ANDREA MCCLATCHEY, PhD
Massachusetts General Hospital

Exploiting macropinocytosis for the development of exosome-mediated drug delivery in NF2 mutant tumors

Merlin, the protein encoded by the NF2 gene, is unique among tumor suppressors because it controls cell reproduction. Merlin controls how growth receptors respond to changes in the mechanical and physical properties of the cellular environment. We have recently discovered that merlin regulates how cells take up fluids and nutrients from the environment, through a process called macropinocytosis. In the absence of merlin, cells take in more fluids and nutrients. This project will seek to take advantage of this feature of NF2-mutant cells by utilizing this pathway for the delivery of drugs for the treatment of NF2 tumors. These studies will provide a foundation for the testing of this kind of treatment delivery for this and other therapies for NF2 patients.

LEI XU, MD, PhD
Massachusetts General Hospital

Reprogramming the Tumor Microenvironment to Enhance Anti-Tumor Immunity in NF2 Vestibular Schwannomas

The hallmark symptom of NF2 is benign bilateral vestibular schwannomas (VS). Over time, these tumors grow and may cause progressive hearing loss, which may lead to social impairment and increased clinical depression. In patients with progressive VS, a thickening of the connective tissue, called fibrosis, correlates with hearing loss. Fibrosis results in high collagen content. Recently, we found that NF2 patients with VS demonstrate elevated collagen content and fibrogenic signaling, and are plagued by hypoxia and immunosuppression. Based on these, we propose to target the fibrogenic signaling pathway to improve hearing and enhance immunotherapy efficacy. Our research will generate important and translatable results for new combination therapy paradigms that are desperately needed for this dreadful disease.

AARON SCHINDLER, PhD
The University of Sydney, Australia

Dietary Intervention for NF1 Muscle Weakness

NF1 is associated with a high tumor burden in adulthood, but for many children it most profoundly impacts their school experiences through learning disabilities, muscle weakness, poor coordination, and fatigue. Recent clinical studies indicate that muscle strength is reduced by 30-50% on average in children with NF1. Our breakthrough research has not only revealed that this weakness is linked to problems in fat metabolism in muscle, but also that dietary changes and supplements can overcome this weakness. We propose to complete a final series of preclinical studies with different diets and supplements to fine-tune our design for a dietary intervention trial in children, scheduled to start in 2018.
Drug Discovery Initiative Awardees

CTF is proud to have recently funded four Drug Discovery Initiative (DDI) awards. We are enthusiastic about these exciting projects!

Jeffrey Field, PhD, University of Pennsylvania, Perelman School of Medicine
MPNST Profiling and Screening: Extension for Exome Sequencing of the Cell Lines Screened
Award amount: $25,000

In a prior DDI award, Dr. Field developed a course for students to do drug screening for 130 relevant NF drugs against 9 NF1 cancer cell lines and two NF2 cell lines. They also screened thousands of drugs against representative NF1 and NF2 cell lines. Further funding will allow Dr. Field and these students to find the mutations for each cell line to more closely correlate the sensitivity to drugs tested, and to identify new drugs to test. Additionally, the data from this project will be made public.

Verena Staedtke, MD, PhD, Johns Hopkins University, School of Medicine
Evaluation of Mebendazole as Chemoprevention in a Neurofibromatosis 1 Transgenic Mouse Model
Award amount: $85,000

This project will explore using chemoprevention, the use of drugs to reduce the risk of cancer development, by repurposing a particular drug for the prevention of MPNST development. The drug, Mebendazole (MBZ), has shown benefits in colorectal cancer syndromes previously, and will be tested in a MPNST mouse model. If successful, the results will have an immediate impact on patient care; the highest death rate among NF1 patients is due to MPNST, and this project hopes to reduce this cancer frequency among NF1 patients.

Andrea Rasola, PhD, University of Padova, Department of Biomedical Sciences
Targeting the Mitochondrial Chaperone TRAP1 to Inhibit Plexiform Neurofibroma Growth
Award amount: $40,000

Changes in cell metabolism constitute a driving force for the growth of many tumor types. Dr. Rasola and his group have also found that TRAP1, a protein that has a crucial function in the control of the energy metabolism of tumor cells, is mandatory for neurofibroma growth. The aim of this project is the identification of molecules that inhibit TRAP1, which might block neurofibroma progression. It is hoped that these new compounds will be the first step in the development of selective and effective anti-neoplastic drugs for NF1 patients.

