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From Process to Progress – 2017 International Conference on Neurofibromatosis 1, Neurofibromatosis 2 and Schwannomatosis

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Abstract

The neurofibromatoses are inherited, tumor suppressor disorders that are characterized by multiple, benign peripheral nerve sheath tumors and other nervous system tumors. Each disease is associated with a distinct genetic mutation and with a different pathogenesis and clinical course. Neurofibromatosis 1 (NF1) is common and epitomized by multiple neurofibromas with widespread complications. NF2 and schwannomatosis are rare diseases that are typified by multiple schwannomas that are particularly painful in people with schwannomatosis. Since 1985, the Children's Tumor Foundation has hosted an international Neurofibromatosis Conference, bringing together international participants who are focused on NF research and clinical care. The

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest

2017 Conference, held in Washington DC, was among the largest gatherings of NF researchers to date and included presentations from clinicians and basic scientists, highlighting new data regarding the molecular and cellular mechanisms underlying each of these diseases as well as results from clinical studies and clinical trials. This article summarizes the findings presented at the meeting and represents the current state-of-the art for NF research.

Keywords

neurofibromatosis 1; neurofibromatosis 2; schwannomatosis

INTRODUCTION

The Children's Tumor Foundation (CTF) hosts the annual international Neurofibromatosis Conference for scientists and clinicians working on the neurofibromatoses - neurofibromatosis 1 (NF1), neurofibromatosis 2 (NF2) and schwannomatosis. The neurofibromatoses are inherited tumor predisposition diseases linked to mutations in distinct genes. NF1 has a birth incidence of 1 in 2,699 to 1 in 3,000 and a prevalence of 1 in 4,560 (Evans et al., 2010). For NF2 the birth incidence is 1 in 27,956 and prevalence is 1 in 50,000, and schwannomatosis has a birth incidence of 1 in 68,956 and a prevalence of 1 in 126,000 (Evans et al., 2018).

In the early 1990s, the focus of neurofibromatosis research was on identifying the molecular pathways underpinning the pathophysiology of these disorders. However, recent advances in molecular biology, the development of animal models, and novel imaging techniques have been matched by an increased understanding of the clinical manifestations and natural history of these conditions. The advent of targeted disease therapy has highlighted the interdependence of the scientist and clinician and the importance of animal models in screening potential new treatments.

The 2017 Washington DC Neurofibromatosis Conference was co-chaired by Dr. Rosalie E Ferner (Guy's and St. Thomas' NHS Foundation Trust, London, UK) and Dr. Larry S. Sherman (Oregon National Primate Research Center, Oregon Health & Science University, Portland, Oregon, USA). The aim was to demonstrate the cellular, molecular, and clinical overlap between NF1, NF2 and schwannomatosis and to highlight that a collaborative approach between scientists and clinicians will facilitate treatment that is tailored to the individual. The conference comprised keynote lectures from leaders in multiple fields, perspective talks and clinical and basic science invited talks with platform presentations selected from submitted abstracts in each session. Highlights of the findings presented in each of the sessions are discussed below.

MEETING SESSIONS

The session entitled Genes, Genotypes and Phenotypes was chaired by Dr. Ludwine Messiaen (University of Alabama, Birmingham, USA) and Dr. Miriam Smith (University of Manchester, UK). An improved understanding of the correlations between diverse genotypic variations in the neurofibromatoses and the complex phenotypic manifestations is important

for enhancing the clinical management of affected individuals and potentially paves the way for targeted therapeutic interventions.

In the opening perspective talk, Dr. Bruce Korf (University of Alabama, Birmingham, USA) discussed the range of treatments currently available for NF1. He drew attention to new techniques that are being developed in the laboratories of Dr. Bob Kesterson and Dr. Deann Wallis (University of Alabama, Birmingham, USA), targeting specific mutations or mutation types in the *NF1* gene (Wallis et al., 2018). The *NF1* gene encodes the protein neurofibromin that negatively regulates the proto-oncogene p21Ras, thereby controlling cell growth and proliferation. Dr. Korf highlighted his group's work on the development of antisense oligonucleotides, designed to induce skipping of affected exons and the use of nonsense read-through drugs to prevent nonsense-mediated decay caused by truncating mutations.

Dr. Hildegard Kehrer-Sawatzki (University of Ulm, Germany) summarized the current knowledge concerning the mechanisms underlying *NF1* microdeletions, as well as their associated genotype-phenotype correlations (Kehrer-Sawatzki et al., 2018). She reviewed the known phenotypic relationships - earlier age at onset, limb overgrowth, tall stature, dysmorphic facial features, cognitive impairment and cardiovascular anomalies. Individuals with NF1 and microdeletions have a higher tumor burden (including subcutaneous, spinal nerve root and plexiform neurofibromas) than people with NF1 without microdeletions. Other genes that are lost in microdeletions and which influence the NF1 phenotype were discussed. For example, when polycomb repressive complex 2 subunit (*SUZ12*) is co-deleted, there is an increase in the development of malignant peripheral nerve sheath tumours (MPNST), which have a 15.8% lifetime risk of occurring in NF1 individuals (Uusitalo et al., 2015). Co-deleted genes are an important area of further study and are of great potential significance for NF1 therapy.

