Bench-to-Bedside Projects Co-funded by the Office of Rare Diseases Research (ORDR) and NIH Institutes and centers, 2010

Project	Investigators Institute(s)/Institution
Targeting Antigen-Antibody Responses in Systemic Capillary Leak Syndrome	**NIAID:** K. Druey; T. Myers; S. Porcella **NCI:** O. Landgren **NIBIB**: A. Gorbach **University of Minnesota:** A. Dudek **Mayo College of Medicine:** P. Greipp
Sympathetic Innervation & Myocardial Injury in Acute Stress Cardiomyopathy	**CC, Radiology and Imaging Sciences:** C. Sibley; D. Bluemke **NINDS:** D. Goldstein **NHLBI:** D. Rosing **Johns Hopkins:** I. Wittstein; F. Bengel; J. Mudd; J. Lima
Preclinical Testing of Targeted Agents for Clinical Development in NF1	**NCI:** A. Kim; B. Widemann; E. Dombi **Children's Hospital Medical Center:** N. Ratner; J. Wu
The DICER1-related Pleuropulmonary Blastoma Cancer Predisposition Syndrome	**NCI:** C. Kratz; B. Alter; P. Rosenberg **National Children's Medical Center:** A. Hill **Children's Hospital & Clinics of MN:** Y. Messinger; K. Schulz
Brain Development in Children with Williams Syndrome, and the LIMK1 Gene	**NIMH:** K. Berman; J. Kleinman **University of Louisville:** C. Mervis
The Role of EGFR in Endolymphatic Sac Tumors	**NCI:** P. Dennis **Yale University:** A. Vortmeyer

1) **Title:** Targeting antigen-antibody responses in Systemic Capillary Leak Syndrome

Summary: The systemic capillary leak syndrome (SCLS, Clarkson syndrome) is a rare disorder of unknown etiology characterized by transient, severe episodes of hypotension, hypovolemia, oliguria and severe edema due to leakage of fluid and macromolecules less than 900 kDa) into tissues. Although no more than 150 cases have been reported since 1960, the disease may be underdiagnosed due to the nonspecific nature of the presenting signs and symptoms and the considerable overlap between other "shock" syndromes including sepsis, anaphylaxis, and angioedema. The most common signs of a classical SCLS episode are hypotension, hemoconcentration, and hypoalbuminemia. Typically the vascular leak resolves after 48-72 hours, with massive tissue fluid mobilization and diuresis. SCLS carries a 5 yr. mortality rate of 30%, due to compartment syndromes and rhabdomyolysis (from leakage of fluid and proteins into muscles), venous and arterial thrombosis (as a result of hemoconcentration), and cardiopulmonary failure due to fluid overload during the recovery phase. Aside from supportive treatment with fluid and vasopressors during the acute episode, there is currently no proven treatment for SCLS. Phosphodiesterase inhibitors such as theophylline have reduced the severity

