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CTF Meeting 2012: Translation of the Basic Understanding of the Biology and Genetics of NF1, NF2, and Schwannomatosis Toward the Development of Effective Therapies

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Abstract

The neurofibromatoses (NF) are autosomal dominant genetic disorders that encompass the rare diseases NF1, NF2, and schwannomatosis. The NFs affect more people worldwide than Duchenne muscular dystrophy and Huntington's disease combined. NF1 and NF2 are caused by mutations of known tumor suppressor genes (*NF1* and *NF2*, respectively). For schwannomatosis, although mutations in *SMARCB1* were identified in a subpopulation of schwannomatosis patients, additional causative gene mutations are still to be discovered. Individuals with NF1 may demonstrate manifestations in multiple organ systems, including tumors of the nervous system, learning disabilities, and physical disfigurement. NF2 ultimately can cause deafness, cranial nerve deficits, and additional severe morbidities caused by tumors of the nervous system. Unmanageable pain is a key finding in patients with schwannomatosis. Although today there is no marketed treatment for NF-related tumors, a significant number of clinical trials have become available. In addition, significant preclinical efforts have led to a more rational selection of potential drug candidates for NF trials. An important element in fueling this progress is the sharing of knowledge. For over 20 years the Children's Tumor Foundation has convened an annual NF Conference, bringing together NF professionals to share novel findings, ideas, and build collaborations. The 2012 NF Conference held in New Orleans hosted over 350 NF researchers and clinicians. This article provides a synthesis of the highlights presented at the conference and as such, is a “state-of-the-field” for NF research in 2012.

Keywords

neurofibromatosis type 1; neurofibromatosis type 2; NF1; NF2; schwannomatosis; tumor suppressor; SMARCB1; merlin neurofibromin; preclinical models

INTRODUCTION

The Neurofibromatosis (NF) Conference, founded and hosted by the Children's Tumor Foundation since 1985, is the world's premier gathering of scientists and clinicians dedicated to advancing research and care for individuals living with NF1, NF2, and schwannomatosis. The conference has grown significantly in recent years, reflecting significant advances in basic pathogenesis and an expanded interest in NF research and development. The 2012 NF Conference was held June 9–12 in New Orleans, LA. The agenda featured well-balanced sessions on basic science, clinical care, and clinical trials addressing all NF manifestations (NF1, NF2, and schwannomatosis).

Unique to this conference were the sessions in which clinicians and researchers exchanged their findings and brainstormed together on certain specific disease manifestations. These mixed sessions aimed at further fostering multi-disciplinary discussions in order to accelerate the translation of basic NF biology research into better informed clinical trials.

The overwhelming highlight of the meeting was how quickly the field of NF has transformed itself into a therapeutic one. Multiple sessions highlighted the identification of driver pathways followed by the preclinical assessment of best in class modulators of these pathways. This has led to multiple actively recruiting interventional clinical trials in the United States and abroad. The expectation is that this number will further increase because of the close collaboration between basic and clinical researchers.

The current report summarizes the work presented at the 2012 NF Conference and as such, an up to date overview of the inspiring field of neurofibromatosis.

KEY NOTE ADDRESSES

Dr. Luis Parada (UTSW, Dallas, TX) demonstrated that the expression of CXCR4 is increased in Skin Derived Precursors (SKPs) from neurofibromas and MPNST when compared to WT SKPs. Knockdown of CXCR4 induced G1/S arrest and attenuated cyclin D1 expression through the beta catenin network. Following these observations he validated the therapeutic validity of CXCR4 by using a known CXCR4 inhibitor (AMD-3100) and showed a cytostatic effect.

Dr. Rhona Mirsky (University College London) discussed about the plasticity of Schwann cells and their response to injury. Dr. Mirsky could show that a single glial transcription factor (c-Jun) is required to reprogram Schwann cells generating a “repair cell” essential for regeneration of an injured nerve [Arthur-Farraj et al., 2012]. Dr. Jan Tuckerman (University of Ulm, Germany) highlighted the advances in understanding the pathophysiology of bone disease in NF1. Using cre-transgenic mouse lines, Dr. Tuckermann discovered glucocorticoids inhibit cytokines independent of GR dimerization and thereby attenuate osteoblast differentiation, which accounts, in part, for bone loss during GC therapy [Rauch et al., 2010]. He further presented screening systems suitable for identifying factors and compounds to enhance osteo-blast function and thus bone formation, opening up new possibilities to treat bone disorders. Dr. Silvia Cappello (Helmholtz Center Munich, Germany) demonstrated that the genetic deletion of the small GTPase RhoA results in migrational disorder in the developing cerebral cortex. She identified that alterations in RhoA in radial glial cells, rather than neurons, caused these migrational defects [Cappello et al., 2012].

Dr. René Bernards (The Netherlands Cancer Institute) built a strong case for the use of functional genetic approaches and serial tumor biopsies to effectively guide the development of targeted therapies in cancer and understand mechanisms of resistance. As an example he described how tumor biopsies in very few patients identified that the feedback activation of EGFR mediates resistance to the BRAF inhibitor vemurafenib in patients with *BRAF* (V600E) mutated colorectal cancer [Prahallad et al., 2012].

ADVANCEMENT IN NF1

Optic Pathway Tumors

Dr. Uri Tabori (University of Toronto) reviewed aberrant activation of the MAPK pathway as the main molecular abnormality noted in non-NF1 pilocytic astrocytoma. The most

common abnormality identified is the *KIAA1549–BRAF* fusion, which is associated with improved progression-free and overall survival compared with “non-fused” pilocytic astrocytomas [Hawkins et al., 2011]. Pre-clinical work suggests that the *BRAF*-induced growth arrest observed in vitro, is due to oncogene-induced senescence, and helps explain how a mutation that results in tumor formation may also account for its benign behavior [Jacob et al., 2011]. This has implications for understanding NF1-associated optic pathway gliomas, which also have active MAPK signaling, rarely transform into higher-grade tumors, and usually spontaneously stop growing without intervention. Dr. David Gut-mann (Washington University, St. Louis, MO) emphasized the importance of the microenvironment (microglia) in the pathogenesis of optic pathway gliomas in *Nf1* mouse models. In addition, he demonstrated that neural stem cells from different germinal zones exhibit differential responses to *Nf1* gene inactivation, which may partly underlie the spatial and temporal distribution of gliomas in children with NF1 [Solga et al., 2013]. A review of prior, present and future clinical trials for low-grade astrocytoma was presented by Dr. Roger Packer (Children's National Medical Center, Washington, DC). Carboplatin-based regimens continue to be the gold standard for NF1-associated low-grade gliomas based on progression free survival (PFS) rates (5-year PFS of 69% at 5 years) and relatively low toxicity in comparison to other regimens. Vinblastine has been shown to be efficacious for recurrent or refractory tumors, but its efficacy for newly diagnosed tumors has not been adequately evaluated. The combination of vinblastine with carboplatin was poorly tolerated because of bone marrow suppression. Other traditional chemotherapy agents (e.g., temozolomide, procarbazine, etc.) are associated with an increased second malignancy risk. Small studies using bevacizumab with or without irinotecan reveal both radiographic and visual responses. Dr. Michael Fisher (Children's Hospital of Philadelphia) presented an update on the international, multi-center; retrospective study of visual outcomes following chemotherapy for NF1-associated optic pathway gliomas [Fisher et al., 2012]. His recent work reveals that in the 3 years following treatment, visual acuity remains stable in only 47% of subjects and 37% require additional therapy during that time. In addition, patients with normal acuity at the start of treatment were less likely to have a significant decrease in vision or to require additional treatment in the subsequent 3 years. Dr. Rob Avery (Children's National Medical Center) updated his work on the use of optical coherence tomography (OCT) to measure retinal nerve fiber layer (RNFL) thickness in children with optic pathway glioma. He previously showed that RNFL thickness correlated with vision in children >6 years of age. However, the OCT equipment used required the cooperation of the children to perform. Preliminary data using hand held OCT for younger and uncooperative children while they were sedated for MRI scans reveals a correlation between RNFL thickness and decreased vision. RNFL thickness is therefore a potential structural marker of visual loss in this population.

NF1 and the Brain

Efforts in the development of biological targeted interventions that improve quality of life (QOL) in patients with NF1 and of biological markersthatmayhaveutilityfortheevaluationofcurrentandfuture interventions were discussed. Kathryn North (Sydney, Australia) presented that some aspects of the NF1 cognitive phenotype are likely due to developmental changes during brain development. Recent

studies in one *Nf1* mouse model suggest that both the Ras/ MAPK and dopaminergic pathways are contributing to working memory deficits in patients with NF1 [Brown et al., 2010]. These observations and differences in the biological mechanism associated with cognitive deficits in NF provide a basis for the development of specific targeted interventions for cognitive deficits in NF1. Ype Elgersma (Rotterdam, The Netherlands), using a novel mouse mutant lacking the neuron-specific *NF1* exon 9a isoform (*Nf1*$^{9a-/9a-}$ mice), reported that the hyperpolarization-activated cyclic nucleotide-gated (HCN) current (I_h) is selectively attenuated in hippocampal parvalbumin-expressing interneurons. In addition, a HCN channel agonist rescued the synaptic plasticity and learning deficits in Nf1$^{9a-/9a-}$ mice, as well as the commonly used Nf1$^{+/-}$ mouse model, confirming the importance of HCN channel dys-function on cognitive function in NF1. Maria T. Acosta (Children's National Medical Center) provided clinical evidence of significant compromise in social cognition in patients with NF1. Autism spectrum disorder symptoms is associated with NF1 in up to 14% per a recent clinical sample in Washington, DC, and replicated in a sample in England (Manchester) [Walsh et al., 2013]. This is the highest frequency reported in NF1 and has important implications for the evaluation of potential biology related mechanism associated with autism spectrum disorder symptoms in NF1. F. Xavier Castellanos (New York University) proposed intrinsic functional connectivity measured by functional MRI (fMRI) as a potential biomarker for NF1 cognitive deficits. Synchronous patterns of intrinsic (spontaneous) low frequency fluctuations in the blood oxygen level dependent (BOLD) signal in brain recapitulate functionalbraincircuitsinamannerthatisreliableacrosstimeandthatis consistent across labs. Evaluation of BOLD in a small subset of typically developing children and in children with NF1 demonstrated that examination of intrinsic functional connectivity might lead to the discovery of biomarkers useful for dissecting the cognitive deficits entailed by NF1 [Chabernaud et al., 2012]. David Gutmann (Washington University) showed evidence that central nervous system (CNS) axonal length is determined by cyclic AMP (cAMP) regulation of Protein Kinase A (PKA)-mediated actin cytoskeleton dynamics. Using novel *Nf1* genetically engineered mice, he observed that agents which restore striatal dopamine levels in the brain can reverse the behavioral abnormalities in these mice [Brown et al., 2010]. He further showed that raclopride positron emission tomography imaging could be used to reveal dopamine defects in *Nf1* mutant mice [Brown et al., 2011].

