The NNFF International Consortium
for the Molecular Biology of NF1 and NF2

June 1-4, 2003
Aspen, CO

A Summary

by
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The National Neurofibromatosis Foundation, Inc. held the 2003 meeting of the *"NNFF International Consortium for the Molecular Biology of Neurofibromatosis 1 and Neurofibromatosis 2"* June 1-4 in Aspen CO.

Two Special Occasions Noted

The Foundation noted two special occasions at this meeting. First, was the fact that the organization is celebrating its 25th Anniversary. NNFF President Peter Bellermann thanked the Foundation's co-founders Dr. Allan Rubenstein, Lynne Courtemanche Shapiro R.N., and Joel Hirschtritt, Esq. Dr. Rubenstein received a standing ovation for his 25 years of dedicated service to the NNFF.

The NNFF also celebrated the 10th Anniversary of the discovery of the gene for Neurofibromatosis Type 2, in the laboratories of Dr. James Gusella (MGH/Harvard), and Dr. Gilles Thomas (Fondation Jean Dausset, Paris) and Dr. Guy Rouleau (McGill University, Montreal). All three were honorary co-chairs of the first session, titled "Neurofibromatosis Type 2-Ten Years Later."

Exceptional Progress During Past Year

Dr. David Gutmann (Washington University) and Dr. Marco Giovannini (Fondation Jean Dausset, France) co-chaired the Consortium Meeting. Mr. Bellermann thanked them both on behalf of the NF Community for a "job well done" in organizing the meeting.

Progress over the past year has been exceptional, and the audience was energized by the information presented during the meeting. Mouse models continue to provide a rich resource for experimental studies to understand the biology of the NF1 and NF2 genes. The spirit of communication and collaboration among NF researchers continues as use of these mouse models expands to new laboratories and research fields. Cells and tissues from these mice are a rich resource for studies in a number of laboratories trying to understand the role of the NF1 or NF2 genes in normal and tumor cell function. There were presentations this year on non-tumor features of NF1, including cardiac and myeloid cell defects, bone abnormalities, and pain. Learning and memory studies were presented for both mouse and fruit fly models. Microarray technology is being used to identify the changes in gene expression in NF1 and NF2 tissues, as a promising avenue toward identifying possible targets for therapeutic development. Many scientists were overheard discussing new data, possible collaborations, or projects that will be done on the return to the laboratory.

Keynote Addresses

There were two keynote speakers at the meeting. Dr. Anton Berns (The Netherlands Cancer Institute, Amsterdam) spoke about technologies that help to

dissect tumorigenesis in mouse models of cancer. These include the annotated mouse genome sequence, the ability to manipulate the mouse germ line, efficient genetic mapping strategies, high throughput mutagenesis screens, RNAi, in vivo imaging technology, pathology analysis, CGH, and gene expression profiling. All of these technologies contribute to the rapid expansion of our knowledge about human tumors. In the mouse models, tumor samples can be used to identify pathways involved in inhibition, maintenance and progression of tumors. Tumors can be monitored using fluorescent imaging technologies. Identified targets can be identified and validated using current technologies, and suitable targets can be entered into the process of drug design and development.

Dr. Mahendra Rao (NIA/NIH) spoke about neural stem cells, and the potential to use them for studying normal neural cell processes, and about the potential of these cells as therapeutic agents in disease models.

Department of Defense and NIH Representatives Participating

The Department of Defense (DOD) and the National Institutes of Health (NIH) were represented at this meeting as well. The DOD Neurofibromatosis Research Program (NFRP) funds a large portion of NF research both nationally and internationally, at a current rate of about $20 million per year. Dr. Rick Kenyon (DOD) spoke about the NFRP and the latest call for applications. New this year is the Clinical Trials Development Award, which will be a fast track process for applicants for a year of funding to develop the resources necessary to propose a clinical trial for DOD funding. He also announced the new policy for allowing for resubmission of proposals, allowing for the first time a response to criticisms of the previous review. He described the NF1 and NF2 story boards, a project that the DOD has undertaken to present milestones and accomplishments in research for NF, and invited comments and corrections from the participants of the meeting. Dr. Robert Finkelstein (NINDS/NIH) was available for consultation about possible funding through the NIH, and to describe new programs that would provide funding opportunities for NF researchers.

