–332.
- Farschtschi, S., Merker, V. L., Wolf, D., Schuhmann, M., Blakeley, J., Plotkin, S. R., . . . Mautner, V. F. (2016). Bevacizumab treatment for symptomatic spinal ependymomas in neurofibromatosis type 2. *Acta Neurologica Scandinavica*, 133, 475–480.
- Fisher, M. J., Loguidice, M., Gutmann, D. H., Listernick, R., Ferner, R. E., Ullrich, N. J., . . . Liu, G. T. (2012). Visual outcomes in children with neurofibromatosis type 1-associated optic pathway glioma following chemotherapy: A multicenter retrospective analysis. *Neuro-Oncology*, 14, 790–797.
- Harris, G. J., Plotkin, S. R., Maccollin, M., Bhat, S., Urban, T., Lev, M. H., & Slattery, W. H. (2008). Three-dimensional volumetrics for tracking vestibular schwannoma growth in neurofibromatosis type II. *Neurosurgery*, 62, 1314–1319.
- Huson, S. M., Harper, P. S., & Compston, D. A. (1988). Von Recklinghausen neurofibromatosis. A clinical and population study in south-east Wales. *Brain*, 111(Pt 6), 1355–1381.
- Korf, B. R. (2000). Malignancy in neurofibromatosis type 1. *The Oncologist*, 5, 477–485.
- Lauchle, J. O., Braun, B. S., Loh, M. L., & Shannon, K. (2006). Inherited predispositions and hyperactive Ras in myeloid leukemogenesis. *Pediatric Blood and Cancer*, 46, 579–585.
- Li, W., Cooper, J., Zhou, L., Yang, C., Erdjument-Bromage, H., Zagzag, D., . . . Giacotti, F. G. (2014). Merlin/NF2 loss-driven tumorigenesis linked to CRL4(DCAF1)-mediated inhibition of the hippo pathway kinases Lats1 and 2 in the nucleus. *Cancer Cell*, 26, 48–60.
- Li, W., & Giacotti, F. G. (2010). Merlin's tumor suppression linked to inhibition of the E3 ubiquitin ligase CRL4 (DCAF1). *Cell Cycle*, 9, 4433–4436.
- Listernick, R., Charrow, J., Greenwald, M., & Mets, M. (1994). Natural history of optic pathway tumors in children with neurofibromatosis type 1: A longitudinal study. *Jornal de Pediatria*, 125, 63–66.
- Malone, C. F., Fromm, J. A., Maertens, O., DeRaedt, T., Ingraham, R., & Cichowski, K. (2014). Defining key signaling nodes and therapeutic biomarkers in NF1-mutant cancers. *Cancer Discovery*, 4, 1062–1073.
- Mayes, D. A., Rizvi, T. A., Titus-Mitchell, H., Oberst, R., Ciraolo, G. M., Vorhees, C. V., . . . Ratner, N. (2013). Nf1 loss and Ras hyperactivation in oligodendrocytes induce NOS-driven defects in myelin and vasculature. *Cell Reports*, 4, 1197–1212.
- Nunes, F. P., Merker, V. L., Jennings, D., Caruso, P. A., di Tomaso, E., Muzikansky, A., . . . Plotkin, S. R. (2013). Bevacizumab treatment for meningiomas in NF2: A retrospective analysis of 15 patients. *PLoS ONE*, 8, e59941.
- Pourtsidis, A., Doganis, D., Baka, M., Bouhoutsou, D., Varvoutsis, M., Synodinou, M., . . . Kosmidis, H. (2014). Malignant peripheral nerve sheath tumors in children with neurofibromatosis type 1. *Case Reports in Oncological Medicine*, 2014, 843749.
- Solomon, J., Warren, K., Dombi, E., Patronas, N., & Widemann, B. (2004). Automated detection and volume measurement of plexiform neurofibromas in neurofibromatosis 1 using magnetic resonance imaging. *Computerized Medical Imaging and Graphics*, 28, 257–265.
- Stivaros, S. M., Stemmer-Rachamimov, A. O., Alston, R., Plotkin, S. R., Nadol, J. B., Quesnel, A., . . . Evans, D. G. (2015). Multiple synchronous sites of origin of vestibular schwannomas in neurofibromatosis type 2. *Journal of Medical Genetics*, 52, 557–562.
- Suva, M. L., Riggi, N., & Bernstein, B. E. (2013). Epigenetic reprogramming in cancer. *Science*, 339, 1567–1570.

How to cite this article: Blakeley JO, Bakker A, Barker A, et al. The path forward: 2015 International Children's Tumor Foundation conference on neurofibromatosis type 1, type 2, and schwannomatosis. *Am J Med Genet Part A*. 2017;9999:1–8. <https://doi.org/10.1002/ajmg.a.38239>