

Discovery of Inhibitors of NSD1 Fusion Proteins as Targeted Therapies for Pediatric Acute Leukemia

Mentor: Andrew Napper, PhD

Background. Gene rearrangements leading to NUP98-NSD1 fusion proteins give rise to aggressive cases of childhood acute myeloid leukemia (AML) by activation of expression of *HOX* oncogenes, which block normal blood cell differentiation. These rearrangements occur in 4-5% of cases of pediatric AML. The prognosis is very poor; event-free survival is less than one year.

Aim. High-throughput screening (HTS) will be used to discover compounds that inhibit the enzymatic activity of NSD1. NSD1 is a histone methyltransferase that specifically methylates lysine 36 on histone 3 (H3K36). The methyltransferase activity of NSD1 is retained in the NUP98-NSD1 fusions, and H3K36 methylation has been linked to *HOX* gene expression and leukemia progression. In future work, selective NSD1 inhibitors will be tested for suppression of proliferation of myeloid progenitor cells transfected with NUP98-NSD1.

Hypothesis. Inhibitors of the methyltransferase activity of NSD1 discovered by HTS will suppress the proliferation of myeloid progenitor cells harboring the NUP98-NSD1 fusion.

Methodology. A miniaturized assay to detect the methyltransferase activity of NSD1 will be validated for HTS, and the Nemours library of 200,000 compounds will be screened for NSD1 inhibitors. Activity of "hits" from HTS will be confirmed by retesting in dose-response to confirm activity and determine IC₅₀ values. The selectivity of confirmed active HTS hits for NSD1 will be determined by profiling against several related histone methyltransferases.

The Natural History of Degenerative Lumbar Disc Disease in Adolescence

Mentor: Joseph Piatt, MD

Background. Lumbar disc disease requiring surgical treatment is much less common in adolescence than in adulthood, but thanks to its origins as an orthopedic institute, the A I duPont Hospital for Children [AIDHC] has a substantial institutional experience with this condition. In addition to pain and potential neurological impairment, patients with this condition and their parents are burdened by fear of the prospect of a lifetime of degenerative spinal disability, but the long-term prognosis is not actually known.

Hypothesis. Patients treated surgically at AIDHC for lumbar disc disease have more lumbar spinal surgery as adults than the general population.

Aim. To obtain long-term follow up of patients treated surgically at AIDHC for lumbar disc disease; to document the incidence of surgical lumbar disc disease in the adult population; and to perform a statistical comparison of the subsequent surgical experiences of former AIDHC patients with the surgical experience of the general adult population.

Methodology. We shall query the AIDHC data warehouse for a list of all patients subjected to lumbar laminectomy for degenerative intervertebral disc disease. We shall trace these patients, all of whom will be adults now, and we shall determine whether they have required additional lumbar spine surgery. We shall employ the National Inpatient Survey and US Census Bureau data to establish the incidence of lumbar disc surgery in life-table format from 21 years of age forward. We shall cast our clinical follow up data in life-table format and use appropriate statistical techniques to test the study hypothesis.

Efferent Auditory Function in Children with Attention-Deficits/Hyperactivity Disorder

Mentor: Kyoko Nagao, PhD

Background. The number of school-aged children who are diagnosed with Attention-Deficits/Hyperactivity Disorder (ADHD) continues to rise in the last decade in the US and so has the number of medicated children. Compared to psychological studies, little attention has been paid on the auditory function in ADHD. Previous behavioral studies suggest that ADHD affects the abilities to perceive and process sounds in noisy environments. Some children with ADHD tend to be sensitive to loud sounds. Although participants in the previous studies had audiometrically normal hearing, audiometric hearing test does not eliminate a potential abnormality in the peripheral components of the auditory function.

Aim. This study aims to investigate the peripheral auditory function and its modulation by the efferent systems in children who present with a diagnosis of ADHD.

Hypothesis. We hypothesize that some children with ADHD present with an impaired efferent auditory function which plays a role in improving a listener's ability to detect signals in noise.

Methodology. We will test this hypothesis by measuring the middle ear muscle reflex (MEMR) and the otoacoustic emissions (OAE) and their suppression, which are main efferent reflexes controlling the auditory periphery. Ten children (7-12 years) with ADHD will be included in this study. We will examine the characteristics of the peripheral auditory system (MEMRs and OAEs) in children with ADHD by comparing the previously collected data from typically developing children. The results will lead us potential alternative objective measures to assess ADHD.

Practicing Safety to Enhance Parenting, Child Development and Support of Family Unity

Please note: Unlike other projects, this project will take place at Thomas Jefferson University Hospital in Philadelphia, PA.