NF2 Clinical and Research Findings

The first session was devoted to the accomplishments of NF2 clinical and research findings. Dr. Mia MacCollin (MGH/Harvard) spoke about NF2 diagnostic criteria. Since 1987, four sets of diagnostic criteria have been published, and while similar, they created the problem of not providing sufficient information for the general clinician to make a diagnosis. The presence of bilateral vestibular schwannomas (VS) is the standard criteria for diagnosis of NF2. However, other symptoms may suggest NF2, in the absence of bilateral tumors. Unilateral VS and the presence of a meningioma, schwannoma or glioma suggests NF2. Mosaicism is a common feature in NF2 founders (nonfamilial patients), and complicates the diagnosis. Other features appear in NF2 patients, but do not appear in the diagnostic criteria. Standard imaging

protocols do not meet the needs for diagnosing NF2, and specific protocols must be described to screen presymptomatic cases. A separate, adjunct meeting was held with a number of top NF2 clinicians, to discuss the NF2 diagnostic criteria and make recommendations for the 2003 consensus on NF2 diagnostic criteria.

Dr. William Slattery (House Ear Institute) described the NF2 Natural History Studies he is conducting with funding from the Department of Defense. The primary purposes of the study were to develop an international consortium of NF2 clinical centers, and to measure the growth rate and clinical course of the disease. In addition, audiology measures were taken. To date, 103 patients have been enrolled. Data have been collected that indicate that there is slow growth over time, and that there is no correlation between right side vs. left side in bilateral tumors. Growth rates are also not related to family history. Audiological data indicate that hearing worsens over time, but newly diagnosed cases have hearing stability for about 2 years. A follow-up study will use 3-D volumetric analysis to detect growth in small tumors.

Dr. Vijaya Ramesh (MGH/Harvard) spoke of the functional significance of NHERF/PDGF receptor binding in lamellipodia and membrane ruffles of cells. Merlin, the NF2 protein, localizes to these structures. In cells which contained mutant NHERF/PDGF interactions, the cytoskeletal elements did form. However, there were spreading defects, reduced cell migration, and F-actin rearrangement and increased focal adhesions. Dr. Andi McClatchey (MGH/Harvard) spoke about signaling defects in NF2-deficient cells. In NF2-deficient cells, there was loss of contact-dependent inhibition of growth, growth factor independence and persistent growth factor receptor signaling, and aberrant differentiation. She also presented data on the effect of the loss of ezrin in intestinal development. Loss of ezrin resulted in high embryonic mortality, with only 16% of NF2 mouse models surviving to birth. Of these, only one has survived to weaning. The ezrin-deficient mice were found to have abnormal intestinal villi, with fusion of microvilli at the brush border. She found that moesin expression in supporting tissues sometimes moved into the surrounding villi in ezrin-deficient mice.

Dr. Marco Giovannini (Fondation Jean Dausset, France) presented information about his NF2 mouse models. In conditional knock-out mice, expressing the cre-recombinase in Schwann cells, 90% of the mice developed Schwann cell hyperplasia, and 40% developed schwannomas. Double mutant mice (with loss of NF2 and NF1 genes) develop a spectrum of tumors related to both NF1 and NF2 tumors. In addition, he showed that by exposing NF2 +/- mice to asbestos fibers, he was able to model malignant mesothelioma, with inactivation of the WT NF2 gene found in 85% of the mesotheliomas. An NF Mouse Models Consortium Meeting was held in February hosting mouse neuropathologists and NF animal models researchers, to develop a consensus on the classification of the mouse peripheral nerve tumors. A paper will be submitted which describes this group's consensus.

Dr. Marcelo Curto (MGH/Harvard) spoke about NF2 conditional mice, crossed with albumin-cre, for liver specific knock-out. In these mice after one month, the livers are greatly enlarged, and there are oval cell tumors.

Dr. Richard Fehon (Duke University) spoke about the function of the ERM proteins in Drosophila. The fruit fly has a gene related to the NF2 protein, termed dmerlin, and only one gene related to the ERM proteins, referred to as dmoesin. In addition, another gene has been shown to be part of the family, "the *expanded* Drosophila gene".. Both dmerlin and dmoesin are expressed in all cells, although merlin has punctate localization and moesin localizes to the apical microvillar and adherens junctions. Both are required to establish anterior/posterior patterning. Both merlin and moesin mutants have overproliferation of cells. Merlin interacts with the *"expanded* protein*"* in a head-to-tail manner, suggesting partial redundancy in function. Moesin does have a role in the Rho pathway, and reducing the level of Rho reduces the moesin mutant phenotype.

