AGENDA

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

May 20 - 23, 2001

Hotel Jerome
Ballroom
330 East Main Street
Aspen, Colorado
Tel. 800-331-7213

Chairpersons

Zach W. Hall, Ph.D.
University of California
San Francisco

Luis Parada, Ph.D.
University of Texas
Southwestern Medical Center

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Sponsors

The National Neurofibromatosis Foundation and the organizers gratefully acknowledge support from:

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Program

5:00PM - 7:00PM Welcome Reception & Blazing Adventure Registration Desk

Session 1: Sunday May 20, 2001 - 8:00 PM -10:20 PM

Topic: CLINICAL STRATEGIES

SESSION CHAIR: Zach Hall

8:00-8:05 PM
Welcome

Peter Bellermann,
NNFF

8:05-8:15 PM
Introduction

Dr. Zach Hall,
UCSF

Dr. Luis Parada,
University of Texas, SW

8:15-8:45 PM
"Nervous System Tumors in NF: Clinical observations and Biologic Questions"

Dr. Robert Martuza,
Harvard Medical School/MGH

8:45-8:50 PM
Q&A

8:50-9:10 PM
"Volumetric MRI of Plexiform Neurofibromas: Measurement of Outcomes for Clinical Trials"

Dr. Bruce Korf,
Harvard Medical School/Partners Ctr.

9:10-9:15 PM Q&A

9:15-9:30 PM Break

9:30-9:50 PM
"The Use of Mouse Models of NF for Preclinical Testing of Novel Therapeutics"

Dr. Kevin Shannon,
UCSF

9:50-9:55 PM
Q&A

9:55-10:15 PM
Clinical Trials

Dr. Judy Small,
NNFF

10:15-10:20 PM
Q&A

Session 2: Monday morning, May 21, 2001 - 7:30 AM-1:00 PM

Topics: ANIMAL MODELS AND GENETICS

SESSION CHAIR: KEVIN SHANNON

7:30-8:30 AM
Breakfast - Antler Bar

8:30-9:00 AM

"Modeling glioma formation with somatic cell gene transfer"

Dr. Eric Holland,
Memorial Sloan Kettering Cancer Institute

9:00-9:05 AM
Q&A

9:05-9:30 AM
"Updates on NF1/p53 modifier screens and Merlin regulation"

Dr. Tyler Jacks,
MIT/ Whitehead Institute

9:30-9:35 AM
Q&A

9:35-9:55 AM
"Conditional lessons in NF1"

Dr. Luis Parada,
University of Texas, SW

9:55-10:00 AM
Q&A

10:00-10:25 AM Break

10:25-10:45 AM
"Function of the NF2 tumor suppressor in tumorigenesis and metastasis"

Dr. Andrea McClatchey,
Harvard Medical School/MGH

10:45-10:50 AM
Q&A

10:50 -11:15 AM
"Mouse models for neurofibromatosis: an update on tumor development and new insights on NF2 gene function"

Dr. Marco Giovannini,
Fondation Jean Dausset - CEPH, Paris, France

11:15-11:20 AM
Q&A

11:20-11:40 AM
"Analysis of Merlin/ERM function in Drosophila"

Dr. Richard Fehon,
Duke University

11:40-11:45 AM
Q&A

12:00 PM Box Lunches at the Antler Bar

Session 3: Monday evening, May 21, 2001 - 8:00 PM-10:15 PM

Topic: SIGNALING

SESSION CHAIR: NANCY RATNER

6:00-7:30 PM Buffet Dinner - Garden Terrace

8:00 - 8:30 PM
"Interactions between Ras and Rho signaling pathways in cell transformation"

Dr. Chris Marshall,
Chester Beatty Labs, United Kingdom

8:30- 8:40 PM
Q&A

8:40 -9:00 PM
"Hyperactivation of p21ras and the hematopoietic specific Rho GTPase, Rac2, cooperate to alter the proliferation of neurofibromin deficient mast cells"

Dr. Wade Clapp,
Indiana University

9:00-9:05 PM

Q&A

9:05 - 9:20 PM BREAK

9:20-9:40 PM "Protein 4.1 tumor Suppressor"

Dr. David Gutmann,
Washington University, St. Louis

9:40 - 9:45 PM
Q&A

9:45 - 10:05 PM
"Gene-expression profiles in meningioma"

Dr. Mladen Golubic,
Cleveland Clinic Fdn.