Dr. Magdalena Koczkowska (University of Alabama, Birmingham, USA) reported a study examining the phenotypes associated with recurrent germline missense mutations affecting one of five adjacent amino acids from codons 844–848 of the *NF1* gene (Koczkowska et al., 2018). This study demonstrated that the mutations affecting this region lead to a more severe clinical presentation, including a higher number of bone abnormalities, optic pathway gliomas and plexiform and spinal nerve root neurofibromas than would be anticipated as part of the NF1 classical phenotype. The research underscores that NF1 missense mutations may not always have a mild phenotype.

Dr. Laura Papi (University of Florence, Italy) gave an overview of new and published results from her laboratory obtained through genetic testing of schwannomatosis patients, who have mutations in the *NF2* gene, which encodes the merlin tumor suppressor protein, as well as other genes including SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (*SMARCB1*), or leucine zipper like transcription regulator 1 *(LZTR1*) (Paganini et al., 2015). Dr. Papi assessed different genotypic groups with their resulting phenotypes and also compared these groups with individuals with schwannomatosis in whom no known genetic cause has been identified. These comparisons may be used for future stratification and prognostic prediction in schwannomatosis.

Dr. Brigitte Widemann (National Cancer Institute, Washington D.C., USA) and Dr. Scott Plotkin (Massachusetts General Hospital, Boston, USA) chaired the session on NF Tumors, which exemplified the progress that has been made towards therapy by a cohesive, co-ordinated multidisciplinary approach to basic and clinical research.

Dr. Wade Clapp (Indiana University School of Medicine) gave an overview of the development of the new therapies for NF1 and NF2. He emphasized that benign and malignant tumor formation in the central and peripheral nervous system is a cardinal manifestation of neurofibromatoses and is associated with significant morbidity and early mortality. Recent genome-wide sequencing studies have detected frequent somatic *NF1* mutations in sporadic cancers including glioblastoma, acute myeloid leukemia and adenocarcinoma of the lung (Philpott, Tovell, Frayling, Cooper, & Upadhyaya, 2017). Mutations in the *NF2* gene are the initiating causes of sporadic schwannomas and meningiomas, and contribute to neurological malignancy (Galani et al., 2017).

Drs. Aerang Kim (Children's National Medical Center Washington D.C., USA) and Anat Stemmer Rachamimov (Massachusetts General Hospital, Boston, USA) highlighted key findings from the 2016 "State of the Science" Conference, sponsored by the National Cancer Institute and the Children's Tumor Foundation (Reilly et al., 2017). The goal was to summarize current knowledge of NF1-related malignant peripheral nerve sheath tumors (MPNST) and to propose strategies for improving diagnosis and management, emphasizing the need for more effective combination therapy trials.

A working group of six expert pathologists reviewed the histology and immunohistochemistry of neurofibromas that are borderline for malignancy and proposed new terminology to replace atypical neurofibroma. Dr. Stemmer Rachamimov described the features of atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP). The definition includes at least two of: loss of neurofibroma architecture, high cellularity, nuclear atypia and <3/10 mitoses per high power field. They usually present as distinct nodular lesions on magnetic resonance imaging (MRI) and may be asymptomatic or enlarge, cause pain or neurological deficit (Higham et al., 2018). They are regarded as precursors of MPNST as they exhibit loss of *CDKNA2A/B* (cyclin dependent kinase inhibitor) and increased uptake on ^{18}fluorodeoxyglucose positron emission tomography. Complete surgical excision is recommended to avoid malignant transformation, unless intervention would cause significant morbidity. Longitudinal evaluation and prospective studies will determine capacity for malignant change in these tumors and potentially lead to early diagnosis of MPNST.

Dr. Pierre Wolkenstein (Hôpital Henri-Mondor, Paris, France) and Dr. Jaishri Blakely (Johns Hopkins Hospital, Baltimore, USA) summarized the findings of a multidisciplinary group investigating cutaneous neurofibromas (cNF) sponsored by the Neurofibromatosis Therapeutic Acceleration Program (NTAP). Dr. Wolkenstein noted that cNF have significant impact on quality of life in NF1 because of pain, itching and bleeding and visibility. He proposed that the term cNF should be used rather than "dermal neurofibroma" to refer to neurofibromas that are limited to the skin. These lesions are localized, but not encapsulated and are not clearly associated with myelinated nerves (Jouhilahti et al., 2011)

Dr. Blakeley also discussed the recommendations of international experts and a funding initiative from NTAP to improve understanding of cNF and develop effective therapy. The working group underlined the complexity of cNF related to the variation in time of appearance, the area of the body affected. The aims were to explore tumor initiation and progression, development of therapies and design, optimization and implementation of clinical trials.

Dr. James Gusella (Harvard, Boston, USA) reported the progress made by the CTF sponsored NF2 Synodos (preclinical) Consortium. A group of international investigators from collaborating laboratories worked together to develop, implement and improve a pipeline for drug testing for NF2-associated tumours. Drugs are screened against normal cells that are the targets for tumor formation, including Schwann cells for schwannoma and arachnoid cells for meningioma. The drugs are also tested against their merlin-deficient counterparts and in genetically modified mouse models or mouse models incorporating a human tumor xenograft. Use of whole genome transcriptomics and active kinome assessment optimizes the pipeline for further drug screens and to make all data available to the research community.

