



V. Neurofibromatosis Research Program



Vision: To decrease the impact of neurofibromatosis and schwannomatosis.

Mission: To promote research directed toward the understanding, diagnosis, and treatment of NF1, NF2, and schwannomatosis to enhance the quality of life for individuals with those diseases.

Congressional Appropriations for Peer Reviewed Research:

- \$90.3M in FY96–02
- \$20M in FY03
- \$20M in FY04

Funding Summary:

- 103 awards from the FY96–02 appropriations
- 14 awards from the FY03 appropriation
- ~21 awards anticipated from the FY04 appropriation

The Disease

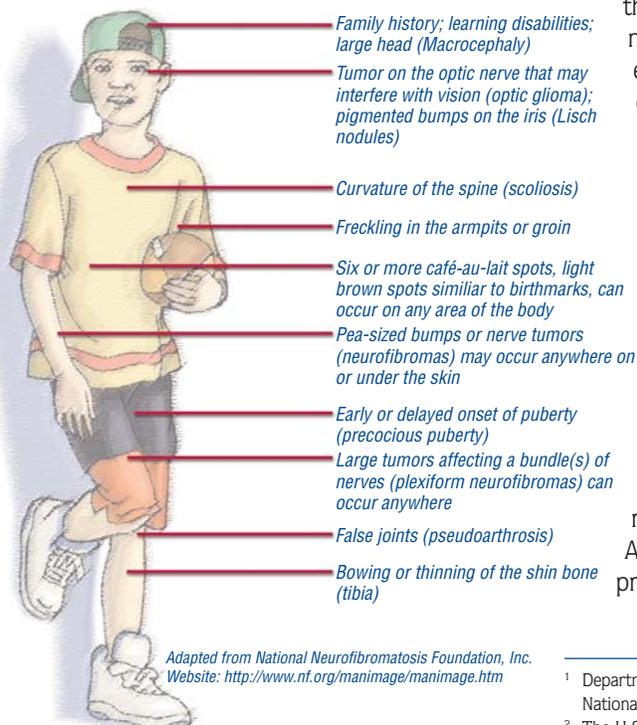
Neurofibromatosis 1 (NF1) and NF2 are distinct genetic disorders of the nervous system. These disorders usually result in tumors involving nerves anywhere in the body; however, non-nervous tissue such as bone and skin can also be affected. Together, these two genetic disorders affect more than 100,000 Americans of both genders and all ethnic groups. NF1 and NF2 are usually inherited as autosomal dominant disorders. Therefore, a parent with NF has a 50% chance of passing the disorder on to his or her child. However, 30% to 50% of NF1 and NF2 cases arise as a result of a spontaneous genetic change. Tumors that develop in individuals with NF can cause disfigurement, deafness, blindness, bone deformation, learning disabilities, and in some cases death. The tumors that appear in NF patients can vary significantly, even among affected individuals in the same family. Surgical intervention can provide palliative relief; however, at this time there is no cure. NF1 is the more common type, affecting about 1 in 4,000 individuals, and is also known as Von Recklinghausen's disease or peripheral NF. A common characteristic of NF1 is the appearance of flat, pigmented markings on the skin called café-au-lait spots. NF1 is also characterized by neurofibromas, which are small, heterogeneous tumors that develop on or just under the skin. Symptoms of NF often appear at birth and usually by the age of 10. Approximately 50% of people with NF1 have learning disabilities. NF2 is rarer than NF1, only affecting about 1 in 40,000 individuals, and is also known as bilateral acoustic NF (BAN). NF2 is characterized by the growth of tumors on nerves of

the inner ear, among other complications. The inner ear neuromas in NF2 patients cause hearing loss and can eventually result in deafness. Hearing loss in NF2 patients can appear as early as the teen years.¹

Schwannomatosis is a rare form of NF that has only recently been discovered. Schwannomatosis is characterized by the growth of multiple, homogeneous tumors, called schwannomas, consisting of Schwann cells or nerve sheath cells. As with NF1 and NF2, schwannomatosis varies greatly among patients. However, more often than not, the first symptom of schwannomatosis is pain.

Program Background

The Congressionally Directed Medical Research Programs (CDMRP) began managing the Department of Defense (DOD) Neurofibromatosis Research Program (NFRP) in response to the fiscal year 1996 (FY96) Senate Appropriations Committee Report No. 104-124, which provided \$8 million (M) for research in NF.² As a leader of NF



¹ Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Neurological Disorders and Stroke, *Report on Neurofibromatosis*. 1993.

