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Project	Investigators Institute(s)/Institution
T cell adoptive therapy for viral infection after stem cell transplantation	**NHLBI:** J. Barrett **NIAID:** D. Douek **CC:** D. Stroncek **Baylor College of Medicine:** C. Bollard
Choroid Plexus-Directed Gene Therapy for Lysosomal Storage Disease	**NICHD:** S. Kaler **NHLBI:** R. Kotin **Children's Hospital of Philadelphia:** J. Wolfe
Elucidation of cancer metabolism by Stable isotope-Resolved Metabolomics	**NCI:** W. Linehan **NICHD:** T. Rouault **University of Louisville:** A. Lane; T. Fan
Treatment of Anti-NMDA receptor encephalitis	**NINDS:** A. Nath; I. Cortese; S. Jacobson; K. Roche **CC:** D. Hammoud **University of Pennsylvania:** J. Dalmau; R. Balice-Gordon
Metabolic phenotyping of Parkin mutation associated Parkinson's Disease	**NHLBI:** M. Sack **NINDS:** R. Youle **University of Central Florida:** X. Han
Metabolic Phenotyping in Methylmalonic Acidemia: Markers and Drug Response	**NHGRI:** C. Venditti; E. Manoli **NIDDK:** K. Chen **Children's National Medical Center:** M. Tuchman
Developing and validating membrane biomarkers in the muscular dystrophies	**NICHD:** J. Zimmerberg; P. Blank; P. Basser; G. Humphrey **NINDS:** C. Bonnemann **University of Massachusetts Memorial Medical Center:** R. Brown **University of Maryland, Baltimore:** R. Bloch

PI: **John Barrett**

Project Title: **T cell adoptive therapy for viral infection after stem cell transplantation**

Abstract:
Recipients of hematopoetic stem cell transplant (SCT) remain highly vulnerable to viral infections, as the currently available treatments are toxic and frequently ineffective. The need to selectively and rapidly restore the immunity to common pathogens is critical in order to achieve radical reduction in transplantation-related morbidity and mortality and in broadening the applicability and availability of SCT. Based on the expertise brought by Dr. Bollard (Baylor College of Medicine [BCM]) we plan to rapidly establish clinical grade manufacture of multi-virus specific T cell for adoptive cell transfer to SCT recipients to treat and prevent opportunistic viral infections in HSCT recipients treated at Clinical Center, NIH, (NHLBI, NCI and NIAID). We intend to generate T cells specific to novel important target BK virus in addition to cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus   and use those cells in a trans-institutional clinical trial. Establishing such a program would provide significant clinical benefit, likely leading to reduction in transplant-related morbidity and mortality. To further understand the factors involved in successful adoptive T cell transfer we will study the fate of infused virus-specific T memory cells in patients treated at BCM using high throughput TCR sequencing in collaboration with Dr. Douek (Vaccine Research Center, NIAID). These advanced techniques will help define the role of novel T cell memory subsets (stem-cell like memory cells, central memory, Th17 cells) in human subjects and would lead to improvement of the therapeutic efficacy of adoptive T cell transfer for the treatment of viruses and malignant disease.

PI: **Stephen Kaler**

Project Title: **Choroid Plexus-Directed Gene Therapy for Lysosomal Storage Disease**

