Bench-to-bedside projects co-funded by Office of Rare Diseases Research (ORDR) and ICs, 2009

Teams	Project	Investigators
NIAID, NCI MD Anderson	"Frequency and TCR Diversity of FOXP3+ Regulatory T Cells in Chronic GVHD"	D. Tran, E. Shevach, NIAID; S. Pavletic, NCI; L. Cooper, MD Anderson
NINDS Univ of Pitt CNMC	"GABAB Receptor Antagonist SGS-742 Treatment in SSADH Deficiency"	W. Theodore, NINDS; K. Gibson, Univ of Pitt; P. Pearl, Children's National Medical Center
NHLBI, NIAID, NIDDK, NHGRI JHU	"Aneurysm Formation in Patients with Mutations in STAT3"	M. Boehm, L. Beltran, A. Walts, S. Han, NHLBI; A. Freeman, S. Holland, NIAID; A. Gharib, NIDDK; J. Davis, NHGRI; H. Dietz, JHU
NIAID, NLM Cincinnati Children's Hosp Univ of Toronto	"Genomic and Stem Cell Approaches to Hemophagocytic Lymphohistiocytosis"	H. Su, M. Lenardo, NIAID; A. Schaffer, NLM; A. Filipovich, Cincinnati Children's Hospital; J. Zuniga-Pflucker, Univ of Toronto
NCI Fred Hutchinson Cancer Ctr JHU	"Leukotriene Inhibition for the Amelioration of Bronchiolitis Obliterans"	R. Gress, NCI; S. Lee, Fred Hutchinson Cancer Ctr; A. Chen, JHU
NCI (CCR) NCI (DCEG) Univ of Toronto	"Repositioning Metformin as an Anti-Cancer Agent in Li-Fraumeni Syndrome"	P. Dennis, C. Harris, NCI (CCR); J. Fraumeni, S. Savage, NCI (DCEG); D. Malkin, Univ of Toronto

1. **Title:** Frequency and TCR diversity of FOXP3+ regulatory T cells in chronic GVHD

   Summary: Allogeneic HSCT is a common procedure given to approximately 7,000 people per year in the US typically for the treatment of blood cancers. Around 30-50% of patients will develop chronic GVHD, which can be life threatening or severely impair quality of life. With improvement in postgrafting immunosuppressive regimens, the number of individuals at risk for cGVHD is increasing. The treatment remains unsatisfactory and steroids are still the mainstay of therapy. The molecular basis and pathophysiology of this disease are still unclear. However, the loss of immune tolerance resulting in autoimmune manifestations may be a major component. Given the dominant role of FOXP3+ regulatory T cells (Tregs) in the maintenance of immune homeostasis and self-tolerance, we hypothesize that the Treg frequency and diversity are affected in these patients. Several studies have evaluated the frequency of Tregs in cGVHD, but the data remains conflicting. However, the diversity of the Treg TCR repertoire has not been evaluated. Tregs develop from the thymus and have been shown to contain a diverse TCR repertoire distinct from conventional CD4+CD25– T cells with a bias toward recognition of self-antigens. In myeloablative patients receiving HSCT, it is unclear how the donors' Tregs develop compared to the effector T cells. It is possible in this environment where the thymus is dysfunctional that the generation of the Treg TCR repertoire compared to that of conventional T cells is differentially affected. The development of cGVHD might

result from a selective loss of diversity in the donor's Treg population. If skewed Treg TCR repertoire is observed, the ultimate goal of these studies would be to use donor-derived Tregs for immunotherapy. We have already established collaboration with Dr. Steven Pavletic (NCI), who is currently conducting a protocol (NCI04C0281) on the natural history of cGVHD. He possesses a large cohort of cGVHD patients with extensive clinical data and blood samples. One new patient with cGVHD is seen at the NCI each week. In addition, we intend to be involved in a multicenter clinical phase 2/3 trials evaluating different therapies in cGVHD headed by Dr. Paul Carpenter at FHCRC. In this trial, we would be analyzing and phenotyping the Tregs during the course of therapy. The primary aim of our study is to determine the frequency and TCR diversity of Tregs based on flow cytometric analysis of intracellular staining of FOXP3 and to utilize the IOTest Beta Mark Kit designed for quantitative determination of the TCR Vβ repertoire by flow cytometry. For more detailed analysis, we will use a methodology based on PCR amplification and sequencing analysis to quantify the size and diversity of the TCR alphabeta repertoire in FACS-sorted CD4+CD25hiCD127− Tregs and CD4+CD25− T cells. If the result indicates a skewing of the TCR repertoire in the Tregs, we hope to analyze and compare the TCR repertoire in the Tregs of the original donors.