10:05 - 10:10 PM
Q&A

Session 4: Tuesday morning, May 22, 2001 - 8:00 AM-12:00 PM

Topic: PLASTICITY

SESSION CHAIR: ANDI MCCLATCHEY

7:30-9:00 AM Breakfast - Antler Bar

9:00-9:35 AM
"Electrophysiological Studies of the learning model long term potentiation (LTP) in wild type and mutant brain tissue"

Dr. Daniel Madison,
Stanford University

9:35-9:45 AM
Q&A

9:45-10:10 AM
"Molecular and cellular mechanisms of learning deficits in NF1 mutant mice"

Dr. Alcino Silva,
UCLA

10:10-10:15 AM
Q&A

10:15-10:40 AM Break

10:40-11:05 AM
"Ras and cAMP pathway defects in
Drosophila NF1 mutants"

Dr. Andre Bernards,
Harvard Medical School/MGH

11:05-11:10 AM
Q&A

11:10-11:35 AM
"NF1- regulated camp pathway in learning & memory"

Dr. Yi Zong,
Cold Spring Harbor Laboratory

11:35-11:40 PM
Q&A

12:00 PM Pizza & Salad - Antler Bar

Session 5: Tuesday evening, May 22, 2001 - 8:00 PM-10:00 PM

Topic: GENOTYPE/PHENOTYPE

SESSION CHAIR: Tyler Jacks

6:00-7:30 PM Buffet Dinner - Garden Terrace

8:00-8:20 PM
"NF1 Genotype/Phenotype Issues"

Dr. Margaret Wallace,
University of Florida, Gainesville

8:20-8:25 PM
Q&A

8:25-8:45 PM
"Expression studies in Schwann cells of the NF1 microdeletion genes"

Dr. Eric Legius,
University of Leuven, Belgium

8:45-8:50 PM
Q&A

8:50-9:10 PM Break

9:10-9:30 PM
"Evidence for a tumor modifying gene in NF1"

Dr. Karen Stephens,
University of Washington

9:30-9:35 PM
Q&A

9:45 PM Wine Tasting - Antler Bar

Session 6: Wednesday, May 23, 2001 - 8:30 AM-11:30 AM

Topic: NEURAL CREST

SESSION CHAIR: LUIS PARADA

7:00-8:30 AM Breakfast - Antler Bar

8:30-9:00 AM
"The role of ErbB receptors and of Sox10 in development of the peripheral nervous system"

Dr. Carmen Birchmeier,
Max Delbrueck Centrum, Germany

9:00-9:10 AM
Q&A

9:10- 9:40 AM
"Neural crest stem cells and peripheral nervous system development"

Dr. Sean Morrison,
University of Michigan

9:40-9:50 AM
Q&A

9:50-10:10 AM Break

10:10-10:30 AM
Do changes in cultured NF1 Schwann cells predict peripheral nerve tumorigenesis"

Dr. Nancy Ratner,
University of Cincinnati

10:30-10:40 AM
Q&A

10:40-11:00 AM
"NF1 neural crest and cardiac development"

Dr. Jon Epstein,
University of Pennsylvania

11:00-11:10 AM
Q&A

11:10-11:30 AM
"Summary: Future Direction"

Dr. James Gusella
Harvard Medical School/MGH

11:30 PM Adjournment

Adjunct Meetings:

Mouse Model Meeting Committee
Hotel Jerome - Board Room on 2nd Floor
Sunday, May 20, 2001, 5:00PM - 7:00PM

Medical Policy Committee - Hosted by Rick Horvitz - Meet in Hotel Jerome Lobby
Monday, May 21, 2001 - 1:00PM - 2:30 PM

International Consortium Summary (2001)

New Progress Reported at Annual Meeting of
The NNFF Intl. Consortium Meeting

"It is fantastic what has been done from last year to this year," so said Dr. James Gusella (Harvard/MGH) in his summary of the 2001 meeting of the NNFF International Consortium for the Molecular Biology of NF1 and NF2 , held in Aspen, CO, from May 20 to 23, 2001.