and frequency of attacks in a limited number of patients, but their use is limited by toxic side-effects. Although the precise cause of the endothelial barrier dysfunction is unknown, evidence points to immune dysregulation in the pathogenesis of SCLS. 75-85% of patients have a monoclonal gammopathy of unknown significance (MGUS). A CD8+CD25+ lymphocytic capillaritis and electron micrographic evidence of endothelial apoptosis were identified in skin biopsies from two SCLS patients. In a separate study, serum from SCLS patients induced apoptosis of cultured endothelial cells in vitro and reactive oxygen species production, suggesting that endothelial death may contribute to the transient vascular breach characteristic of acute SCLS episodes. Initial transcriptional profiling of peripheral blood mononuclear cells (PBMCs) from SCLS patients shows evidence of B lymphocyte activation during an attack. In addition, SCLS lymphocytes stimulated with autologous monoclonal paraprotein, but not control IgG, upregulate activation markers such as CD154 and CD86. When considered with earlier findings, our preliminary results suggest that anti-idiotypic lymphocyte activation mediated by the monoclonal paraprotein could evoke transient endothelial injury in SCLS. Research Proposal This proposal will enable us to expand our patient base to further investigate SCLS mechanisms. We have now obtained specimens from 10 SCLS patients admitted to the NIH Clinical Center under a new protocol (09-I-0184), which has a target accrual of 30 patients over 3 years. First, we plan to explore how activated lymphocytes might induce endothelial permeability in SCLS. Secondly, we are expanding blood-outgrowth endothelial cells (BOEC) from patients in collaboration with Dr. Dudek for transcriptome profiling and functional studies. Such cells may provide insight into whether intrinsic endothelial cell dysfunction contributes to SCLS and could be used as a platform to test the efficacy of new therapeutic agents. Third, in collaboration with Dr. Gorbach, we are developing a non-invasive infrared imaging system to assess blood flow perturbations and endothelial function that is equivalent to currently used invasive methods. These studies may ultimately enable development of an early warning system to detect impending leak episodes. Finally, a new treatment regimen for SCLS will be explored. Several SCLS patients whose MGUS evolved into frank multiple myeloma saw complete abatement of their vascular leak symptoms after chemotherapy for myeloma. Taken together with our studies, this finding suggests that the monoclonal paraprotein and/or abnormal B cell/plasma cell clone has a direct function in SCLS pathogenesis. Therefore, we hypothesize that therapeutic agents targeting the monoclonal paraprotein or clonal B cell population may prevent or reduce the severity of acute SCLS episodes. Specifically, we will conduct an open-label pilot study of lenalidomide for the treatment of SCLS. This drug is highly toxic to malignant plasma cells, is now used as upfront therapy for myeloma, and has been used successfully in other MGUS-related syndromes mediated by cytoxic paraproteins (e.g. POEMS). It is hoped that by exploring the etiology and treatment of SCLS, we will not only be able to better manage the disease, but will also gain insight into both the mechanisms controlling endothelial cell growth and permeability under physiological conditions and the endothelial damage and dysfunction characterized by diverse conditions such as atherosclerosis, Ebola virus infection, septic shock, and pulmonary hypertension.

2) **Title:** Sympathetic Innervation & Myocardial Injury in Acute Stress Cardiomyopathy

Summary: Acute stress cardiomyopathy (ASC) is a syndrome of profound ventricular dysfunction in the setting of emotional or physical stress. First recognized in a subset with apical akinesis and basal hypercontractility referred to as apical ballooning, dysfunction involving all portions of the myocardium have been described. The pathogenesis of ASC is unknown. Significant elevations in plasma catecholamines occur in ASC compared with myocardial infarction and biopsies have shown contraction band necrosis suggestive of catecholamine injury. Acute b-adrenergic overload causes myocyte injury via ryanodine receptor 2 mediated calcium efflux. Regional variation in myocardial sympathetic innervation may explain the patterns of dysfunction in ASC. Ten percent have recurrence, but little else is known about the sequelae of ASC. I dentification of subclinical myocardial injury would suggest an elevated long-term risk of heart failure and arrhythmia, permitting targeted follow-up. PET with radiolabeled epinephrine is a powerful non-invasive means to assess cardiac sympathetic function. Cardiac MR tagging permits assessment of regional systolic function. MR gadolinium enhancement is the current standard for assessment of myocardial fibrosis but is insensitive to diffuse myocardial abnormalities. Imaging with T2 weighted techniques and T1 mapping methodology appear to be the most promising approach to non-invasive assessment of diffuse injury in ASC. These techniques allow definitive noninvasive phenotyping of myocardial structure, function and innervation. We plan to evaluate 25 patients with ASC using cardiac MR, PET and endomyocardial biopsy. Patients will be enrolled from the heart failure service at the Johns Hopkins Hospital, a major referral center for ASC. B lood samples for systemic catecholamine levels will be obtained. Endomyocardial biopsy will be performed at JHH. At discharge from index hospitalization, participants will be transported to the NIH Clinical Center for imaging. Follow-up will occur at the Johns Hopkins Outpatient Center. Bench: Dr. Bengel is internationally known for translational cardiovascular molecular imaging. This expertise has not previously been available at the NIH campus. The Clinical Center has 2 state of the art 128 slice PET/ MDCT scanners. Leveraging this capability will enhance implementation of molecular techniques at NIH. Drs Sibley and Bluemke are actively engaged in the development of cardiac T2 and T1 mapping techniques in tissue specimens, healthy volunteers and in a large cohort study (EDIC), providing ideal reference populations. The combination of the mature imaging techniques in MR and PET and the gold standard of histology is a unique opportunity to rapidly advance this novel and evolving technique. The imaging resources at the NIH Clinical Center hospital are ideal for optimization and refinement of T2 and T1 mapping methodology and image analysis. Dr David Goldstein is a world authority on catecholamine biochemistry and sympathetic regulation. His laboratory pioneered many of the methods for catecholamine measurement that will be employed in this project. Bedside: Johns Hopkins Hospital is an internationally recognized center of expertise in cardiomyopathy and Dr. Wittstein follows one of the largest cohorts of acute stress cardiomyopathy patients in existence. Our close relationship with Johns Hopkins will permit rapid deployment of the technical imaging development performed at the NIH Clinical Center hospital to the clinical care of these patients. Dr Rosing directs the cardiology consultation service at the Clinical Center, and will identify and recruit participants at the NIH.