Dr. Gelareh Zadeh (Toronto Western Hospital, Canada) described an integrative genomic analysis to determine the somatic landscape of sporadic and NF2-associated schwannomas (Agnihotri et al., 2016). Analysis of 125 samples detected disruption of *NF2* and recurrent mutations in ARID1A (AT-Rich Interaction Domain) and ARID1B, both subunits of the SWI/SNF chromatin remodelling complex that are often mutated in cancer cells (Shain & Pollack, 2013) in DDR1 (Discoidin domain receptor family, member 1), a receptor tyrosine kinase activated by collagens and often expressed in highly invasive cancer cells (Alves et al., 1995) and other novel mutations. In addition, genome-wide methylation profiling identified four molecular sub-groups, each with unique molecular signatures. Finally, in 12/125 tumours (10%) RNA sequence analysis showed an in-frame fusion of SH3PXD2A, which has been implicated in multiple cancers e.g. (Stylli, I, Kaye, & Lock, 2012) and HTRA1, the high temperature requirement serine peptidase A1 gene, arising from a balanced inversion on chromosome 10q. Expression of the SH3PXD2A-HTRA1 fusion resulted in elevated phosphorylated-extracellular signal-regulated kinases (ERK) and increased tumorigenesis, which could be inhibited by blocking ERK through mitogen-activated protein kinase (MEK) inhibition.

Dr. Lei Xu (Massachusetts General Hospital, Boston, USA) discussed the potential role of the hepatocyte growth factor (HGF) / c-MET pathway in the progression of schwannomas (Zhao et al., 2018). In a previous clinical trial of the vascular endothelial growth factor (VEGF) inhibitor bevacizumab, an inverse relationship between hearing response and baseline HGF levels was observed. Radiotherapy combined with c-MET blockade in a preclinical schwannoma model enhanced the efficacy of radiation and resulted in improved hearing and decreased vestibular schwannoma growth, suggesting a possible novel therapeutic approach.

Dr. Jianqiang Wu (Cincinnati Chidren’s Hospital, Ohio, USA) showed significant reduction of neurofibroma volume in an $Nf1^{flox/flox;DhhCre}$ neurofibroma mouse model by inhibiting

both mitogen active protein kinase (MAPK) and the Signal Transducer and Activator of Transcription (STAT) pathways. The combination therapy inhibits cell proliferation and also induces CD68+ cells and the overexpression of a macrophage (M2) marker which inhibits the induction of pro-inflammatory cytokines. The long-term effects of this therapy are still being evaluated.

Dr. Adrienne Watson (Recombinetics Inc, St. Paul, Minnesota, USA) provided an update of a swine model of NF1 that harbors a known human *NF1* premature termination codon. The resulting pig model replicates to a high degree the broad spectrum of disease that develops in individuals with NF1 (Meyerholz et al., 2017). She demonstrated that the NF1 mini pig meets the diagnostic criteria for NF1 manifesting café au lait patches, cutaneous neurofibromas and is associated with ras hyperactivation. The pigs are being monitored longitudinally to characterize the NF1 phenotype. Pharmacological studies are evaluating pain medication and MEK inhibitors in this novel model. The aim is that the model will help discover new treatments and serve as a superior platform to evaluate the safety and efficacy of novel NF1 therapies prior to Phase 1 clinical trials.

Optic pathway gliomas occur as low grade pilocytic astrocytomas in NF1 and multiple pathways are involved in the development of these tumours, including the ras / MAPK pathway (de Blank et al., 2017). Advances in molecular biology have facilitated targeted treatment of these tumors. On behalf of the Paediatric Brain Tumor consortium, Dr. Roger Packer (Children's National Medical Center, Washington D.C., USA) presented the results of a prospective, phase two study of the MEK inhibitor Selumetinib in children with NF1-assocatied optic pathway gliomas. Inclusion criteria included either refractory or progressive tumor on neuroimaging or decline in visual acuity. Ten of 25 (40%) patients achieved a partial response with a two-year progression free survival of 96% +/− 4%. Visual assessments were ongoing and toxicity included raised creatine phosphokinase, gastrointestinal disturbance, rash, paronychia and neutropenia. Large prospective studies were planned with validated functional outcome measures to determine the usefulness of this therapy.

Dr. Brian Weiss (Cincinnati Children's Hospital, Cincinnati, USA) reported the findings of an NF Clinical Trials Consortium phase 2 trial of the MEK inhibitor PD-0325901 in NF1-related plexiform neurofibromas, which contribute significantly to the morbidity of many NF1 patients. Nineteen individuals were enrolled age 16–39 years (median 24 years). Eight (42.1%, 95% CI 20%, 67%) had a partial response by cycle 12. The drug was well tolerated and the most common dose limiting toxicity was an acneiform rash in 3/19 patients (16%). It is possible that volume changes of plexiform neurofibromas may be smaller in adults compared to children because the tumors grow faster in younger people. Further research should determine whether other functional outcome measures such as motor function and pain improve with treatment in adults.