Dr. Gusella's enthusiasm was echoed by the other participants of this year's gathering of the world's leading scientists working on NF. New and exciting results were reported by a number of different studies, from animal models to tumors to learning disabilities. The meeting was chaired by Dr. Zach Hall (UCSF) and Dr. Luis Parada University of Texas, SW). Among the major sponsors of the meeting were four NIH Institutes: NINDS, the National Cancer Institute, the National Institute of Deafness and Other Communication Disorders, the National Heart, Lung and Blood Institute, as well as the March of Dimes.

The list of this year's participants was unusual. Instead of having the customary, one eminent scientist from outside the field as a platform speaker, a major effort had been made by the co-chairs to bring in five such presenters from other fields that have relevance to NF research. The aim was to bring major, new talents to the field.

Moreover, the twenty-five speakers had each been given a full scholarship to bring one of his/her brightest young scientists from their laboratories to the meeting. The intent was again to attract exceptional young and new talents to the field. The latter is an on-going goal the Foundation pursues also with its annual prizes for young scientists and with its annual Young Investigator Awards.

Leading off the three-day meeting was neurosurgeon Dr. Robert Martuza (Harvard/MGH) who described the types of tumors found in NF1 and NF2. He explained the difficulties in surgically removing many of the tumors. He expressed the hope that rational treatments

would be developed soon to "put me out of business." Dr. Bruce Korf (Harvard/Partners) described the natural history study for plexiform neurofibromas in NF1. He emphasized the need for this type of study to detail the growth patterns of these unpredictable tumors, which will be critical to defining tumor response to treatment in NF1 clinical trials.

Next, Dr. Kevin Shannon (UCSF) described his involvement in the NF Mouse Model Consortium, and the larger NCI-sponsored Mouse Models of Human Cancer Consortium. The goals are to establish pathologic diagnosis of mouse tumors and relating them to human tumors, building a repository of mutant strains, enhancing other technologies for studying mouse tumors and pre-clinical testing of therapeutics.

Dr. Judy Small (NNFF) described clinical trials for NF1 and NF2. The rationales for developing new therapies includes using chemotherapy to kill rapidly growing tumor cells, preventing blood vessel formation needed to sustain large tumors, developing agents that will prevent tumor cells from growing or causing them to program their own death, and targeting specific cell types in the tumors. A detailed list of currently active clinical trials can be found online at The NF Website: http://www.nf.org/clinical_trials/.

The next session moved to animal models, which are important for the development of therapies in NF1 and NF2. Drugs can be tested in these models before they are tried in human clinical trials. Dr. Eric Holland (Memorial Sloan Kettering) described his research with mouse models of gliomas. These mice develop tumors after being treated with retroviruses that contain certain tumor genes. Different types of tumors can be obtained depending on the type of genes being used.

Dr. Tyler Jacks (MIT/Whitehead) followed and spoke about his NF1/p53 mice which develop leukemia and pheochromocytoma, a rare tumor found in people with NF1. He finds that by crossing these mice with other strains of mice, he can manipulate the type and timing of the tumors. Dr. Jacks hopes to use this information to find modifying genes that would affect the types of tumors found in human NF1.

Dr. Luis Parada (University of Texas SW) then described a system for creating mutated copies of the NF1 gene in mice in specific cell types or at specific times in the mouse. The procedure is called conditional knock-outs. Dr. Parada has created mice with NF1 mutations that develop tumors similar to plexiform neurofibromas. These mice will be very useful for pre-clinical testing of treatments for neurofibromas.

Dr. Andrea McClatchey (Harvard/MGH) spoke about experiments using tissues from NF2 mutant mice. NF2 mutant cells grow faster than normal cells, do not require special growth factors to grow, and lack some of the other features of normal cells. While NF2 mutant cells are not "transformed," i.e. they are not "cancer cells," the NF2 mutant mice do develop metastatic osteosarcoma, a bone abnormality not seen in humans..

Dr. Marco Giovannini ((Fondacion Jean Dausset, Paris, France) described work on "conditional knock-outs" for the NF2 gene. He is able to manipulate these mice so that they develop Schwann cell abnormalities, schwannomas, sarcomas, or meningiomas. These mice will again be useful for pre-clinical testing of drugs.