Mentor: Diane Abatemarco, PhD

Background: Parents recovering from drug addiction may lack the benefits and supports of traditional extended families and cohesive communities, and are often isolated in their parenting. These vulnerable new parents receive little education and support to deal with the added stress of a newborn or their very young children. The Maternal Addiction Treatment Education and Research (MATER) center at Thomas Jefferson University provides comprehensive treatment services for substance-abusing pregnant and parenting women and their children in outpatient (Family Center) and residential (My Sister's Place) settings.

Hypotheses. 1) Substance-using women who receive the intervention will show an increase in knowledge, attitudes, and behavioral intention toward their children, and 2) Women attending the 12-week Mindfulness Based Parenting (MBP) course will report less stress and a greater ability to positively parent their young children. MBP and Practicing Safety, a set of parenting education and evaluation modules, will be supported by enhanced case management linking the pediatric clinic, Family Center and community services for care management.

Aim. We propose an integrated intervention to improve parenting in a population of pregnant and parenting women with substance use disorders.

Methodology. The intervention consists of Practicing Safety, strengthening the 'Family Medical Home', coupled with MBP to enhance parental resilience in the face of stress.

Characterization of the Interaction between SMN and FUS: Potential Interplay amongst Motor Neuron Diseases

Mentor: Matthew E. R. Butchbach, PhD

Background. Spinal muscular atrophy (SMA) is a leading genetic cause of infant death in the world. SMA primarily targets motor neurons. SMA results from reduced levels of the ubiquitously expressed survival motor neurons (SMN) protein. How can a protein expressed in all cell types selectively affect motor neurons when its expression is reduced? One explanation is that SMN has a unique function in motor neurons that is essential for the normal functioning of these cells. Recently, SMN expression and localization has been shown to be altered in cells from patients with amyotrophic lateral sclerosis (ALS), another motor neuron disease. Specifically, ALS cells harboring mutant FUS protein exhibit mislocalization of SMN within their nuclei.

Hypothesis. We hypothesize that SMN and FUS do physically interact and this interaction may be essential for normal functioning of motor neurons.

Aim. This study will provide novel insights into the biology of SMN and FUS in motor neurons and may also identify a potential link between two different motor neuron diseases, SMA and ALS.

Methodology. In this study, we will examine the interaction between SMN and FUS using in vitro binding assays, co-immunoprecipitation and immunofluorescence colocalization.

Expression of Novel Genes Controlling Steroid Production

Mentor: Kamin Johnson, PhD

Background. The interest of our lab is the etiology of congenital reproductive system defects which occur in over 2% of all male newborns. Steroid hormones control a variety of physiological processes including stress responses, blood pressure, pregnancy, and fetal organ development. Fetal testis-derived steroids (androgen) direct fetal male reproductive development, and we have identified a number of candidate genes that may regulate fetal testis androgen production. In the testis, the cell type producing steroids is termed the Leydig cell, and Leydig cell steroid production is induced by the gonadotropin leutinizing hormone.

Hypothesis. We hypothesize that 1) the expression of candidate steroidogenic genes correlates with Leydig cell differentiation during fetal testis development and 2) candidate steroidogenic gene expression is regulated by leutinizing hormone.

Aim. These two hypotheses will be tested by quantifying the mRNA levels of candidate genes and the levels of their protein products during fetal mouse testis gonad development as well as in cultured Leydig cell lines. If our hypotheses are true, fetal testis candidate gene expression will increase coincident with steroid production and will be induced by leutinizing hormone.

Methodology. An ontology series of mouse male and female fetal gonads will be microdissected for analysis. Tissue will be collected before and after differentiation of fetal Leydig cells. In addition, fetal mouse testes and a Leydig cell line will be cultured with or without stimulation by leutinizing hormone. From these samples, mRNA levels of candidate genes will be assayed using genome-wide expression microarrays or quantitative reverse transcription polymerase chain reaction (qRT-PCR). Protein products of candidate genes will be quantified by Western blot and immunofluorescent staining of cells and tissues.

The Use of a Portable Instrumented Postural Stability System (PIPSS) for Balance Testing in Post-Concussion Athletes

Mentor: Kathleen O'Brien, MD

Background. Sports-related concussion is a growing epidemic in youth and collegiate athletes. The risk of returning an athlete to sport prior to full recovery can have significant consequences. As such, return to play testing should be both sensitive and valid in determining readiness. The Balance Error Scoring System (BESS) is the most widely used clinical assessment of postural stability with post-concussion athletes, yet it lacks the quantitative aspect needed to detect subtle changes in stability and has only moderate reliability. The most accurate assessment of stability relies on center of pressure measures acquired from the NeuroCom Instrumentation system during a Sensory Organization Test (SOT). The high cost of this technology limits feasibility for clinicians and coaches. The Portable Instrumented Postural Stability System (PIPSS) is a new, low-cost assessment system that measures postural stability using mass-produced, inexpensive gaming technology having the potential to be readily available for field testing in physician offices and training rooms.

