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First International Conference on RASopathies and Neurofibromatoses in Asia: Identification and Advances of New Therapeutics

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Abstract

The neurofibromatoses, which include neurofibromatosis type I (NF1), neurofibromatosis type II (NF2), and schwannomatosis, are a group of syndromes characterized by tumor growth in the nervous system. The RASopathies are a group of syndromes caused by germline mutations in genes that encode components of the RAS/mitogen-activated protein kinase (MAPK) pathway. The RASopathies include NF1, Noonan syndrome, Noonan syndrome with multiple lentigines, Costello syndrome, cardio-facio-cutaneous syndrome, Legius syndrome, capillary malformation arterio-venous malformation syndrome and SYNGAP1 autism. Due to their common underlying pathogenetic etiology, all these syndromes have significant phenotypic overlap of which one common feature include a predisposition to tumors, which may be benign or malignant. Together as a group, they represent one of the most common multiple congenital anomaly syndromes estimating to affect approximately one in 1000 individuals worldwide. The subcontinent of India represents one of the largest populations in the world, yet remains underserved from an aspect of clinical genetics services. In an effort to bridge this gap, the *First International Conference on RASopathies and Neurofibromatoses in Asia: Identification and Advances of New Therapeutics* was held in Kochi, Kerala, India. These proceedings chronicle this timely and topical international symposium directed at discussing the best practices and therapies for individuals with neurofibromatoses and RASopathies.

Keywords

Clinical trial; diagnostic criteria; neurofibromatoses; RASopathy; RAS/MAPK; signal transduction pathway; therapy

INTRODUCTION

The neurofibromatoses, which include neurofibromatosis type I (NF1), neurofibromatosis type II (NF2) and schwannomatosis, are a group of syndromes characterized by tumor growth in the nervous system (Blakeley & Plotkin, 2016). The RASopathies are a group of syndromes caused by germline mutations in genes that encode components of the Ras/mitogen-activated protein kinase (MAPK) pathway (Rauen, 2013; Tidyman & Rauen, 2016).

Together these genetic syndromes are one of the most common multiple congenital anomaly syndrome groups affecting more than 1 in 1000 individuals, but they may be under-identified especially in countries where medical genetics expertise and molecular testing are limited. To some extent, the neurofibromatoses and RASopathy mutations affect the RAS pathway which is a signal transduction pathway that is a well-known driver in oncogenesis but is also critical in human development and cellular homeostasis. Although each of these syndromes are distinctive, due to the common underlying pathogenetic mechanisms of RAS/MAPK pathway dysregulation, these syndromes have overlapping phenotypes which may include craniofacial dysmorphology, cardiac malformations, cutaneous, musculoskeletal, and ocular abnormalities, neurocognitive impairment, and neoplasia both benign and malignant. The advantage of discussing the neurofibromatoses and the RASopathies together as a group of syndromes allows for the consideration of common therapeutic options.

India represents one of the largest populations in the world, yet remains underserved from an aspect of clinical genetics services. Clinical geneticists and neurologists who may specialize in these syndromes typically practice in the larger cities. In an effort to bridge this gap the *First International Conference on RASopathies and Neurofibromatoses in Asia: Identification and Advances of New Therapeutics* was convened on November 27th to November 29th, 2017 in Kochi (Cochin), Kerala, India. With years of planning, prestigious content experts and attendees including clinicians, basic scientists, physician-scientists, trainees and students from more than 20 countries around the world participated. The meeting was chaired by Meena Upadhyaya along with an international team of co-organizers which included Ashok Pillai, Gareth Evans, Joshi George and Stuart Enoch. The overarching goal of this inaugural three-day symposium was to provide an open forum for participants to discuss basic science and clinical issues of neurofibromatoses and RASopathies focusing on therapy. These proceedings provide the clinical and scientific communities with an executive summary of this clinical translational symposium.