The lessons learned will translate readily with broad applicability in all forms of cardiomyopathy with similarly diffuse myocardial injury. Specific Aims are to: 1) Examine the relationship between sympathetic innervation imaged with PET and myocardial injury on biopsy. (Hypothesis: The severity of sympathetic innervation abnormality correlates directly with the severity of histologic injury.) 2) Characterize myocardial T2 and T1 characteristics in acute stress cardiomyopathy and determine the relationship of MRI findings to injury on endomyocardial biopsy. 3) Determine the spatial relationship between sympathetic innervation, injury on imaging, and regional dysfunction on MRI. 4) Determine the relationship between PET defined impairment of innervation and systemic catecholamine levels. 5) Determine the relationship between patterns of regional function and sympathetic innervation during an episode of ASC and patterns after recovery of global left ventricular function .

3) Title: Preclinical Testing of Targeted Agents for Clinical Development in NF1

Summary: A debilitating complication of Neurofibromatosis Type 1 (NF1) is the development of peripheral nerve sheath tumors called plexiform neurofibromas (PN). There is no known effective medical treatment for PN. Research focused on the biology of NF1 and pathogenesis of PN has identified potential molecular targets shared with cancers for which new agents are being developed. Selection and prioritization of agents for clinical trials is a key challenge in drug development for NF1 as only few agents can be tested in the clinical setting due to small patient numbers, time, and cost. Lack of relevant models precluded preclinical testing in the past. However, preclinical models of PN have become available and may have utility in the rational development of drugs for NF1. We propose testing targeted agents with scientific rationale in a genetically susceptible mouse model for NF1-associated neurofibroma developed by Dr. Nancy Ratner [Cincinnati Children's Hospital (CCHMC)]. Data from treatment trials performed at CCHMC in mice will be analyzed for tumor volume, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) at the NCI and CCHMC utilizing similar methods currently used in clinical trials. Promising agents will be prioritized for phase I/II trials developed and conducted under the direction of the Pediatric Oncology Branch (POB) by Drs. AeRang Kim and Brigitte Widemann (NCI) in their active clinical trials program for NF1-related tumors. These studies will allow for more rational drug development and serve to validate the utility of this mouse model in predicting for response in NF1 clinical trials.

4) Title: The DICER1-related Pleuropulmonary Blastoma Cancer Predisposition Syndrome

Summary: MicroRNAs are small nucleotide sequences that fine-tune the expression of protein coding genes by modulating their translation. These molecules are involved in a broad variety of cellular pathways and play an important role in cancer pathogenesis. DICER1 is a crucial RNase that participates in microRNA biogenesis by cleaving precursor microRNAs to produce mature microRNA sequences. Germline DICER1 mutations cause familial Pleuropulmonary Blastoma (PPB) (Hill et al., Science. 2009;325:965), a rare pediatric tumor of the lung that is part of a cancer predisposition syndrome which includes a unique spectrum of benign and malignant

