Pediatric Thalassemia Preclinical Landscape - DRAFT

Organization Name	Project Leader	Project Title	OniX Summary

BAYLOR COLLEGE OF MEDICINE	SHEEHAN, VIVIEN ANDREA	A Genomics Approach to Gamma-Globin Regulation	**Research Question:** How does FOXO3 regulate fetal hemoglobin (HbF) levels in patients with Sickle Cell Disease (SCD)?
			Stage: In vitro (using human primary erythroid culture) with some preliminary in silico analysis (WES data).
			Methods:
			• WES data analysis of 171 SCD patients to identify variants associated with HbF levels.
			• Functional studies in human primary erythroid culture to validate the role of FOXO3 in HbF regulation.
			• Planned future studies include:
			  ○ Analysis of WES data from a larger cohort (1000 patients) to confirm FOXO3-HbF association.
			  ○ Investigating the effect of FOXO3 knockdown on HbF expression (mimicking patient variants).
			  ○ Examining the role of FOXO3 pathway genes in HbF regulation.
			  ○ Identifying FOXO3 binding sites through ChIP-Seq.
			  ○ RNA-Seq analysis to assess the effect of FOXO3 on other erythroid genes.
			Drug Development: The ultimate goal is to develop novel HbF-inducing therapies for Hemoglobinopathies based on the understanding of FOXO3 and its pathway in HbF regulation.

JACKSON LABORATORY	PETERS, LUANNE L	Genetic Modifiers of Beta-like Globin Gene Switching	**Research Question:** How can we identify novel genes that regulate the switch from fetal hemoglobin (HbF) to adult hemoglobin in humans?
			Stage: In vivo with animals (mice)
			Methods:
			• **Utilizing two mouse models:**
			  ○ Nan mice with a mutation in KLF1 gene leading to abnormal HbF regulation.
			  ○ Diversity Outbred (DO) mice with high genetic variation in HbF expression.

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			• Performing Quantitative Trait Locus (QTL) mapping to identify genetic regions influencing HbF levels in both models. • ChIP-seq to compare DNA binding sites of normal and mutant KLF1 protein. • RNA-seq and phospho-proteome profiling to analyze gene expression and protein activity in erythroid cells. • Data integration to identify and prioritize candidate genes for further functional studies. **Drug Development:** This research aims to discover new genes involved in HbF regulation, potentially leading to novel therapeutic targets for Hemoglobinopathies like Sickle Cell Disease (SCD) and beta-thalassemia.
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE	KATO, GREGORY	Pulmonary Hypertension and the Hypoxic Response in SCD (PUSH)	This is not a single research project, but rather a summary of findings from a larger observational study (PUSH) on Sickle Cell Disease (SCD) in children and adolescents. **Stage:** In vivo with human subjects **Methods:** • Enrolled children and adolescents with SCD (Sickle Cell Anemia) and followed them over a period. • Collected clinical data, performed tests like echocardiography, and analyzed blood samples. • Analyzed data retrospectively to identify associations between various factors and disease severity. **Drug Development:** Not directly applicable here, this study focused on observing and understanding disease progression in SCD. **Key Findings:** • Elevated tricuspid regurgitant velocity (TRV) in Echocardiography is associated with a decline in exercise capacity. • Higher hemolytic activity and elevated left ventricular filling pressure (E/Etdi) predict increased TRV, which in turn predicts reduced exercise capacity.

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			• Several factors are associated with frequent severe vaso-occlusive pain episodes, including age, alpha-thalassemia trait, higher hemoglobin, lower lactate dehydrogenase, and higher TRV. • Genome-wide association study (GWAS) identified some genetic loci affecting fetal hemoglobin (HbF) levels, but a significant portion of the variation remains unexplained. • Patients with Chuvash polycythemia (increased hypoxia sensing) have elevated pulmonary artery pressure, and iron deficiency further worsens it. • Patients with SCD have increased osteoclast activity, potentially due to inflammation rather than iron overload. • There may be a link between bone complications and pulmonary complications in SCD.