Hypothesis. PIPSS will have better sensitivity than BESS and good criterion validity with SOT.

Aim. To determine the validity of the PIPSS in evaluating postural stability, using the BESS as a comparison field test and the SOT as the gold standard.

Methodology. Prospective data will be collected from 60 children ranging from 12 to 17 years of age from two groups: children 1-4 weeks post-concussive episode and healthy individuals. Subjects will undergo PIPSS, BESS, and SOT testing.

The Effect of Injury Factors on Recovery from Pediatric Concussion

Mentor: Jane Crowley, PhD

Background. Traumatic brain injury (TBI) is a leading cause of morbidity and mortality in children. Concussion or mild traumatic brain injury comprises 80-90% of all TBI injuries in this age group. Interest in the detection and treatment of this group has exploded and as clinicians our role in effective management.

Hypothesis. There is a relationship between primary aspects of injury and the course of recovery from concussion. Aspects of injury used will be cause (e.g. a fall, sports, motor vehicle accident) and presence of amnesia.

Aim. The aim is the identification of an "at risk" group that requires alterations in conventional management to reduced morbidity. In clinical experience to date, there are indications of such factors, but no studies exist that examine such factors in a pediatric population.

Methodology. Method will be a retrospective analysis of 200 to 300 records of patients seen in the clinical course of patient care in the Concussion Clinic. Patients will be between the ages of 10 and 18. Statistical analysis will be logistic regression. There would be two analyses. One would use the relationship of symptom resolution (self-report and patient/parent ratings) as the recovery indicator investigating its relationship to characteristics of the patient and injury. The second analysis will use the scores on neuropsychological testing (ImPACT computerized cognitive test) as the dependent variable.

Quantifying Forces on Bone While Wearing a Cast

Mentor: Tariq Rahman, PhD

Background. The purpose of this project is to determine if a loose cast has a detrimental effect on bone healing after internal fixation subsequent to a supracondylar fracture in the upper arm. The cast may become loose as swelling subsides, and this may impart additional stresses on the bone that may interfere with healing.

Hypothesis. We surmise that the difference in the forces translates to a difference in outcome.

Aim. 1) To develop a reproducible method of evaluating the effect of casting on forces imparted to a joint while wearing a cast. 2) To develop an objective method of studying the effect of the “tightness” of a cast on the forces across an immobilized joint. 3) To determine if there is a difference in the forces imparted to the elbow joint when a person is wearing a loose long arm cast versus a well fitted cast.

Methodology. We will measure forces inside the cast as subjects actively move their elbow, first with a well fitting cast and then with a loose cast and compare the magnitude of the two forces over time. We will recruit ten subjects who will be fitted with the two types of casts and then will be asked to walk on a treadmill while we monitor forces in their arm casts. The forces sensor will be specially designed to measure elbow torques.

Role of Mcl-1 Protein in Leukemia Cell Survival and Chemoresistance

Mentor: Sonali Barwe, PhD

Background. Acute lymphoblastic leukemia (ALL) is the most frequent cancer in children and adolescents. Although recent advances in the treatment of childhood ALL have helped achieve cure rates of over 80%, the remaining 20% of patients who suffer from a relapse of the disease usually face a poor prognosis. Resistance to glucocorticoid induced apoptosis is one of the major risk factors for relapse and poor outcome in childhood ALL. The expression of Mcl-1, an anti-apoptotic protein belonging to the Bcl-2 family, is increased in glucocorticoid resistant ALL cells. Mcl-1 plays a crucial role in the survival of leukemia stem cells. Specific inhibitors against other members of the Bcl-2 family are currently in clinical trials for chronic leukemia.

Hypothesis. We hypothesize that reducing the levels of Mcl-1 protein, either by knockdown or by use of a specific inhibitor will impart glucocorticoid sensitivity and induce ALL cell death.

Aim. Test the role of Mcl-1 in ALL cell survival and glucocorticoid resistance.

Methodology. We will test the levels of Mcl-1 protein in ALL cell lines with differing glucocorticoid sensitivities. Survival and glucocorticoid sensitivity of ALL cells with or without specific knockdown of Mcl-1 protein will be compared. Finally, we will characterize the effect of inhibitors that disrupt Mcl-1 protein function on ALL cell survival in vitro and in vivo.