neoplasms. PPB represents the first human disease linked to germline mutations in a gene involved in microRNA processing. I t is unknown what fraction of all PPB patients actually has a genetic disease, and what tumors or other medical conditions are directly attributable to DICER1 mutations. Moreover, the penetrance of DICER1 mutations and the frequency of inherited versus *de novo* mutations are undetermined. Finally, the mechanism by which DICER1 haploinsufficiency contributes to PPB susceptibility is unknown. In the bedside component of this application, we propose to study a cohort of families with PPB or associated neoplasms in the NIH Clinical Center in order to address three major objectives: (1) to determine the frequency of DICER1 germline mutations in patients with PPB (or neoplasms from the PPB spectrum) and their family members; (2) to characterize the phenotype and study the incident and prevalent cancer rates in these patients and their family members for all cancers combined and for each type of cancer, and to identify and confirm the specific types of cancer and benign neoplasms associated with this disorder; and (3) to identify differences between DICER1 mutation-positive patients who do develop cancer and those who do not develop cancer. The bench component, which is included in an R01 grant proposal by the extramural investigators, will investigate DICER1's role in PPB initiation, and evaluate potential mechanisms of oncogenesis. These studies will be conducted in Dr. Hill's laboratory at the Children's National Medical Center. The proposed project has the potential to result in better understanding of an important and newly-discovered pathway to human malignancy and to create a resource for the development of novel therapies.

5) Title: Brain Development in Children with Williams Syndrome, and the LIMK1 Gene

Summary: Williams syndrome (WS), a rare neurodevelopmental disorder caused by a 1.6 MB hemizygous deletion on chromosome 7 (7q11.23), is characterized by distinctive cognitive features, including severe impairment in spatial cognition, with relative strengths in language and face processing, as well as characteristic "hypersocial" personality and lack of fear of strangers; in contrast to this social fearlessness, WS patients have significant anxiety in nonsocial circumstances and high incidence of specific phobia. Since the affected genes are known and the behavioral/cognitive abnormalities are relatively circumscribed and specific, WS offers a privileged setting for investigating the mechanism by which genetic abnormalities are translated in the brain to produce such clinical problems. We previously undertook an extensive multimodal *in vivo* neuroimaging study of adults with WS that has provided important new information about the brain phenotype in this rare disorder. While these findings offer important new insights, it is clear that the cognitive and behavioral disturbances result from a complex interplay of altered neural systems that occurs in the specific genetic environment of WS. In order to achieve a broader understanding of relevant neurogenetic mechanisms, they must be studied from a developmental and translational perspective, an approach that also offers potential for impacting early intervention. To meet this imperative, we will bring together experts in neuroimaging of genetic and cognitive disorders (Dr. Berman), childhood neurodevelopmental disorders (Dr. Mervis), and postmortem brain investigation of allelic variation (Dr. Kleinman) to initiate a study of WS children along with an incisive investigation of

the effects of allelic variation in a key gene affected in WS. We will build on a confluence of exceptional opportunities for these endeavors: the NIH's superb imaging sciences facilities, our access to a unique cohort of WS children WS, and the availability of a world-class brain bank and analytical methods. Two scientific advances also provide impetus: 1) Our success in delineating the brain phenotype in adult WS, which will provide the crucial context within which to view the emergence and modification of these neural circuit abnormalities from a developmental perspective in WS children and from which to launch translational studies of specific gene effects; and 2) recent understanding arising from knockout mouse models and from our own neuroimaging work of the roles of specific genes in 7q11.23 (particularly LIMK1) in the genesis of the WS brain phenotype. The clinical component of this award would bring children with classic WS deletions, smaller deletions, and duplications to the NIH for comprehensive study. Concurrently, the basic component of this award would enable a focused, developmental postmortem brain investigation of the effects of LIMK1 genotype across the lifespan from 14 weeks of gestation to age 85.