Session 1: Introduction to the RASopathies and Neurofibromatosis Type 1

Session 1 was chaired by Bronwyn Kerr and Eric Legius. The session began with a welcome from Meena Upadhyaya followed by the lamp lighting ceremony symbolizing purity and the new insights to which the conference aspired. The Keynote speaker was Katherine (Kate) Rauen who set the stage for the meeting by providing a comprehensive overview discussing the Ras/mitogen activated protein kinase (MAPK) pathway and the common underlying pathogenetic mechanism of the RASopathies. Dysregulation of the pathway during human development results in overlapping clinical features and medical issues seen in these individuals. Because the RAS/MAPK pathway has been studied extensively in cancer, many small molecules that target this pathway already exist and show great promise as possible treatment options. Susan Huson then provided an overview of NF1 including the diagnosis, natural history and management of the syndrome. The diagnostic criteria for NF1 have traditionally relied on café-au-lait macules (CALMs), intertriginous freckling, neurofibromas, Lisch nodules, bone lesions, optic pathway gliomas (OPGs) and family history. The most common misdiagnosis is pigmentary miscegeny which is abnormal pigmentation arising from parents of different ethnicity. Challenges with the current diagnostic criteria arise from segmental NF1, Legius syndrome (LS) meeting NF1 criteria,

fading of pigmentation with age, congenital mismatch repair deficiency syndrome, NF2 and ataxia telangectasia. Notably, the lifetime malignancy risk is significantly increased. Ratna Puri next discussed the RASopathy clinical experience in New Delhi, India over an 8-year period. During this period, 41 NF1 individuals were diagnosed with pathogenic variants identified in 46%. The sex ratio was not significantly different and 34% had a positive family history. Of the clinical features, CALMs were present in 89%, Lisch nodules in 31% and neurofibroma in 55%. The age of presentation of patients with Noonan syndrome (NS; n=28) varied from 3 months to 22 years. Short stature was a consistent feature in 87% patients and characteristic facial dysmorphism was present in 88%. *PTPN11* mutation accounted for the majority of molecularly confirmed cases. Cardio-facio-cutaneous syndrome (CFC) was seen to a lesser extent. Ashok Pillai discussed the clinical aspects of the Neurofibromatoses Clinic in Kochi, India. Their centralized clinic facilitates a personalized and comprehensive approach to improve both the quality and duration of life in NF patients. Research from this clinic has identified a 17-fold elevation in cerebral spinal fluid medium chain hyaluronan (CSF HA) levels in NF2 subjects. Schwannoma cell secretion of medium chain HA is commensurate to increased proliferation *in vitro* suggesting that CSF HA may have a central role in the tumorigenesis of schwannoma and perhaps other tumors of NF2.

The session concluded with molecular discussions by Ludwine Messiaen and Rick van Minkelen. Ludwine Messiaen described *NF1* mutational spectra and genotype-phenotype correlations. A few clinically relevant genotype-phenotype correlations have been reported to date: 1) individuals with a constitutional *NF1* microdeletion more frequently present with a large number of neurofibromas at a young age, dysmorphic facial features and developmental delay, 2) individuals with p.Met992del present with a milder pigmentary phenotype without externally visible cutaneous neurofibromas (CNs) or plexiform neurofibromas (PNs), 3) missense mutations affecting amino acid 1809 do not develop external PNs or CNs or symptomatic OPGs, and more frequently have NS-like features and pulmonic stenosis, 4) individuals with missense mutations affecting codons 844-848 have a high prevalence of severe phenotype including PNs and symptomatic spinal neurofibromas, symptomatic OPGs, other malignant neoplasms and bone abnormalities. Together, the above mentioned groups may affect counseling and surveillance in a significant fraction of the NF1 population. Rick van Minkelen ended the session discussing pathogenic variant databases and sharing genetic data. DNA variant databases are categorized in three groups: 1) central databases like OMIM, dbSNP, ExAC (gnomAD) or HGMD, 2) gene/disease specific databases like LOVD, ClinVar or Decipher and 3) ‘other’ sources like Genome browsers, Orphanet, GeneCards and SwissProt. There are several gene specific databases for RASopathy related genes that use the LOVD format. The most actively used RASopathy databases are those for *NF1* (www.lovd.nl/NF1; 3892 variants) and *SPRED1* (www.lovd.nl/spred1; 262 variants). Furthermore, databases are available for *NF2*, *LZTR1*, *PTPN11*, *SOS1* and, although more limited, for *SMARCB1*, *HRAS*, *KRAS*, *BRAF*, *NRAS* and *RAF1*. All gene specific databases have some limitations.