Abstract:
The choroid plexuses are highly vascularized structures that project into the ventricles of the brain. The polarized epithelia of the choroid plexuses produce cerebrospinal fluid by transporting water and ions into the ventricles from the blood and secrete a large number of proteins. We demonstrated recently that lateral ventricle administration of recombinant adeno-associated virus serotype 5 (rAAV5) resulted in selective gene transfer to the choroid plexus epithelia and rescued a mouse model of Menkes disease, a lethal pediatric disorder of copper transport. In this proposal, we detail experiments to assess the feasibility of targeting choroid plexus epithelia with rAAV gene therapy vectors for treatment of a broader category of neurometabolic diseases, lysosomal storage disease (LSD). Lysosomes are compartments that function as the primary digestive units within cells and specific enzymes within lysosomes normally break down nutrients. However, patients with LSDs are unable to metabolize these nutrients, resulting in greatly diminished lifespans and reduced quality of life. There are no ideal therapeutic options currently available, especially for the CNS manifestations of LSD. Brain-directed enzyme replacement has shown great promise for several LSDs but requires repeated intrathecal administration due to short enzyme half-lives. In contrast, rAAV-mediated gene transfer of missing lysosomal enzymes to choroid plexus epithelia should enable continuous synthesis and secretion of enzymes into the CSF and penetration to cerebral and cerebellar structures. Recombinant AAV transduction results in sustained episomal transgene expression, choroid plexus epithelia do not turnover, and CSF flow carries molecules throughout the ventricular system into the subarachnoid space from which molecules ultimately reach the entire brain. Thus, we will study the efficacy of two known AAV serotypes (AAV5 and AAV4), as well as a novel choroid plexus-specific AAV capsid under development in the Kaler lab, in a well-characterized LSD, alpha-mannosidosis. We will use both mouse and guinea pig genetic models of alpha-mannosidosis available in our respective labs to evaluate choroid plexus transduction by the three AAV vectors, and determine post-treatment alpha-mannosidase concentration and distribution in brain. The studies in the mouse model will require less virus and be easier to breed, whereas the guinea pig features a partially gyrencephalic brain more similar to the human brain. Thus, the two models will be complementary. In parallel, we will embark on a natural history study of 10-15 affected individuals to assess physical signs and CSF biomarkers potentially useful as outcome measures in future clinical trials. The two lead investigators' labs are well-versed in the techniques needed to complete these experiments and have access to additional expertise and vector production capacity through Dr. Kotin (NHLBI), an Intramural expert in AAV biology, and the NICHD Biomedical Mass Spectroscopy Facility for the CSF proteomic analyses proposed. The potential impact on clinical practice in the field of LSD is high since, if the proposed aims are successfully achieved, the largest current barriers to health for patients with LSDs would be circumvented. The principles of gene transfer and CSF protein transport being investigated in this project will potentially be useful for other neurometabolic diseases with global effects on brain.

PI: **W. Marston Linehan**

Project Title: Elucidation of cancer metabolism by Stable isotope-Resolved Metabolomics

Abstract:
The proposed research will involve a unique collaboration between Dr. W. Marston Linehan (NCI) and Drs. Teresa Fan and Andrew Lane (Louisville) that combines cutting-edge clinical research on rare familial renal carcinomas at NCI with the state-of-the-science biochemical and analytical technologies developed at CREAM, Louisville.

Background

It is now becoming clear that metabolic reprogramming induced by oncogenic mutations is a key to human cancer development, particularly for the rare, hereditary renal cell carcinomas. One intriguing outstanding question is how germline mutations in metabolic enzymes such as mitochondrial fumarate hydratase (FH) result in reprogramming of cell metabolism to promote the development of very aggressive hereditary Leiomyomatosis papillary renal cell carcinoma (HLRCC). Such cells have a truncated Krebs cycle, which in principle limits their oxidative phosphorylation potential and their ability to produce biosynthetic precursors for growth. Recently it was shown that in FH knockout kidney epithelial cells, a new pathway from glutamine to oxidized heme was induced. However, this intriguing finding does not account for the aggressive growth of human FH(-/-) cells derived from HLRCC, which would require much more extensive metabolic reprogramming. Papillary renal cell carcinoma occurs in 15% of patients with kidney cancer; however, papillary kidney cancer occurs in over 40% of U.S. African American patients with kidney cancer.

Stable Isotope Resolved Metabolomics

This question can be best and most efficiently answered using stable isotope tracers coupled with metabolomics technologies, e.g. Stable Isotope Resolved Metabolomics or SIRM. At Univ. of Louisville, Fan and Lane have successfully developed the SIRM approach for translational cancer research from cells through mouse models to human patients. The SIRM approach offers an unprecedented opportunity to elucidate altered metabolic networks in surgery-eligible patients with rare cancers such as HLRCC. Our collaborative SIRM efforts on one of the FH(-/-) HLRCC tumor cell lines (UOK262) points to new paradigms in metabolic reprogramming that may account for unusually aggressive tumor behavior. In particular, using 13C glucose and 13C/15N glutamine as tracers, we have identified the sources of carbon and nitrogen and their transformation pathways for energy production and biosynthesis of glycogen, nucleotides, and both the glycerol and fatty acyl chains of a large number of lipids in UOK262 cells. This work is leading to the design of anticancer agents that target the growth susceptibilities of UOK262 cells, which can be tested readily in cell culture and animal models.