Dr. Marco Giovannini, MD, PhD, University of California, Los Angeles
Preclinical Safety and Efficacy Evaluation of Long-Term Anti-VEGFA Treatment Administration in a GEM Model of NF2-Related Schwannoma
Award amount: $84,999

Case reports and clinical trials have reported that bevacizumab (Avastin), can induce both tumor regression and hearing improvement in patients with NF2-associated vestibular schwannomas. Dr. Giovannini will test Avastin in an NF2 mouse schwannoma model to analyze its efficacy in terms of tumor shrinkage and hearing performance. Setting the Avastin response baseline in mice will allow prioritization of new drugs by comparing their efficacy, and will therefore aid the choice of new drug candidates for clinical trials in NF2 patients.

NEW FUNDER-PUBLISHER PARTNERSHIP
PLOS ONE

The Children’s Tumor Foundation and the scientific journal PLOS ONE are collaborating on a new funding program in the area of neurofibromatosis research. The new initiative, called the Drug Discovery Initiative Registered Report (DDIRR) Awards, is a funder-publisher partnership to integrate the Registered Reports model in the grant application process.

Registered Reports pre-determine the research question, methodology, and design of a study to be carried out, and are designed to enhance the rigor, reproducibility, and transparency of the science produced. Upon thorough review of the study design at the time of grant application, awardees are guaranteed an in-principle acceptance to publication in the journal PLOS ONE. Provided the study is conducted according to the plan, acceptance in principle is honored regardless of study outcome—as such the Registered Report model contributes to eliminate publication bias. This new award will serve as a pilot and will evolve from the Foundation’s classic Drug Discovery Initiative Award program.

The Request for Applications for the 2017 DDIRR Awards is now open. Learn more at ctf.org/research.
2015 **DRUG DISCOVERY INITIATIVE Awardees:**

CTF awarded five Drug Discovery Initiative (DDI) awards in its first of two calls for applications in 2015. Two of the awards will target novel therapies for NF1-related tumors, specifically malignant peripheral nerve sheath tumors (MPNSTs), and three for NF2-related tumor therapies. We are enthused to be able to fund these exciting projects!

**Alexander Schulz, MD, PhD,** of Leibniz Institute for Age Research, Germany, received an $85,000 *in vivo* award for his proposed study, "Establishing a protein replacement therapy for the treatment of Schwann cell-derived nerve sheath tumors." This proposal aims to establish an innovative approach using recombinant proteins to prevent schwannoma development by altering the interaction of Schwann cells and axons (long nerve cell protrusions).

**Andrea McClatchey, PhD,** of Massachusetts General Hospital/Harvard University, received a $40,000 award allowing her to continue to work on her 2014 project, "Expanded testing of centrosome-unclustering drugs in NF2-mutant tumors." Centrosomes are so-called cellular organelles that are essential for normal cell division, and their overduplication is a feature in tumor cells. The goal in this expanded study is to investigate the sensitivity of other NF2-mutant tumor cells, particularly meningioma, to centrosome targeting drugs and to test an expanded panel of these drugs that act in different ways on all NF2 tumor types.

**Lei Xu, MD, PhD,** of Massachusetts General Hospital, received an $85,000 award for her proposed study "Combining immunotherapy and antiangiogenic therapy in an NF2 schwannoma model." The use of bevacizumab, a so-called antiangiogenic drug, in the treatment of NF2 vestibular schwannomas has shown an ability to improve hearing in some patients. The proposed study will combine the use of bevacizumab with immunotherapy, and if the results are superior to either treatment alone, Dr. Scott Plotkin of MGH will use the results to design a clinical trial for NF2 patients.

**Steven Lewis Carroll, MD, PhD,** of the Medical University of South Carolina, received an $85,000 *in vivo* award for his proposed study "Combinatorial therapy with receptor tyrosine kinase inhibitors for MPNST." This study will identify three drugs (all currently in clinical use or clinical trials for other cancer types) that effectively inhibit MPNST proliferation. These drugs will be tested in various combinations in hopes of generating sufficient data to attract follow-on funding from the NIH or DOD to expand testing of RTK therapies for the difficult-to-treat MPNSTs.

**Jeffrey Field, PhD,** of University of Pennsylvania, received a $40,000 *in vitro* award for his proposal "MPNST profiling and screening: an experiment in research-based education." This project will create the first ever college course in drug screening, and will specifically screen for drugs for NF1 MPNSTs. Students will screen drugs, both known and novel, against NF tumor cell models, primarily cancer models. The known drugs will serve as a starting point for comparison with other screening efforts.
A. THOMAS LOOK, MD, of the Dana-Farber Cancer Institute, was granted an in vivo DDI Award for his proposal, “Drug discovery for NF1-associated malignant peripheral nerve sheath tumors using the zebrafish model.”