Session 2: Neurofibromatosis Type 2

Session 2 focused on NF2 and was chaired by Jaishri Blakeley and Bruce Korf. Gareth Evans presented the epidemiology, genetics, natural history and diagnostic criteria of NF2 with new data about the genotype-phenotype relationships seen in NF2. Truncating mutations are associated with more meningiomas, earlier onset of symptoms and an overall worse prognosis. In contrast, missense mutations result in the mildest disease course. Large deletions and in-frame deletions are also often associated with mild disease course. Splice site mutations are variable, but there is evidence for worse prognosis if the mutation is at the 5' end of the gene. Unilateral vestibular schwannomas (VS) associated with at least two other schwannomas may be due to LZTR1 schwannomatosis rather than NF2 illustrating the overlap in presentation between NF2 and schwannomatosis. Raji Anup discussed the role of clinical nurse specialists (CNSs) in the care of individuals with NF2. Given that NF2 presents most commonly in the young adult with multiple cranial neuropathies, peripheral neuropathies and myelopathy, many specialists are required for optimal management. CNSs can have a pivotal role in supporting patients as they manage progressive hearing loss, visual impairment and neurological disability. Management should focus on medical or surgical interventions along with quality of life and helping patients adjust to the diagnosis. Vijaya Ramesh presented advances in efforts to identify pathways and targets critical to NF2-associated meningioma and schwannoma formation. Multiple cell systems to model the effects of various *NF2* variants on the behavior of arachnoidal and schwann cells are used with the goal to identify pharmacologic targets that abrogate the influence of *NF2* on these cell types. They discovered that the mTOR pathway is critical to meningioma propagation and showed pre-clinically that mTORC1/mTORC2 inhibitor is effective in halting arachnoidal cell proliferation. This work supported the launch of a clinical trial for individuals with *NF2*-driven meningiomas (NCT02831257). Jaishri Blakeley closed the session with a discussion of the current management strategies for the various manifestations of NF2, including the role of clinical trials. Surgery is the mainstay of treatment for sporadic VS, however, surgery for NF2-associated VS can be more complex. Radiation therapy is the next most common therapy for sporadic VS but has uncertain benefit for control of tumor progression and introduces the possibility of both neurologic dysfunction and a small, but measurable risk for late malignant conversion. Drugs have been tried off-label with some sign of activity for VS resulting in clinical trials. Meningiomas are another major concern in NF2 as these can be multiple and affect critical areas causing multiple neurologic morbidities. Schwannomas can occur on any myelinated nerve and there is a high incidence of cranial neuropathy and multifocal motor and sensory neuropathy. Ependymomas are the rarest tumor in NF2 and generally do not require intervention.

Session 3: Tumors of the Neurofibromatoses

Session 3, moderated by Nancy Ratner and Brigitte Widemann, began with Scott Plotkin providing a historical perspective of schwannomatosis, the third form of the neurofibromatoses. Schwannomatosis can be caused by germline mutations in the *SMARCB1* or *LZTR1* genes; additional genes remain unidentified. Schwannomatosis patients have a predisposition to schwannomas and meningiomas, although bilateral VS are rare and lumbar schwannomas appear to predominate. Tumors show biallelic mutations in *NF2*. More than half of schwannomas show mixed schwannoma/neurofibroma hybrid

histology. Chronic pain is the presenting and main issue for >60% of schwannomatosis patients driving the development of clinical trials for schwannomatosis pain. Eric Legius next discussed atypical neurofibromas referred to as Atypical Neurofibromatous Neoplasms of Uncertain Biologic Potential (ANNUBP), and like benign neurofibromas show the biallelic loss of *NF1* in Schwann cells but in addition they also have loss of the *CDKN2A/B* genes. ANNUBP show features including hypercellularity, nuclear atypia, mitotic activity and loss of neurofibroma architecture, and often arise in pre-existing PNs. Risk for transformation to lethal malignant peripheral nerve sheath tumors (MPNST) is increased in patients with ANNUBP, but if removed surgically, ANNUBP do not recur. Individuals with whole *NF1* gene deletions are also predisposed to MPNST, but appear not to show lesions involving ANNUBP on whole body MRI, suggesting that more than one pathway can lead to MPNST. Next, Michael Fisher discussed OPGs which are pilocytic astrocytomas that occur in 15-20% of children with NF1. In many cases, OPG growth is indolent, but in up to half of individulas, visual deficits occur. Obtaining quantitative measures of visual function (especially acuity) is crucial in management of these patients. New potential biomarkers under investigation to assist in evaluation of patients include Diffusion Tensor Imaging to assess the integrity of the optic radiations and Optic Coherence Tomography (OCT) to measure the thickness of the retinal nerve fiber layer. New therapeutic modalities are being explored for tumors that recur following upfront carboplatin-based chemotherapy regimens including RAS pathway inhibitors.