Vascular Anomalies in NF1

Jan Friedman (University of British Columbia) reviewed the main cardiovascular features associated with NF1. Congenital heart defects are observed in at least 2.4% of patients. The prevalence of hypertension is not well characterized but is thought to be higher than expected and may be associated with echocardiographic changes. Renal artery stenosis and pheochromocytoma are observed infrequently in patients with NF1. Systemic vasculopathy is often asymptomatic and may present with life-threatening medical complications or sudden death. Physicians caring for patients with NF1 should have a high index of suspicion for congenital heart defects, hypertension and systemic vasculopathy. David Ingram (Riley Hospital for Children, Indiana University School of Medicine) presented his work using genetically engineered neurofibromin-deficient mouse models to study genetic and cellular evidence for vascular inflammation and its contribution to cardiovascular disease in NF1

patients. His two murine models of NF1 vasoocclusive and aneurysm disease closely recapitulate the vascular disease observed in NF1 patients, and demonstrate that *Nf1* heterozygous myeloid cells are the critical cellular effectors of both NF1 vaso-occlusive and aneurysm disease. Finally, Dr. Ingram presented preliminary data demonstrating that statins may inhibit the progression and/or initiation of NF1 vaso-occlusive or aneurysm disease in vivo. These data provide insights into potential therapies for NF1 patients with vascular disease. Nicole Ullrich (Boston Children's Hospital, Harvard Medical School) reviewed cerebrovascular disorders in NF1, which represent a serious and under recognized complication with a putative prevalence rate of 6% for cerebral vasculopathy. Types of observed vascular changes include stenoses, occlusions/compressive vasculopathy, moyamoya syndrome, aneurismal lesions, major artery ectasia and arteriovenous fistulae/ malformations. Risk factors are thought to include younger age, known optic pathway glioma and prior cranial irradiation. Many patients with cerebrovascular arteriopathy are asymptomatic at time of presentation and vascular changes are thought to be progressive in 1/3–1/2 of patients. Dr. Ullrich presented data that suggest early surgical intervention may prevent the development of future strokes and fixed neurologic deficits. A low threshold for further vascular imaging is recommended in patients with NF1 who present with symptoms suggestive of vasculopathy. Edward Smith (Boston Children's Hospital, Harvard Medical School) reported findings from a study of children with asymptomatic vascular changes, which demonstrated variable onset of arteriopathy; however, once narrowing was detected, slow arterial flow was quickly followed by radiographic progression in almost 60% within 5 years. This suggests that there is a narrow window for intervention before strokes ensue. Dr. Smith also presented a potential method to detect urinary biomarkers of angiogenesis, including matrix metalloproteinases and vascular endothelial growth factor, in children with moyamoya compared to matched control subjects as well as in source tissue of the affected blood vessels. These markers may have utility in the detection of vascular changes as well as in predicting outcomes after surgical intervention.

NF1 Bone Abnormalities

Dr. Schorry reviewed the clinical bone phenotype including generalized (osteopenia, short stature, and macrocephaly) and focal(tibial dysplasia/pseudarthrosis, sphenoid wing dysplasia, dystrophic scoliosis, non-ossifying fibromas, and pectus deformities of the chest) osseous abnormalities. Dr. Florent Elefteriou, reviewed that various mouse models have shown that *Nf1* plays a role in regulation of collagen synthesis and bone mineralization. The $Nf1^{prx-/-}$ mouse model affects chondrocytes and osteoblasts, and results in abnormal cartilage formation and deficient bone mineralization, which can be partially rescued with bone morphogenic protein-2 (BMP2). Correction of bone mineralization may be needed for better healing of tibial pseudarthrosis. Potential targets for future therapeutics include transforming growth factor (TGF)-beta1 and extracellular inorganic pyrophosphate. Dr. David Little (Sydney, Australia) reviewed the medical and surgical management of tibial pseudarthrosis,including the importanceof excision of the fibrous hamartoma atthe site offracture,whichisthoughttohavebiallelicinactivationof *NF1*. The rationalefor using acombination ofBMP2 and bisphosphonates in NF1 tibial pseudarthrosis was presented. Dr. Ndong (Vanderbilt University) showed that osteoprecursor cells from $Nf1^{col2-/-}$ mice have severe defects in mineralization and impaired differentiation. BMP2 corrected the

differentiation defect, but not the mineralization defect. Interestingly, treatment with a combination of BMP2 and recombinant human TNSALP (Asfotase) rescued both the differentiation and mineralization defects. Dr. Kolanczyk reported on the cortical humerus findings in his *Nf1Prx1* tibial dysplasia mouse model, which suggest that neurofibromin is required for normal osteocyte function. Dr. Rhodes discussed the role of osteoclasts in NF1 pseudarthrosis. Using *Nf1*$^{flox/+}$;*LysMCre* mice there was an increased frequency of osteoclast progenitors, osteoclastogenesis, and bone resorption in vitro. To examine the role of *Nf1*$^{+/-}$ osteoclasts on fracture healing in vivo, lethally irradiated *Nf1*$^{flox/flox}$;*Col2.3Cre* mice were transplanted with bone marrow cells from wild-type or *Nf1*$^{flox/+}$;*LysMCre* mice. Compared to wild-type bone marrow cells, *Nf1*$^{flox/+}$;*LysMCre* bone marrow cell reconstituted *Nf1*$^{flox/flox}$;*Col2.3Cre* mice with an induced tibial fracture, exhibited significantly reduced callus bone volume fraction showing a deficit in fracture repair. Dr. Yang showed that TGF-beta1 serum levels were higher in *Nf1*$^{flox/-}$;*Col2.3Cre* mice compared to wild-type mice, and that a TGF-beta1 receptor inhibitor rescued some osteoporotic and fracture phenotypes in this mouse model. These animal studies document the rapid progress that has been made using preclinical mouse models to better understand the pathophysiology ofskeletalabnormalitiesinNF1 withpotential therapeutic targetsfor treatment. Dr. Arrington provided a now published detailed case seriesofindividualswithNF1withawidearrayofskullabnormalities highlighting that skull abnormalities in NF1 is an understudied area [Arrington et al., 2013]. Although the primary focus of the session was on bone abnormalities, muscle–bone interactions are under-studied in NF1 and Dr. Schindler reported on some interesting findings of the effects of conditional double inactivation of *NF1* in skeletal muscle, which lead to a severe muscle myopathy and abnormal metabolic function. His data demonstrate that muscle expression of NF1 is critical for viability in mice. Investigation of muscle and metabolic function in NF1 is a new area of research with potential for significant biologic insights.

NF1 Mouse Models, and Preclinical Studies Guiding Clinical Trial Development

Alison Lloyd (UC London, UK) presented mouse models, which demonstrated a central role for the ERK signaling pathway in controlling Schwann cell behavior in the normal adult peripheral nervous system. In mature, quiescent myelinating Schwann cells activation of theERKsignalingwassufficientto drivethese cells back to a progenitor/stem-like state. Moreover, activation of this signaling pathway led to a transcriptional response that, via the secretion of multiple chemokines, orchestrated the multicellular response required for nerve repair and implicated in NF1 tumor formation. NancyRatner (CincinnatiChildren's Hospital, USA)presented now published [Jessen et al., 2013] direct evidence that blocking MEK signalingwassufficienttoprofoundlyshrinkexistingneurofibromas in a mouse model, correlating with modulation of a transcriptional negative feedback loop. Clinical trials with MEK inhibitors for plexiform neurofibromas are ongoing or in development and will allow to evaluate the utility of the mouse model in predicting for response in humans. In contrast, MEK inhibition was only transiently effective in treatment of MPNST models, in which blockade of Aurora kinases provided a durable response [Patel et al., 2012]. Yuan Zhu (U. Michigan, USA) extended the analysis of MEK inhibition to the central nervous system. He found that biallelic, but not heterozygous, inactivation of *Nf1* led to an enlarged corpus callosum—a manifestation recently linked to cognitive deficits in individuals with NF1.

Treatment with a MEK inhibitor during neonatal stages rescued the formation of an enlarged corpus callosum in an *Nf1*-deficient mouse model. Karlyne Reilly (NIH) highlighted the importance of sex in her presentation of an interaction of sex and genotype on brain tumor (astrocytoma) susceptibility in an NF1-associated mouse model. She also presented an update on the development of schweinfurthin as a candidate anti-NF1 therapeutic. Dr. Frank McCormick (UCSF) described a novel mechanistic connection between *NF1* and *SPRED1*, the gene mutated in Legius syndrome [Stowe et al., 2012]. He showed that the SPRED1 protein directly interacts with neurofibromin and recruits it to the membrane, where it can suppress Ras. These findings not only provide a satisfying explanation for the overlapping features of NF1 and Legius syndrome but reveal novel mechanistic insight into how neurofibromin is regulated. For example, SPRED1 binds directly to c-kit and M-CSF-receptor, suggesting that neurofibromin may regulate Ras in the context of these signaling proteins specifically. Dr. McCormick also shared insight into how different Rasisoforms maydifferentially function, which willultimately affect how we think about NF1 and Ras-driven tumors. Dr. Gideon Bollag from Plexxikon reported on the development of PLX4032, a potent and selective inhibitor of oncogenic B-RAF kinase activity. Given favorable pharmacology properties and improved overall survival compared to standard-of-care chemotherapy in malignant melanoma, PLX4032 was approved by US and European regulatory agencies, but resistance frequently develops on treatment. Next generation compounds aimed to combat the development of resistance were presented. These studies illustrate of how kinase inhibitors that target a Ras effector pathway (BRAF) can be developed as a cancer therapy. Dr. Bollag also discussed an additional approach to treat hyperproliferative diseases by targeting the tumor microenvironment. PLX3397 is a selective inhibitor of FMS and KIT kinases. FMS is the receptor for CSF-1, which plays a critical role in a variety of cells within a tumor's microenvironment. PLX3397 has shown anti-tumor activity in a variety of in vivo studies, and is currently in clinical testing in cancer patients. Notably, loss of the *NF1* gene and consequent activation of the Ras pathway in Schwann cells results in secretion of both CSF-1 and SCF, and macrophages and mast cells, which are important constituents of neurofibromas. As such the potential of PLX3397 as a therapeutic for NF1 could be explored in future studies.

Dr. Chris Moertel, from the University of Minnesota described the current status of brain tumor immunotherapy and its relevance to the NF population. An early analysis of the phase I clinical trial (NCT01171469, IND 13887) utilizing GBM6 and autologous dendritic cells was shown, including evidence of immune response in a subset of patients and radiographic evidence of tumor regression in one patient. These early results are encouraging and the progress in the field of immunotherapy for cancers may result in the evaluation of these treatment approaches for patients with NF. Dr. Benjamin Braun, from UCSF, discussed the preclinical development of signal transduction therapy for juvenile myelomonocytic leukemia (JMML). This rare but lethal form of leukemia is strongly associated with NF1 [Loh, 2011]. Sporadic cases in other children are associated with activating mutations in *KRAS*, *NRAS*, and *PTPN11*. Dr. Braun described efforts to use *Nf1* and *Kras* JMML mouse models for preclinical therapeutic studies, in collaboration with Kevin Shannon and the CTF Preclinical Consortium. The MEK inhibitor PD0325901 was found to cause disease regression in both systems, correcting both hyperproliferation of myeloid cells and the

aberrant differentiation that causes anemia [Chang et al., 2011; Lyubynska et al., 2011]. Importantly, MEK inhibition does not selectively eradicate mutant stem cells in either mouse model. Thus, other strategies may be required for a definitive cure of JMML. To broaden the search for active agents, the Braun lab has developed a novel in vitro screening platform using primary myeloid progenitors sorted by FACS into multi-well plates allowing the screen of hundreds of compounds for selective toxicity against mutant cells. Dr. Karen Cichowski, from Brigham and Women's Hospital and Harvard Medical School described her laboratory's efforts in developing new potential therapies for malignant peripheral nerve sheath tumors (MPNSTs), the most common lethal feature of NF1. Her laboratory has shown that the mTOR pathway is critically deregulated in NF1 mutant MPNSTs. mTOR inhibitors such as rapamycin suppress the growth of these tumors but do not cause tumor regression [Johannessen et al., 2005; Johannessen et al., 2008]. However, when combined with HSP90 inhibitors, potent tumor regression is observed [De Raedt et al., 2011]. This therapeutic combination is currently being evaluated in clinical trials. Dr. Cichowski also presented a platform by which her laboratory has been evaluating the combined effects of numerous drug combinations in this tumor model, with the goal of identifying more promising combinations that can be evaluated in NF1 patients.