NF1-related MPNSTs are very aggressive tumors with poor prognoses for the patients who are diagnosed with it. Surgery to remove MPNSTs is not effective because they often recur and metastasize. Chemotherapy regimens are not only ineffective, but toxic to the patient. Dr. Look and his team have developed a zebrafish model, through which they will rapidly screen drugs that are already in use in humans, obviating the need to perform expensive and time-consuming toxicology studies. They predict that they will be able to identify one or more already-FDA-approved drugs, which have been developed for other diseases, that will show activity against MPNSTs. These drugs could potentially be “repurposed” to more effectively treat this small subset of NF1 patients.

JOSEPH KISSIL, PhD, of the Scripps Research Institute, was granted an in vivo DDI Award for his project, “Assessing the anti-tumor activity of crizotinib in NF2-deficient meningioma.”

Dr. Kissil and his team have identified an already-FDA-approved drug, known as crizotinib, as having anti-tumor activity against NF2-related schwannomas. This drug is already in use in patients with lung cancer and has demonstrated few side effects, and is therefore safe. A clinical trial is currently being initiated to test crizotinib against schwannoma in NF2 patients. The group will now assess whether crizotinib can also be useful against another NF2-related tumor, meningioma, by testing this drug in cell and animal models. Should this show a desirable effect, it would indicate that the trial being initiated should be expanded to include meningioma in addition to schwannoma.

NANCY RATNER, PhD, of Cincinnati Children’s Hospital, was granted an in vitro DDI Award for her study, “Mechanisms of resistance to MEK inhibition in neurofibroma.”

This study aims to find drugs that reduce neurofibroma size and are potentially curative. We already know that drugs that target MEK proteins shrink most neurofibromas. In patients with NF1, the mutated gene, neurofibromin, can no longer do its proper function of turning off a protein called Ras. When Ras is on, downstream pathways (that include MEK) are also active, contributing to neurofibroma formation. By using a drug to inhibit MEK, the over-active pathway is turned off, which can shrink neurofibromas. However, both in humans and in preclinical trials in mice, inhibiting MEK doesn’t always work and some neurofibromas show resistance to MEK inhibition. Dr. Ratner and her team will work to determine what else is being turned on during MEK inhibition so that it can also be targeted, prevent drug resistance, and identify an increasingly successful treatment for patients with NF1.
Plastic Surgeon’s Work on NF1 Patients Promising

In September, the French plastic surgeon Professor Laurent Lantieri gave a spectacular presentation at the European NF meeting in Barcelona during which he showed the incredible benefit of surgery in certain cases of NF1. Dr. Lantieri has operated on over 600 patients including two face transplants in extreme cases. His work is able to show clear statistics on outcomes. Dr. Annette Bakker invited him to New York and organized a meeting with Dr. Michael Fisher, a top neuro-oncologist in the NF field, on November 4th to identify next steps.

“A dream is the bearer of a new possibility, the enlarged horizon, the great hope.” — HOWARD THURMAN
2014 DRUG DISCOVERY INITIATIVE AWARDS: Round 1 Recipients

The Drug Discovery Initiative (DDI) awards program is focused on seed funding preclinical drug testing studies on neurofibromatosis in cell or animal models, and is one of the most successful Children’s Tumor Foundation programs to date. The Foundation is pleased to announce the most recent recipients of this important grant.

Miriam Smith, PhD
University of Manchester

Treatment of Neurofibromatosis Type 2 (NF2) by Exon Skipping

Neurofibromatosis type 2 (NF2) is a neurogenetic disorder that predisposes patients to develop tumors of the nervous system. It is known that NF2 disease is caused by mutation of the NF2 gene. Dr. Smith will use the DDI award to develop a cutting-edge method to ‘rescue’ mutations in coding regions of NF2, where 98-99% of small mutations are found.

Gregory Riggins, MD PhD
Johns Hopkins University

Testing Combinations of FDA-approved Agents with and without Radiation Therapy in an NF2 Schwannoma Murine Model

Dr. Riggins will use the DDI award to examine the safety and efficacy of radiation combined with compounds that effect NF2 tumor growth through multiple pathways, including kinase inhibitors and mTOR inhibitors. He will first test the toxicity and efficacy of each compound alone and then will test their effect together with and without radiation therapy.