The *NF1* somatic mutational landscape in sporadic human cancers was reviewed by Meena Upadhyaya. In the 27 years since cloning of the *NF1* gene, it has become clear that *NF1* mutations in sporadic tumors cause resistance to multiple drugs. *NF1* mutations co-occur with mutations in other RAS pathway genes in NF1 mutant melanomas which suggests co-operatively. Conversely, somatic *NF1* mutations in melanomas can drive carcinogenesis in the absence of *BRAF* and *NRAS* mutations. *NF1* microdeletions (co-deletion of 14 genes) as well as *NF1* duplication have been identified in both blood and solid tumors. Whether mutations are biallelic or heterozygous remains unclear, as does whether there are reasons, in addition to its large size, why the *NF1* gene is especially mutable. The identification of *NF1* somatic mutations in a wide spectrum of tumors indicates neurofibromin plays a key role in tumorigenesis far beyond its evident role in NF1 disease. To complement this, Ian Frayling presented his work on NF1 patients with breast cancer looking for possible genotype-phenotype correlations. NF1 patients who develop breast cancer after age 50 show twice the increase in incidence of nonsense mutations, as compared to all NF1 patients. Conversely specific types of missense mutations were present in NF1 patients with breast cancer who were less than 50 years old, while no patients with whole *NF1* gene deletion developed breast cancer. In addition, the predominant somatic *NF1* mutation in sporadic breast cancers is amplification. The data suggest genotype-phenotype correlations for predisposition to breast cancer in NF1, which may depend on the subtleties of gain or loss of function conferred by different mutations. Sheetal Sharda then presented mutation data of 115 RASopathy patients using a RASopathy gene panel. The detection rate for pathogenic/likely pathogenic variants was 81%. The variants detected included missense (53%), nonsense (31%), frameshift (12%) and splice site (4%). Twenty eight percent were classified as variants of unknown significance (VOUS). The major reasons for reporting VOUSs were

lack of phenotype correlation, prediction by *in silico* tools as likely benign, variants found with another significant variant (such as NF2 with NF1) or unavailability of the literature to prove pathogenic/likely pathogenicity of the variant.

Session 4: Translational Research and Clinical Application

Session 4 was chaired by Vijya Ramesh and Michael Fisher and opened with an inspiring presentation from Luis Parada. MPNSTs are the major cause of mortality in NF1 and standard treatments have limited success. These tumors are thought to arise from PNs as a consequence of acquiring additional mutations in either the *p53* or *INK4-ARF* (*CDKN2A/B*) tumor suppressor genes. Genetically engineered mouse models for MPNST harboring *NF1* and *p53* mutations readily form tumors and have an identifiable cancer stem cell (CSC) population. Ablation of CSCs specifically abrogates the ability of the tumors to transplant. These results have therapeutic implications and suggest inhibitors targeting CSCs. Next, Nancy Ratner discussed cross-species transcriptome analyses of mouse and human neurofibromas and MPNSTs which identified global negative feedback of genes that regulate RAS/MAPK signaling in both species. Mouse models provided strong rationale for testing MEK inhibitors in NF1 clinical trials. In addition to dysregulated RAS signaling, macrophages and mast cells infiltrate neurofibroma. The identification of a chemokine-receptor pathway necessary for neurofibroma formation revealed that neurofibroma formation correlates with T cell and dendritic cell (DC) recruitment uncovering an unappreciated role for T cell/DCs in neurofibroma initiation suggesting that targeted anti-inflammatory therapy might slow or prevent neurofibroma development in NF1 patients.