The non-Tumor manifestations of NF session was chaired by, Dr. Aaron Schindeler (University of Sydney, Australia) and Dr. Nicole Ullrich (Boston Children's Hospital, Boston, USA). Although NF1 patients have the greatest number of non-tumor-associated manifestations directly linked to their disease, patients with NF2 and schwannomatosis also

experience non-tumor associated complications. Dr. Schindeler described that muscle weakness and fatigue can have profound effects on quality of life and is an under-recognized challenge for people with NF1. Intramyocellular lipid deposits have been found in both *NF1*-deficient human and *Nf1*-null mouse muscle, consistent with myopathy. Weakness and lipid build-up were rescued in a knock-out mouse model with a medium-chain fatty acid and carnitine-enriched diet (Summers et al., 2018), suggesting a potential therapy for patients.

Dr. Juliana Souza (Federal University of Minas Gerais, Brazil) presented her work on resting metabolic rate (RMR) and body composition (BC) in NF1, which are known to vary according to health status. Predictive equations were used to estimate energy requirements to avoid high cost and time-consuming procedures. Adiposity was measured by dual X-ray absorptiometry (DXA). RMR was measured by indirect calorimetry (mRMR) in 26 NF1 adults, mean age 34.3 years ± 6.1, and compared with eight different predictive equations (pRMR). The mean mRMR was 1633.9 ± 471.1 kcal and the pRMR ranged from 1244.6 ± 239.9 kcal to 1519.9 ± 271.1. Energy expenditure equations in general use underestimated RMR with large differences and low accuracy compared with the indirect calorimetry and the dual X-ray absorptiometry method. The efficacy of using these methods to assess NF1 patients will require additional investigations.

Dr. David Stevenson (Stanford University, California, USA) discussed the clinical spectrum of scoliosis in NF1, noting that the aetiology of dystrophic scoliosis has not been established. In two NF1 individuals with scoliosis, vertebral tissue and matched blood samples showed that loss of heterozygosity (LOH) was in the intervertebral disc and in a paraspinal neurofibroma respectively. The bone adjacent to the paraspinal neurofibroma demonstrated significant osteoid suggesting a mineralization defect that is likely to be secondary to a local paracrine effect of the neurofibroma on adjacent bone.

Dr. Seyedmohammad Tahaei (Vanderbilt University, Nashville, Tennessee, USA) shared his research on the osteogenic differentiation potential of *NF1*-deficient osteoprogenitors (Tahaei et al., 2018). Pseudarthrosis is a long bone dysplasia that manifests in young children with NF1 and causes significant morbidity through bone fracture and delayed healing. Multiple studies have shown that Nf1-deficient osteoprogenitors are characterized by a reduced response to osteogenic cues. Dr. Tahaei and colleagues revealed that pro-epiregulin (EREG) and epidermal growth factor receptor (EGFR) were significantly over-expressed in NF1 associated pseudarthrosis cells but not TGFB1 (transforming growth factor B1) and its receptor TGFBR1. EGFR stimulation is known to inhibit osteogenic differentiation and the researchers postulated that chronic EGFR stimulation in Nf1-deficient skeletal progenitors contributed to their reduced osteogenic differentiation potential. Inhibition of Egfr signalling using Poziotinib (tyrosine kinase inhibitor) or AG-1478 (a selective EGFR inhibitor) or blocking the epiregulin ligand, did not correct the differentiation deficit of Nf1-deicient bone marrow stromal cells. Inhibition of EGFR signalling is therefore unlikely to promote bone healing in children with NF1 associated pseudarthrosis and further studies are needed to determine the cause of impaired osteogenic differentiation and bone repair.

Generalized peripheral neuropathy is independent of local tumor compression and is a recognized manifestation of NF1 and NF2 and has been described recently in schwannomatosis (see below). Dr. Helen Morrison (Leibniz Research Institute for Aging, Germany) presented her work on neuropathy demonstrating that merlin haploinsufficiency in neurons in an Nf2 mouse model contributes to atrophic, damaged axons and to neuropathic symptoms over time (Schulz et al., 2013; Schulz, Zoch, & Morrison, 2014). These findings support the hypothesis that loss of NF2 function can induce peripheral neuropathy directly.

Dr. Victoria Williams (Guy's and St Thomas' NHS Foundation Trust, London, UK) gave a platform presentation on clinically significant neuropathy in 175 patients attending the London national NF2 service and assessed frequency, clinical manifestations and neurophysiology. Clinically significant, symmetrical length-dependent, axonal neuropathy was found in 33 of 175 (19%) NF2 patients and was not present in children. NF2 neuropathy was associated with more severe genetic mutations and a higher burden of lumbosacral nerve root schwannomas. Dr. Williams highlighted the importance of distinguishing generalized peripheral neuropathy from cauda equina tumors to inform treatment decisions.

Dr. Matthew Evans (Guy's and St Thomas' NHS Foundation Trust, London UK) gave a retrospective review of 60 schwannomatosis patients identifying a length-dependent, axonal sensorimotor neuropathy in 6.7% of this cohort, confirmed on neurophysiology. Common causes of axonal peripheral neuropathy including diabetes mellitus were excluded. Mutation, age, gender and self-reported pain did not differentiate patients with schwannomatosis neuropathy from individuals with schwannomatosis and no neuropathy. This is the first report of generalized peripheral neuropathy in schwannomatosis and expands the clinical phenotype.