David Largaespada, PhD
University of Wisconsin-Madison

Targeting Hyaluronic Acid for NF1-associated Tumors

Malignant peripheral nerve sheath tumors (MPNST) remain the leading cause of death for NF1 patients and most therapies have failed to demonstrate effectiveness against plexiform neurofibromas and MPNSTs. Recently, Dr. Largaespada and colleagues showed that a combination of two drugs, RAD001 and PD-901, were effective at treating mice that develop Schwann cell tumors. To improve drug delivery to the tumors, Largaespada will combine these drugs with PEGPH20, which has been shown to safely and effectively improve drug delivery and efficacy of chemotherapy in patients.

Andrea McClatchey, PhD
Harvard Medical School

Preclinical Investigation of Centrosome Unclustering Drugs in NF2-mutant Schwannoma

Excess numbers of centrosomes, a part of the cell that is essential for normal cell division, occurs in many different tumor types and is a feature of tumors that differentiate them from normal cells. Merlin has a key role in controlling the number of centrosomes within cells. Dr. McClatchey will use the DDI award to test if NF2 tumors are more sensitive to drugs that target excess centrosomes.

For more information, please visit www.ctf.org/ddi.

More Children’s Tumor Foundation Resources Available in Spanish

This fall, CTF is introducing two new features for the Spanish-speaking members of our community. People whose first language is Spanish will now be able to go to www.nfregistry.org and access the surveys in Spanish. In addition, our popular Newly Diagnosed with NF1 booklet is now available in Spanish. For copies, or a link to this publication on our website, please email info@ctf.org.
2013
DRUG DISCOVERY INITIATIVE FUNDING AWARDED

The Drug Discovery Initiative (DDI) awards program was designed to provide a critical early-stage doorway to the NF preclinical pipeline. These small scale awards, with a quick application and turn-around process, allow the Foundation to fuel the therapeutic pipeline for a relatively small investment. This year, the DDI program funded four proposals in the first round and CTF is in the process of evaluating with reviewers the second round of applications.

The first four funded labs are:

Jean Nakamura, MD
University of California San Francisco
Identification of Novel Targets in NF1 Cancers by Drug Sensitivity Profiling

TEST DIFFERENTIALLY MUTATED SUBTYPES OF NF1 TUMORS FOR DRUG SENSITIVITY TO DEFINE SPECIFIC SIGNALING PROFILE SIGNATURES AND EFFECTIVE DRUGS FOR CLINICAL APPLICATION

The Neurofibromatosis Syndrome, characterized by loss of the NF1 gene, confers increased risk of cancer development. The investigators have developed a unique mouse model of cancers caused by NF1 loss. One of the goals of this study is to analyze these tumors to define how mutations in the NF1 gene lead to cancer, and how these processes can be stopped. The project uses a 94-compound drug library of established chemotherapeutic agents, representing multiple cancer signaling pathways, against the tumors generated to identify critical biological processes that work with NF1 to cause cancer. This information will help classify subtypes of NF1 tumors by characteristic mechanisms of cancer formation helping direct patients to appropriate and effective therapies.

Lei Xu, MD, PhD
Harvard Medical School, Massachusetts General Hospital
Effect of TGF-beta Blockade in Recurrent NF2 Vestibular Schwannoma

TEST OF NOVEL DRUG IN COMBO WITH RADIATION THERAPY FOR VESTIBULAR SCHWANNOMA

The hallmark of NF2 is bilateral vestibular schwannomas (VS). Ionizing radiation has become a standard treatment for VS. Despite the initial response to radiation, most patients with NF2 ultimately relapse and develop resistance to further radiation therapy. This project will focus on the effect of TGF-β, a particular cell signaling pathway, as the most potent inducer of fibrosis in general and fibrosis correlated with hearing loss in VS using an established VS model that mimics human disease by progressing after radiation. The data generated in this proposal will provide insights into the potential use of TGF-β blockade as a new adjunct to radiation therapy.