6) Title: The role of EGFR in endolymphatic sac tumors

Summary: Epithelial neoplasms of the ear are rare, and can originate in the endolymphatic duct and sac (ELSTs) or middle ear (middle ear adenocarcinomas). When sporadic, these tumors are difficult to detect and may present with severe audiovestibular or facial nerve dysfunction. Complete resection is often difficult because of location and the late stage at diagnosis. Although ELSTs can occur sporadically, they are commonly associated with von Hippel-Lindau (VHL) disease, where bilateral tumors are frequent. Research in ELSTs is lacking. Other than loss of VHL, there has been little molecular analysis of ELSTs, no mouse model for preclinical testing and no medical therapies to minimize the extent of surgery or treat unresectable patients. Our proposal has three aims: 1. Characterize a new mouse model driven by activation of EGFR that recapitulates features of epithelial ear neoplasms, 2. Determine activation of the EGFR pathway in human ELSTs, and 3. Design a clinical trial for subjects with epithelial ear neoplasms using EGFR-targeted drugs. Our mouse model arose serendipitously during efforts to generate models of lung cancer driven by activating mutations in EGFR (EGFRL858R/T790M). We used two lung-specific promoters to drive expression of EGFRL858R/T790M, and found that if a Clara cell-specific promoter was used (CC10), 87/130 mice developed lung tumors. In contrast, if a type II pneumocyte promoter was used (SP-C), 0/54 mice developed lung tumors. By 15-30 wk, 21/54 SP-C mice developed head tilt and exhibited abnormal gait. CT and MRI scans of mice with head tilt showed that all mice had invasive bilateral inner and middle ear tumors, but no other tumors. Histology of the ear tumors showed adenocarcinoma, and EGFRL858R/T790M was expressed in the tumors. Because combinations of EGFR-directed therapies have been shown to decrease the size of lung tumors driven by EGFRL858R/T790M, we treated three mice with head tilt and bilateral ear tumors with cetuximab, a monoclonal antibody against EGFR, and an irreversible EGFR tyrosine kinase inhibitor. All three mice had complete regression of ear tumors and normalization of gait. These studies provide preliminary evidence that this model recapitulates features of ELSTs and show that these tumors are dependent upon activation of

EGFR. In this aim, we will expand the colony of mice to determine the cell of origin for these tumors, to assess activation of pathway components downstream of EGFR such as Akt and ERK in these tumors, and to expand our drug treatment studies. Expanding treatment studies is important because we want to validate our preliminary results. In addition, we want to assess whether combinations of EGFR-targeted drugs are necessary and whether inhibition of components downstream of EGFR might be effective. The results of these studies will impact the design of clinical trials to treat patients with epithelial ear neoplasms. The fact that our mouse model of ELSTs depends upon EGFR raises the question as to whether human ELSTs from VHL patients have activation of EGFR. This hypothesis is supported by the fact that loss of VHL increases levels of TGF-α and activates EGFR in renal cancer cells, and silencing of EGFR decreases VHL-dependent renal cancer growth. Therefore, in our second aim we will evaluate VHL, EGFR and downstream components in fresh and paraffin-embedded ELSTs from a collection in NINDS. Our analysis will include immunohistochemistry as well as sequencing. If EGFR is activated but not mutated in human ELSTs, it would suggest that activating mutations in EGFR in our mouse model bypass the need for loss of VHL. We will confirm this by assessing VHL status in our murine tumors. More importantly, detection of active EGFR in human ELSTs will prompt clinical testing of EGFR-based therapies in ELST. Although complete resection of ELSTs can be curative, there is a need for medical therapies. In particular, the locally aggressive nature makes gross resection difficult without jeopardizing critical structures. Combining medical therapies with partial resection might allow for effective tumor control in patients with large tumors. In aim three of this proposal, we will test EGFR-based therapies in patients with ELST. The combination we will employ is cetuximab and erlotinib, because it is similar to the effective combination used in our preclinical studies, and both drugs are FDA-approved and available for purchase. Phase I studies of this combination have been completed, and it is well tolerated. Because these tumors are so rare, we will propose a pilot trial to test cetuximab and erlotinib in patients prior to surgery and in those that are unresectable. The primary endpoint will be the response rate. If objective responses are observed, it would prompt future trials with newer EGFR-based therapies. Other trials with inhibitors of downstream components such as Akt, mTOR, or MEK could also be considered if preclinical data are promising.