Animal Models

Mouse models of NF1 and NF2 have become very important for understanding not only tumor formation but also many of the other symptoms of the diseases. Dr. Kevin Shannon (University of California, San Francisco) provided an update on the NF Mouse Models Consortium, established to produce and characterize models of NF1 and NF2, to identify biochemical pathways and targets, and to provide the host for preclinical intervention studies. The Consortium continues to interact with the NCI's Mouse Models of Human Cancer Consortium, although it is funded through the Department of Defense NF Research Program. There are some hurdles that must be overcome in order for preclinical testing to move ahead. There are also intellectual property concerns, among both pharmaceutical and academic organizations. It is difficult to identify and obtain lead compounds. At this point, preclinical testing in the laboratory is limited to small numbers of animals, and does not yet involve high throughput testing. Mouse model scientists in academic settings typically lack the necessary expertise and resources. There will be an NF Mouse Models Consortium Preclinical Therapeutics meeting in 2004 where a number of these issues will be discussed. Participating will be academic scientists ands well as biotech and pharmaceutical experts.

Dr. Shannon spoke about his research involving mouse models of leukemia. By using the NF1$^{flox/flox}$ mouse (created in the Parada lab) and crossing it with a bone marrow-specific expressor of the cre-recombinase, he has been able to demonstrate myeloproliferative disease in the mice by three months of age. He is testing the GM-CSF inhibitor PD184352 in these mice. In JMML, patients have GM-CSF hypersensitivity, although only 25%, have activated Ras or NF1 mutations. In Noonan Syndrome, a genetic disease related to NF1, there are reports of cases with JMML. There are mutations in the gene PTPN11 in 50% of

Noonan cases, and patients with JMML without NF1 mutations or activated Ras, 80% have PTPN11 mutations.

Dr. David Gutmann (Washington University, St. Louis) described studies related to astrocytoma modeling in the mouse. The NF1$^{flox/flox}$ mouse crossed to an astrocyte-expressing cre mouse resulted in no tumor formation in the mice, even after 20 months of age. This suggested that NF1 loss in astrocytes is not equivalent to Ras activation. When NF1^{flox}-astrocyte mice were crossed with a lox-stop-lox Ki-Ras mouse, the NF1-deficient astrocytes had impaired cAMP signaling. Crossing NF1^{flox}-astrocyte mice with NF1 heterozygous mice resulted in a swelling in the optic nerve and chiasm, reflecting more closely the NF1 optic pathway glioma.

Dr. Karlyne Reilly (NCI/NIH) spoke about genetic modifiers in mouse susceptibility to astrocytoma. In Nf1:p53 cis/C57Bl/6 mice, tumors arise with an average latency of 7 months, and there is loss of p53 and increase in Ras expression resulting from chromosome loss. When this mutation was crossed into a 129S4/Sv strain, the mice were resistant to tumor formation, and small tumors that did grow were very low grade. She plans to cross into other strain backgrounds, and to identify strains with higher risk for astrocytoma or lower occurrence of MPNST. With the proper genetic crosses, she will be able to identify genes that act as modifiers of tumor expression.

Dr. Kristine Vogel (University of Texas, San Antonio) spoke about mutation frequencies and tumor progression in NF1 and Nf1(+/-);Trp53(+/-) mouse tissues. Mutations in NF1 or Trp53 affect the DNA repair mechanisms, resulting in increased mutation frequency. Tumor cells lines have been generated, and will be a valuable resource for testing a variety of DNA-damaging agents and culture conditions for changes in the mutation frequency.

Dr. Kelly Monk (University of Cincinnati) described experiments designed to test the relevance of the observation that there were EGFR(+) Schwann cells in dermal and plexiform neurofibromas. In a transgenic mouse overexpressing EGFR in Schwann cells, there were enlarged, fibrotic nerves, mast cell accumulations, and disrupted axon-glial interactions, all features of human neurofibromas. In a c-kit mutant with mast cell ablation, the nerve ultrastructure could be restored. Dr. George Perrin (University of Florida) described a xenograft mouse model of NF1 plexiform neurofibroma. Xenografts were obtained by transplantation of MPNST cell lines into the nerves of Scid mice. These tumors grew rapidly and reproducibly. He plans to use these mice to test therapeutic agents.