Experimental Approach to Patient Studies at the Clinical Center

We aim to bring to bear these SIRM approaches directly to studies of human patients with HLRCC or other renal carcinomas driven by germline mutations such as FH(-/-) or SDH (-/-). Patients will be infused via i.v. 13C6-glucose or 13C5,15N2-glutamine preoperatively, and cancerous (CA) and non-cancerous (NC) kidney tissues are resected in the operating room, and flash frozen within 5 minutes of resection. The tissues are then prepared for analysis by NMR and mass spectrometry (MS) to determine how the CA and NC tissue have metabolized the stable isotopic tracers. Concurrently, small pieces of the tumor tissue are used to establish stable cell lines and for implantation in nude mice for subsequent SIRM studies. Thin (0.5 to 1 mm thick) CA and NC tissue slices will also be prepared at OR for ex vivo SIRM studies for comparison with the in vivo and in vitro studies. This multipronged approach will enable the translation of cell-based mechanistic findings (bench) directly to clinical settings (bedside). The animal,

human tissue slice, and cell models will also be used for testing new drugs against the metabolic enzyme targets uncovered by the SIRM approach.

We plan to also perform this metabolic analysis on the tumor tissue of African American patients with kidney cancer. They have a much higher likelihood of developing aggressive type 2 papillary kidney cancer than do Caucasian patients and those who are sickle trait positive are at risk for the development of medullary kidney cancer. This work has the definite potential to identify novel approaches to therapy for fumarate hydtratase- and succinate dehydrogenase-deficient kidney cancer as well as other forms of papillary kidney cancer. We have recently shown a remarkable reprogramming of the metabolic pathways in cells derived from kidney cancers from patients who had fumarate hydratase-deficient kidney cancer (HLRCC) that were very sensitive to agents which targeted the metabolic abnormalities (metformin). Preliminary studies suggest that targeting the glutamine pathway holds significant promise for targeted therapeutic approaches for these and other tumors characterized by aerobic glycolysis.

PI: **Avindra Nath**

Project Title: Treatment of Anti-NMDA receptor encephalitis

Abstract:
We propose to optimize the treatment of anti-NMDA receptor (NMDAR) encephalitis, determine the clinical, neuroimaging, and immunological features that predict clinical recovery and risk of relapses, and how these features correlate with the cellular and synaptic effects of patients' antibodies. This disease has two stages, (1) acute presentation of behavioral change and psychosis followed by coma, catatonia, seizures, dyskinesias, and autonomic instability with central hypoventilation; (2), usually lasting >6 months, in which patients do not require intensive care but have prominent alteration of executive functions, social interactions, and amnesia. In about 40% of cases the trigger of the disorder is a tumor with ectopic expression of NMDAR (usually a teratoma); for the rest of the patients the trigger is unknown. Treatment in stage 1, is based on intensive care, search for an occult teratoma (and removal if found), and immunotherapy including, corticosteroids, plasma exchange and/or IVIg (first line treatment, effective in ~50% cases). Rituximab and/or cyclophosphamide (second line therapies) are effective and increasingly used, replacing first line therapies. With this approach, most patients transition from stage 1 to stage 2. Treatment in stage 2 is largely unknown.

In CSF, there is rapid and robust intrathecal synthesis of antibodies and oligoclonal bands, with pathological studies showing brain and meningeal plasma cell infiltrates. Brain MRI is often normal but most patients have abnormal brain FDG-PET findings similar to those identified in models of NMDAR antagonists (fronto-temporal hypermetabolism, occipital hypometabolism). Experiments using cultured dissociated rodent hippocampal neurons and cerebroventricular infusion of antibodies to rodents show a dramatic and specific decrease of synaptic NMDAR clusters by a mechanism of receptor cross-linking and internalization. These effects correlate with antibody titers and reverse upon removing the antibodies from cultures. The study of this disorder is important because: Since its discovery in 2007, it has been identified as one of the most frequent autoimmune encephalitis in children and young adults; 50% of patients are younger than 18 years (median 20 years); the disorder is potentially lethal but 90% of patients survive (60% full recovery, 40% with residual cognitive-psychiatric deficits); the process of recovery is protracted and requires a multidisciplinary treatment approach; prompt treatment associates with better outcomes and fewer relapses.