² The U.S. Army Medical Research and Materiel Command, but not the CDMRP, was also responsible for managing congressional appropriations in FY92 for NF research.

“It is difficult to overstate the impact of the [NFRP], and hard to imagine where our research efforts would be today without it.”

Bruce Korf, M.D., Ph.D., FY04 NFRP IP Chair

research funding worldwide, the NFRP has managed \$130.3M from FY96 to FY04, and 117 awards have been made through FY03 across the categories of research, research resources, and training/recruitment. The NFRP has supported the development of critical resources and collaborations, innovative basic research, the training of tomorrow’s leaders, and clinical research in an effort to improve the understanding, diagnosis, and treatment of NF and schwannomatosis and enhance the quality of life of persons with those diseases. (Refer to the storyboard on pages V-4–V-5 for a comprehensive review of NF1 research and the contributions of the NFRP to the field.) Appendix B, Table B-3, summarizes the congressional appropriations and the investment strategy executed by the NFRP for FY03–04.

The Fiscal Year 2003 Program

Congress continued the DOD NFRP in FY03 with a \$20M appropriation. The program challenged the scientific community to advance basic research and bring laboratory research to the clinic. Six award mechanisms were offered, including Clinical Trial Development, Clinical Trial, Idea, Investigator-Initiated (with optional nested postdoctoral traineeships), New Investigator, and Therapeutic Development Awards. The Clinical Trial Development Award represented a new feature in FY03 to establish the necessary research resources needed for a multi-institutional clinical trial. Both of the proposals received under this award mechanism were funded. Additional summary information about the number of proposals received, number of awards, and dollars invested for the FY03 NFRP can be found in Table V-1. As illustrated in Figure V-1, the FY03 NFRP has developed a research portfolio that encompasses basic, clinical, and population-based research.

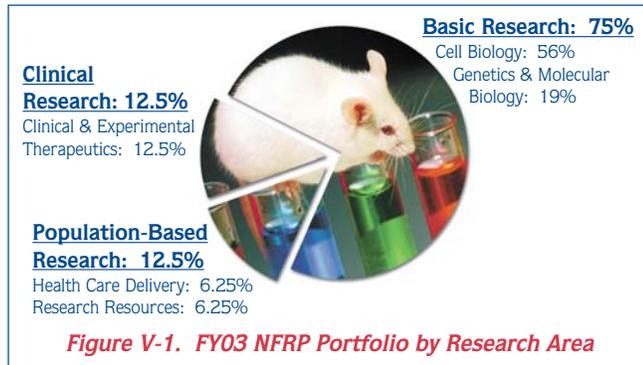


Figure V-1. FY03 NFRP Portfolio by Research Area

The Vision for the Fiscal Year 2004 Program

The DOD NFRP was continued through an FY04 congressional appropriation of \$20M. A total of 95 proposals were received across six award mechanisms, as detailed in Table V-2, and approximately 21 awards are anticipated. Five of the award mechanisms were previously established by the NFRP (Clinical Trial Development, Clinical Trial, Investigator-Initiated, New Investigator, and Therapeutic Development Awards) and one is new to the program (Concept Awards). The intent of the Concept Award mechanism is to encourage the exploration of untested, high-risk questions relevant to NF1, NF2, and/or schwannomatosis research.

Table V-1. Funding Summary for the FY03 NFRP

Category & Award	Proposals	Awards	Investment
Research			
Clinical Trial	2	0	\$0.0M
Idea	23	1	\$0.7M
Investigator-Initiated	17	6	\$10.6M
New Investigator	15	3	\$2.0M
Therapeutic Development	4	2	\$3.3M
Research Resources			
Clinical Trial Development	2	2	\$0.3M
Total	63	14	\$16.9M

Table V-2. Award Mechanisms Offered and Proposals Received for the FY04 NFRP

Category & Award	Proposals Received
Research	
Concept	61
Clinical Trial	3
Investigator-Initiated Research	18
New Investigator	11
Therapeutic Development	2
Research Resources	
Clinical Trial Development	0
Total	95

Encapsulating a Field of Research: The NF1 Storyboard

The NFRP has developed a storyboard that depicts key advances in NF1 basic and clinical research. The storyboard provides a graphic representation of particular research areas, such as cellular biology and experimental therapeutics with the major breakthroughs within each research area listed on a time line. A snapshot of the NF1 storyboard is shown below starting with the establishment of the NFRP in 1996 through the current year. Color coding schemes delineate NFRP-funded advances and indicate linked research.