Three speakers were selected to give platform presentations in the Basic Science Session. Dr. Thomas De Raedt (Harvard Medical School, Boston, USA) showed that programmed cell death protein 1 (PD1) checkpoint blockade, as a single agent, is ineffective in a genetically engineered mouse model for MPNST, even though these mouse tumors have a reasonable mutation burden. Surprisingly, with combined inhibition of MEK and bromodomain protein 4 (BRD4), a rapid (5 days) influx of cytotoxic T lymphocyte (CTLs), (CD8 positive T-cells) in the tumor is observed. Excitingly, adding the anti-PD1 antibody to the MEKi/BRD4i therapy, significantly enhanced tumor regression in the MPNST model because of a more favorable immune microenvironment. However, PD1-checkpoint blockade does not cooperate with MEK or BRD4 inhibitors as single agents. Dr De Raedt also showed that the enhanced tumor regression is CD8 dependent, and performed a detailed study of how these compounds reshape the immune microenvironment.

Dr. Kyle Williams (University of Minnesota, USA), used Clustered Regularly Interspaced Short Palindromic Repeat /CRISPR associated protein 9 (CRISPR/Cas9) and created immortalized human Schwann cell lines deficient for the *NF1* gene. These were paired with isogenic wild-type parental cells to identify synthetic lethal interactions and exploitable vulnerabilities. Using these cells, a large-scale screen identified novel drug classes that selectively kill the *NF1*-deficient cells. Dr. Williams is also performing synthetic lethal

genetic screens to identify therapeutic targets in an unbiased manner. This is a novel approach for identifying potential NF1 therapies.

Ashley Turner (University of Alabama, Birmingham, USA) presented her research on exploring new therapies that directly target specific genetic mutations in NF1. Her aim is to assess the ability of nonsense suppression therapies to treat *NF1* nonsense mutations, as they comprise nearly 20% of *NF1* mutations characterized to date. She presented data on a novel *Nf1* loss-of-function mouse model with acute loss of gene function systemically, which illustrates that neurofibromin is essential to sustain life in the adult mouse. A preliminary nonsense suppression drug study showed rescue of the lethality in the acute knockout adult mice. Further *in vitro* and *in vivo* nonsense suppression therapy studies are ongoing.

The session on Learning, Memory and Behavior Through the Ages focused on the clinical spectrum, measurement and underlying mechanisms of the cognitive deficits that are associated with NF1. The chairs were Dr. Eric Legius (Center for Human Genetics, Leuven, Belgium) and Dr. Ype Elgersma (Erasmus MC University Medical Center, Rotterdam, The Netherlands). The session began with a presentation from Dr. Legius, who summarized the current knowledge of cognitive impairment in NF1 and pointed out that until now the emphasis has been on children with little focus on the problems encountered by adults, particularly elderly individuals. The clinical variability of the NF1 cognitive phenotype has not yet been explained and it is uncertain whether this variability is also encountered in the general population. Dr. Legius hypothesized that the range of NF1 associated cognitive problems could be related to second hit mutations in the brain. He posed the question as to whether the loss of *SUZ12* in *NF1* microdeletion, (a core component of polycomb repressive complex 2 that functions as a histone methyltransferase that promotes epigenetic gene silencing), contributes to the more severe cognitive phenotype in these individuals.

Dr. John Constantino (Washington University, St. Louis, USA) described the results of a large, international, multi-site study that assessed autistic traits in 531 people with NF1; the median age was 11 years (range 2.5 to 83.9 years), (Morris et al., 2016). Quantitative autistic trait scores (QAT) were continuously distributed and pathologically shifted with 13.2 % of patients having scores in the most severe range. The male to female ratio (1.6:1) was less pronounced than in sporadic (4:1) autistic spectrum disorder (ASD). First degree relatives with NF1 showed higher correlation for ASD than families with autism in the general population, implying a strong effect of the *NF1* gene on the disorder. Future research on NF1 as a quantitative trait locus for autistic social impairment may contribute to understanding the underlying pathophysiology of ASD.

Dr. Jonathan Payne (Murdoch Children’s Research Institute, Melbourne, Australia) reported that NF1 children have reduced social information processing, theory of mind, abnormal visual scanning of faces and attention and inhibitory control problems (Pride, Korgaonkar, North, Barton, & Payne, 2017). He reviewed data from recent functional magnetic resonance imaging (fMRI) studies with the aim of unraveling the underlying neural mechanisms of inhibitory control in NF1 children and matched controls. Impaired response inhibition is a cardinal feature of neuropsychiatric disorders and the right inferior frontal gyrus is a crucial structure for successful response inhibition. Abnormal NF1 activation patterns were

identified in several cortical locations including hypoactivation in the right inferior frontal gyrus. Children with NF1 also exhibited hypo-activation in key components of the ventral attention system. These findings provide evidence of dysfunction within critical cognitive networks in NF1 and may prove to be useful surrogate outcome markers for treatment responses in targeted therapeutic trials.