Intramural studies: In this open label pilot study 12 patients will be recruited in two arms. For all patients, baseline studies at disease onset (stage 1) will be available, including CSF analysis, brain MRI, EEG, tumor screening with chest/abdomen and pelvic CT. At arrival to NIH, patients will have a comprehensive neurological/neuropsychological assessment. Patients with detectable NMDAR antibodies will be randomized to receive rituximab (375 mg/m2, weekly for 4 weeks, every 3 months). Analysis of CSF oligoclonal band will be performed at NIH. Serum and CSF samples will be sent to the extramural investigators for studies described below. Periodic clinical, serologic and EEG follow-up will be performed every 2 months. FDG-PET will be obtained every 4 months for 12 months. Patients with residual deficits after the first year, will be followed clinically, serologically, and with EEG every 4 months, and with FDG-PET every 6 months, for 1 additional year. Prolonged follow-up is needed because current studies indicate that patients continue to improve for at least 2 years. If patients continue to relapse despite rituximab; they will receive cyclophosphamide (750 mg/m2 monthly for 4 months).
Extramural studies: Determination of antibody titers in serum/CSF and analysis of structural and functional effects of the antibodies in cultured neurons and in vivo will be performed at the University of Pennsylvania as an extension of current NIH funded studies. This work, which includes confocal microscopy and electrophysiology on dissociated hippocampal neurons and rodent brain slices, is being

conducted to determine how patients' antibodies lead to spatial and temporal changes in hippocampal synaptic structure, function and plasticity, and what are the dynamics of recovery of synapse structure/function after exposure to patient antibodies is discontinued. The proposed studies will link the titers and effects of antibodies with symptoms, FDG-PET findings, response to treatment, risk of relapses, and elucidate subgroups of patients (e.g., children versus older individuals; stage 1 versus stage 2 or relapse; treatment responders versus non-responders, extended versus non-extended immunotherapy). Results of these studies will transform the understanding and treatment of anti-NMDAR encephalitis.

PI: **Michael Sack**

Project Title: **Metabolic phenotyping of Parkin mutation associated Parkinson's Disease**

Abstract:
Parkin mutations associate with autosomal recessive early-onset Parkinson's disease (EOPD). As murine genetic deletion of Parkin (an E3-ubiquitin ligase) does not faithfully replicate the human syndrome, it is proposed that parkin mutations increase biological susceptibility to, rather than directly causing substantia nigra neurodegeneration. The characterization of Parkin function is therefore instrumental in delineating the pathophysiology underpinning EOPD.

An important function of Parkin that is now well characterized shows that Parkin translocates from the cytosol to the mitochondria under severe mitochondrial stress conditions to mediate the mitophagic mitochondrial quality control program to facilitate recycling of defective mitochondria and that Parkin mutations attenuate this biology. However, the mechanisms underlying 'de-energizing mitochondria' to initiate this Parkin relocation in the context of Parkin mutations has not been clearly established.

We propose that perturbations in lipid biology may contribute towards this mitochondrial dysfunction. This hypothesis is suggested, in part, by epidemiologic evidence showing that lower levels of cholesterol and fatty acids increase the risk of Parkinson's Disease (PD) and that high dietary intake of omega-3 polyunsaturated FAs (n-3 PUFAs) decreased PD risk. Furthermore, the phospholipid and glycerophospholipid content of mitochondria are instrumental in maintaining mitochondrial integrity and function. At the experimental level, this lipid hypothesis is supported by our recent study where we show that Parkin stabilizes fatty acid transport protein levels to facilitate fat uptake, and that this diminished fat uptake in Parkin knockout mice result in systemic effects including resistance to obesity, hepatosteatosis and insulin resistance.