ST. JUDE CHILDREN'S RESEARCH HOSPITAL	WARE, RUSSELL E	Genetic Modifiers in Children with Sickle Cell Anemia	**Research Question:**
			• How can genetic variations outside the globin genes predict the development of cerebrovascular and hepatobiliary disease in children with Sickle Cell Disease (SCA)?
			Stage: In silico (using DNA samples)
			Methods:
			• Analyze DNA samples from over 400 children with SCA who participated in past clinical trials (CSSCD, STOP) and an upcoming trial (BABY-HUG).
			• Look for specific polymorphisms (variations) in genes related to:
			  ○ Thrombosis (blood clotting)
			  ○ Brain injury repair
			  ○ Bilirubin metabolism
			  ○ Iron accumulation
			• Correlate identified polymorphisms with patient data like clinical events, lab results, and imaging studies.
			Drug Development: Not the direct focus, but the long-term goal is to identify genetic risk factors to develop targeted interventions for children with SCA.

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DUKE UNIVERSITY	WARE, RUSSELL E	Genetic Modifiers in Children with Sickle Cell Anemia	**Research Question:** Can genetic variations outside the globin genes predict the development of complications in children with Sickle Cell Disease (SCA)?
			Stage: In silico (using DNA samples)
			Methods:
			• Analyze DNA samples from over 400 children with SCA who participated in past clinical trials.
			• Look for specific polymorphisms (variations) in genes related to various pathways potentially affecting disease severity, including:
			  ○ Blood clotting (thrombosis)
			  ○ Brain injury repair
			  ○ Bilirubin metabolism
			  ○ Iron accumulation
			• Correlate identified polymorphisms with patient data like disease complications, lab results, and imaging studies.
			Drug Development: Not the main focus, but the long-term goal is to identify genetic risk factors to develop targeted interventions for children with SCA.

ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI	SUCHY, FREDERICK J	MOLECULAR PEDIATRICS AND CHILD HEALTH	**TBA**
CRADLE GENOMICS, INC.	ARMANT, DAVID RANDALL	A Platform for Safe, Noninvasive Prenatal Genetic Testing at Five Weeks of Pregnancy	**TBA**
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE	FITZHUGH, COURTNEY	Nonmyeloablative haploidentical peripheral blood stem cell transplantation in congenital anemias	**Research Question:** Can Alemtuzumab (anti-CD52 antibody) combined with total body irradiation (TBI) and post-transplant cyclophosphamide (PT-Cy) improve engraftment rates in high-risk patients with Sickle Cell Disease (SCD) and beta-thalassemia undergoing bone marrow transplantation (BMT)? **Stage:** In vivo with human subjects (clinical trial) **Methods:**

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			• Phase 1 & 2 clinical trial with escalating doses of PT-Cy after transplant. • Enrolled 23 patients with SCD and beta-thalassemia with severe complications. • Conditioning regimen included Alemtuzumab and TBI. • Evaluated engraftment rates, patient survival, and Graft-versus-Host Disease (GVHD) rates. **Drug Development:** Not directly applicable, this study focused on improving a bone marrow transplant protocol for SCD and beta-thalassemia. **Key Findings:** • Higher PT-Cy doses (100mg/kg) improved engraftment rates (83%) compared to lower doses (33% & 63%). • Overall survival rate was 60.9% with a median follow-up exceeding 8 years. • No mortality before 100 days post-transplant. • 50% of patients in the highest PT-Cy dose group remained disease-free. • No significant acute or chronic GVHD observed. **Future Directions:** • New protocol testing additional immunosuppression to further improve success rates. • Investigate clinical and genetic risk factors for post-transplant complications. • Identify biomarkers to assess graft tolerance and personalize immunosuppression. • Explore non-genotoxic conditioning regimens to reduce long-term side effects.
UNIVERSITY OF MARYLAND BALTIMORE	GLADWIN, MARK T	Sickle Cell Disease and Cardiovascular Risk-Red Cell Exchange SCD-CARRE	**Research Question:** Can regular automated exchange blood transfusions improve morbidity and mortality in high-risk adult patients with Sickle Cell Disease (SCD)? **Stage:** In vivo with human subjects (planned clinical trial)