Assessment of resting state networks can reveal differences in the functional architecture of the developing brain related to NF1 mutation and neurofibromin deficiency. Dr. Ben Shofty (The Gilbert Israeli NF Center, Tel Aviv, Israel) described automated clustering analysis of resting state networks. An abnormal organization of association networks, particularly deficient long-distance, functional connectivity was observed in NF1 children. Most notably cortico-striatal functional connectivity was altered, demonstrating diminished functional connectivity between striatum and the ventral attention network, as well as the posterior cingulate area, which is associated with the default network (Pride, Korgaonkar, North, & Payne, 2018). Conversely, somato-motor functional connectivity with the striatum was increased. The suggestion is that abnormal striatal function may play an important role in the pathogenesis NF1 cognitive problems. These findings are particularly noteworthy because cortico-striatal dysfunction might play a role in the etiology of ASD.

Dr. Michaela Fenckova (Radboud University, Nijmegen, The Netherlands) reported that genetic studies in flies identified the ras-MAPK signaling pathway as a pivotal pathway for habituation, an evolutionary conserved fundamental form of learning. Habituation defects associated with NF1 and increased ras-MAPK signaling originated from inhibitory, GABAergic neurons. This is significant because it recapitulates previous findings in mice that GABAergic dysfunction underpins the cognitive deficits in NF1. However, decreased ras-MAPK signaling in excitatory neurons specifically inhibits the plastic habituation response. This binary mechanism may have implications for treatment of rasopathies and suggests that the aim should be to restore circuit function and balances, rather than decreasing ras activity per se. The habituation defects of the Nf1 drosophila model can be partially corrected with lamotrigine, a promising novel therapy that rescued the cognitive deficits in an Nf1 mouse model (Omrani et al., 2015). A randomized controlled trial of lamotrigine in NF1 children is in progress in Rotterdam, The Netherlands and Leuven, Belgium. Drosophila habituation testing permits high-throughput screening and establishes this method as a platform for unbiased screening of novel drugs that might be beneficial for cognitive impairment in NF1 and related disorders.

The session on New Advances in NF2 and Schwannomatosis was chaired by Dr. Marco Giovannini (UCLA, California, USA) and Dr. Gareth Evans (University of Manchester, UK). Model systems were the focus for investigating the pathogenesis of schwannomatosis and NF2 at molecular and cellular levels. The first speaker in the session was Dr. Jeremie Vitte (University of California, Los Angeles, USA), who reported on a mouse model of schwannomatosis. Germline pathogenic variants of the *SMARCB1* gene predispose to two distinct tumor syndromes: rhabdoid tumor predisposition syndrome and familial schwannomatosis. The first is associated with malignant tumors mostly developing in the central nervous system and kidney in children. Familial schwannomatosis is characterized by a typically adult onset of benign tumors involving mainly the spinal nerve roots and

peripheral nerves, but cranial nerves are also affected. To understand the origin of these two types of *SMARCB1*-associated tumors, Dr. Vitte and co-workers generated different tissue and developmental stage-specific conditional knockout mice carrying *Smarcb1* and/or *Nf2* deletion. *Smarcb1* loss in early neural crest was necessary to initiate tumorigenesis in the cranial nerves and meninges with typical histological features and molecular profiles 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 developing schwannomas with the same underlying gene variants 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.

Dr. Michel Kalamarides (Hôpital de la Pitié-Salpétrière, Paris, France) generated mice with specific smoothened (*SMO)* pathogenic variants found in 3–5% of patients' meningiomas. Smoothened is a component of the embryonic sonic hedgehog (SHH) signaling pathway. Conditional activation of these SMO variants in these animals leads to meningothelial meningioma formation. Furthermore, Dr. Kalamarides reported on in vitro evidence supporting the efficacy of using the SMO-inhibitor Sonidegib for treating these tumors (Boetto, Apra, Bielle, Peyre, & Kalamarides, 2018). These findings warrant further investigation to determine the therapeutic potential for Sonidegib to treat NF2 meningiomas.

Dr. Steven Matsumoto (Oregon Health and Science University, Portland, Oregon, USA) presented work from his laboratory and the laboratory of Dr. Larry Sherman (Oregon National Primate Research Center, Beaverton, Oregon, USA) on the pain associated with schwannomatosis. They found that inducible conditional disruption of the *Smarcb1* gene in adult mouse Schwann cells does not lead to changes in peripheral nerve morphology, Schwann cell proliferation or alterations in cell cycle-related gene expression in peripheral nerves. However, mice with targeted disruption of *Smarcb1* in Schwann cells demonstrate behavioral phenotypes consistent with increased pain sensitivity. They showed that dorsal root ganglion (DRG) neurons from mice with Schwann cell-targeted disruption of *Smarcb1* express elevated levels of the transient potential cation channel subfamily V member 1 (TRPV1), a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin. They also found that TRPA1 and the calcitonin gene related peptide (CGRP) are elevated in the DRG neurons of mice with Schwann cell-targeted disruption of *Smarcb1*. TRPA1 is an ion channel that acts as a sensor for environmental irritants and CRGP has been implicated in pain signaling. Wild type DRG cells grown in *Smarcb1*-null Schwann cell conditioned media demonstrated elevated cobalt uptake, a marker of TRPV1 activity, compared to cells grown with wild type Schwann cell conditioned media. Consistent with these findings, DRG cultures treated with *Smarcb1*-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients expressed elevated levels of TRPV1, TRPA1 and CGRP as indicated by immunocytochemistry. Collectively, these data indicate that loss of *SMARCB1* in Schwann cells leads to the secretion of a factor or factors that induce the expression of pain mediators in sensory neurons, and suggest a mechanism for schwannomatosis pain.