Clinical Highlights for NF1

Dr. Eva Dombi (Pediatric Oncology Branch, National Cancer Institute) presented data from a phase II clinical trial of pegylated interferon alpha 2b (pegintron) in the treatment of plexiform neurofibromas in NF1 patients. In thirty patients with progressive plexiform neurofibromas the median PFS using volumetric MRI analysis on treatment with pegintron was 22.6 months compared with 10.6 months in a historical placebo control group. Two patients had a ≥20% decrease in tumor volume and eight had volume reduction of 11–16%. It was concluded that the treatment had activity by prolonging the time to progression of progressive plexi-form neurofibromas. Dr. Bruce Korf (University of Alabama at Birmingham) reported the results of a multicenter natural history study of the rate of growth of unselected plexiform neurofibromas not receiving treatment directed at the plexiform neurofibroma usingvolumetricMRI.Atotalof261patientswereenrolled,ofwhich at least two MRIs were available for 131. Median age at first MRI was 13.3years(range,2.5–55.5years)andthemedianobservationperiod was24.1months(range,6.4–76.9months).Plexiformneurofibroma growth wasseen more frequently in youngerpatients andthe growth rate did not correlate with tumor location or morphology. These data will be useful in counseling of individuals regarding the likelihood of growth of plexiform neurofibromas as a function of age and in the design of future clinical trials for plexiform neurofibromas. Children with NF1 have a high frequency of deficits in executive function—a set of higher level cognitive abilities such as working memory, planning and organization that regulate and control other cognitive functions and behavior. Dr. Kristina Hardy (The Jennifer and Daniel Gilbert NF Institute, Children's National Medical Center/George Washington University School of Medicine) presented results of a pilot study in 10 children with NF1 and working memory deficits of the feasibility and efficacy of home-based computerized cognitive training (Cogmed®). Primary outcomes included compliance as well as change in attention and working memory scores from baseline to post-intervention. Treatment compliance was high with all participants completing at least 92% of training

sessions with no adverse events. Participants exhibited post-treatment improvement in attention and executive function on a number of performance-based measures, but not on the parent-rated questionnaire of executive dysfunction. These preliminary data suggest that home-based computerized training is feasible for children with NF1 and a larger, randomized clinical trial to determine efficacy appears warranted. Dr. Rosa Nguyen and coworkers (University of Maryland and University Hospital Hamburg-Eppendorf) retrospectively analyzed clinical and MRI data of 52 NF1 patients with plexiform neurofibromas who had surgery to debulk the tumors. The aim of the study was to determine tumor growth rate and identify prognostic features for tumor progression in post-operative PNFs. Surgical indications included cosmetic disfigurement (n = 18), functional deficits (n = 13), and pain (n = 10). Eleven patients (21%, 9 children vs. 2 adults) with residual tumor had repeated surgery due to tumor progression. Tumor type was significantly associated with tumor progression; specifically diffuse plexiform neurofibromas progressed faster than nodular ones ($P < 0.005$). Patients aged 21 and younger had the highest progression rate($P < 0.01$).Thirteen plexiform neurofibromaswere visually completely resected and did not recur during observation (mean 3.7 years, 1.0–7.5). The authors concluded that age, tumor type, location, and depth may predict progression of partially resected plexiform neurofibromas, and that patients may benefit from elective surgery of small and completely removable plexiform neurofibromas.

Dr. Kent Robertson (Indiana University) presented preliminary now published results of an open-label, phase 2 study of imatinib in 36 NF1 patients with plexiform neurofibromas [Robertson et al., 2012]. Of the 23 patients who received imatinib for at least 6 months, 69 plexiform neurofibromas were evaluated and 26% of these patients had a 20–38% decrease in volume of one or more plexiform neurofibromas. Pediatric patients tended to have a first measurable response earlier than adults (median 4 vs. 8 months) suggesting that future trials on these slow growing tumors will require a minimum of 12 months therapy to accurately assess response. Increased plasma levels of VEGF and MCP-1 correlated with non-response to imatinib. Follow up studies are examining the activity of imatinib, sunitinib, and cabozantinib. The biomarkers being studied will include measurement of circulating cytokines as well as flow cytometric analysis of circulating pro-angiogenic progenitor cells and inflammatory monocytes.

ADVANCEMENT IN NF2

Deciphering Pathways of NF2

A signaling-focused session opened with Cristina Fernandez-Valle (University of Central Florida) describing a pilot NF2 high-throughput screening campaign of a 1,280 compound library (LOPAC) using merlin-null mouse Schwann cells to identify probes that reduced cell viability. Nearly half of the 40 identified compounds modulate components in merlin-dependent pathways. These compounds will be validated using multiple assays and selectivity will be tested using normal mouse Schwann cells.

Merlin interacts with the E3 ubiquitin ligase $CRL4^{DCAF1}$ in the nucleus and inhibits DCAF1. Wei Li (Memorial Sloan-Kettering Cancer Center) presented data describing a functional link between merlin, DCAF1, and YAP, and provided a novel mechanism for

merlin in the activation of the Hippo tumor suppressor pathway [Li et al., 2010]. Dr. Sylwia Ammoun (University of Plymouth, United Kingdom) demonstrated a strong overexpression of three TAM receptors (TAM family receptor tyrosine kinases—Tyro3 (Sky), Axl, and Mer) and their ligand Gas6 in human schwannomas. Gas6 is mitogenic and increases schwannoma cell–matrix adhesion and survival acting via Axl. Gas6/Axl signaling stimulates FAK and Src but not ERK1/2, JNK1/2 or AKT, and converges with IGFBP-1/integrin β1-mediated signaling in schwannoma toward increased proliferation [Ammoun et al., 2013]. A specific antibody targeting the Axl receptor is currently being used in clinical trials for other tumors and could potentially be tested for schwannoma and other NF2-related tumors.

Dr. Susann Schirmer (Leibniz Institute for Age Research, Germany) described the complex formation between merlin and p120RasGAP and its influence in mediating contact inhibition of growth through restriction of Ras activity. The association between merlin, p120RasGAP and Ras was characterized and a direct interaction between merlin and p120RasGAP as well as Ras was detected. It was shown that p120RasGAP preferentially binds the active (S518A) but not the inactive (S518D) form of merlin. Furthermore, p120RasGAP knockdown in Schwann cells leads to loss of contact-mediated inhibition of growth, mimicking the loss of merlin phenotype. Future experiments will focus on the functional relationship between merlin and p120RasGAP in vivo, including analysis of the tumorigenic potential of p120RasGAP-deficient Schwann cells in vivo.

Dr. Robert Hennigan (Cincinnati Children's Hospital) reported on the findings of his recently published article on the role of merlin in microtubule-based vesicular transport through control of Rho/ Rac family of GTPases activity [Hennigan et al., 2013]. The decrease in Rac-dependent anterograde trafficking of exocytic vesicles as a result of loss of merlin could represent a possible mechanism for the control of growth factor receptors at the cell surface.

Dr. Nic Tapon (London Research Institute, United Kingdom) described the generation of a biosensor for Hippo signaling using the Split-TEV protein–protein interaction reporter system and application to a genome-wide RNAi screen in Drosophila. This work uncovered a number of new pathway members as well as a potential link between nutrient sensing and Hippo signaling [Wehr et al., 2013].

Dr. Georg Halder (University of Leuven, Belgium) discussed the role of the merlin-regulated Hippo pathway in cell polarity, showing that when all cells of *Drosophila* imaginal discs lose epithelial cell polarity (undergo EMT), they hyperproliferate and form large tumorous masses due to upregulation of the Hippo pathway effector Yki, the *Drosophila* homolog of YAP and TAZ. Strikingly, when discs also contain normal cells, the mutant cells do not hyperproliferate nor do they upregulate Yki but rather die by apoptosis. Therefore, the normal cells act to suppress the tumorigenic phenotype of mutant cells, elegantly showing how homeo-static mechanisms can eliminate the abnormal cells to prevent cancer through regulation of the Hippo pathway [Chen et al., 2012].

Dr. Duojia Pan (Johns Hopkins University) investigated the feasibility of targeting the Hippo pathway oncoprotein YAP as a potential therapeutic approach against NF2 and showed that disruption of the YAP–TEAD complex potently suppresses hepatomegaly/tumorigenesis resulting from *Nf2* inactivation in the mouse liver. Screening of a library of FDA-approved drugs identi fied verteporfin as a small molecule that inhibits TEAD–YAP association and YAP-induced liver overgrowth, providing proof of principle for YAP inhibitors as molecular-targeted therapeutics for NF2 [Liu-Chittenden et al., 2012].

Concerning Merlin's regulation of mTORC1 and mTORC2 signaling via the Rheb/TSC1/TSC2 proteins, Dr. Vijaya Ramesh (Massachusetts General Hospital) proposed that additional mutational events or compensatory mechanisms that may contribute to tumor growth and progression. Rapamycin differentially affects Akt signaling in NF2-suppressed arachnoidal and Schwann cells, while the mTOR kinase inhibitor Torin1 is more effective than rapamycin in blocking signaling and proliferation in meningioma cells. These findings suggest that inhibitors targeting both mTORC1 and mTORC2 may be more effective than rapamycin alone for treating NF2-associated tumors [Han et al., 2008; James et al., 2012].

Dr. Joe Kissil (Scripps Research Institute) detailed the identification of a novel group of merlin-interacting proteins—the angiomotins—associated with tight and adherens junctions and interactors of several small G-protein modulators. The findings presented provide a potential mechanism to explain how merlin regulates signaling through the Rac and Ras/MAPK pathways from cell–cell junctions. These studies implicate the angiomotins as potential therapeutic targets in NF2 [Yi et al., 2011].

Therapeutic Frontiers: Preclinical Use of NF2 Mouse and Cellular Models Guiding Novel Therapeutics Development

NF research has benefited over the past few years from an array of progressively more sophisticated genetically engineered mouse (GEM) models. These models continue to provide an understanding of how the manifestations of NF2 emerge, and should help to identify new candidate targets for therapeutic interventions. Several presentations at the conference provided updates on the characterization and analysis of NF mouse and cellular models. Dr. Marco Giovannini (House Research Institute) reported on the use of mouse models of NF2 to identify new candidates to test in clinical trials. For example, RAD001 (everolimus) limited the growth of NF2-related schwannomas in his model. House Research Institute is now sponsoring a Phase II clinical trial, evaluating the efficacy of RAD001 in NF2 patients (ClinicalTrials.gov Identifier: NCT01345136). Dr. Toshifumi Tomoda (City of Hope) identified a novel role of merlin tumor suppressor in autophagy, a cellular catabolic pathway that has been implicated in tumorigenesis. First, evidence that merlin forms a complex with autophagy regulating proteins (i.e., LC3, Atg1, Atg16, dynein) upon autophagy induction was presented. Toshifumi showed that in Schwann cell cultures as well as in a xenograft model, loss of merlin caused an accumulation of cellular metabolic stress (i.e., hypoxia, DNA damage response), a condition known to accelerate tumorigenesis. Rapamycin and additional autophagy inducers were able to suppress this metabolic stress and tumor burden in a NF2 xenograft model suggesting that the metabolic stress caused by attenuated autophagy could be targeted as a new therapy against NF2. Dr. David Parkinson