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			Methods:
			• Compare the effects of monthly automated exchange blood transfusions to standard care in 150 high-risk adult SCD patients.
			• High-risk will be defined by elevated levels of two biomarkers:
			  ○ Tricuspid regurgitant jet velocity (TRV) measured by Doppler echocardiography (estimates pulmonary artery pressure).
			  ○ NT-proBNP levels measured by laboratory test.
			• Evaluate outcomes including:
			  ○ Disease progression
			  ○ Exercise capacity
			  ○ Frequency of vaso-occlusive pain crises and acute chest syndrome
			Drug Development: Not directly applicable, but the study aims to evaluate the effectiveness of a specific blood transfusion procedure.
			Background:
			• SCD patients develop progressive cardiopulmonary complications as they age.
			• Elevated TRV and NT-proBNP levels are strong predictors of mortality in SCD patients.
			• Exchange transfusions are effective for treating acute SCD complications but not routinely used for prevention.
			Rationale:
			• Regular exchange transfusions may slow disease progression, improve exercise tolerance, and reduce complications**.**

VANDERBILT UNIVERSITY MEDICAL CENTER	BICK, ALEXANDER	Clonal hematopoiesis and inherited genetic variation in sickle cell disease	**TBA**
ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI	BERNSTEIN, EMILY	Mechanisms and Modeling of Neuroblastoma-	**Research Question:** How do alterations in the ATRX protein contribute to neuroblastoma (NB) development, and can drugs targeting a specific enzyme (EZH2) be used for treatment?

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		Associated ATRX Alterations	**Stage:** In vitro and in vivo (using human cancer cells and potentially animal models) **Methods:** • Functional, epigenomic, and proteomic studies to investigate the role of ATRX alterations (specifically in-frame fusion proteins) in NB development (Aim 1). • Analyze gene expression patterns in NB cells with and without ATRX alterations, as well as the effects of EZH2 inhibition (EZH2i) on these patterns (Aim 2). **Drug Development:** EZH2 inhibition (EZH2i) is explored as a potential treatment strategy for NB with ATRX alterations. **Background:** • Mutations and alterations in the ATRX protein are found in various cancers, including NB. • The specific consequences of these ATRX alterations and their potential vulnerabilities are not fully understood. • Preliminary data suggests NB cells with ATRX in-frame fusion proteins have distinct gene expression and may be sensitive to EZH2 inhibition. **Future Directions:** • Identify how ATRX alterations promote NB development through functional studies. • Define the effects of EZH2i on gene expression and key targets in NB cells. • Provide evidence for EZH2i as a potential treatment approach for NB with specific ATRX alterations.
UNIVERSITY OF TENNESSEE HEALTH SCI CTR	ZAHR, RIMA	Genetic modifiers of Sickle Cell Kidney Disease	**Research Question:** • How do genetic variations influence the development and progression of chronic kidney disease (CKD) in patients with Sickle Cell Disease (SCD)? **Stage:** Not directly applicable (career development proposal) **Methods:**

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			• Not directly applicable (research plan outlining future directions) **Drug Development:** Not directly applicable, but the long-term goal is to develop methods to identify patients at high risk for CKD. **Background:** • African Americans have a higher risk of CKD compared to whites, and genetic factors are likely involved. • APOL1 gene variations are a major risk factor for CKD in SCD, but not the only one. **Future Directions (proposed research):** • Investigate the combined effects of multiple genes (including APOL1, -a3.7, HMOX1, and BCL11A) on CKD development and progression in SCD patients. • Analyze the influence of known CKD risk factors from the general population on CKD development in SCD. • Develop a multi-gene risk profile to identify patients at high risk for CKD. **Career Development Goals:** • Gain expertise in genetic epidemiology and genomic analysis methods. • Use a large patient cohort with existing genetic data to study CKD in SCD. • Establish herself as a leading researcher in the field of SCD-associated kidney disease.
UNIVERSITY OF MICHIGAN AT ANN ARBOR	CASTRO, MARIA G	Uncover the role of H3.3-G343R mutation in shaping the DNA damage response, anti-tumor immunity and mechanisms of resistance in glioma.	**Research Question:** How does the H3F3A G34R mutation affect response to treatment and the tumor microenvironment in pediatric high-grade gliomas (pHGG)? **Stage:** In vivo with animals and in vitro with human cells **Methods:** • Utilizing genetically engineered mice with H3F3A G34R mutation, alongside TP53 and ATRX knockdown, to model pHGG. • Comparing gene expression in these mice with and without the H3F3A mutation.