2. **Title:** GABAB Receptor Antagonist SGS-742 treatment in SSADH Deficiency

Summary: This proposal studies a rational intervention (GABAB receptor antagonist SGS-742) in succinic semialdehyde dehydrogenase deficiency (SSADH--OMIM 271980; Aldh5a1 gene at 6p22) a heretofore untreatable disorder of GABA metabolism with high neuropsychiatric morbidity. This autosomal-recessive disorder impairs the major conversion of succinic semialdehyde to succinic acid leading eventually to excessively high GABA and y-hydroxybutyric acid (GHB) concentrations along with neurotoxic effects involving GABAergic neurotransmission. Clinical features include seizures (often intractable to medical therapy), severe and persistent deficits in expressive language, hypotonia, and neuropsychiatric disturbances. This proposal covers complementary bench and bedside components. We assess the effect of SGS-742 on electrophysiology and survival in Aldh5a1-/- mice. We also propose a phase II clinical trial in SSADH-deficient patients, employing neurological and neuropsychological evaluations, transcranial magnetic stimulation (TMS), and flumazenil (FMZ)-positron emission tomography (PET) co-registered with MRI. The planned intervention, SGS-742, is a selective, orally active GABABR antagonist which has demonstrated good safety and tolerability in clinical trials for cognitive impairment. Due to the rare occurrence of the disease, we will administer SGS-742 to a small number of patients with SSADH deficiency (n=6). We predict that 1) application of SGS-742 to Aldh5a1-/- mice improves seizure control, electrocorticography, and survival, and 2) We predict that patients will show: 1) improvement in the areas of attention, reaction time, visual information handling, and working memory, and 2) improvement in baseline abnormalities in cortical silent period and long-interval intracortical inhibition as measured by TMS, and may alter GABAAR binding measured by FMZ-PET.

3. Aneurysm formation in patients with mutations in STAT3

Summary: Autosomal dominant (AD) Hyper-IgE syndrome (HIES) is a rare deficiency

arising from mutations in STAT3 and characterized by elevated IgE, dermatitis, recurrent infections, and skeletal and connective tissue abnormalities. Arterial tortuosity and aneurysms are common, but the signaling pathways linking STAT3 to these abnormalities are unknown. In general, aneurysm formation is the result of multiple factors affecting that arterial segment and its local environment. Recently, two independent mutations in TGF-b signaling have been identified and linked to aneurysm formation. Marfan syndrome (MFS) is an AD disorder with ocular, skeletal and cardiovascular manifestations due to mutated Fibrillin (FBN)-1. FBN-1 is homologous to latent TGF-b binding proteins and is essential for connective tissue elastic fiber formation. Loeys-Dietz syndrome (LDS) is an AD disease overlapping with both MFS and HIES. LDS is caused by TGF-b receptor (TGBFR)-1 or -2 mutations. While significant inroads into our understanding of the role of TGF-b signaling in aneurysm formation have now been made, the role of STAT3 remains completely unknown. However, STAT3 and TGF-b are involved in distinct but inter-related signaling pathways. TGF-b appears to inhibit STAT3 activity via smad2/3, while STAT3 inhibits TGF-b via smad7 activation. Therefore, STAT3 mutations may be associated with increased TGF-b signaling, similar to MFS and LDS, and we hypothesize that aneurysm formation in HIES is related to the same signaling pathways as in MFS and LDS. We propose to explore the STAT3-mutation mediated TGF-b dependent and -independent signaling pathways leading to pathological vascular remodeling in HIES. We will use patient derived vascular cell systems to explore these mutations. Also, mice with mutated STAT3 and with conditional knock out of STAT3 will be used to understand the impact of these gene mutations and ablation on aneurysm formation via the modulation of specific signaling pathways. These mice (all already in existence) will be used to test treatment strategies to retard aneurysm progression. We will integrate our human and murine findings with our bedside practice, with the aim of advancing our diagnostic and therapeutic tools to develop novel treatments, as already done in MFS. This project involves the NIAID, NHLBI, NIDDK and Johns Hopkins University School of Medicine as follows: HIES clinical evaluation and genotyping by NIAID (A. Freeman, S. Holland); pre-clinical treatment strategies in mouse aneurysm models (with the objective of achieving clinical translation of these studies) by Center for Genetic Medicine, John Hopkins Hospital (H. Dietz); vascular imaging of HIES by NIDDK (A. Gharib); isolation and characterization of patient vascular cells, STAT3/TGF-b signaling mechanisms and murine models with mutated STAT3 by NHLBI (M. Boehm).

4. **Title:** Genomic and Stem Cell Approaches to Hemophagocytic Lymphohistiocytosis

Summary: Familial hemophagocytic lymphohistiocytosis (FHLH) is a rare autosomal recessive childhood disease in which immune hyperactivation with impaired natural killer (NK) cell and cytotoxic T lymphocyte (CTL) function, precipitated by viral infection, is invariably fatal. We propose to integrate new genomic and stem cell approaches to study this disease. Our goals are to 1) extend fundamental knowledge of the biology of cytotoxic lymphocyte function and development; 2) improve diagnosis and guide clinical decision making for FHLH; 3) establish a new research tool for studying immune function in previously transplanted patients; and 4) explore the feasibility of using autologous inducible pluripotent stem (iPS) cells with gene targeting, to correct defective

lymphocyte function in vitro for inherited immunodeficiencies. In addition, our studies have broader relevance to chronic viral infections, hematopoiesis, tumor surveillance, and transplantation.