(University of Plymouth, United Kingdom) presented his novel discovery that the loss of SOX10, a known transcription factor vital for Schwann cell development contributes independently to the phenotype of schwannoma cells [Doddrell et al., 2013] potentially a key pathway/modifier to the pathology of merlin-null schwannoma tumors. Dr. Oliver Hanemann (University of Plymouth, United Kingdom) discussed the targeting of the PDGFR pathway in NF2 from bench to bedside. He presented the rationale for choosing PDGFR as a target [Ammoun et al., 2008; Zhou et al., 2011] and showed that targeting PDGFR with Sorafinib is more effective than targeting ErbB2 with lapatinib or MEK alone [Ammoun et al., 2010a] or in combination with selumetinib [Ammoun et al., 2010b]. Dr. Hanemann presented the "fast track" concept of jumping to Phase 0 studies from primary human cells and illustrated the design of two recruiting Phase 0 studies with nilotinib and sorafenib. Dr. Andrea McClatchey (Harvard Medical School) presented new insight into the roles of merlin and the related protein ezrin in organizing the cell cortex and showed that their activities are essential for centrosome/mitotic spindle orientation and function during epithelial morphogenesis [Hebert et al., 2012]. Her group found that an unexpected consequence of this activity is that *NF2*-mutant tumor cells are prone to forming multipolar spindles, which can lead to genome instability. Dr. Alexander Schulz (Leibniz Institute for Age Research, Germany) from the Morrison group reported that axons of the peripheral nervous system predominantly express the less well-characterized merlin isoform 2, and this specific type of merlin impacts on axon structure maintenance [Schulz et al., 2013]. Genetically engineered mice that specifically lack merlin isoform 2 display abnormal axons and these mice suffer from polyneuropathy-like symptoms of axonal origin. This finding is of clinical interest since 70% of NF2 patients show signs of polyneuropathy, often occurring in the absence of nerve damaging tumors [Sperfeld et al., 2002]. Dr. Jeff Gehlhausen from the Clapp laboratory at Indiana University presented a new mouse model of schwannoma formation that utilizes a periostin-Cre transgene to conditionally delete *Nf2* in a subset of the neural crest lineage. These mice develop multiple schwannomas of the dorsal root ganglia and peripheral nerves. Most importantly, similar to patients with NF2, they also exhibit progressive hearing loss. It will be very interesting to determine whether progressive hearing loss in these mice is caused by bona fide vestibular schwannomas (VSs), which have not been seen in other *Nf2*-mutant mouse models. Moreover this exciting new mouse model will be an excellent tool to screen new candidate targets to speed up therapeutic interventions.

Clinical Highlights in NF2

Dr. Ashok Asthagiri (NIH, Bethesda, MD) summarized the data now published showing longitudinal growth of intracranial tumors (mostly meningiomas) with MRI volumetric analysis in NF2 patients (mean follow up of 9.5 years) [Dirks et al., 2012]. Three different growth patterns were seen: saltatory, linear, and exponential. Over a 10-year period, 50% of the meningiomas identified by imaging were de novo tumors. Female sex and younger age were associated with increased growth rate. Dr. Asthagiri also summarized the data in which hearing loss develops as a result of cochlear aperture obstruction and accumulation of intra labyrinthine protein [Asthagiri et al., 2012]. It was proposed, that MRI based identification of elevated intra labyrinthine protein may help identify the ear at-risk for developing hearing loss. Dr. Mathias Karajannis (New York University) gave an overview of current trials in NF2 and presented data on his now published single institution phase II study with lapatinib

in NF2 patients with progressive VS [Karajannis et al., 2012]. The study design included volumetric MRI response analysis as a primary endpoint and hearing as a secondary end point. Four of 17 evaluable patients experienced an objective volumetric response (reduction in VS volumes from – 15.7% to – 23.9%). Four of 13 patients evaluable for hearing met hearing criteria for response. Median time to overall progression (i.e., volumetric progression or hearing loss) was 14 months. Dr. Brad Welling (Ohio State University) reported the data using AR42, a novel histone deacetylase inhibitor, and AR12, a PDK1/Akt inhibitor. Dr. Welling showed potency of AR-42 in human schwannoma and meningioma cultures, in a xenograft model of schwannomas and in a transgenic schwannoma model (*P0Cre*;*Nf2flox2*/*flox2* mice) [Giovannini et al., 2000]. Preliminary clinical data of AR-42 in solid tumor patients suggest that AR-42 is well tolerated and OSU is planning a phase 0 study in NF2 patients to better understand the pharmacodynamics of AR-42 in NF2-related tumors [Bush et al., 2011; Jacob et al., 2012].

Diagnosis of NF2 can present a major challenge in individuals who are affected de novo, that is, without previous family history of the disorder. Dr. Amanda Bergner and coworkers (Johns Hopkins Comprehensive NF Center) evaluated the utility of criteria set forth by Baser et al. [2011] to identify individuals who do not fulfill classical criteria but who may in fact be affected. Among 10 patients referred for evaluation of NF2 but not meeting classical criteria, 40% had a Baser score indicative of possibly being affected. Use of the Baser score might thus increase the ability to provide an early diagnosis and genetic counseling of individuals with NF2 who do not yet meet classical criteria.

Dr. Jaishri Blakeley (Johns Hopkins, Baltimore) presented on decision making for patients with NF2. Discussion of experimental trials is now routinely part of clinic visits for patients with NF2. Currently, there are nine clinical trials open or in active development for patients with NF2. Many additional trials are available for patients with tumor types associated with NF2. Awareness of clinical trial options is a priority for all clinicians caring for patients with NF2. Conversely, clinical scientists should design studies with consideration of standard clinical care and engage all parties during treatment with an experimental drug.

Anat Stemmer-Rachamimov's studies of angiogenesis in NF2-associated and sporadic schwannomas showed that VEGF pathway is an important driver of angiogenesis in NF2 schwannomas. This finding was further supported by the treatment effect of anti-VEGF therapy on VSs in NF2 patients [Plotkin et al., 2009]. Analysis of a series of NF2-associated and sporadic meningiomas with a panel of immunohistochemical stains directed at the VEGF pathway components isongoing. The results of the VEGF pathway analysis will be correlated to the microvascular density in these tumors.

Speeding Up Interventions for NF2

This session began with two invited speakers. The first Dr. Antony Bretscher (Cornell University) challenged the current model of merlin conformations/phosphorylation and how they affect the regulation of cell growth. He showed data that the tumor suppressor merlin controls growth in its open state and phosphorylation converts merlin to a less-active more-closed state [Sher et al., 2012]. The second speaker, Dr. Samuel Rabkin, introduced new and different strategies for possibly treating NF2 tumors. Drawing on his experience in NF

research he described how oncolytic viruses, which selectively replicate in cancer cells, are a novel therapeutic strategy for cancer. He showed the use of oncolytic herpes simplex virus (HSV) for the treatment of tumors arising in NF, demonstrating the efficacy of this strategy, a new orthotopic MPNST model generated by implanting human NF1 MPNST stem-like cells into the sciatic nerve of immune-deficient mice was described [Messerli et al., 2006]. Oncolytic HSV is also efficacious in NF2 meningiomas andschwannomas. “Arming”oncolyticHSVwiththerapeutictrans-genes, such as IL-12, can enhance anti-tumor immunity and inhibition of tumor growth. He also discussed that combinations with chemotherapeutic drugs can further enhance efficacy.

This following brainstorming session intentionally mixed a panel of scientists and clinicians with different backgrounds and interests including clinical experts Drs. Scott R. Plotkin (Harvard Medical School), Jaishri Blakely (The Johns Hopkins Hospital), Victor-Felix Mautner (University Clinic Hamburg-Eppendorf, Germany), and basic researchers: Alison Lloyd (University College London), Larry Sherman (Oregon Health and Science University), Filippo Giancotti (Memorial Sloan-Kettering Cancer Center), and Samuel Rabkin (Massachusetts General Hospital). The idea was to initiate questions, foster new ideas, and possibly challenge the scientific state-of-the-art.

The panel initiated an interactive discussion with the audience participation. Dr. Alison Lloyd opened up the discussion by asking whyschwannomas predominantly grow on the eighth cranialnerve. Apparently there is no general consensus on why this area is so susceptible to tumor growth, some thoughts were discussed focusing on possible nerve injury susceptibility but the lack of any appropriate animal models to test any hypothesis would hinder progress. Also discussed was the merlin pathway “chaos.” An animated discussion unfolded pointing toward the continual need to unravel merlin biology. However, most urgently there is still a high demand to define the tumor-relevant pathways in NF2 specific tumors. The recent SYNODOS announcement by the Children's Tumor Foundation (http://www.ctf.org/Research/Synodos.html) aims to fund and create such a NF2 consortium with basic, translational and clinical scientist teams joining forces to fight NF2. Also Drs. Mautner and. Plotkin presented and discussed the benefits of sharing NF2 treatment experiences: “Do we need a platform to share treatment experiences with bevacizumab or other drugs in NF2 patients?” Bevacizumab has demonstrated activity in NF2 VSs with hearing improvement and decrease in tumor volume in case series around the world [Plotkin et al., 2009]. A prospective study is ongoing to confirm the initial responses observed. The need for prolonged administration of bevacizumab in patients with NF2 combined with concerns for potential chronic or cumulative toxicities have resulted in administration of bevacizumab outside of clinical trials at varying schedules (every 2–3 weeks, use of drug holidays) and doses (5–10 mg/kg). Experiences with the use of bevacizumab and its effects on meningiomas and spinal tumors in addition to VSs were discussed. It was concluded that a platform for clinical observations during drug treatments outside of clinical trials could accelerate research in this area.

Microstructural Peripheral Nerve MRI Is a Powerful Tool to Study NF2-Associated Neuropathy

Apart from deafness, reduced visual acuity, and neurological deficits after surgery, NF2 patients are at increased risk of developing peripheral neuropathy, which is an additional burden for them [Sperfeld et al., 2002]. Although it is known that nerve compressing schwannomas can cause NF2 neuropathy, there is often no mass lesion which can explain the distribution of clinical symptoms of polyneuropathy frequently involving multiple nerves. Sural biopsies have shown that intraneural tumorlets are correlated with NF2-associated neuropathy. Microstructural peripheral nerve MRI (performed by large coverage T2 sampling) allows the precise localization and quantification of peripheral nerve pathology. A pilot study has detected the diffuse presence of fascicular microlesions (diameter < 2 mm) and intermediate lesions (diameter ≥ 2 but < 5 mm) along the entire extremities. This cumulative lesion burden was found to closely correlate with the clinical severity of the NF2 neuropathy.

ADVANCEMENT IN SCHWANNOMATOSIS

Schwannomatosis Genetics

The current state of knowledge of involvement of *SMARCB1*/*SNF5 INI1* (gene ID 6598) in inherited and sporadic schwannomatosis and the investigations to identify further mechanisms for causation of the condition were summarized. Dr. Miriam Smith (University of Manchester, United Kingdom) described her work in documenting the proportion of schwannomatosis due to *SMARCB1* in the UK and then summarized the published literature [Boyd et al., 2008; Hadfield et al., 2008; Sestini et al., 2008; Smith et al., 2012b,c; Plotkin et al., 2013]. Dr. Smith also alerted the community to the relatively high proportion of sporadic mutations (45%) due to mosaic NF2,highlighting the needto examinemore than onetumor genetically in individuals with multiple non-VSs to distinguish between mosaic NF2 and schwannomatosis. Dr. Laura Papi (University of Florence, Italy) reported on the *SMARCB1* mutational spectrum in schwannomatosis in Italy. Dr. Papi presented unpublished data on the proportion of families and sporadic cases with *SMARCB1* mutations, which was very similar to the report from the previous speaker. She then described her laboratory's work in undertaking exome sequencing in schwannomatosis families and cases not due to *SMARCB1*. Arkadiusz Piotrowski (University of Alabama) reported that his laboratory has found no convincing evidence of germline methylation of *SMARCB1* as a cause of schwannomatosis. He also described his laboratory's work in undertaking exome sequencing in schwannomatosis families and cases not due to *SMARCB1*. Gareth Evans (University of Manchester) gave an overview of the clinical and molecular heterogeneity of schwannomatosis. He emphasized the need to rule out NF2 as thoroughly aspossible by tumor analysis and MRI scanning of the head. Even then, there is evidence that VSs may occur in schwannomatosis [Smith et al., 2012a]. Families have recently been described with multiple meningiomas, with *SMARCB1* mutations [Christiaans et al., 2011]. However, *SMARCB1* is only a minor contributor to patients and families with meningioma disease [Hadfield et al., 2010]. At least three undiscovered mechanisms for schwannomatosis were hypothesized: (1) an undiscovered locus on chromosome 22q; (2) a gene on a different chromosome; and (3) a mechanism possibly not transmissible that predisposes to mitotic

recombination. CTF currently offers to sponsor a collaborative schwannomatosis effort to validate or de-validate the above hypotheses.