Taken together, we propose that an intriguing concept, linking Parkin mutations with perturbed lipid biology and impaired mitochondrial integrity in the development of EOPD, is emerging.
Hypothesis: Disruption in lipid handling by parkin mutations predisposes to paradoxical systemic and neuronal disease risk. These include improved systemic insulin sensitivity with reduced adipose mass in parallel with, perturbed neuronal mitochondrial membrane lipid composition resulting in increased susceptibility to mitochondrial and neuronal dysfunction.

1. To test this hypothesis we will pursue the following specific aims:
   Delineation of the systemic metabolic profile of Parkin mutant subjects versus controls.
2. Characterize whether parkin mutation-mediated impaired fat uptake disrupts mitochondrial membrane phospholipid content and mitochondrial function.
3. Determine the biochemistry of fat uptake and the fidelity of mitochondrial function and its genome (mtDNA) in iPS cell-derived dopaminergic neurons from EOPD subjects harboring parkin mutations compared to age-, gender- and body-mass indexed matched controls.

PI: **Charles Venditti**

Project Title: **Metabolic Phenotyping in Methylmalonic Acidemia: Markers and Drug Response**

Abstract:
Methylmalonic acidemia (MMA) is a heterogeneous inborn error in metabolism regarded as one of the most common inborn errors of organic acid metabolism. Isolated MMA is caused by complete or partial deficiency of the enzyme methylmalonyl-CoA mutase (mut0 or mut- type, respectively), or a defect in the synthesis of its cofactor, adenosylcobalamin (cblA, cblB, or cblD-variant 2 type). MMA presents along a clinical spectrum, typically with onset in the neonatal period and can feature: hyperammonemia, vomiting, hypotonia, hypothermia, respiratory distress, severe ketoacidosis, pancytopenia, developmental delay, and failure to thrive. Surviving patients are prone to severe metabolic decompensations and can develop secondary complications including neurocognitive impairment, progressive renal failure, metabolic stroke, growth failure, functional immune impairment and optic nerve atrophy. Therapeutic approaches are limited in vitamin B12 non-responsive patients and mainly include dietary restriction of propiogenic precursors (valine, isoleucine, methionine, and threonine), carnitine supplementation, and vigilant inpatient monitoring during periods of infection.

Among the most severe complications seen in the initial presentation and with recurrence of metabolic imbalance in patients with MMA is hyperammonemia. In many affecteds, this can be severe and require hemodialysis. While the long-term effects of hyperammonemia in MMA are not fully understood, it is likely that brain injury occurs when MMA patients experience hyperammonemia. In MMA and the related disorder, propionic acidemia (PA), the propensity to develop hyperammonemia stems from inhibition of N-acetylglutamate synthase (NAGS) by propionyl-CoA accumulation in the mitochondria. While other mechanisms may operate to predispose patients with MMA and PA to hyperammonemia, maximizing the function of the urea cycle to aid nitrogen disposal regardless of the underlying mechanism is critical.

A promising small molecule therapy that increases hepatic ureagenesis has been described: N-carbamyl-L-glutamate (NCG). Oral administration of this structural analogue of N-acetylglutamate has been demonstrated to be virtually curative of the urea cycle defect N-acetylglutamate synthase (NAGS) deficiency. A small number of reports have described the use of NCG in cases of PA and MMA to help control ammonia levels in the acute setting. More recently, Dr Mendel Tuchman's research group (Children's National Medical Center) has developed an in vivo tracer method that reliably predicts the effect of NCG on the rate of ureagenesis and demonstrated that NCG is effective in improving ureagenesis and reducing nitrogen load in 7 patients with propionic acidemia. This research has led to FDA to issue an IND (#68,185) to Dr Tuchman for the use of NCG (Carbaglu, Orhpan Europe) in the study of PA and MMA. Whether NCG would benefit some patients more than others seems likely and hence there is a practical need to easily determine which patients might respond and to what extent.