The NF1 storyboard serves multiple purposes. It provides a comprehensive review of NF1 research and assists the NFRP Integration Panel in identifying gaps in the portfolio of funded projects and designing new funding mechanisms to fill those gaps. The storyboard is also featured at scientific conferences to showcase the contributions of the NFRP in advancing the field. In the future, the NF1 storyboard may be used to identify interdisciplinary collaborations that might move the field forward more rapidly.

Analysis of the storyboard reveals important trends. Clearly, greater advances have been made in basic research than in clinical or translational studies. The work of basic scientists has improved the understanding of the molecular and cellular mechanisms underlying NF pathogenesis substantially, but this knowledge has been slow to be translated into the development of new therapeutics. The NFRP has offered and continues to emphasize several clinically oriented award mechanisms to encourage the translation of laboratory research into the clinic.

	● ★ ■ Linked Research	§ NFRP Funded Research				
Molecular Biology & Genetics	Identification of a point mutation in a dermal neurofibroma – supports the tumor suppressor hypothesis		Structure of the catalytic domain of <i>NF1</i> determined			Homologous REP-mediated recombination between chromosomes proposed as cause of LOH in patients with deletions §
Cellular Biology	Ras-GTP to Ras-GDP ratio elevated in neurofibromas and MPNSTs	<i>NF1</i> ^{-/-} Schwann cells proliferate in response to forskolin; <i>NF1</i> ^{+/-} and <i>NF1</i> ^{-/-} Schwann cells do not §	<i>NF1</i> ^{-/-} Schwann cells have a growth advantage and are easily transformed § Neurofibromin regulates Protein Kinase A ●			Schwann cells proposed as source of LOH and tumor formation in NF1 § <i>NF1</i> ^{-/-} mast cells show increased proliferation and hyperactivation of Ras
Pathobiology	Loss of <i>NF1</i> associated with the development of leukemias and MPNSTs	Microdeletion of <i>NF1</i> and surrounding genes associated with facial anomalies and early onset §	<i>NF1</i> ^{+/-} and <i>NF1</i> ^{-/-} Schwann cells are both angiogenic and invasive in culture ★	Sera from NF1 patients shows increased mitogenic activity on Schwann cells in culture	p53 is expressed in MPNSTs, but not in neurofibromas	<i>INK4A</i> is frequently mutated in MPNSTs, but not neurofibromas <i>NF1</i> ^{-/-} mice develop astrocytomas § Loss of <i>NF1</i> associated with astrocytomas and neurofibromas Nerve grafting wounding causes hyperpigmentation in <i>NF1</i> ^{+/-} mice § ★
Technology/Animal Models	Improved mouse model of myeloid leukemia developed	Mouse model of learning and memory defects developed	<i>Drosophila</i> model of NF1 developed ●			<i>NF1</i> ^{-/-} chimeric mice developed-develop multiple neurofibromas that are <i>NF</i> ^{-/-} <i>NF1</i> ^{-/-} -p53 ^{+/-} mouse developed-model of MPNST development (as well as astrocytomas and glioblastomas) §
Behavioral & Cognitive Biology	1st research addressing the molecular basis of learning disabilities in NF1	<i>Drosophila</i> <i>NF1</i> involved in growth, learning, and memory ●	Cognitive defects detected in <i>NF1</i> ^{+/-} mice § ★			<i>NF1</i> regulates the learning pathway in <i>Drosophila</i> ●
Imaging, Detection & Diagnosis						
Epidemiology						Epidemiologic study of 4,402 NF1 patients from 3 databases identifies specific associations between features §
Experimental Therapeutics		Translational Research: compound testing for NF1 treatments begun				Farnesyl Transferase Inhibitors (FTIs) tested for therapeutic effects on NF1 §
Symptom Management		Assessment of surgical removal of plexiform neurofibromas: 56% of tumors did not progress; 20% found improvement				
Important Meetings & Symposia		NNFF Clinical Care Advisory Board: Diagnostic Evaluation and Management of NF1 and NF2				NINDS Workshop: Defining the Future of Neurofibromatosis Research
	1996	1997	1998	1999	2000	

For instance, the FY03 program offered two different award mechanisms to assist investigators during the earlier stages of drug development and increase the likelihood of clinical trial success: The Therapeutic Development Award was launched in FY01 to fund preclinical drug development, while the Clinical Trial Development Award was introduced in FY03 to support the establishment of trial research resources and the development of clinical protocols. The Clinical Trial Award, which debuted in FY99, funds Phase 1 and Phase 2 clinical studies. Additionally, in FY97, the NFRP offered the Natural History Study Award to fund studies on the growth of plexiform neurofibromas in NF1 patients and vestibular schwannomas in NF2 patients; these data can aid in the evaluation of the efficacy of therapeutics in clinical trials.