Schwannomatosis Pathophysiology and Pain Management Approaches Use of Animal Models of *Snf5* loss to Understand Schwannomatosis Pathophysiology

Dr. Jeremie Vitte (House Research Institute) described the phenotypes of mice with conditional loss of *Snf5* (gene ID 6598) from crosses of P0-Cre animals with *Snf5*-double-floxed mice. Constitutive homozygous deletion of *Snf5* results in embryonic lethality, while mice with heterozygous loss of *Snf5* develop malignant rhabdoid tumors and do not show any signs of schwannomas. P0-Cre-mediated deletion of *Snf5* results in viable mice; approximately 80% of these mice die between 1.5 and 4.5 months. These mice develop tumors in the meninges and various aggressive peripheral nerve tumors, including the olfactory nerve, trigeminal nerve, optic nerve, and vestibular nerve. Tumors display a mosaic pattern of SNF5 expression along with intact NF2 expression, and activation of the mTOR pathway. The oncogenic interaction of *Snf5* and *Nf2* was tested using conditional alleles of *Snf5* and *Nf2* (*Snf5fl/ fl* × *Nf2fl/fl*) bred with P0-Cre ERT2. These mice do not exhibit any obvious tumors and pain behavior testing detected no significant difference between the wild-type and mutant mice. By 20–24 months, these mice demonstrate an onion bulb phenotype in the trigeminal nerve and cauda equina. This feature requires further characterization. Dr. Larry Sherman (Oregon Health and Science University) described mice with tamoxifen-inducible disruption of Snf5 in adult Schwann cells (with Cre recombinase under the control of the PLP promoter). Tumors do not develop in these mice nor is there evidence of altered cell cycle progression in Schwann cells in vivo or in vitro. However, the mice do develop enhanced thermal sensitivity, accompanied by an increased expression of the TRPV1 capsaicin receptor in dorsal root ganglion neurons. TRPV1 expression could be induced in wild-type dorsal root ganglion cells by conditioned medium from *Snf5*-null Schwann cells, indicating that loss of *Snf5* induces the expression of a secreted factor from Schwann cells that in turn increases TRPV1 expression and thermal sensitivity. These findings suggest a mechanism for the systemic pain often experienced by schwannomatosis patients and have the potential to reveal novel approaches to pain management for affected patients. Dr. Ganjam Kalpana (Albert Einstein College of Medicine) presented her findings on the transcriptional regulation of inflammatory genes by SNF5 [Lee et al., 2011; Smith et al., 2011]. SNF5-expressing rhaboid cells were found to upregulate interferon genes and genes involved in inflammation along with a downregulation of genes involved in mitosis. Targeting cyclin/Cdk with a combination of fenretinide and flavopiridol was found to be effective in treating $Snf5^{flox/+}$ mice bearing malignant tumors. These findings highlight a novel therapeutic strategy for tumors with loss of *Snf5*.

Surgery, Therapeutic Opportunities, and Non-Operative Approaches to Manage Schwannomatosis-Associated Pain

The current approach for managing the pain experienced by patients with schwannomatosis is either surgery or analgesics. Dr. Allan Belzberg (Johns Hopkins University) presented the available data on the efficacy of surgery for both successful surgical resection of schwannomas and pain control. He observed that the tumors seen in schwannomatosis are often more complicated than the sporadic schwannomas, and hence require specialty care to

ensure successful resection without nerve injury. Although the resection frequently results in dramatic improvement in pain, in a handful of patients, the more tumors resected, the less effective surgery is for pain control. There have been some anecdotal reports by both patients and their physicians that a secondary form of whole body pain develops, especially after a patient has been through multiple tumor resections. It is not clear how often this occurs and if the development of diffuse pain is related to the number of surgeries a patient has had for painful schwannomas. This lack of data is one example of the relatively limited natural history data available concerning schwannomatosis. Therefore CTF sponsored the creation of the International Schwannomatosis Database (ISD). In the ISD, sites around the world can register their patients in a deidentified manner to allow global collaboration to better address natural history questions about schwannomatosis.

Dr. Sanjog Pangarkar of UCLA spoke about the currently available strategies to address pain in general and neuropathic pain in particular. There are no available data on the types of medications that are most effective for the types of pain associated with schwannomatosis. However, there are several general pain management strategies that can be applied to manage local pain, diffuse pain, or both. For localized pain (often related to a specific tumor and involving one limb), systemic drug options are often the first choice. However, these agents are accompanied by systemic side effects and therefore some patients will opt for a local therapy. Local pain control options include regional nerve block, radio frequency ablation, and spinal cord stimulation. These are invasive approaches, but can be performed quickly and safely by physicians experienced in interventional pain management and often result in regional pain relief at least temporarily. It is important to note that placement of a spinal cord stimulator can limit future MRI and hence should be considered carefully in light of this limitation. For diffuse pain, there are multiple medications that are intended to treat neuropathic-like pain. These include antidepressants (such as amitriptyline, nortriptyline, duloxetine), calcium channel alpha 2-delta ligands (such as gabapentin, pregabalin), and medications that address inflammation (such as meloxicam or celecoxib). Used alone or in combination, these agents may be effective at managing the pain associated with schwannomatosis. Another class of drugs that can be considered is opiates, which act in both the peripheral and central nervous systems to decrease the perception of pain. However, opiates have several concerning side effects and properties that limit their usefulness as a long-term pain management strategy. Importantly, chronic use of opiates can result in habituation and in some cases addiction. Hence, in general, it was recommended to use opiate-based medications only intermittently for “breakthrough” pain. In some cases, the pain associated with schwannomatosis is resistant to all non-opiate therapies and long-term opiate use is required. In these cases, long-acting forms of opiate medications, such as methadone, levorphanol, fentanyl, and possibly ketamine-NMDA antagonist (in bolus dosing daily for 1 week), are recommended. Maximal benefit with minimized harm is likely to be accomplished with a thoughtful multidisciplinary team approach that is customized to the individual patient. A promising advancement in the last 4 years has been the development and optimization of whole-body magnetic resonance imaging (WBMRI) for the assessment of schwannomas in the setting of schwannomatosis. Cai et al. [2009] showed that WBMRI with 3D segmentation was feasible in patients with NF1, NF2, and schwannomatosis and that details of tumor burden and distribution were readily available

with this technique. This has subsequently been applied to evaluate tumor burden in schwannomatosis. Dr. Scott R. Plotkin (Massachusetts General Hospital/Harvard Medical School) presented the evolving data available on WBMRI and how WBMRI can be integrated into the management of schwannomatosis patients [Plotkin et al., 2012]. Specifically, given that patients with schwannomatosis often have tumors distributed throughout their bodies, it becomes impractical to obtain multiple regional MRIs over time. Absence of WBMRI leads to inconsistent data acquisition with each serial evaluation, marring both clinical decision-making and the learning about tumor behavior over time in general. WBMRI uses standard MRI scanners with commercially available software to create a single DICOM image that extends from head to ankle in most patients in under an hour. Dr. Plotkin presented his team's data on the patients with schwannomatosis imaged to date. WBMRI was found to identify internal nerve sheath tumors in 71% of patients. For patients with tumors, the median tumor count was determined to be 4 (range: 1–27 tumors) and the median whole-body tumor volume was reported as 39.4 ml (range: 7.0–1,371.5 ml). Hence, WBMRI can reliably detect internal tumors in patients with schwannomatosis with a short scan time. An important limitation to recognize is the limited resolution for spine lesions. Therefore, if a nerve root lesion or myelopathy is clinically suspected, dedicated spine MRI is recommended. However, WBMRI could reliably detect lesions ≥ 2 cm outside of the spine and skull. Thus, this is a technology that should be considered for increased clinical application for patients with schwannomatosis to allow more consistent tumor surveillance and potentially as a tool to measure the effects of therapies as new drugs are developed specifically targeting schwannomatosis-associated schwannomas.

Dr. Vanessa Merker and coworkers (Massachusetts General Hospital, Boston, MA) presented a retrospective analysis of the clinical features of 87 patients who fulfilled either research or clinical diagnostic criteria for Schwannomatosis [Merker et al., 2012]. The most common presentation was pain unassociated with a mass (46%). Peripheral schwannomas were present in 89% patients, spinal schwannomas in 74%, and intracranial meningiomas in 5%. Chronic pain was the most common symptom (68%) and usually persisted despite aggressive surgical and medical management, and was often associated with anxiety and depression. These findings support a proactive surveillance plan in which neurological symptoms and signs are actively investigated by MRI to identify tumors. Early referral to specialty care and active pain management is warranted.

Schwannomatosis Clinical Highlights

Dr. Anat Stemmer-Rachamimov (MGH, Boston,MA)summarized data on a correlative study of immunohistochemical staining of schwannomas with *INI1/SMARCB1* and mutation analysis of the *SMARCB1* gene. A mosaic pattern of INI1 expression was seen in the great majority of tumors from familial schwannomatosis and NF2 patients, in about half of the tumors from sporadic schwannomatosis, and only in 5% of solitary, sporadic schwannomas [Patil et al., 2008]. These findings (based on protein expression) show a much higher rate of *SMARCB1* involvement in schwannomatosis than the rate reported when the gene is analyzed by mutation analyses (30% in familial schwannomatosis; 15% in sporadic schwannomatosis). This discrepancy may be due to differences in the tumor sets studied or in differences in the sensitivity of the methods used; but it may also reflect epigenetic

changes or loss of other proteins that interact with *SMARCB1*. A study that analyzes a series of schwannomas by both immunohistochemistry and mutation analyses is underway.

LATEST ADVANCES IN BASIC AND CLINICAL SCIENCE IN NF

Dr. Rebecca Dodd from David Kirsch's laboratory at Duke University presented her findings on the anti-tumor activity of MEK and mTOR inhibitors in a new mouse model of soft tissue sarcoma. Injecting adenovirus-Cre into the sciatic nerve or the musculature of $NF1^{fl/fl}$;$Ink4a^{fl/fl}$ mice generates either MPNSTs or a spectrum of high-grade myogenic sarcomas. Cell lines from these tumors and tumor allografts were found to be sensitive to MEK or mTOR inhibition, with combination therapy demonstrating the greatest response. The MEK inhibitor PD0325901 delayed tumor growth without inducing apoptosis, thus implying a cytostatic mechanism and identifying MEK as a potential therapeutic target in NF1-associated sarcomas. Current CTF Young Investigator Award recipient Dr. Maryam Jahanshahi from Cathie Pfleger's laboratory at the Mount Sinai School of Medicine presented her screen for novel Hippo pathway effectors. Using an in vitro expression cloning approach, she identified the Cdh1/fzr inhibitor Rae1 as a novel Wts substrate and presented genetic evidence implicating Rae1 in mitosis and organ size homeostasis. Dr. James Walker from the Bernards laboratory at Massachusetts General Hospital presented results of a genetic screen aimed at identifying modifiers of a *Drosophila* NF1 organismal growth defect. The screen identified Ras and cAMP/PKA pathway components, as well as several genes implicated in synaptic functioning. Additional gene profiling and metabolomic analyses revealed aberrant expression of the glucagon ortholog adipokinetic hormone in the brain of *dNf1* mutants, resulting in altered trehalose and triglyceride levels. Interestingly, among the genes identified in the size modifier screen, genetic or pharmacological inhibition of *Drosophila* Alk was also found to restore the *dNf1* learning deficit, implicating the human ortholog of this neuronal receptor tyrosine kinase as a potential therapeutic target.