Dr. Alcino Silva (University of California, Los Angeles) described studies on learning and memory in NF1 mutant mice. He has demonstrated hyperactivation of Mapk in NF1 mice. The NF1 mutation causes deficits in synaptic plasticity, resulting in spatial learning deficits. Decreasing Ras function by genetic

manipulation or pharmacological intervention rescues these deficits. To determine the location of the deficits, mice were engineered that had loss of NF1 expression specifically in excitatory neurons or in inhibitory neurons. Loss in excitatory neurons resulted in no deficits, while loss of NF1 in both excitatory and inhibitory neurons disrupted learning. Studies were done which showed that increased GABA inhibition is not caused by post-synaptic changes in excitatory neurons, and that NF1 regulates signaling around axonal terminals. Dr. Steven Kushner (UCLA) described studies of Ras/Mapk signaling and the role in cognition. Ras/Mapk acts presynaptically through Synapsin 1, and there is also an increase in LTP in these mice.

Dr. Yuan Zhu (University of Texas, Southwestern Medical Center) spoke about the use of conditional knock-out mice to study CNS tumors. Loss of p53 and activation of the receptor tyrosine kinase pathway (RTK) occur during astrocytoma formation. To answer the question of whether loss of NF1 and p53 is sufficient to induce astrocytoma, mice were generated with both p53 and NF1 mutations. Loss of only NF1 was not sufficient to cause astrocytoma formation. Loss of both p53 and NF1, however, resulted in 100% astrocytoma formation. It was also demonstrated that order was important, and that p53 loss must occur first in order for tumors to form. It was also shown that the progenitor cells for these tumors were neural stem cells.

Dr. Jon Epstein (University of Pennsylvania) spoke about the roles of NF1 and Ras in cardiac development. The NF1flox/flox mouse was crossed with a mouse expressing cre through the Tie-2 promoter, causing loss of NF1 expression in endothelial cells. The resulting mice had heart and vascular defects, and can serve as a model of the cardiovascular effects seen in patients with NF1.

Dr. Wade Clapp (Indiana University) described his studies on the role of mast cells in NF1. Heterozygous NF1 (+/-) mice have increased numbers of peritoneal and cutaneous mast cells. In other cancer models, inflammatory mast cells upregulate angiogenesis, and mast cells localize to the tumor microenvironment. In cell culture studies, embryonic day 13.5 Schwann cells from either wild type (+/+) or double mutant NF1 (-/-) animals were mixed with heterozygous NF mast cells. Conditioned media from the -/- mice caused increased migration of mast cells, which was mediated through the α4-B1 integrin.

Dr. Cynthia Hingtgen (Indiana University) is interested in understanding how growth factors affect pain response (hyperalgesia) in NF1 patients, using NF1 (+/-) mice. There is increased Ras in these mice in response to growth factors. Stem cell factor treated NF1(+/-) mice demonstrated mechanical hyperalgesia compared to wild type (+/+) or untreated (+/-) animals. Central sensory neurons also had a 3-4 fold increase in the release of neuropeptides substance P (SP) and in calcitonin gene-related protein (CGRP) in response to capsaicin stimulation. These neuropeptides are an indication of sensory neuron

sensitization and are important in initiation of the pain signal. Changes in levels correlate with hyperalgesia. Further studies are needed in this area.

Dr. Janet Hock (Indiana University), a bone biologist and endocrinologist, is studying the abnormalities in bone in NF1. Mouse models can be used to study trabecular bone. There were no differences in bone between (+/+) and (+/-) mice for bone mineral density, skull size, bone length, or trabecular bone histomorphometry. There were differences in the biomechanical properties of strength, and a decreased vertebral height. In NF1 (+/-) primary bone, osteoclasts are hyperactivated, and there is an increase in p21Ras, Akt, and apoptosis is enhanced in bone cells, although not in bone marrow stromal cells.

Dr. James Walker (MGH/Harvard) spoke about his work using Drosophila microarrays to study changes in gene expression. He has identified 100 genes that are downregulated greater than 2-fold, and 69 genes upregulated at least 2-fold, and will study these genes to identify modifiers that may play a role in NF1 pathology. Dr. Yi Zhong (Cold Spring Harbor Laboratory) spoke about learning in Drosophila. Overexpression of NF1 in flies results in an enhancement in memory.