Following this, Uday Khire presented data on the synthesis of a novel allosteric, macrocyclic MEK1/2 inhibitor, CIP-137401, that potently inhibits ERK1/2 activity in cultured cells and tissues of mice after systemic administration. Mice with dilated cardiomyopathy caused by *laminA/C* gene mutation have abnormally increased cardiac ERK1/2 activity. In mice, CIP-137401 was well-tolerated resulting in improved heart and skeletal muscle and prolonged survival. CIP-137401 also displayed potent activity in tumor xenograft models suggesting that CIP-137401 may find clinical utility in the treatment of rare diseases related to Laminopathies and RASopathies. The session concluded with Brigitte Widemann reviewing the landscape of prior clinical trials for PNs and described the recent success with selumetinib, a specific MEK inhibitor, which resulted in PN shrinkage in 71% of subjects in a phase I study for NF1 children with inoperable PN. Subsequently, phase II trials will further characterize the effects of selumetinib in children and adults with PNs or CNs. In addition, a phase II trial combining selumetinib and a mTOR inhibitor has been developed for patients with refractory MPNST.

Session 5: Translational Research and Clinical Application - Continued

This session was moderated by Suma Shankar and Girisha KM and opened with Ype Elgersma speaking on factors underlying the cognitive variance associated with NF1. The cognitive deficits associated with NF1 are generally perceived as highly variable, but detailed studies addressing the extent of the variability, as well as the underlying factors, are lacking. Variability of NF1 cognitive performance highly resembles the general population. Moreover, monozygotic twin pairs with NF1 show the same high interclass correlation as

neurotypical monozygotic twins, indicating that the variance in IQ is mainly genetically determined. Hence, the variability of cognitive function in NF1 is largely determined by natural variation in genetic background, and that the influence of T2-hyperintensities, second-hit mutations, allelic imbalance or genetic modifiers of NF1 is minimal with respect to cognitive function. Next, Pierre Wolkenstein provided a comprehensive overview of the neurofibromatoses. Early research was dedicated to nosology leading to clinical descriptions, then *NF1* and *NF2* genes were identified in 1990 and 1991, respectively. Modifying genes were hypothesized in 1993 to cause variability leading to the identification of *ANRIL* in 2011. In 2007, an NF1-like syndrome, Legius syndrome, was linked to *SPRED1*. In 2008, some familial schwannomatoses were correlated to alteration in *SMARCB1* and a few years later to *LZTR1*. Beside the progress in molecular biology of neurofibromatoses, treatments emerged as highlighted by the increase number of clinical trials. Animal models are numerous and readily used for pre-clinical studies with the hope of ending neurofibromatoses thanks to the dynamism of the community. This two-part session was concluded with Bruce Korf speaking on genome-guided therapeutics for NF1. Most therapeutic efforts interfere with components of RAS pathway signaling or with intercellular signaling. An alternative approach based on restoration of function of the mutated gene or abnormal gene product may be considered. Stop mutations, which comprise ~21% of germline *NF1* mutations, may be approached using drugs that promote nonsense mutation read through. Missense mutations, ~11% of the total, may be amenable to small molecules that alter protein folding. Exon skipping is also under consideration. The development of NF1 model systems will help test genome-guided therapies.

Session 6: Clinical and Molecular Diagnoses: Management of RASopathies from Around the World