2011 CTF Young Investigator Awardee, Dr. Adrienne Watson from David Largaespada's laboratory (University of Minnesota), presented another novel mouse model of MPNST. She used the Sleeping Beauty transposon technology to identify genetic modifiers of neurofibroma formation in mice expressing both activated *p53*and *Egfr*transgenes.Transposase activationwasfound to lead to an increase in the onset, frequency, and malignant behavior of tumors in this model, and in particular, to a large increase in MPNSTs. Mutation of either the *Nf1* or *Pten* gene was found to be common in neurofibromas, with MPNSTs frequently exhibiting mutation of both *Nf1* and *Pten*. Subsequent modeling and preclinical studies support the therapeutic targeting of these pathways in MPNST.

Next-Generation Sequencing in NF: Massively Parallel Sequencing—Massively Parallel Results

The precipitous decline in sequencing costs due to massively parallel or “next-gen” sequencing technologies permits the routine characterization of NF-associated tumors at the highest possible resolution (individual base pairs) and the interrogation of the exomes or genomes of individuals with unusual NF-like pheno-types that do not arise secondary to mutations in *NF1* or *NF2*. Dr. Douglas Stewart(National Cancer Institute)and Dr. Eric

Legius (Catholic University of Leuven, Belgium) co-chaired a session exploring how whole-exome and whole-genome sequencing will affect diagnosis, prognostication and management in NF. Dr. Melinda Merchant (National Cancer Institute) discussed the promises, pitfalls and progress in molecularly targeted treatment. Treatment decisions need to be made promptly, however signifi-cant hurdles remain in the timely processing, analysis and interpretation of large-scale genomics data from a tumor biopsy. Important regulatory issues remain unresolved as detailed in the recent Institute of Medicine report [Academies, 2012]. Well-designed, prospective trials will be required to determine the potential benefit using these approaches. Dr. Stewart then discussed preliminary efforts in his lab to whole-exome sequence NF1-associated plexiform neurofibromas and matching germline DNA. In the discovery stage, DNA from Schwann cell cultures from plexiform neurofibromas from the laboratory of Dr. Peggy Wallace [Muir et al., 2001] (plus matching germline DNA) are sequenced and undergo single nucleotide polymorphism (SNP) chip genotyping. Preliminary results from 11 tumor/normal pairs identified germline mutations in *NF1* in 11/11 (100%) matched pairs, of which 7/8 (88%) were concordant with previously identified *NF1* mutations. Somatic mutations of *NF1* were identified in 9/ 11 (82%) of samples, of which 5/5 (100%) were concordant with previously identified NF1 mutations. Dr. Legius then discussed work in his lab to identify the gene underlying a recently reported syndrome characterized by multiple orbital neurofibromas, distinctive face, marafnoid habitus, and painful peripheral nerve tumors [Babovic-Vuksanovic et al., 2012]. The affected individuals were sporadic cases. Comprehensive mutation analysis was performed, but no mutations were found in these candidate genes (*NF1*, *NF2*, *SMARCB1*, *RET*, *PRKAR1A*, *PTEN*). Dr. D. Gareth Evans (University of Manchester) described the preliminary identification of a familial meningioma gene and echoed many of the issues raised by Drs. Stewart and Legius. In the identification of novel syndrome variants, the investigator does not want to miss the single variant that is causing the disorder. Therefore the filtering process of the sequence reads isless stringent,resulting in many false positive but also false negative variants. Massively parallel sequencing permits gene identification for rare syndromes given a correct genetic hypothesis, sufficient coverage of the responsible gene and no important genetic heterogeneity.

RESPONSE EVALUATION IN NF AND SCHWANNOMATOSIS (REINS)

Drs. Plotkin and Widemann initiated this international collaboration in 2010 with the goal to develop standardized response criteria to determine treatment response in clinical trials for patients with NF1, NF2, and schwannomatosis. REiNS working groups are open to all interested in contributing to the effort. Current REiNS working groups (and their leaders) include: Tumor size (Brigitte Widemann, Eva Dombi, NCI, Bethesda, MD), functional outcomes (Scott R. Plotkin, MGH, Boston, MA), visual outcomes (Michael Fisher, Children's Hospital of Philadelphia), patient reported outcomes (Pamela Wolters, NCI, Bethesda, MD), neurocognitive outcomes (Karen Walsh, CNMC, Washington, DC), whole body MRI (Jaishri Blakeley, Johns Hopkins, Baltimore, MD), and bio-markers (Oliver Hanemann, University of Plymouth, UK). Response criteria will continue to be modified as experience in trials for NF is gained, and working groups will be added. The hope is that once published, the consensus criteria will be incorporated into future clinical trials and will

improve the ability to determine and compare treatment efficacy. The preliminary recommendation of the tumor size working group is to use volumetric MRI for the measurement of plexiform neurofibromas, VSs, and meningiomas. Imaging of these tumors should be performed consistently to allow for volumetric analysis and cover the entire lesion. The functional outcomes group focuses on the evaluation of hearing, facial functioning, walking performance and respiratory, bladder and bowel functions. While much of this work is still in progress, recommendations for hearing evaluation are complete with emphasis on maximum word recognition score. The visual outcomes working group supports the use of visual acuity testing by age appropriate measures as the primary outcome for the evaluation of visual pathway integrity. Optic pallor should be considered as a contributing variable. The patient reported outcomes committee is working on the selection of outcome measures for four areas: general QOL, disease specific QOL, pain and functional disability. The group has so far rated functional disability measures and recommends the Health Assessment Questionnaire-Disability Index and Child Health Assessment Questionnaire (HAQ-DI and CHAQ) for assessment of general functional disability and the Pediatric Outcomes Data Collection Instrument (PODCI) to assess functional disability after orthopedic surgery in children. http://www.rein-scollaboration.org is a web-site where the working groups exchange ideas and pose updates. Public access will be available in the near future.

NF MANAGEMENT SERVICES—ENGLAND's EXPERIENCE

Dr. Susan Huson (St. Mary's Hospital/University of Manchester) and Rosalie Ferner (Guy's and St. Thomas' NHS Foundation Trust, United Kingdom) chaired a clinical session on England's NF services funded by the National Specialist Commissioning Group which commissions highly specialized services nationally for the National Health Service to provide optimal care for children and adults in England with rare medical conditions. Prof. Ferner stated that the "complex NF1" service, established in 2009 at Guy's and St. Thomas' NHS Foundation Trust London and Central Manchester University Hospitals NHS Foundation Trust, aims to offer holistic, standardized, multidisciplinary care for NF1 individuals of all ages with rare complications that are serious and potentially life-threatening (e.g., extensive, symptomatic, disfiguring plexiform neurofibromas; neurofibromatous neuropathy; atypical neurofibromas and MPNST; neurovascular complications; multiple sclerosis; sphenoid wing dysplasia and pseudarthrosis of the long bones; atypical phenotypes and a prenatal counseling service for people with segmental NF1). The remit is to provide access for all patients to the resources and expertise of the designated national centers and to ensure regular national audit and succession planning. Karine Lascelles (Guy's and St. Thomas' NHS Foundation Trust) noted that the pediatric service was fully integrated within the adult clinic and described the role of amputation in the management of carefully selected children with very extensive, painful plexiform neurofibromas involving the lower limb. Dr. Guy Leschziner (Guy's and St. Thomas' NHS Foundation Trust) reported on a prospective study of sleep quality and disturbance in 100 consecutive NF1 clinic patients. There was ahigh frequency of sleep disturbance, poor sleep quality, and excessive daytime sleepiness; however, daytime sleepiness and sleep disturbance were not related. Dr. Leschziner postulated that some patients have organic

sleep pathology such as sleep apnea or periodic limb movement disorder causing daytime sleepiness, while others might have sleep disturbance due to multiple factors (anxiety, depression or cognitive status). Dr. Judith Eelloo (St. Mary's Hospital, United Kingdom) explained the role of NF1 specialist nurses as advocates and educators of patients and their families, forming an important link between the patient, national center, local clinics, allied professionals, the Neuro Foundation, and the community. The complex NF1 service facilitates a solid infrastructure of specialist clinicians with expertise in managing rare NF1 manifestations. The development of robust outcome measures will allow meticulous evaluation of interventions and provide the best possible care for patients.

Dr. Huson described England's national NF2 service, established in 2010 and led by Prof. Gareth Evans. Prior to this, management in a specialist NF2 center was associated with a significant decrease in disease-related mortality [Baser et al., 2002], although standards of care varied among centers despite UK consensus NF2 management guidelines published in 2005 [Evans et al., 2005]. The four centers with the largest multidisciplinary NF2 experience therefore developed a model for NF2 care to provide lifelong, holistic care for all of England's NF2 patients and their at-risk family members. The centers are located in Cambridge (lead Mr. Patrick Axon), London (lead Prof. Rosalie Ferner), Oxford (leads Dr. Allyson Parry and Dr. Dorothy Halliday), and Manchester (lead Prof. Gareth Evans). All NF2 radio-surgery is performed in one national center in Sheffield (lead Mr. Jeremy Rowe), while auditory brainstem implant insertion is performed in Cambridge and Manchester. The mainstay of care is the same-day multidisciplinary out-patient assessment which commences with a holistic overview and examination by a physician or nurse specialist. This informs a radiological review meeting and relevant members of the surgical team then see the patients. Each center has separate adult and pediatric clinics. National commissioning also enables rapid implementation of new treatments for which there is adequate evidence. Bevacizumab treatment is now funded for patients with rapidly growing schwannomas that threaten hearing or other neurological functions depending on their location. The four centers collect standard datasets for all aspects of NF2 care.

Clinical nurse specialists liaise with local health care professionals and families between clinic appointments. They also help families decide when and in what setting at-risk children will first learn about NF2. There is funding for specialist care of facial nerve palsies and for patients and their families to attend NF2 courses organized by Hearing Link, a UK charity assisting people with adult onset hearing loss. From a research viewpoint, the English NF2 cohort provides a unique opportunity for monitoring outcomes of changes in treatment and the natural history of all aspects of NF2.

DOD NF CLINICAL TRIALS CONSORTIUM

Dr. Roger Packer (CNMC, Washington, DC) updated conference attendees about the Department of Defense (DOD) NF Clinical Trials Consortium. In 2012, the Consortium was awarded funding for an additional 5 years with a goal of running phase 2 trials for NF1, NF2, and schwannomatosis. In order to achieve this goal, additional sites with expertise in NF2 and schwannomatosis were recruited to the Consortium through a competitive application process. In 2013, the Consortium hopes to report the results for ongoing studies,

including STOPN (Phase II Study of the mTOR Inhibitor sirolimus in NF1 related plexiform neurofibromas) and STARS (a randomized-placebo controlled study of lovastatin in children with NF1). The results of a phase II study of RAD001 for children with NF1 and chemotherapy-refractory progressive low-grade gliomas will be reported subsequently. Upcoming trials include a phase 2 study of bevacizumab in children and young adults with NF2 and progressive VSs and a phase 2 study of bone morphogenetic protein 2 and pamidronate at surgical treatment of tibial pseudarthrosis in NF1. A phase 2 study of bevacizumab and RAD001 for adults with progressive malignant peripheral nerve sheath tumor (MPNST), being performed in collaboration with the Sarcoma Alliance (SARC), will to open in late 2012. Plans are well underway for one to two studies for NF1 patients with plexiform neurofibromas.