Molecular Genetics & Biology

Dr. Jan Friedman (University of British Columbia, Canada) spoke about the natural history and risk factors for NF1. The course of NF1 is not predictable, as symptoms vary widely among individuals, even within the same family. He reported on studies that show that there is a correlation between certain features of NF1 among families, including pigmentation, tumor burden, and risk for malignancy. He described the data about a slightly increased risk for malignancy and the shorter life expectancy for patients with NF1. These data, however, were based on a set of patients who were followed at clinics which might see more of the complicated cases of NF1.

Dr. Bruce Korf briefly described mutation analysis for NF1 germline and somatic mutations. He also described the natural history study for plexiform neurofibromas in NF1, funded by the DOD NFRP. The study "Natural History of Plexiform Neurofibromas in NF1" is in its fourth year. The major goal of the study is to use volumetric MRI to measure the growth rate of plexiform neurofibromas. Although data are still being collected, and hence a final analysis has not been done, we have begun to look at longitudinal growth data. The volumetric protocol has been validated and produces volume data accurate to about 10%. We have also identified imaging differences between superficial and deep plexiform neurofibromas. Finally, it is hoped that the consortium of clinical data collection centers will serve for future clinical studies, including clinical trials.

Dr. Margaret McLaughlin (MIT Center for Cancer Research) described her studies on the hormonal regulation of neurofibroma growth. Hers is one of the

first studies of hormonal influences in neurofibromatosis, a phenomenon often noted by patients with NF1. Estrogen receptors were not present on the tumor cells. Progesterone receptors (PR), however, were found in 75% of the tumors. The PR were found in every subtype of neurofibroma, but were not present in schwannomas or MPNST. They were also not present in peripheral nerve. There are two isoforms of PR, and both types were found in the tumors. Cells that express PR could also express neurofibromin, but not S100. The PR+ cells were either fibroblast or perineurial cells, not Schwann cells. Progesterone has been shown to promote myelination in the CNS, and Schwann cells can produce progesterone. These data suggest that the PR+ cells are non-neoplastic, but may support the neoplastic cells. It is possible that anti-progestins, such as RU486, may be therapeutic against these tumors.

Dr. Andre Bernards (MGH/Harvard) described a project looking for genetic modifiers that might control the number or density of cutaneous neurofibromas in NF1 patients. He is looking for patients with a very low or very high burden of tumors, and will use genetic analyses to determine whether there are distinct genes expressed (or not expressed) which lead to either of the tumor phenotypes. Dr. Shyra Miller (University of Cincinnati) described experiments using expression profile analysis to identify genes that are changed during progression of neurofibromas into MPNST. From 8 cells lines, 6 NF1-related and 2 sporadic, she has identified a gene, p16/INK4 which is lost in NF1 associated tumors. Dr. Karen Stephens (University of Washington) described her work on NF1 microdeletions, in which 1.5 megabases are deleted in a region containing the NF1 gene. She found that 70% of these microdeletions are recurrent and occur at discrete recombination sites within the flanking repeat sequences (NF1REP). Patients with this microdeletion genotype have a higher burden and earlier appearance of cutaneous neurofibromas.

Dr. Jan Dumanski (Uppsala University, Sweden) described the use of high resolution mapping to profile gene copy numbers in NF2-related samples using a full-coverage chromosome 22 microarray. This array allows for accurate detection of homozygous/heterozygous deletions, amplifications/gains, and breakpoints of imbalanced translocations. This microarray may be useful in detecting deletions in other genes, including the locus predisposing to schwannomatosis, and future studies are focused on this study.

Dr. Karen Cichowski (BWH/Harvard) described studies on neurofibromin, which is dynamically regulated by the proteosome. She is dissecting the signals that contribute to neurofibromin stabilization or degradation. Neurofibromin is degraded rapidly by the proteosome in response to growth factor stimulation, but reappears shortly after growth factor treatment, playing a critical role in terminating Ras signaling. Neurofibromin is phosphorylated as it is re-expressed, and this phosphorylation is induced by expression of constitutively activated Ras, Raf, and MEK, but not activated AKT. MEK inhibitors, but not PI3K inhibitors block the phosphorylation. These data suggest a novel negative feedback model

where activation of the Ras/Raf/MEK pathway induced neurofibromin phosphorylation, increasing its activity and stability.

Dr. Kelly Morgan (University of Minnesota) spoke about studies on a myeloproliferative disorder associated with NF1. Data suggested that blocking of the GM-CSF receptor or downstream signals may be of therapeutic benefit in NF1-associated myeloid diseases. Reintroduction of the GRD domain of the NF1 gene reduced the amount of activated K-Ras and N-Ras in myeloid cells, however, the GRD was strongly selected against in the transduced cells, limiting its use as a therapeutic agent. Inducible GRD and small interfering RNA against GRD will be used to modulate the level of expression.