Dr. Richard Fehon (Duke University) described studies using Drosophila Melanogaster, the fruit fly, to analyze the function of Merlin, the NF2 protein. Merlin is part of a larger family

of related proteins called 4.1, which contain Merlin-like proteins called ERM. In the fruit fly, however, there is only one ERM protein, which makes studies of function much easier. It is also easier to make mutations in the fly's Merlin gene. In a series of experiments, Dr. Fehon was able to show that inserting the human Merlin protein into the mutated fly will result in a normal fly.

To function properly, cells must be able to communicate with neighboring cells and also with cells that are quite distant. To that end, cells produce "signaling" molecules, which are secreted and transported to other sites. Cells contain specific targets on their surface that are called receptors. When a signal molecule binds, the receptor activates a signaling process inside of the cell, where there are a number of other "signal" proteins. These proteins interact in a cascading fashion, and tell the cell what it should do. The cell may be told to grow, stop growing, die, move, etc. When one of the signal molecules has been changed due to a mutation, the cell cannot get the right series of signals, and may change its normal behavior. Such is the case when a tumor suppressor gene, such as NF1 or NF2, is mutated. The cells begin to grow and divide rapidly because they don't receive the information that tells them to stop. This results in tumor formation.

Dr. Chris Marshall (Chester Beatty Labs, UK) described the action of two signal proteins, Ras and Rho, which are involved in controlling cell growth. He has identified a number of other signaling molecules that interact with Ras or Rho, and are changed either in structure or concentration in cancer cells. Dr. Wade Clapp (Indiana University) described studies showing that Neurofibromin, the NF1 protein, modulates Ras activity through another signaling pathway, C-kit. In mice that contain one defective copy each of NF1 and C-kit, there are defects in melanocyte and mast cell properties. Melanocytes are the cells that give the café-au-lait macules their color. Mast cells have been shown to be present in neurofibromas.

Dr. David Gutmann (Washington University, St. Louis) spoke about a 4.1 family protein called DAL-1. This protein is expressed in high levels in adenocarcinoma in lung. It is also highly expressed in the brain. DAL-1 is lost in some central nervous system tumors, such as meningiomas, but is not lost in sporadic schwannomas. Dr. Mladen Golubic (Cleveland Clinic) spoke on gene expression profiling in meningioma. He described the targeting of the NF2 and Ras-regulated pathways as a means to develop future treatments for these tumors.

Learning disabilities are a common feature of NF1, occurring in about 50% of people affected by NF1.. Studies on learning and memory may shed light on the reasons behind this problem. Use of NF1 mutant mice can help identify specific changes that result in learning deficits. Dr. Daniel Madison (Stanford University) described the biology of the learning process, called long term potentiation, or LTP. He defined LTP as the long-lasting increase in the strength of the brain's excitatory path after heavy use. Simply, when a neuron is given a long-term signal, it changes its response to other signals of the same type, or has "learned" the signal.

Dr. Alcino Silva (UCLA) described learning deficits in NF1 mutant mice. These mice are slower to learn tasks than are normal mice. Since it is known that Ras is elevated in NF1, he studied the effect of Ras in the mice. He found that he could "rescue" the learning deficits by decreasing Ras activity, using Ras mutations or drugs targeted to inhibit Ras function. He has also been able to demonstrate that mutations in NF1 specific to neurons resulted in disrupted

learning. Moverover, defects in NF1 Ras signaling also underlie behavioral deficits in NF1 mutant mice.

Dr. Andre Bernards (Harvard/MGH) described NF1 mutant fruit flies as having a mutant appearance. They were smaller than normal and had Ras signaling defects. These mutant flies also had a learning and memory deficit. He showed that human NF1 could rescue the size deficit. He also showed that the NF1 gene interacts with another signaling pathway, the Cyclic-AMP system. Dr. Yi Zhong (Cold Spring Harbor Laboratory, NY) also spoke about the learning deficits in the flies. He has developed a system to test the flies using shock avoidance and is studying whether the NF1 gene can rescue the learning deficit in flies.