Cristina Fernandez-Valle, PhD
University of Central Florida
Creation of Human Merlin-Null Schwann Cells for NF2 Studies

FIRST ATTEMPT TO EMPLOY HUMAN SCHWANN CELL LINES FOR DRUG SCREENING - NOW MOST RESEARCHERS USE MOUSE LINES

A major roadblock to developing drug therapies for NF2 is the lack of human Schwann cell lines with reduced or no expression of the merlin tumor suppressor. The investigators of this proposal are both Schwann cell biologists with combined expertise in NF2 and the cultivation of human Schwann cells. Together they propose to create a set of human Schwann cells having reduced levels of merlin protein using two different strategies. The cell lines will be characterized and their response to a panel of compounds that have known anti-proliferative effects on mouse merlin-deficient Schwann cell lines will be carried out. The end result should be creation of human Schwann cell lines lacking merlin that can be used in larger drug screens.

Charles W. Yates, MD
Indiana University School of Medicine
Testing Periostin-Cre NF2 Conditional Knockout Mouse for Potential Treatment Compounds Useful for NF2

NEW NF2 ANIMAL MODEL GENERATION: FIRST NF2 ANIMAL THAT DEVELOPS VS AND BECOMES DEAF, NEED FOR VALIDATION AS PRECLINICAL MODEL – USE EXISTING EFFECTIVE DRUGS THAT WERE TESTED IN THE CLINIC AND SEE IF THE MODEL PREDICTS CORRECTLY

This group developed a genetically engineered mouse (a mouse with changes to the DNA similar to the genetic changes seen in people with the disease) by causing the gene, merlin, which is responsible for development of NF2, to have mutations early in development. Changes in the gene in this mouse led to some of the tumors similar to those most commonly seen in humans (vestibular schwannomas, which are otherwise known as acoustic neuromas). Using this mouse as a model would be helpful because testing compounds developed to treat NF2-related tumors could also test some of the specific endpoints similar to what is seen in people. To determine if this is a useful model, the group proposed testing AR42, a specific class of medication called an HDAC inhibitor and a compound that in lab studies of cells and other mouse models may potentially be useful in people with NF2 because it reduces or shrinks the size of vestibular schwannomas. The project will examine if the treated mice will respond with preservation of hearing and decrease in tumor size over a three month period of treatment.
Children’s Tumor Foundation Funds Six Drug Discovery Initiatives

The Children’s Tumor Foundation Drug Discovery Initiative (DDI) program, launched in 2006, provides a drug screening mechanism for researchers with a concept that may advance therapies for the manifestations of NF. DDI awards invest relatively small amounts of funding into projects that could provide exponential return in follow-on funds from government and industry sources.

Below are the six most recent DDIs funded by the Children’s Tumor Foundation:

**Chris Maxwell, University of British Columbia** and **Conxi Lazaro, Catalan Institute of Oncology-IDIBELL**: Targeting NF1 Associated MPNST with Aurora Kinase Inhibitors

**Filippo Giancotti, Memorial Sloan-Kettering Cancer Center**: Preclinical Efficacy of the Neddylation Inhibitor MLN4924 in Neurofibromatosis Type 2

**Nancy Ratner, Cincinnati Children’s Hospital Medical Center**: In Vivo Testing of Anti-Oxidants in NF1 CNS

**Andrea McClatchey, Massachusetts General Hospital**: Heterogeneity of Drug Response in NF2-deficient Schwannomas

**Rajesh Khanna, Indiana University**: Assessment of Peptide-based Disruptors of the Neurofibromin and CRMP-2 Interaction as Novel Analgesics for NF1

**Michael Brownstien, Pisces Therapeutics, LLC**: Small Molecule Ras Inhibitor for the Treatment of Neurofibromatosis Type 1

**Looking Forward**

Here are a few events that may be of interest. For a complete listing or more information, please see www.ctf.org/calendar

12/15 - Detroit, MI

Detroit Pistons Basketball Game CTF Fundraiser

12/15 - Salt Lake City, UT

NF Symposium at Shriners Hospitals for Children

01/16 - Portland, OR

NF/Schwannomatosis Support Group Meeting

01/19 - El Nido, CA

Casino Night

01/25 - Daytona, FL

Rolex 24 Hours at Daytona Race

02/02 - Wilbraham, MA

Family Dinner Event

02/09 - Cupid’s Undie Run in 15 Cities

www.cupidsundierun.com

02/16 - Cupid’s Undie Run in 2 Cities

www.cupidsundierun.com

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**Cupid’s Undie Run**

Cupid’s Undie Run registration is now available at www.CupidsUndieRun.com

Cupid’s Undie Run is a one mile fun run in which participants race in their underwear, outdoors, on Valentine’s Day weekend. There will be a Cupid’s Undie Run in 17 cities across the U.S. Please go to www.cupidsundierun.com for more information and to register.