Dr. Michael Stern (Rice University) described studies concerning Ras regulation of perineurial glial growth in Drosophila. Data indicated that Ras activity is necessary and sufficient for increased perineurial glial growth, and that Ras can promote this growth cell nonautonomously.

Dr. Eva Dombi (NCI/NIH) described the use of longitudinal automated volumetric MRI analysis of plexiform neurofibromas as part of a Phase II clinical trial for NF1. The trial is an ongoing double-blinded, placebo-controlled, cross-over phase II trial using a farnesyltransferase inhibitor. At disease progression (increase of tumor volume >20%), patients are crossed over to the second phase. Automated volumetric MRI analysis reliably detects tumor progression based on quantification of smaller changes in tumor size than can be detected by either of the two standard tumor response criteria.

Dr. Westley Friesen (PTC Therapeutics) described the use of nonsense suppression as a novel therapy for NF1 or NF2. More than 30% of NF1 patients, and about 20% of NF2 patients have nonsense mutations. PTC is developing compounds that allow for read-through of the nonsense mutation to produce active, full length protein. Small molecules, such as gentamycin, have been shown to read through nonsense mutations in Cystic Fibrosis, Hurlers Syndrome, p53 and Duchenne Muscular Dystrophy. PTC is developing compounds that function in a similar way, and will test them in preclinical studies to determine if they are capable of restoring full-length neurofibromin and merlin to tumor cells. Such compounds may provide therapeutic value to patients with NF1 or NF2.

Cell Biology

Dr. Michael Bennett (University of Cincinnati) spoke about the role of the NF1 gene in oligodendrocyte differentiation. He found that an NF1 mutation increased the number of oligodendrocyte precursors, and that the precursors showed increased Ras-GTP. This increase in cell number could be inhibited by farnesyltransferase inhibitors.

Dr. Laurence Goutebroze (Fondation Jean Dausset, France) described the association of CASPR/paranodin with merlin and B1-integrin in the central nervous system. This association is thought to be interdependent. Dr. Dominique Lallemand (MGH/Harvard) spoke about the role of merlin in tumorigenesis. NF2-deficient mouse embryo fibroblasts (MEF) do not undergo contact-dependent inhibition of proliferation. He showed that NF2 MEF lack "adherens junctions";and that if NF2 function is restored, "adherens junctions" form and contact inhibition is restored.

Dr. Oliver Hanemann (University of Ulm, Germany) described the role of Rac1/JNK signaling in schwannomas. In merlin-deficient schwannoma cells, there is high Rac1 activity. This protein also phosphorylated merlin through PAK activation. In primary human schwannoma cells, Rac1 and PAK are translocated to the membrane, and there are increased levels of JNK in the nucleus. This suggests that merlin regulates Rac1 activation, and downstream signaling, which is important for schwannoma cell de-differentiation.

Dr. Nancy Ratner (University of Cincinnati) spoke about gene expression in NF1-deficient Schwann cells. Schwann cells derived from NF1 null mice have enhanced migration compared to wild type controls. Migration is not inhibited by treatment with a farnesyltransferase inhibitor or a dominant negative H-Ras adenovirus. However, two other Ras proteins, R-Ras and TC21, both affected cell migration. Using a dominant negative R-Ras decreased migration 50%, while TC21 activation increased migration 2-fold.

Dr. Feng-Chun Yang (Indiana University) discussed how loss of NF1 in Schwann cells provides a stimulant for mast cell migration via the secretion of a Kit ligand. The stimulus was potent for NF1+/- mast cells, but not for wild type cells. Using protein arrays, candidate chemokines were identified. He showed that hyperactivation of the Ras-Class1$_A$-PI-3 kinase-Rac 2 pathway is directly responsible for the increased migration of NF1+/- mast cells.

Posters

In addition to the oral presentations, there were 36 abstracts presented as posters. These posters covered a number of areas of both NF1 and NF2 research, and added to the overall scientific contributions to the meeting.