The storyboard also illustrates the importance of identifying and establishing cross-disciplinary collaborations. For example, researchers created a *Drosophila* model of NF1 in 1997, but the NF1 protein was not demonstrated to regulate learning in *Drosophila* until 2000. An early collaboration between the animal modelers and the behavioral scientists may have expedited this discovery.

NF1 was first described in the 19th century. However, most of the key advances in the NF field have been made in the past 15 years. The primary challenge for the NFRP in the 21st century will be to maintain this momentum by assisting investigators in establishing interdisciplinary collaborations and applying the lessons learned from that collaborative research to the development of new therapeutics for this often devastating disease.

<p><i>Epi1</i> identified as second hit involved in AML development and progression \$</p>							
<p><i>NF1</i>^{+/+}:p53^{+/+} tumors overexpress EGFR-possible therapeutic target</p>		<p>Ras signaling is dynamically regulated by ubiquitin-mediated proteolysis of neurofibromin \$</p>	<p>Brain lipid binding protein regulates Schwann cell-axon interactions in peripheral nerve tumors \$</p>	<p><i>NF1</i>^{-/-} mouse Schwann cells secrete Kit ligand, which stimulates migration of <i>NF1</i>^{-/-} mast cells \$ ★</p>	<p><i>NF1</i>-deficient mast cells have reduced surface Fas antigen expression in response to Kit ligand and are resistant to Fas-ligand mediated apoptosis \$ ★</p>	<p>Expression of activated TC21/R-Ras2 enhances migration of <i>NF1</i>-deficient mouse Schwann cells ★</p>	<p>Neurofibromin contains a nuclear localization signal</p>
	<p>Specific ablation of <i>NF1</i> in Schwann cells causes the formation of plexiform neurofibromas \$ ■</p>	<p>Invasive phenotype of MPNST cells does not involve Ras but is dependent on Src kinase activity and Src-dependent elevation of CD44 expression \$</p>	<p>Specific ablation of <i>NF1</i> in endothelial cells causes multiple cardiovascular abnormalities that are associated with elevated ras signaling \$ ■</p>	<p><i>NF1</i>^{-/-} mice lacking <i>NF1</i> in astrocytes develop optic nerve gliomas \$ ■</p>		<p>Somatic inactivation of <i>NF1</i> in hematopoietic cells causes progressive myeloproliferative disease \$ ■</p>	
<p><i>NF1</i>^{23a-/-} mouse developed that lacks only <i>NF1</i> splice variant type II – not susceptible to tumors but has learning disabilities \$</p>	<p>Conditional <i>NF1</i>^{-/-} mouse developed that allows targeting of specific cells \$ ■</p>	<p>Mouse model of plexiform neurofibromas developed \$ ■</p>					
			<p>Cognitive ability does not improve as children with NF1 develop into adulthood, despite decreases in MRI abnormalities \$</p>				
	<p>No correlation found between (1) the type and location of <i>NF1</i> gene mutations and (2) the type, location, and size of plexiform neurofibromas \$</p>	<p>Analysis of statistical associations among NF1 clinical features – nine features grouped into three distinct sets \$</p>		<p><i>NF1</i> microdeletions associated with increased risk of developing MPNSTs</p>	<p>No correlation found between the development of café-au-lait spots and neurofibromas in individual body segments of NF1 patients, but the number of body segments affected is influenced by genetic factors \$</p>	<p>Deletion of the <i>NF1</i> gene responsible for disease phenotype in at least 4.4% of a cohort of 500 unrelated unselected NF1 patients</p>	<p>Optic pathway gliomas often present in older children with NF1 and may progress years after diagnosis</p>
	<p>Children with NF1 and low-grade visual pathway gliomas respond well to vincristine and carboplatin and do not develop progressive disease</p>		<p>Open label Phase 1 trial of thalidomide in NF1 patients with plexiform neurofibromas – drug was well tolerated and minor responses were observed in four patients</p>				
<p>2001</p>	<p>2002</p>	<p>2003</p>	<p>2003</p>			<p>2004</p>	
<p>NINDS Workshop: Developing Therapies for the Neurofibromatoses</p>							

“The CDMRP, through innovative funding mechanisms...provides important research support...for the prevention, diagnosis, and treatment of neurofibromatosis.”