Session 6 focused on clinical management from around the world and was facilitated by Susan Huson, Bronwyn Kerr, Kate Rauen and Shubha Phadke. Shubha Phadke first described the clinical and molecular diagnoses of RASopathies in India has revealed genotypic and phenotypic spectrum similar to that seen in other populations. Abeer Alsaegh described the attributes and challenges of the Oman Genetics and Developmental Medicine Clinic, an active service providing clinical genetics service to all tertiary and secondary hospitals in the country. Challenges included finding a primary physician for follow up, lack of multidisciplinary clinics and screening for complications. Sheela Nampoothiri described the NF1 clinic which is jointly conducted with neurosurgery and neurology at the Amrita Institute of Medical Sciences & Research Center, India. Additionally, there is a large cohort of individuals with RASopathies followed in this clinic. Joanne Ngeow described panel testing of 25 cancer susceptibility genes including *NF1* performed on 220 Asian breast cancer patients or their family members referred for genetics risk assessment. Challenges and barriers for genetic testing in Singapore were discussed. Isabel Cordeiro described their genetic service in Portugal including a monthly multidisciplinary Neurofibromatosis Outpatients Clinic. They organize an annual meeting with scientists and patients and have a close collaborative relationship with the NF advocacy group. Shay Ben Shachar (Israel) described an algorithm using retrospective data generated by machine learning-based techniques. Children >29 months old with at least one atypical CALM or less than six CALMs have a 0.9% (95% confidence interval [CI] 0-2.6%) risk for constitutional NF1

while children <29 month-old with greater than 6 CALMs have a high 80.4% risk (95% CI 74.6%-86.2%). Yemima Berman from Australia ended the session describing a pilot program initiating breast MRI screening in NF1 women aged 30-39. This study aims to examine acceptability of breast screening for women with NF1 and develop preliminary experience with breast MRI.

Session 7: Clinical Aspects of RASopathies

The clinical session on RASopathies was chaired by Kate Rauen and Miikka Vikkula. Bronwyn Kerr opened the session discussing NS, a common RASopathy characterized by the triad of congenital heart disease, short stature and recognizable facial characteristics. Although ~50% of cases are due to mutations in *PTPN11*, it is a highly heterogeneous disorder, with at least 12 other genes of the RAS/MAPK pathway known to be mutated in a significant number of cases, and the responsible gene/s remaining unknown in a sizeable proportion of clinically diagnosed patients. Although most of these genes can cause a classic phenotype, other aspects such as hypertrophic cardiomyopathy (*RIT1*, *RAF1*), lymphoedema (*RIT1*) or myelodysplasia (*CBL*) may dominate the clinical picture. Next, Eric Legius discussed that 3-4% of individuals with a clinical diagnosis of NF1 have LS, caused by heterozygous mutations in the *SPRED1* gene. Individuals with LS have multiple CALMs with or without freckling but they do not show the increased tumor risk seen in NF1. They do not show the typical choroidal noduli seen by OCT in NF1 and this feature can be used to differentiate both conditions in children. It has been shown that SPRED1 binds with neurofibromin and recruits neurofibromin to the plasma membrane where neurofibromin can downregulate active RAS. Bronwyn Kerr then discussed Costello syndrome (CS) which is a developmental disability syndrome associated with moderate developmental delay, short stature and macrocephaly, congenital heart disease, loose skin on the hands and feet, distinctive facies and warts at moist surfaces. CS is due to activating mutations in *HRAS.* Most CS patients have a missense mutation resulting in p.G12S, with p.G12A being the next most common. Cancer in early life presents a significant health risk with a 15% chance of developing cancer. Expert opinion has suggested a screening protocol. CFC, discussed by Kate Rauen, is characterized by cardiac abnormalities, distinctive craniofacial appearance and cutaneous abnormalities. Like other RASopathies, CFC shares many overlapping phenotypic features, but mostly with NS and CS. The diagnosis is based on clinical findings and molecular genetic testing. The four genes known to be associated with CFC syndrome are *BRAF*, *MAP2K1*, *MAP2K2* and rarely *KRAS*. These genes are known oncogenes in the RAS/MAPK pathway. Because of the complexity of care, medical management guidelines have been published. Miikka Vikkula wrapped up the session explaining that there are at least two inherited vascular RASopathy syndromes: capillary malformation-arteriovenous malformation 1 and 2 (CM-AVM1 and CM-AVM2). About 50-60% of CM-AVM patients have a loss-of-function mutation in *RASA1* (CM-AVM1) and another 30% in *EPHB4* (CM-AVM2). The overall incidence of fast-flow malformations is about the same in the two forms but the frequency of intracerebral AVMs is lower in CM-AVM2. Loss-of-function of either one seems to lead to activation of RAS signaling. Additional models, preclinical studies and clinical trials are warranted to test the same approach for other vascular anomalies, including the RASopathies.