Clinical studies and case reports were presented for both NF1 and NF2. Lan Kluwe (University Hospital Hamburg-Eppendorf) reported on analysis of NF1 mutations in families with NF1 and spinal tumors. The results indicated that this group of NF1 patients exhibited the same degree of variability of symptoms as most other NF1 patients. Dr. Ina Vandenbrouke (Ghent University Hospital, Belgium) identified a novel splice variant of NF1 lacking exon 43, expressed at high levels in a number of tissues. She showed that cellular localization was

affected by the presence or absence of this exon. With exon 43, there was strong nuclear localization, with faint cytoplasmic localization. Without exon 43, there was no nuclear localization, and homogenous cytoplasmic localization. She suggests that exon 43 controls localization to the nucleus, and that there is a functional role in neurofibromin in the nucleus.

Dr. Bradley Welling (The Ohio State University) described the use of microarrays to analyze gene expression profiles in human vestibular schwannomas. Forty-two genes were upregulated 3-fold or more in 5 of 7 tumors, compared to normal vestibular nerve. Included in this group were osteonectin, an angiogenesis mediator, and RhoB GTPase, which is important in cellular signaling. Among genes that were downregulated, were the apoptosis-related LUCA-15 genes, and CDK2. Dr. Michael Baser described a series of studies evaluating genotype-phenotype correlations in NF2 patients. Symptoms included vestibular schwannoma growth rates, intracranial meningiomas, cataracts, spinal tumors, and peripheral nerve tumors. In addition, he compared the association of constitutional NF2 mutation type with non-8th nerve nervous system tumors and symptoms. Finally, he presented information about the need to modify the diagnostic criteria for NF2, in cases where there were not bilateral vestibular schwannomas.

Dr. Eva Sujansky (University of Colorado) presented an interesting case of three generations of patients with neurogenic tumors. Tumors were identified pathologically as neurofibromas, however, the patients did not meet diagnostic criteria for NF1. She suggested that this might represent an atypical NF. Dr. Anat Stemmer-Rachamimov (MGH/Harvard) presented a case report of a patient with multiple sporadic meningiomas in the brain and lung. This patient had no clinical or family history of NF2, however, the tumors exhibited loss of NF2 function. Lung tumors were thought to be due to metastasis of brain meningiomas, a rare occurrence in NF2.

Dr. Jussi Koivunen (University of Oulu, Finland) described studies assessing epidermal wound healing in NF1 patients. Since NF1 expression occurs in keratinocytes, it is possible that loss of this expression in NF1 patients would result in changes in healing of epidermal wounds. She found, however, that there was no difference in the rate of healing, or in the long term outcomes between NF1 and non-NF1 controls. Dr. Andreas Kurtz (MGH/Harvard) described work in his laboratory on the evaluation of midkine serum levels and age and onset of neurofibroma development in NF1 patients. His data suggest that there is an elevation of midkine serum levels that correlate with age, number of cutaneous tumors, presence of astrocytomas, optic gliomas, and malignant peripheral nerve sheath tumors. He proposes analysis of serum-based factors as useful diagnostic markers for NF1 tumorigenesis.

Dr. Victor Mautner (Klinikum Nord, Hamburg) described studies evaluating attention problems in adults with NF1. NF1 adults with attention problems

reported more interpersonal distress and poorer psychosocial functioning, not related to the severity of disfigurement. These studies confirm findings in children, and suggest that treatment continue through adulthood.

Learning disabilities research for NF1 was described using NF1 mouse models described earlier by Dr. Alcino Silva. Learning and memory are controlled by a part of the brain called the hippocampus. Dr. Yijun Cui (UCLA) described the use of the NF1 (+/-) mice to study neurotransmitter GABA inhibition in the brain. She found that GABA signaling inhibition was caused by an increase in GABA release in hippocampal neurons. Dr. Steven Kushner (UCLA) described studies described studies using an activated Ras mutation (RasG12V) expressed in the glutamatergic forebrain neurons resulted in enhanced learning of two different types of hippocampal-dependent tasks: spatial learning and fear conditioning. The mechanism for this enhanced learning was through increased Ras/ERK/Synapsin I signaling in excitatory forebrain neurons.

Dr. George Mashour (MGH/Harvard) presented studies on the effect of fatty-acid (FA) modulation on the growth of MPNST. Docosahexaenoic acid (DHA) and arachidonic acid (AA) were studied. A low dose of DHA was found to stimulate growth of cells in vitro, and high doses induced cell death. High dose also reversed the stimulatory effect of a number of growth factors, including EGF, PDGF-beta, bFGF, and IGF-1. AA was found to have a reciprocal effect, stimulating growth at high doses. Dr. Gail Deadwyler (Loyola University Chicago) hypothesized that increased expression of MAPK leads to activation of phospholipase A_2, leading to prostaglandin secretion and activation of prostaglandin E_2. Preliminary data support this hypothesis. Dr. Stacey Thomas (Loyola University Chicago) determined that in neurofibromin-deficient Schwann cells, there is an altered expression of angiogenic factor VEGF and anti-angiogenic factor pigment epithelium derived factor (PRDF), which may promote a pro-angiogenic state in Schwann cells.