Robert S. Greenwood, M.D., FY02-04 NFRP Peer Review Panel Member

Scientific Outcomes and Advances

The DOD NFRP award outcomes are exciting and present promise for the future. Advances are being made in basic and clinically oriented research in NF and schwannomatosis. The following projects are a testimony to the dedicated investigators working to support the program’s vision of decreasing the impact of these diseases.

Dissecting the Molecular and Genetic Mechanisms of Neurofibroma Formation in NF1

Karen Stephens, Ph.D., University of Washington

Dr. Karen Stephens, an investigator at the University of Washington in Seattle, has received four awards from the NFRP since 1996 to conduct research into the molecular genetics of NF1. Her latest work, supported by an FY02 NFRP Investigator-Initiated Research Award, focuses on the genetic and molecular mechanisms underlying the formation of skin neurofibromas, the most common tumors in individuals with NF1. She is studying a group of NF1 patients who carry deletions of the *NF1* gene and several adjacent genes of unknown function. Because these patients tend to develop large numbers of skin neurofibromas at an early age, she hypothesizes that deletion of one of the genes adjacent to *NF1* favors the development of skin neurofibromas and possibly other tumors. Dr. Stephens will evaluate tumor characteristics and other clinical features in patients with *NF1* deletions. She also will look for specific genetic changes in skin neurofibromas in patients with *NF1* deletions to provide insight into how the tumors develop. She has already determined how the large *NF1* deletions occur on the chromosome. Ultimately, Dr. Stephens’ research has the potential to improve our understanding of neurofibroma formation, which may aid in the development of new therapies to slow or halt the growth of potentially devastating tumors.

Targeting Angiogenesis for the Treatment of NF1 Tumors

David Muir, Ph.D., University of Florida

NF1, a common genetic disorder that affects about 1 in 4,000 people worldwide, is characterized by developmental abnormalities in the nervous system, skin, bones, and other tissues. Many NF1 patients develop plexiform neurofibromas, nerve tumors that often grow very large and can be

debilitating or fatal. A more complete understanding of what causes neurofibromas to grow is needed to develop better therapies to manage NF1 tumor growth. Dr. David Muir of the University of Florida, a recipient of an FY02 NFRP Investigator-Initiated Research Award, is using mice lacking the *NF1* gene (*NF1*^{+/-} mice) to investigate the mechanisms by which NF1 tumors induce angiogenesis, the formation of new blood vessels required for tumor growth. The ultimate goal of this research is to discover effective therapies for the treatment of plexiform neurofibromas by blocking angiogenesis. Dr. Muir found that angiogenesis in response to low oxygen levels is higher in the retinas of *NF1*^{+/-} mice than in normal control mice. Additionally, he showed that formation of new blood vessels in response to a protein called fibroblast growth factor 2 is enhanced in the corneas of *NF1*^{+/-} mice compared to control mice. Importantly, Dr. Muir also developed a new animal model of NF1 by implanting Schwann cells from human NF1 neurofibromas into the nerves of *NF1*^{+/-} mice. The effects of anti-angiogenic agents on tumor vascularity and growth will be assessed in living mice using noninvasive magnetic resonance imaging. These ongoing studies may lead to the development of improved treatments for plexiform neurofibromas in NF1 patients.

A New Model for Merlin Localization and Function

Wallace Ip, Ph.D., University of Cincinnati College of Medicine, Ohio

NF2, an inherited disorder that affects 1 in 40,000 individuals, is characterized by the formation of bilateral schwannoma of the 8th cranial nerve and predisposition to other nervous system tumors. NF2 is caused by mutations in a tumor suppressor gene called merlin, a member of a family of



proteins that bind to the structural support network in cells (known as the cytoskeleton). Recent work by Dr. Wallace Ip at the University of Cincinnati College of Medicine, a recipient of an FY02 NFRP Idea Award, provides new insight into merlin localization and function. Dr. Ip's group demonstrated that most merlin within cells is attached to specialized areas of the cell membrane called lipid rafts. His data also suggest that merlin activation is accompanied by dissociation of merlin-containing lipid rafts from the cytoskeleton. Merlin is the first tumor suppressor to be localized to lipid rafts, which contain a high concentration of signaling molecules that regulate cell growth. These findings suggest that the ability of merlin to disrupt growth-promoting signaling pathways originating from the cell membrane may be dependent on its association with the rafts. Future studies will examine whether mutant merlin proteins modeled after mutations known in NF2 patients are defective in lipid raft targeting and whether forced localization of the mutant proteins to lipid rafts can restore normal function. Dr. Ip's research may provide explanations for the loss of function associated with some merlin mutations and ultimately help investigators develop new therapeutics for the affected individuals.