5. **Title:** Leukotriene inhibition for the amelioration of bronchiolitis obliterans

Summary: Bronchiolitis obliterans (BO) is a rare and morbid manifestation of chronic graft-versus-host disease (cGVHD) following hematopoietic stem cell transplantation (HSCT). The mortality of BO after HSCT exceeds 80% at five years and is unchanged by recent advances in immunosuppressive therapies. In part, the development of novel therapies has been hindered by the lack of understanding of the pathogenesis of BO after HSCT. In contrast, the mechanisms underlying similar immune-mediated lung destructive processes are better elucidated, including rejection following allogeneic lung transplantation (LTBO) and scleroderma lung disease (SLD). In all of these diseases, there is evidence for both infiltration of activated immune cells and increase in fibrosis of lung tissue. Increased leukotriene production has recently been implicated in the development of both LTBO and SLD in animal models and clinical studies. Leukotrienes are lipid molecules that enhance chemotaxis and activation of immune cells. Thus, inhibition of leukotrienes may diminish alloimmune lung destruction by redirecting immune cells away from lung tissues. Leukotrienes have also been implicated in the development of lung fibrosis, stimulating fibroblast proliferation and deposition. Inhibition of leukotrienes has led to lung remodeling in asthma patients and may decrease fibrosis in post-HSCT BO. Montelukast (singulair) is an approved, well-tolerated, oral agent with an excellent safety profile that inhibits leukotriene action in lung inflammation. We have recently initiated a prospective trial to evaluate the efficacy of montelukast for the treatment of BO after HSCT. Due to the rare incidence of BO after HSCT, we have initiated discussions with the Fred Hutchinson Cancer Research Center (Hutch CRC) to open our trial at this site in Seattle. The feedback has been positive and the trial has already passed an initial evaluation by the Long Term Follow-up Committee at that institution. In addition to the clinical assessment of benefit of montelukast, we have begun work in the laboratory to study the pathogenesis of BO. We have done pilot experiments using a preclinical model of BO after BMT. We hope to use this model to study the role of leukotrienes in this genesis of this disease and to identify the immune elements implicated in BO. Finally, from a translational standpoint, we are studying multiple immunologic parameters on patients enrolled on this trial. Leukotriene production is being measured in the blood, urine, and bronchoalveolar lavage fluid (BALF). Using flow cytometry, the leukotriene receptor levels are quantified on immune cells from the blood and BALF to identify potential key players in the pathogenesis of BO after HSCT.

6. **Title:** Repositioning metformin as an anti-cancer agent in Li-Fraumeni syndrome

Summary: p53 is somatically mutated in 50% of sporadically occurring cancers. Germline mutations in p53 are frequently observed in patients with the cancer-susceptibility syndrome, Li-Fraumeni syndrome (LFS). LFS patients develop early onset cancers, and no existing drugs address loss of p53 function in this rare population.

However, mouse models that recapitulate the phenotype of LFS have potential to identify therapies for the treatment or prevention of cancer in these patients. The oral anti-diabetic drug metformin is a promising candidate because it preferentially inhibits growth of tumor cells that lack p53 and activates AMP-activated protein kinase (AMPK), an emerging target in cancer. Moreover, metformin is FDA approved, well tolerated, and associated with decreased cancer incidence in diabetic patients. Specific Aim 1. Preclinical studies. Metformin will be given to p53 null and p53 mutant mouse models that genocopy and phenocopy LFS. A schedule of metformin that achieves plasma levels similar to that observed in humans and that activates AMPK in mouse tissues will be used. Metformin will be administered before tumors form or after they are established to determine if metformin is effective in cancer prevention and/or treatment. Tumor incidence, latency and size will be measured at various time points. Markers of apoptosis and cell cycle arrest will be studied. Activation of AMPK will be assessed in tumor and surrogate tissues as a pharmacodynamic marker and to gain insight into mechanisms that might underlie responsiveness to metformin. Tissues will also be analyzed to determine if other pathogenic events that have been identified in LFS patients such as changes in telomere length, copy number variation, and microRNA expression can be identified in these mouse models. These studies will be performed in the laboratories of Drs. Malkin and Harris. Specific Aim 2. Clinical Studies. A clinical protocol will be designed and implemented at the Clinical Research Center by Drs. Dennis and Bernstein in CCR, Drs. Fraumeni and Savage in DCEG, and Dr. Malkin at the Hospital for Sick Children in Toronto. The protocol will have a natural history component where LFS families who meet clinical criteria will be followed and sequencing of p53 will be offered. LFS patients with p53 mutations will be eligible for enrollment. Metformin will be administered for 6 months to patients who do not have cancer, and longer to LFS patients with cancer who benefit clinically. The primary endpoint will be safety and tolerability. Secondary endpoints will include activation of AMPK in PBMCs, tumor regression as assessed by MRI scan, and decreases in tumor metabolism as assessed by PET scan. Other analyses will include preliminary association of tumor responses with plasma metformin levels, p53 genotype, and genetic anticipation within families. When safe, tumor biopsies will be performed to validate biomarkers identified in preclinical studies.