Two signaling pathways consistently unregulated in *NF2* mutant tumors are yes associated protein (YAP) and target of rapamycin (TOR). Dr. Shannon White (Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington D.C., USA) and coworkers examined the metabolic profiles of SN12C and SC4 *NF2*-deficient tumor cell lines and their dependency on YAP/ transcriptional co-activator with PDZ-binding motif (TAZ) signaling. They showed that in *NF2*-deficient cells, aberrantly activated YAP/TAZ promotes phosphatidylinositol 3-kinase (PI3K)/Protein Kinase B (AKT) signaling to shift the cell's energetics to a glycolysis-driven phenotype, while dampening mitochondrial respiration. This YAP/TAZ-mediated metabolic rewiring prevents excess mitochondrial reactive oxygen species (ROS) accumulation and oxidative stress-induced cell death under nutrient-deprived conditions. Notably, induction of YAP/TAZ knockdown in pre-established orthotopic *NF2* mutant kidney tumors caused tumor regression due to oxidative stress-mediated cell death. These results have revealed not only a previously unappreciated role for the Merlin-Hippo-YAP/TAZ pathway in regulating cell metabolism, but also new metabolic vulnerabilities that could be exploited to selectively kill *NF2*-mutant tumor cells.

Aberrant activation of mammalian target of rapamycin complex 1 (mTORC1) signaling upon NF2 loss in arachnoidal cells (ACs), the cell of origin for meningiomas, had led to clinical trials using rapalogs (RAD001/everolimus) for NF2. Roberta L. Beauchamp in Dr. Vijaya Ramesh's laboratory (Massachusetts General Hospital, Boston, USA) demonstrated that NF2 loss also activates a distinct mTORC2-serine threonine-protein kinase-N-myc downstream regulated gene (SGK1-NDRG1) signaling axis. She also reported that treatment of NF2-null meningiomas using the dual mTORC1/2 inhibitor AZD2014 decreased proliferation with greater efficacy than rapamycin. This has led to ongoing clinical trials with AZD2014 for meningiomas.

This group investigated additional dysregulated pathways in NF2 tumor cells, particularly druggable kinases, by performing a large-scale kinome screen. They identified increased expression/activation of kinases including *1)* robust phospho-erythropoietin-producing hepatocellular receptors EPHA2 (S897) and EPHB1(Y594), downstream Src family kinase pSrc/SFK(Y416), and c-KIT in NF2-null meningioma cells by immunoblotting, and *2)* increased *EPHA2* and *EPHA4* transcription in NF2-null ACs. EPHA2 is often overexpressed/upregulated in human cancers, and their mechanistic studies revealed phosphorylation of oncogenic EPHA2(S897) upon NF2 loss to be MEK/MAPK-dependent. Importantly, treatment with the multi-kinase inhibitor dasatinib significantly inhibited pEPHA2, pEPHB1, c-KIT and Src/SFK in NF2-null ACs and meningioma cells with minimal effect on mTORC1/2 signaling. Conversely, AZD2014 strongly blocked mTORC1/2 pathways with no effect on EPH-receptor tyrosine kinase (RTK) or Src/SFK targets. This activation of independent downstream pathways led them to test effects of combined AZD2014+dasatinib on cell viability/proliferation. Using dose matrix screening, they found that combined AZD2014+dasatinib treatment inhibited proliferation rate and exhibited synergy in NF2-null ACs and primary meningiomas. In addition to mTORC1/2, they showed for the first time that EPH-RTK, SFK and c-KIT pathways are upregulated upon NF2 loss. This supports the potential to co-target mTORC1/2 and EPH-RTK/SFK pathways as a novel, more effective treatment strategy for NF2-deficient meningioma.

Dr. Filippo Giancotti (Memorial Sloan Kettering Cancer Center, New York, USA) and colleagues had previously shown that Merlin suppresses YAP by inhibiting the cullin E3 ubiquitin ligase CRL4-DCAF1, which in turn targets Large Tumor Suppressor (LATS1 and 2) (Li et al. Cell 2010 and Cancer Cell 2014). At the conference, Giancotti reported that the Nedd8-activating enzyme (NAE) inhibitor MLN4924 effectively blocks the activity of CRL4-DCAF1, thus inhibiting YAP. However, neither the inhibitor nor siRNA-mediated knock down of DCAF1 affected TOR activation, suggesting that loss of Merlin activates TOR through a separate mechanism. Intriguingly, MLN4924 inhibited the growth of PDX models of NF2 loss-driven malignant mesothelioma but it did not induce their stasis or regression. However, a combination of MLN4924 and the PI-3K/TOR inhibitor GDC-098 caused either tumor stasis or regression in these models (Cooper et al., 2017). These findings suggest that MLN4942 and TOR kinase inhibitors may exhibit efficacy in *NF2* mutant tumors.