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REFERENCES

Academies IoMotN. Evolution of translational omics: Lessons learned and the path forward. 2012. www.iom.edu/translationalomics

Ammoun S, Flaiz C, Ristic N, Schuldt J, Hanemann CO. Dissecting and targeting the growth factor-dependent and growth factor-independent extracellular signal-regulated kinase pathway in human schwannoma. Cancer Res. 2008; 68:5236–5245. [PubMed: 18593924]

Ammoun S, Cunliffe CH, Allen JC, Chiriboga L, Giancotti FG, Zagzag D, Hanemann CO, Karajannis MA. ErbB/HER receptor activation and preclinical efficacy of lapatinib in vestibular schwannoma. Neuro Oncol. 2010a; 12:834–843. [PubMed: 20511180]

Ammoun S, Ristic N, Matthies C, Hilton DA, Hanemann CO. Targeting ERK1/2 activation and proliferation in human primary schwannoma cells with MEK1/2 inhibitor AZD6244. Neurobiol Dis. 2010b; 37:141–146. [PubMed: 19804833]

Ammoun S, Provenzano L, Zhou L, Barczyk M, Evans K, Hilton DA, Hafizi S, Hanemann CO. Axl/Gas6/NFkappaB signalling in schwannoma pathological proliferation, adhesion and survival. Oncogene. 2013 Advance online publication 14 January 2013; doi: 10.1038/onc.2012.587.

Arrington DK, Danehy AR, Peleggi A, Proctor MR, Irons MB, Ullrich NJ. Calvarial defects and skeletal dysplasia in patients with neurofibromatosis Type 1. J Neurosurg Pediatr. 2013; 4:410–416. [PubMed: 23414129]

Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rahman M, Turmaine M, Wicher GK, Mitter R, Greensmith L, Behrens A, Raivich G, Mirsky R, Jessen K. c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. Neuron. 2012; 75:633–647. [PubMed: 22920255]

Asthagiri AR, Vasquez RA, Butman JA, Wu T, Morgan K, Brewer CC, King K, Zalewski C, Kim HJ, Lonser RR. Mechanisms of hearing loss in neurofibromatosis type 2. PLoS ONE. 2012; 7:e46132. [PubMed: 23049959]

Babovic-Vuksanovic D, Messiaen L, Nagel C, Brems H, Scheithauer B, Denayer E, Mao R, Sciot R, Janowski KM, Schuhmann MU, Claes K, Beert E, Garrity JA, Spinner RJ, Stemmer-Rachamimov

A, Gavrilova R, Van Calenbergh F, Mautner V, Legius E. Multiple orbital neurofibromas, painful peripheral nerve tumors, distinctive face and marfanoid habitus: A new syndrome. Eur J Hum Genet. 2012; 20:618–625. [PubMed: 22258529]

Baser ME, Friedman JM, Aeschliman D, Joe H, Wallace AJ, Ramsden RT, Evans DG. Predictors of the risk of mortality in neurofibromatosis 2. Am J Hum Genet. 2002; 71:715–723. [PubMed: 12235555]

Baser ME, Friedman JM, Joe H, Shenton A, Wallace AJ, Ramsden RT, Evans DG. Empirical development of improved diagnostic criteria for neurofibromatosis 2. Genet Med. 2011; 13:576–581. [PubMed: 21451418]

Boyd C, Smith MJ, Kluwe L, Balogh A, Maccollin M, Plotkin SR. Alterations in the SMARCB1 (INI1) tumor suppressor gene in familial schwannomatosis. Clin Genet. 2008; 74:358–366. [PubMed: 18647326]

Brown JA, Emnett RJ, White CR, Yuede CM, Conyers SB, O'Malley KL, Wozniak DF, Gutmann DH. Reduced striatal dopamine underlies the attention system dysfunction in neurofibromatosis-1 mutant mice. Hum Mol Genet. 2010; 19:4515–4528. [PubMed: 20826448]

Brown JA, Xu J, Diggs-Andrews KA, Wozniak DF, Mach RH, Gutmann DH. PET imaging for attention deficit preclinical drug testing in neurofibromatosis-1 mice. Exp Neurol. 2011; 232:333–338. [PubMed: 21963652]

Bush ML, Oblinger J, Brendel V, Santarelli G, Huang J, Akhmametyeva EM, Burns SS, Wheeler J, Davis J, Yates CW, Chaudhury AR, Kulp S, Chen CS, Chang LS, Welling DB, Jacob A. AR42, a novel histone deacetylase inhibitor, as a potential therapy for vestibular schwannomas and meningiomas. Neuro Oncol. 2011; 13:983–999. [PubMed: 21778190]

Cai W, Kassarjian A, Bredella MA, Harris GJ, Yoshida H, Mautner VF, Wenzel R, Plotkin SR. Tumor burden in patients with neurofibromatosis types 1 and 2 and schwannomatosis: Determination on whole-body MR images. Radiology. 2009; 250:665–673. [PubMed: 19244040]

Cappello S, Bohringer CR, Bergami M, Conzelmann KK, Ghanem A, Tomassy GS, Arlotta P, Mainardi M, Allegra M, Caleo M, van Hengel J, Brakebusch C, Gotz M. A radial glia-specific role of RhoA in double cortex formation. Neuron. 2012; 73:911–924. [PubMed: 22405202]

Chabernaud C, Mennes M, Kardel PG, Gaillard WD, Kalbfleisch ML, Vanmeter JW, Packer RJ, Milham MP, Castellanos FX, Acosta MT. Lovastatin regulates brain spontaneous low-frequency brain activity in neurofibromatosis type 1. Neurosci Lett. 2012; 515:28–33. [PubMed: 22433254]

Chang T, Krisman K, Harding-Theobald E, Braun BS, Shannon K. MEK inhibition modulates the growth of Nf1 mutant hematopoietic cells and induces clinical improvement in a murine model of juvenile myelomonocytic leukemia (JMML). ASH Annu Meet Abstr. 2011; 118:797.

Chen CL, Schroeder MC, Kango-Singh M, Tao C, Halder G. Tumor suppression by cell competition through regulation of the Hippo pathway. Proc Natl Acad Sci USA. 2012; 109:484–489. [PubMed: 22190496]

Christiaans I, Kenter SB, Brink HC, van Os TA, Baas F, van den Munckhof P, Kidd AM, Hulsebos TJ. Germline SMARCB1 mutation and somatic NF2 mutations in familial multiple meningiomas. J Med Genet. 2011; 48:93–97. [PubMed: 20930055]

De Raedt T, Walton Z, Yecies JL, Li D, Chen Y, Malone CF, Maertens O, Jeong SM, Bronson RT, Lebleu V, Kalluri R, Normant E, Haigis MC, Manning BD, Wong KK, Macleod KF, Cichowski K. Exploiting cancer cell vulnerabilities to develop a combination therapy for ras-driven tumors. Cancer Cell. 2011; 20:400–413. [PubMed: 21907929]

Dirks MS, Butman JA, Kim HJ, Wu T, Morgan K, Tran AP, Lonser RR, Asthagiri AR. Long-term natural history of neurofibromatosis Type 2-associated intracranial tumors. J Neurosurg. 2012; 117:109–117. [PubMed: 22503123]

Doddrell RD, Dun XP, Shivane A, Feltri ML, Wrabetz L, Wegner M, Sock E, Hanemann CO, Parkinson DB. Loss ofSOX10 function contributes to the phenotype of human Merlin-null schwannoma cells. Brain. 2013; 136:549–563. [PubMed: 23413263]

Evans DG, Baser ME, O'Reilly B, Rowe J, Gleeson M, Saeed S, King A, Huson SM, Kerr R, Thomas N, Irving R, MacFarlane R, Ferner R, McLeod R, Moffat D, Ramsden R. Management of the patient and family with neurofibromatosis 2: A consensus conference statement. Br J Neurosurg. 2005; 19:5–12. [PubMed: 16147576]

Fisher MJ, Loguidice M, Gutmann DH, Listernick R, Ferner RE, Ullrich NJ, Packer RJ, Tabori U, Hoffman RO, Ardern-Holmes SL, Hummel TR, Hargrave DR, Bouffet E, Charrow J, Bilaniuk LT, Balcer LJ, Liu GT. Visual outcomes in children with neurofibromatosis type 1-associated optic pathway glioma following chemotherapy: A multicenter retrospective analysis. Neuro Oncol. 2012; 14:790–797. [PubMed: 22474213]

Giovannini M, Robanus-Maandag E, van der Valk M, Niwa-Kawakita M, Abramowski V, Goutebroze L, Woodruff JM, Berns A, Thomas G. Conditional biallelic Nf2 mutation in the mouse promotes manifestations of human neurofibromatosis type 2. Genes Dev. 2000; 14:1617–1630. [PubMed: 10887156]

Hadfield KD, Newman WG, Bowers NL, Wallace A, Bolger C, Colley A, McCann E, Trump D, Prescott T, Evans DG. Molecular character-isation of SMARCB1 and NF2 in familial and sporadic schwannomatosis. J Med Genet. 2008; 45:332–339. [PubMed: 18285426]

Hadfield KD, Smith MJ, Trump D, Newman WG, Evans DG. SMARCB1 mutations are not a common cause of multiple meningiomas. J Med Genet. 2010; 47:567–568. [PubMed: 20472658]

Han S, Witt RM, Santos TM, Polizzano C, Sabatini BL, Ramesh V. Pam (protein associated with Myc) functions as an E3 ubiquitin ligase and regulates TSC/mTOR signaling. Cell Signal. 2008; 20:1084–1091. [PubMed: 18308511]

Hawkins C, Walker E, Mohamed N, Zhang C, Jacob K, Shirinian M, Alon N, Kahn D, Fried I, Scheinemann K, Tsangaris E, Dirks P, Tressler R, Bouffet E, Jabado N, Tabori U. BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. Clin Cancer Res. 2011; 17:4790–4798. [PubMed: 21610142]

Hebert AM, DuBoff B, Casaletto JB, Gladden AB, McClatchey AI. Merlin/ERM proteins establish cortical asymmetry and centrosome position. Genes Dev. 2012; 26:2709–2723. [PubMed: 23249734]

Hennigan RF, Moon CA, Parysek LM, Monk KR, Morfini G, Berth S, Brady S, Ratner N. The NF2 tumor suppressor regulates microtubule-based vesicle trafficking via a novel Rac, MLK and p38(SAPK) pathway. Oncogene. 2013; 32:1135–1143. [PubMed: 22525268]

Jacob K, Quang-Khuong DA, Jones DT, Witt H, Lambert S, Albrecht S, Witt O, Vezina C, Shirinian M, Faury D, Garami M, Hauser P, Klekner A, Bognar L, Farmer JP, Montes JL, Atkinson J, Hawkins C, Korshunov A, Collins VP, Pfister SM, Tabori U, Jabado N. Genetic aberrations leading to MAPK pathway activation mediate oncogene-induced senescence in sporadic pilocytic astrocytomas. Clin Cancer Res. 2011; 17:4650–4660. [PubMed: 21610151]

Jacob A, Oblinger J, Bush ML, Brendel V, Santarelli G, Chaudhury AR, Kulp S, La Perle KM, Chen CS, Chang LS, Welling DB. Preclinical validation of AR42, a novel histone deacetylase inhibitor, as treatment for vestibular schwannomas. Laryngoscope. 2012; 122:174–189. [PubMed: 22109824]