Session 8: Selumetinib (MEK1/2 inhibitor) in Treating Patients with NF1 and other RASopathies

A stimulating round table discussion of international experts on NF1 and MEK inhibition was facilitated by Gareth Evans and was comprised of 10 researchers and clinicians. Panel members included Jaishri Blakeley (USA), Michael Fisher (USA), Bruce Korf (USA), Eric Legius (Belgium), Luis Parada (USA), Scott Plotkin (USA), Nancy Ratner (USA), Kate Rauen (USA), Brigitte Widemann (USA) and Pierre Wolkenstein (France). The remit was to assess the potential for selumetinib (MEK1/2 inhibitor) in treating NF1 pediatric patients with PN. The panel was confident that selumetinib slows or prevents PN growth and shrinks the majority of PNs. However, there is a need to demonstrate that PN shrinkage is associated with improved patient-reported and functional outcomes. The potential benefit of selumetinib in NF1 is being explored in the US National Cancer Institute-sponsored Phase I/II SPRINT trial in pediatric patients with symptomatic NF1-related PNs. The SPRINT trial will validate patient-reported and functional endpoints, and confirm their utility for future clinical trials. Once efficacy of selumetinib in NF1 has been demonstrated by SPRINT, the optimum treatment and dosing regimen should be defined. It is proposed to follow-up with patients for 7 years after treatment initiation, or 5 years after treatment discontinuation (whichever is longer).

Treatment of PNs is important in order to minimize the risk of progression to aggressive tumors such as MPNSTs. Growth of PNs is most rapid in children, and slows into adulthood (age ~16–18 years onwards). Morbidity may be prevented by treating patients from an early age until the time at which PN growth is expected to have slowed. It is unclear whether treatment with selumetinib could change the natural history of the condition, leading to continued growth of PNs into adulthood. Long-term patient follow-up will be important to understand the impact of chronic selumetinib administration on tolerability. It is conceivable that patients may require drug holidays with long-term selumetinib administration and it is unclear whether a lower dose of selumetinib will be effective as maintenance therapy. There are reports of tumor re-growth following dose reduction. The panel also concluded that selumetinib should also be considered for clinical trial in other RASopathies.

Session 9: Treatments and Future Directions

Session 9 was chaired by Luis Parada and Joshi George and began with an overview of spine surgery for neurofibromas by Anant Tambe who discussed the challenges of spinal surgery in NF1 and the current management strategies devised at the National Complex NF1 Center in Manchester. Surgery is challenging because of anatomical abnormalities, poor bone stock and dural ectasia. Further challenges include increased risk of blood loss, physiological issues due to hypertension related to renal artery stenosis and/or pheochromocytoma. Cognitive impairment and learning difficulties add to the complexity of the consent process. The results of surgery are improving with 1) better understanding of the above, 2) use of spinal cord monitoring reducing neurological injury, 3) better blood preservation strategies and use of cell salvage and 4) technological advances like the ultrasonic bone scalpel and 3D CT modeling to help plan surgery. Newer surgical techniques like trap door laminoplasty are also being used. Suma Shankar next discussed targeting reduction in the activity of RAS signaling using farnesyl transferase inhibitors, and RAF and MEK inhibitors in treating

RASopathies. Several mouse models of RASopathies were presented whereby administration of a MEK inhibitor in both pregnant and nursing female mice normalized phosphorylation levels of ERK1/2 and improved cranial dysmorphia and cardiac defects. The complexity of bringing these potential treatments to clinical trials and the need for collaboration among multiple teams of researchers, clinicians, private industry, and advocacy organizations for eventual therapies was discussed. Bruce Korf concluded with discussing future directions for the RASopathies. He emphasized the need for research and development in the areas of clinical care and therapeutic development, and outlined three "wishes" for future work. In the area of research, he noted the need to better understand in detail the interactions of proteins involved in the RAS pathway. He also suggested the creation of a registry for genotype-phenotype correlations and further need to define pathophysiology. In therapeutic development, there is a need to define endpoints for measurement of efficacy, increased attention to preclinical testing, and the conduction of clinical trials. Finally, in the area of community engagement, he emphasized the need for practice guidelines, the use of patient-facing apps and the value of patient coaches in guiding clinical management.

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