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			• Investigating the effects of H3F3A G34R on:
			  ○ DNA repair processes
			  ○ Response to DNA damaging agents (radiotherapy)
			  ○ Reprogramming of immune cells within the tumor microenvironment
			• Analyzing chromatin accessibility and its potential role in DNA repair.
			• Using single-cell RNA sequencing to identify different cell types within the tumor microenvironment.
			Drug Development: Not directly applicable, but the goal is to identify vulnerabilities in H3F3A G34R pHGG for improved therapies.
			Background:
			• pHGG is an aggressive brain tumor with poor prognosis.
			• H3F3A G34R mutation is a common subtype associated with poor outcomes.
			• Standard treatment (surgery, radiation, and chemotherapy) has limited effectiveness.
			Future Directions:
			• Understand how H3F3A G34R mutation affects DNA repair and immune response.
			• Investigate the role of chromatin accessibility in DNA repair for H3F3A G34R tumors.
			• Identify different cell populations within the tumor microenvironment.
			• Develop more effective treatment strategies for H3F3A G34R pHGG based on its unique vulnerabilities.

CASE WESTERN RESERVE UNIVERSITY	CAO, KAIXIANG	Exploring novel regulatory mechanisms underlying enhancer activation and cell fate transition	**Research Question:** How does the protein Mll4 regulate enhancer activity, chromatin structure, and gene expression in stem cell maintenance and differentiation? **Stage:** Not directly applicable (career development proposal) **Methods:** • Not directly applicable (research plan outlining future directions)

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			Drug Development: This is a basic research proposal, but the long-term goal is to develop therapies for diseases caused by malfunctioning epigenetic modifiers. **Key Findings (of previous work):** • Mll4 is a critical enzyme for depositing H3K4me1 (histone methylation) at enhancers, which are regulatory regions controlling gene expression. • Mll4 has both catalytic activity-dependent and independent functions in regulating enhancers and stem cell differentiation. • There might be competition between different epigenetic regulators in controlling gene expression. **Future Directions (proposed research):** • Investigate how H3K4me1 and other epigenetic marks influence enhancer function and cell fate decisions. • Explore the role of higher-order chromatin structures in stem cell biology. • Identify new factors and pathways involved in regulating enhancers and cell fate determination. **Career Development Goals:** • Gain expertise in biochemistry, proteomics, bioinformatics, stem cell biology, and genome-wide screening techniques. • Strengthen grant writing and leadership skills. • Transition to an independent research career focused on the epigenetics of development and disease.
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE	FITZHUGH, COURTNEY	Nonmyeloablative haploidentical peripheral blood stem cell transplantation in congenital anemias	**TBA**
UNIVERSITY OF PITTSBURGH AT PITTSBURGH	GLADWIN, MARK T	1/2 Sickle Cell Disease and CardiovAscular	**TBA**

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		Risk - Red cell Exchange Trial (SCD-CARRE Trial)	

UNIVERSITY OF MINNESOTA	YONG, JEONGSIK	Functional crosstalk between the Fanconi Anemia and ATRX/DAXX histone chaperone pathways	**Research Question:** How do ATRX and FANCD2 proteins cooperate in DNA repair mechanisms?
			Stage: In vitro (using human knockout cell lines)
			Methods:
			• Utilizing human cell lines with mutations in ATRX, FANCD2, or both.
			• Investigating the interaction between ATRX, FANCD2, and the MRN (MRE11-RAD50-NBS1) nuclease complex.
			• Analyzing the role of this complex in:
			  ○ Homologous recombination (HR)-dependent repair of stalled replication forks
			  ○ Repair of directly induced DNA double-strand breaks (DSBs)
			  ○ DNA interstrand crosslink (ICL) removal
			Drug Development: Not directly applicable, this research focuses on understanding fundamental DNA repair mechanisms.
			Key Findings (preliminary):
			• ATRX and FANCD2 form a complex with MRN nuclease.
			• This complex promotes HR-mediated repair of replication forks and DSBs.
			• Loss of ATRX or FANCD2 function increases ICL sensitivity, suggesting additional, independent roles for ATRX in ICL removal.
			Future Aims:
			• Understand the structure and composition of the ATRX-MRN-FANCD2 complex.
			• Elucidate the mechanisms by which this complex promotes replication fork recovery.
			• Distinguish between FANCD2-dependent and independent roles of ATRX in ICL repair.