James MF, Stivison E, Beauchamp R, Han S, Li H, Wallace MR, Gusella JF, Stemmer-Rachamimov AO, Ramesh V. Regulation of mTOR complex 2 signaling in neurofibromatosis 2-deficient target cell types. Mol Cancer Res. 2012; 10:649–659. [PubMed: 22426462]

Jessen WJ, Miller SJ, Jousma E, Wu J, Rizvi TA, Brundage ME, Eaves D, Widemann B, Kim MO, Dombi E, Sabo J, Hardiman Dudley A, Niwa-Kawakita M, Page GP, Giovannini M, Aronow BJ, Cripe TP, Ratner N. MEK inhibition exhibits efficacy in human and mouse neurofibromatosis tumors. J Clin Invest. 2013; 123:340–347. [PubMed: 23221341]

Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K. The NF1 tumor suppressor critically regulates TSC2 and mTOR. Proc Natl Acad Sci USA. 2005; 102:8573–8578. [PubMed: 15937108]

Johannessen CM, Johnson BW, Williams SM, Chan AW, Reczek EE, Lynch RC, Rioth MJ, McClatchey A, Ryeom S, Cichowski K. TORC1 is essential for NF1-associated malignancies. Curr Biol. 2008; 18:56–62. [PubMed: 18164202]

Karajannis MA, Legault G, Hagiwara M, Ballas MS, Brown K, Nusbaum AO, Hochman T, Goldberg JD, Koch KM, Golfinos JG, Roland JT, Allen JC. Phase II trial of lapatinib in adult and pediatric patients with neurofibromatosis type 2 and progressive vestibular schwannomas. Neuro Oncol. 2012; 14:1163–1170. [PubMed: 22844108]

Lee S, Cimica V, Ramachandra N, Zagzag D, Kalpana GV. Aurora A is a repressed effector target of the chromatin remodeling protein INI1/hSNF5 required for rhabdoid tumor cell survival. Cancer Res. 2011; 71:3225–3235. [PubMed: 21521802]

Li W, You L, Cooper J, Schiavon G, Pepe-Caprio A, Zhou L, Ishii R, Giovannini M, Hanemann CO, Long SB, Erdjument-Bromage H, Zhou P, Tempst P, Giancotti FG. Merlin/NF2 suppresses tumorigenesis by inhibiting the E3 ubiquitin ligase CRL4(DCAF1) in the nucleus. Cell. 2010; 140:477–490. [PubMed: 20178741]

Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, Liu JO, Pan D. Genetic and pharmacological disruption of the TEAD–YAP complex suppresses the oncogenic activity of YAP. Genes Dev. 2012; 26:1300–1305. [PubMed: 22677547]

Loh ML. Recent advances in the pathogenesis and treatment of juvenile myelomonocytic leukaemia. Br J Haematol. 2011; 152:677–687. [PubMed: 21623760]

Lyubynska N, Gorman MF, Lauchle JO, Hong WX, Akutagawa JK, Shannon K, Braun BS. A MEK inhibitor abrogates myeloproliferative disease in Kras mutant mice. Sci Transl Med. 2011; 3:76ra27.

Merker VL, Esparza S, Smith MJ, Stemmer-Rachamimov A, Plotkin SR. Clinical features of schwannomatosis: A retrospective analysis of 87 patients. Oncologist. 2012; 17:1317–1322. [PubMed: 22927469]

Messerli SM, Prabhakar S, Tang Y, Mahmood U, Giovannini M, Weissleder R, Bronson R, Martuza R, Rabkin S, Breakefield XO. Treatment of schwannomas with an oncolytic recombinant herpes simplex virus in murine models of neurofibromatosis type 2. Hum Gene Ther. 2006; 17:20–30. [PubMed: 16409122]

Muir D, Neubauer D, Lim IT, Yachnis AT, Wallace MR. Tumorigenic propertiesof neurofibromin-deficient neurofibroma Schwann cells. AmJ Pathol. 2001; 158:501–513. [PubMed: 11159187]

Patel AV, Eaves D, Jessen WJ, Rizvi TA, Ecsedy JA, Qian MG, Aronow BJ, Perentesis JP, Serra E, Cripe TP, Miller SJ, Ratner N. Ras-driven transcriptome analysis identifies aurora kinase A as a potential malignant peripheral nerve sheath tumor therapeutic target. Clin Cancer Res. 2012; 18:5020–5030. [PubMed: 22811580]

Patil S, Perry A, Maccollin M, Dong S, Betensky RA, Yeh TH, Gutmann DH, Stemmer-Rachamimov AO. Immunohistochemical analysis supports a role for INI1/SMARCB1 in hereditary forms of schwannomas, but not in solitary, sporadic schwannomas. Brain Pathol. 2008; 18:517–519. [PubMed: 18422762]

Plotkin SR, Stemmer-Rachamimov AO, Barker FG II, Halpin C, Padera TP, Tyrrell A, Sorensen AG, Jain RK, di Tomaso E. Hearing improvement after bevacizumab in patients with neurofibromatosis type 2. N Engl J Med. 2009; 361:358–367. [PubMed: 19587327]

Plotkin SR, Bredella MA, Cai W, Kassarjian A, Harris GJ, Esparza S, Merker VL, Munn LL, Muzikansky A, Askenazi M, Nguyen R, Wenzel R, Mautner VF. Quantitative assessment of whole-body tumor burden in adult patients with neurofibromatosis. PLoS ONE. 2012; 7:e35711. [PubMed: 22558206]

Plotkin SR, Blakeley JO, Evans DG, Hanemann CO, Hulsebos TJ, Hunter-Schaedle K, Kalpana GV, Korf B, Messiaen L, Papi L, Ratner N, Sherman LS, Smith MJ, Stemmer-Rachamimov AO, Vitte J, Giovannini M. Update from the 2011 International Schwannomatosis Workshop: From genetics to diagnostic criteria. Am J Med Genet Part A. 2013; 161A:405–416. [PubMed: 23401320]

Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature. 2012; 483:100–103. [PubMed: 22281684]

Rauch A, Seitz S, Baschant U, Schilling AF, Illing A, Stride B, Kirilov M, Mandic V, Takacz A, Schmidt-Ullrich R, Ostermay S, Schinke T, Span-broek R, Zaiss MM, Angel PE, Lerner UH, David JP, Reichardt HM, Amling M, Schutz G, Tuckermann JP. Glucocorticoids suppress bone formation by attenuating osteoblast differentiation via the monomeric glucocorticoid receptor. Cell Metab. 2010; 11:517–531. [PubMed: 20519123]

Robertson KA, Nalepa G, Yang FC, Bowers DC, Ho CY, Hutchins GD, Croop JM, Vik TA, Denne SC, Parada LF, Hingtgen CM, Walsh LE, Yu M, Pradhan KR, Edwards-Brown MK, Cohen MD, Fletcher JW, Travers JB, Staser KW, Lee MW, Sherman MR, Davis CJ, Miller LC, Ingram DA,

Clapp DW. Imatinib mesylate for plexiform neurofibromas in patients with neurofibromatosis type 1: A phase 2 trial. Lancet Oncol. 2012; 13:1218–1224. [PubMed: 23099009]

Schulz A, Baader SL, Niwa-Kawakita M, Jung MJ, Bauer R, Garcia C, Zoch A, Schacke S, Hagel C, Mautner VF, Hanemann CO, Dun XP, Parkinson DB, Weis J, Schroder JM, Gutmann DH, Giovannini M, Morrison H. Merlin isoform 2 in neurofibromatosis type 2-associated polyneuropathy. Nat Neurosci. 2013; 4:426–433. [PubMed: 23455610]

Sestini R, Bacci C, Provenzano A, Genuardi M, Papi L. Evidence of a four-hit mechanism involving SMARCB1 and NF2 in schwannomatosis-associated schwannomas. Hum Mutat. 2008; 29:227–231. [PubMed: 18072270]

Sher I, Hanemann CO, Karplus PA, Bretscher A. The tumor suppressor merlin controlsgrowthin its open state, andphosphorylationconverts it to a less-active more-closed state. Dev Cell. 2012; 22:703–705. [PubMed: 22516197]

Smith ME, Cimica V, Chinni S, Jana S, Koba W, Yang Z, Fine E, Zagzag D, Montagna C, Kalpana GV. Therapeutically targeting cyclin D1 in primary tumors arising from loss of Ini1. Proc Natl Acad Sci USA. 2011; 108:319–324. [PubMed: 21173237]

Smith MJ, Kulkarni A, Rustad C, Bowers NL, Wallace AJ, Holder SE, Heiberg A, Ramsden RT, Evans DG. Vestibular schwannomas occur in schwannomatosis and should not be considered an exclusion criterion for clinical diagnosis. Am J Med Genet Part A. 2012a; 158A:215–219. [PubMed: 22105938]

Smith MJ, Walker JA, Shen Y, Stemmer-Rachamimov A, Gusella JF, Plotkin SR. Expression of SMARCB1 (INI1) mutations in familial schwannomatosis. Hum Mol Genet. 2012b; 21:5239–5245. [PubMed: 22949514]

Smith MJ, Wallace AJ, Bowers NL, Rustad CF, Woods CG, Leschziner GD, Ferner RE, Evans DG. Frequency of SMARCB1 mutations in familial and sporadic schwannomatosis. Neurogenetics. 2012c; 13:141–145. [PubMed: 22434358]

Solga AC, Gianino SM, Gutmann DH. NG2-cells are not the cell of origin for murine neurofibromatosis-1 (Nf1) optic glioma. Oncogene. 2013 Advance online publication 14 January 2013; doi: 10.1038/onc.2012.580.

Sperfeld AD, Hein C, Schroder JM, Ludolph AC, Hanemann CO. Occurrence and characterization of peripheral nerve involvement in neurofibromatosis type 2. Brain. 2002; 125:996–1004. [PubMed: 11960890]

Stowe IB, Mercado EL, Stowe TR, Bell EL, Oses-Prieto JA, Hernandez H, Burlingame AL, McCormick F. A shared molecular mechanism underlies the human rasopathies Legius syndrome and Neurofibromatosis-1. Genes Dev. 2012; 26:1421–1426. [PubMed: 22751498]

Walsh KS, Velez JI, Kardel PG, Imas DM, Muenke M, Packer RJ, Castellanos FX, Acosta MT. Symptomatology of autism spectrum disorder in a population with neurofibromatosis type 1. Dev Med Child Neurol. 2013; 55:131–138. [PubMed: 23163951]

Wehr MC, Holder MV, Gailite I, Saunders RE, Maile TM, Ciirdaeva E, Instrell R, Jiang M, Howell M, Rossner MJ, Tapon N. Salt-inducible kinases regulate growth through the Hippo signalling pathway in Drosophila. Nat Cell Biol. 2013; 15:61–71. [PubMed: 23263283]

Yi C, Troutman S, Fera D, Stemmer-Rachamimov A, Avila JL, Christian N, Persson NL, Shimono A, Speicher DW, Marmorstein R, Kissil JL. A tight junction-associated Merlin-angiomotin complex mediates Merlin's regulation of mitogenic signaling and tumor suppressive functions. Cancer Cell. 2011; 19:527–540. [PubMed: 21481793]

Zhou L, Ercolano E, Ammoun S, Schmid MC, Barczyk MA, Hanemann CO. Merlin-deficient human tumors show loss of contact inhibition and activation of Wnt/beta-catenin signaling linked to the PDGFR/Src and Rac/PAK pathways. Neoplasia. 2011; 13:1101–1112. [PubMed: 22247700]