Dr. Min Wu (University of Florida) described studies creating an alternative mouse model of tumorigenesis. Mouse NF-/- Schwann cells were transplanted into the sciatic nerve of an adult Scid/NF1+/- mouse. Engrafted nerves exhibited extensive cell proliferation, hypercellularity, proliferation independent of axonal contact, and mast cell infiltration. This model can be used to study the potential contribution of other genetic or epigenetic factors to tumor formation in NF1. Dr. Laura Fishbein (University of Florida) described the effects of steroid hormones on the growth of NF1 tumors and Schwann cell culture. Data was inconclusive using in vitro and xenograft models of NF1.

Dr. Frances Hannan (Cold Spring Harbor Laboratory) presented data on the regulation of adenylyl cyclase in learning in Drosophila. Since cAMP pathways are not involved in pathogenesis of NF1 in human studies, she suggests that the Ras pathway appears to be the pathway important for both pathogenesis and memory defects in human NF1.

Dr. Klaus Scheffzek (European Molecular Biology Laboratory, Heidelberg) described work on comparison of Ras-specific GTPase activating proteins (GAPs), neurofibromin and p120, based on their crystal structure. Three prominent regions of the RasGAPs, the finger loop, and phenylalanine-leucine-arginine (FLR-) region, and the alpha7/variable loop, contain the structural fingerprints that that dictate the GAP function. In addition, other regions of neurofibromin are being studied in crystal form to identify other domains important for neurofibromin function.

Dr. John Stickney (University of Cincinnati) presented studies designed to identify the targeting of merlin to the cell membrane lipid rafts. His results indicated that both halves of the protein contain information for lipid raft targeting, and each half plays a role by interacting with each other. Dr. Marianne James (MGH/Harvard) described studies on the morphological and cytoskeletal abnormalities in merlin-deficient meningioma cells compared to normal arachnoidal cells. The major accomplishment for this study at MGH is having the four sets of matched tumor and normal cell samples for comparison. There are distinct alterations in the cytoskeletal organization in the merlin-deficient cells. Other studies are being performed to determine the alterations in cell signaling, membrane interactions and association of merlin with actin.

Dr. Annie Chan (MGH/Harvard) reported results on how the loss of merlin results in overgrowth and tumorigenesis in Schwann cells derived from the dorsal root ganglia. In merlin-deficient cells, the Schwann cells switch their survival dependency from axonal-derived neuregulin to Schwann cell autonomous signals. Dr. Marisa Loeffler (MGH/Harvard) described the role of merlin in bone development. Primary cultured osteoblast (bone precursor) cells required merlin to form adherens junctions and to undergo contact-dependent inhibition of growth. Loss of merlin also conferred unique growth advantages to the osteoblasts, which may explain the tumorigenic properties of the NF2-/- osteoblasts in mice and humans.

Dr. Taru Muranen (University of Helsinki, Finland) described the role of merlin in controlling growth of cells via the cell cycle. Merlin was shown to be shuttled between the nucleus and cytoplasm, and the nucleus has the most merlin just after cell division. Nuclear merlin is strongly reduced in cells grown to confluency. Dr. Lidiya Lebedeva (Russian Academy of Sciences) presented information on the role of Drosophila merlin in the control of mitosis exit and development. Cells lacking dmerlin showed asynchronous chromosome condensation at anaphase in mitosis. In addition, there was meiotic nondysjunction demonstrated in testis cells. This effect on mitosis may be in part responsible for the abnormalities in development observed in merlin deficient flies.

Dr. Kiran Mantripragada and Dr. Patrick Buckley (Uppsala University, Sweden) described high-resolution profiling of an 11-megabase segment of human chromosome 22 in sporadic schwannoma using array-CGH. This technology allowed them to differentiate between loss of the entire 11 Mb segment in some tumors, and the presence of some normal tissue in some tumors, which will provide a way to determine the important parts of chromosome 22 involved in schwannoma formation.