Genetic studies are used to identify the mutations that occur in the NF1 gene in patients. While a large number of mutations have been identified, and some of them cluster at certain sites, for the most part it has not been possible to link these mutations to the symptoms in a patient. This is called the genotype (gene mutation)/phenotype (symptoms) correlation.

Dr. Margaret Wallace (University of Florida/Gainsville) spoke of this issue. She has found that patients with the same mutation, either by inheriting it from a family member or as a spontaneous event often have different symptoms. She suggests that these differences are due to modifier genes, environmental effects, or a second mutation in the cell that may vary between patients and even between tumors in the same patient. She emphasized the need for more studies of mutations, to continue to look for common features that may help to predict the course of the disease and suggest appropriate treatments.

Dr. Eric Legius (University of Leuven, Belgium) spoke about a specific mutation, a "microdeletion", where a large piece of DNA, about 1.5 megabases, is missing in the chromosome, including the NF1 gene and many other genes as well. Patients with this mutation tend to have more severe manifestations of the disease, with learning and behavioral deficits, numerous cutaneous neurofibromas, and often plexiform neurofibromas. These patients are more at risk for malignant transformation of the plexiform tumor. Dr. Legius is studying the genes that are located in the repeat sequences near the breakpoints of the deletion that may affect the symptoms of NF1.

Dr. Karen Stephens (University of Washington, Seattle) also spoke of the microdeletion. She reported that she is studying genes that are found within the deletion, but not in the repeat sequences. Dr. Stephens previously identified the first genotype/phenotype correlation involving entire gene deletions in NF1.

Neurofibromatosis 1 is considered to be a disease of neural crest cells. These are cells that migrate out of the spinal column as it is forming in very early, fetal development. Neural crest cells form all of the structures of the peripheral nervous system, the nerves and associated cells outside of the brain and spinal cord. Dr. Carmen Birchmeier (Max Delbrück Centrum, Berlin, Germany) spoke about genes involved in the development of the peripheral nervous system. Again, there are signaling molecules that tell the cells where to go and what types of cells and structures to form. Dr. Birchmeier) studies erbB and Sox10, and a gene "neuregulin" that is involved in the signaling pathway. Mice that are mutant for erbB2 cannot develop a nervous system and die before birth. The same effect is seen for mice with mutations in erbB3 or neuregulin. In erbB mutants, the neural crest cells get "stuck" in the spinal cord and do not

migrate. In neuregulin mutants, the cells do not migrate properly. Sox10 was found to regulate the expression of erbB3, and is a key regulator in the development of the peripheral nervous system.

Dr. Sean Morrison (University of Michigan) studies the biology of neural crest cells in mice. He removes the stem cells that will become neural crest, and treats them to find out at which time the cells become fixed as to the type of cell they will become. While neural crest migration occurs in a brief 24 hour period early in development, the neural crest stem cells are capable of forming different cell types until the 14th day after birth.

Dr. Nancy Ratner (University of Cincinnati) presented research on the Schwann cells from NF1 mutant mice, and the identification of a protein called BLBP(for brain lipid binding protein) which regulates the growth of the cells. She can purify two types of Schwann cells from the mice, one which acts like normal cells, growing slowly, and another which is called txf that grows quickly and expresses a growth factor receptor not found on normal Schwann cells. BLBP is found at higher levels in the txf cells. Since only cells that grow slowly can become associated with neurons, the txf cells do not. Treating the cells with an antibody to BLBP allows the cells to associate with neurons. This suggests that BLBP may play a role in NF tumorigenesis.

Dr. John Epstein (University of Pennsylvania) described work on the effect of the NF1 mutation on the development of the heart. The NF1 mutant mouse dies before birth because of a cardiac abnormality. The heart has enlarged endocardial cushions, which affect the ability to form the heart valves. Also, the heart may have abnormal vessel formation, with both the aorta and the pulmonary artery in the right ventricle. Dr. Epstein is also studying a different developmental gene called pax3 where mutations can result in similar cardiac defects.

Dr. Jim Gusella (Harvard/MGH) summarized the meeting and provided a vision for future directions for NF research. He diagrammed a research cycle that involves patient populations, clinical and molecular analysis and diagnosis and management. He also diagrammed how the different levels of research all contribute toward understanding of the disease, which is necessary for treatment.