This project aims to: 1) develop optimal stable isotope methods for measuring oxidative capacity using 13C isotopomers of propionate, branched chain amino acids, and glycine in mice and patients with MMA compared to controls; 2) to jointly investigate with Dr Mendel Tuchman's research group (Children's National Medical Center) a subset of patients with MMA who are candidates to receive NCG to help manage their underlying disease.

PI: Joshua Zimmerburg

Project Title: Developing and validating membrane biomarkers in the muscular dystrophies

Abstract:
Limb-Girdle Musculodystrophy, Type II B (LGMD2B) is a rare, debilitating, recessive disorder characterized by progressive loss of muscle without any FDA-approved therapy. The genetic disorder consists in mutations or loss of the protein dysferlin; such diseases include Myoshi myopathy and are collectively termed dysferlinopathies. At the heart of LGMD2B is impaired sarcolemma repair and subsequent muscle fiber death, ultimately without regeneration. Dysferlin is hypothesized to mediate the rapid repair of the sarcolemma after muscle damage. To treat patients in this situation, we would like to fortify the natural tendency of membranes to reseal after damage, and minimize the inflammation that impedes regeneration and magnifies tissue destruction. Our "bench" biophysics teaches us that membrane resistance to damage will be a function of the lipid composition of the sarcolemma. Since a significant part of our membrane lipids comes from diet, it is reasonable to try and bypass the requirement for dysferlin by changing the diet such as to alter the lipid composition of the sarcolemma. However, to rigorously test the effect of such therapy, we need to develop methods to quantify in patients membrane damage and its relationship to disease progression.

The aims of this study are:

1) To develop an index based on a change in a specific biomarker with exercise. It should be feasible to measure this biomarker at high enough frequency to follow its time course, and the exercise should be repeated enough times in each patient to provide reproducible data that can be indexed to muscle strength.

2) Once the index described in aim (1) is achieved, to administer dietary supplementation and measure this index periodically during therapy and during withdrawal of therapy, to follow patient response to therapeutic intervention.

3) To vary the level of supplementation to determine a dose-response curve for determining the proper therapeutic dosage for future, larger scale studies.

To determine therapeutic efficacy for LGMD2B and other muscular dystrophies characterized by membrane damage, we will develop a reproducible measure of muscle membrane damage. Our hypothesis that the there is an index based either on the alteration in serum CK levels with exertion, or alterations in diffusion tensor MRI, that is a clinical correlate to the efficacy of therapy. For example, it is well established that CK is temporarily elevated after exercise, and that enzyme levels correlate with the severity of cardiac infarction. While these patients are characterized by high CK, random sampling is very variable, because of muscle mass, severity of disease progression, and renal function. CHANGES in serum CK with exercise (e.g. before and after) may be a more robust measure of sarcolemma membrane damage. For this we propose to perform frequent draws for CK determinations, determined by the individual patients CK clearance. We will also investigate metabolites, enzymes, and peptides in serum and urine, as well as a variety of novel quantitative MRI techniques. This approach will directly correlate potential biomarkers with therapeutic efficacy evaluated by muscle performance testing, appearance of edema, and patient directed questionnaires. Our initial "bench" work will include testing the hypothesis (based on our and others preclinical studies on rodents) that certain fatty acids can reduce muscle damage. We would then identify and validate additional MFA in primary cell culture and identify and

validate potential sarcolemma injury biomarkers based upon the published proteomic screen for dysferlin protein binding partners.

Dr. Brown follows a large cohort of LGMD muscular dystrophy patients; he will serve as the referring physician although we will also accept other unsolicited referrals. Dr. Bonnemann will coordinate patient testing at the NIH Clinical Center and serve as admitting physician. Brown and Bonnemann will be responsible for the safety and design of the exercise protocols, and the rigor of the endpoint assessments for muscle strength and disease progression. Dr. Bloch, the developer of an eccentric wounding protocol in mouse will correlate CK-MB and other potential biomarkers with injury and dietary supplementation. Dr. Basser, an inventor of diffusion tensor MRI, will coordinate correlative studies of muscle edema following exercise in both patient and animal models with and without dietary supplementation, as well as design and test novel methods to follow muscle damage. Drs. Blank, Humphrey and Zimmerberg will design, evaluate, and verify MFA activity on membrane damage in cultured tissues.