For additional information about this research, please refer to the following publication:

- Stickney JT, Bacon WC, Rojas M, et al. 2004. Activation of the tumor suppressor merlin modulates its interaction with lipid rafts. *Cancer Research* 64:2717-2724.



Bottom Line

Since 1996, the DOD NFRP has been responsible for managing \$130.3M in congressional appropriations, which has resulted in 117 awards for FY96-03. The NFRP is dedicated to improving and enhancing patient quality of life of persons with NF and Schwannomatosis, and NFRP-supported studies offer the potential to revolutionize the management of these diseases. Research highlights, award data, and abstracts of funded NFRP proposals can be viewed on the CDMRP website (<http://cdmrp.army.mil>).

Fiscal Year 2004 Integration Panel Members

Bruce Korf, M.D., Ph.D. (Chair), University of Alabama, Birmingham

Judy Small, Ph.D. (Chair Emeritus), The National Neurofibromatosis Foundation, Inc.

Jackson Gibbs, Ph.D. (Chair Elect), Merck Research Laboratories

Peter Adamson, M.D., University of Pennsylvania School of Medicine

Peter Bellermann, M.P.A., The National Neurofibromatosis Foundation, Inc. and International Neurofibromatosis Association

Brenda Duffy, M.A., Neurofibromatosis, Inc.

Robert Finkelstein, Ph.D., National Institute of Neurological Disorders and Stroke

Nancy Fisher, R.N., M.D., M.P.H., Washington State Health Care Authority

William Johnson, M.D., University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School

Eric Legius, M.D., Ph.D., Catholic University Leuven, Belgium

Arie Perry, M.D., Washington University School of Medicine

Tina Young Poussaint, M.D., Harvard Medical School

The FY05 NF Consortium Development Awards: Advancing Basic Science into the Clinic

The work of dedicated investigators has resulted in monumental advances in our understanding of the basic molecular and cellular mechanisms underlying NF. However, despite strong interest and need, this knowledge has been slow to be translated into the development of new clinical therapeutics. The NFRP, in collaboration with the National Institutes of Health, sponsored a meeting with the NFRP Integration Panel and expert consultants in September 2004 to discuss critical barriers to the successful clinical translation of basic NF research and develop strategies to overcome those barriers. This 2-day meeting included leading experts in the areas of human subjects research protection, bioethics, NF clinical care, NF natural history, oncology clinical trials, pathology, radiology, and statistics.

Meeting participants determined that the formation of an NF-specific clinical consortium would address many of the identified barriers, facilitate clinical research, and ultimately lead to new treatments for the disease. This consortium would consist of a network of exceptional investigators at research institutions who would collaboratively develop and conduct clinical studies of new therapies, as well as a central Operations Center to provide necessary administrative and management support to the research sites.

Based on the recommendations provided at the meeting, the NFRP developed two new award mechanisms for FY05 that will fund the establishment of the necessary collaborations and the development of key resources for the consortium. It was agreed that the consortium should initially focus on NF1 with the option of later expanding to conduct NF2- or Schwannomatosis-focused studies. The NF Consortium Development Site Award will select and support 5 to 10 consortium member institutions, whereas the NF Consortium Development Operations Center Award will select and fund the Consortium Operations Center. Investigators from each institution and the Operations Center will collaborate to develop standard operating procedures, communications and management plans, a draft charter, and clinical protocols, and will submit a proposal to the FY06 NF Consortium Award mechanism to support the established consortium.

The ultimate goal of the NF Consortium Development Awards and subsequent NF Consortium Awards is to accelerate the progression of novel therapies from bench to bedside, thus furthering the NFRP's mission of enhancing the quality of life of individuals with NF.

“The peer review environment creates an overwhelming sense of duty to responsibly defend and support research most likely to impact the lives of children and adults dealing with the...diagnosis of NF.”

*Rhonda Mahacek, FY01-04 NFRP
Consumer Peer Review Panel Member*