The session on Signaling in Neurofibromatosis was chaired by Dr. Alison Lloyd (University College London, UK) and Dr. Karlyne Reilly (National Cancer Institute, Bethesda, Maryland, USA). Researchers presented the latest results on how signaling pathways are regulated by NF1 and NF2, reporting on the identification of novel targets and mechanisms of action. Dr. Kirill Martemyanov (Scripps Institute, Florida, USA) reported that morphine receptors in the striatum activate Ras signalling by a novel pathway. In a combination of *in vitro* and *in vivo* studies, he found that NF1 is a direct target of the morphine G-protein coupled receptor (GPCR) receptor via the inhibitory binding of Gβγ. These findings indicate a novel mechanism involving NF1, whereby GPCR receptors can regulate Ras activity with important implications for reward pathways and potentially other pathways controlled by similar receptors.

Dr. Rebecca Burdine (Princeton University, New Jersey, USA) described the power of zebrafish and Drosophila models to rapidly assess within an organism the “strength” of mutations associated with the rasopathies, which encompasses a large class of human developmental abnormalities including NF1. Surprisingly, she found that a particular MEK-1 variant could have opposing effects on downstream ras signaling pathways in different tissues- with findings consistent between the two organisms. These findings demonstrate the power of these model organisms for studying the complexities of ras signaling and indicate that these findings are likely to be applicable across species.

Robert Allaway (Sage Bionetworks, Washington, USA) presented studies on the molecular and immunologic characterization of a large study of cutaneous neurofibromas. A consortium consisting of the Children’s Tumor Foundation (CTF), Sage Bionetworks, Mount Sinai and HudsonAlpha collaborated to profile a panel of cNF samples paired with patient matched whole blood samples donated to the CTF biobank. These data have been released as a resource for the community at www.synapse.org/cutaneousneurofibromas. Common signatures include resting mast cell and M2 macrophage infiltration together with mutation within CREB-binding protein and CDC27 pathways. Integrative analysis confirmed the activation of these pathways within the tumors. This is likely to be a valuable resource for the community for further research and to explore treatment options for these poorly understood tumors.

Many tumors have a distinct metabolism that might provide susceptibilities to therapeutic interventions. However, the metabolic features of NF1 tumours have not been well explored. Dr. Rasola (University of Padova, Italy) reported that cells lacking NF1 exhibit enhanced glycolysis and decreased mitochondrial respiration and identified a mechanism that offers therapeutic potential. The mechanism involves a fraction of active ERK1/2 associating with the enzyme succinate dehydrogenase (SDH) and TRAP1, a molecular chaperone of the heat shock protein 90 (HSP90) family. This causes an inhibition of SDH, decreased succinate levels and a resulting decrease in mitochondrial respiration. The interaction is dependent on TRAP1 and disrupting this complex, abrogates tumorigenicity providing a potential new molecular target and approach for the treatment of these tumours.

The product of the *NF2* gene, merlin, has been shown to have many diverse activities in cell signalling, with much research focused on the role in cytoskeletal remodelling and cell-cell junction signalling. Dr. Chunling Yi (Georgetown University, Washington D.C., USA) expanded on a recently identified role of merlin in regulating the redox state of the cell. Merlin acts upstream of ras-related C3 botulinum toxin substrate 1 (Rac1) to inhibit Rac1-dependent upregulation of ROS. Using a mouse genetically-engineered liver tumor model of *Nf2* loss, her group has shown that Rac1 has both tumor-promoting and tumor-inhibiting properties due to upregulation of tumor-promoting cytokines through the NFκB signalling pathway and activating the DNA damage response, leading to activation of p53 and p16 checkpoints and senescence. She discussed the role of glutathione and thioredoxin in scavenging ROS in this system and the implications for treatment of merlin deficient tumors.

As discussed above, merlin plays a role in the Hippo-YAP pathway. Dr. Joseph Kissel (The Scripps Research Institute, Florida, USA) discussed how merlin interacts with YAP and the scaffold protein angiomotin (Amot). Because the role of Amot in regulation of YAP has been controversial, his talk provided a new fundamental understanding of the regulation of YAP signalling through a shift in its localization upon phosphorylation of Amot on serine 176. Depending on the phosphorylation state of Amot, the YAP-merlin complex can localize to the nucleus, the cytoplasm, or tight junctions in the plasma membrane. At the tight junction, the Amot-YAP-merlin complex associates with E-cadherin and Proteins Associated with Lin 7 (Pals1)/ PALS1- associated tight junction protein (PATJ), whereas in the nucleus the complex facilitates YAP's association with Tea domain family member (TEAD) and the activation of genes involved in cell proliferation. These data resolve previous conflicting studies on the role of Amot by showing the importance of phosphorylation state in regulating Amot's function. These data also highlight how merlin's tumor suppressor function is regulated by subcellular localization.

CONCLUDING REMARKS

Our understanding of the pathophysiology of the neurofibromatoses has grown tremendously over the past three decades. As demonstrated by the presentations summarized here, basic science studies have revealed novel mechanisms underlying both tumorigenesis and other manifestations of NF1, NF2 and schwannomatosis. These studies have also provided innovative platforms for identifying new therapeutic targets that have, in several instances, been advanced to pre-clinical and clinical trials. Ongoing clinical studies have

resulted in refined criteria for the diagnosis and management of patients by their physicians. Outcome measures for novel therapy have been proposed, including the possibility of developing strategies to tailor treatment to the individual. Collectively, the work summarized here offers new directions for understanding NF1, NF2 and schwannomatosis and how to manage and treat these diseases.

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