

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2019

### 2019 Awardee

**Laura Calvi, M.D.**

University of Rochester

*Role of Myeloid Populations in Microenvironmental Regulation of Myelodysplastic Syndromes*

7/1/2019 - 6/30/2022

Previous data from our laboratories and others demonstrate a supportive role of the bone marrow microenvironment (BMME) in myelodysplastic syndromes (MDS). Using preclinical models and bone marrow cells from patients with MDS and controls, we have identified novel direct and indirect effects of marrow myeloid populations that modulate hematopoiesis. We find that myeloid cells are activated and defective with respect to phagocytosis and that, through their contribution to erythroblastic islands, they directly disrupt the process of erythropoiesis. In addition, since in MDS marrow professional phagocytic cells are defective, unexpectedly marrow mesenchymal stem and progenitor populations acquire phagocytic capacity that disrupts their ability to support hematopoietic stem cells. Based on these findings, the central hypothesis of this project is that impaired macrophages in myelodysplastic syndrome are novel direct and indirect mediators of defective microenvironmental support of erythropoiesis and hematopoietic stem cells. With the integrated approaches permitted by the current close collaborations already established between the Project PIs, using genetic and pharmacologic tools, clinically-relevant MDS murine models and human marrow samples from MDS patients and age-matched volunteers, we propose the following overall specific aims: (1) To define MDS-related changes in phagocytosis in marrow mesenchymal stem and progenitor cells and their dependence on macrophage defects and inflammation; (2) To determine defects in the myeloid components of the specialized erythroid BMME that direct progression of anemia in MDS.

The complex reciprocal interactions between BMME populations and MDS could only be defined and selected for translation efficiently through a team approach. This project brings together complementary expertise into a new multidisciplinary team to impact MDS care. While we have already identified Interleukin 1 signaling as a key targetable pathway that impacts these mechanisms, uncovering novel dynamic interactions of MDS with its BMME that contribute to disease progression will provide additional novel therapeutic strategies in MDS.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2019

### 2019 Awardee

**Jason Cheng, M.D., Ph.D.**

University of Chicago

*Dissecting RNA Cytosine Methyltranscriptomes for Effective Treatment of Myelodysplastic Syndrome (MDS)*

7/1/2019 - 6/30/2022

The broad, long-term objectives of this project are 1) to elucidate the role of RNA cytosine methyltranscriptomes in normal hematopoiesis and hematologic malignancies; 2) to dissect the RNA cytosine methyltranscriptome-mediated chromatin structures and drug response and resistance; and 3) to develop novel RNA cytosine methyltranscriptome-based tools to predict and/or overcome drug resistance. Drug resistance is a major obstacle to successful epigenetic therapy for myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), but the mechanisms governing the selectivity and resistance of epigenetic drugs remain elusive. We recently demonstrated that RNA cytosine methylation (RNA:m5C) and its methyltransferases (RCMTs) mediate the formation of unique active chromatin structures and methyltranscriptomes that regulate the response and resistance to azacitidine in MDS/AML cells. Based on our data, we propose the central hypothesis that RNA:m5C/RCMTs regulate chromatin structures and gene expression as well as the drug response/resistance in MDS. We will test our hypothesis by the three specific aims: 1) to define the interacting and functional domains of RCMTs that regulate the drug response/resistance in MDS/AML cells; 2) to identify new factors, including proteins, DNA and RNA, in the drug-resistant methyltranscriptomes/chromatin structures in MDS/AML; 3) to identify small molecule compounds that can disrupt the drug-resistant methyltranscriptomes/chromatin structures in MDS/AML. For aim 1, we will perform a series of RCMT domain-swapping and mutation/deletion experiments and the newly developed 5-ethynyluridine clicking chemistry and stimulated emission depletion confocal microscopy as described in our study. For aim 2, we will perform 1) immunoprecipitation mass spectrometry to identify new proteins; 2) RNA-CLIP-sequencing to identify the RCMT-associated RNA and DNA; and 3) high sensitivity mass spectrometry to precisely assess the levels and distribution of m5C in total RNA, tRNA, rRNA and mRNA. A time-resolved Förster resonance energy transfer assay will be used in high throughput screening in aim 3.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2019

### 2019 Awardee

**Jaroslav Maciejewski, M.D., Ph.D.**

The Cleveland Clinic

*Induction of Synthetic Lethality by DNA Dioxygenase Inhibition as a Targeted Therapy in MDS*

8/1/2019 - 7/31/2022

In this Taub Foundation research application we propose to develop a targeted therapy approach for TET2MT MDS involving aKG-based TET inhibitors (TETi). We will test the hypothesis that TET inhibition leads to synthetic lethality and may be a potential therapeutic principle for TET2MT MDS that will lead to development of a new class of dioxygenase-inhibiting agents. Our objectives are i) to generate and test TETi in cell-free, ex vivo and in in vivo pre-clinical models in both preventive and therapeutic modes; ii) to provide a preclinical proof of concept for development of drugs that affect TET function for rational application in TET2MT myeloid neoplasia, in particular MDS.

We plan to perform structure-guided refinement of TET inhibitors. A focused small molecule library modelled on the lead TET inhibitor to preferentially bind/inhibit TET DNA dioxygenase. These will be tested in TET-activity assays using liquid chromatography mass spectroscopy (LCMS) and DNA dot blot to detect hmC/mC levels as a readout.

We will establish the effects of TETi compounds in vitro human and murine models. Selected TETi will be tested for their efficacy in specific genetic context. Experiments will be performed with TET2 KD human cell lines, natural TET2MT cell lines (SIGM5, OCI-AML5, HEL and MEG01), genetically manipulated normal CD34+ cells, patient-derived CD34+ cells and murine cells derived from tet2+/- and tet2-/- mice.

We will also characterize TETi preventative and therapeutic efficacy as well as tolerability in pre-clinical murine models. We will evaluate TETi's abilities selected in SA2 to delay/prevent MDS/MPN evolution and to retard progression of transplanted MPN/MDS cells including those arising spontaneously in tet2-/- mice and those arising sooner in crosses with more aggressive phenotypes e.g., tet2+/-flt3, NUP98/HOX9A/tet2+/-.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2019

### 2019 Awardee

**Xiaochun Yu, M.D., Ph.D.**

Beckman Research Institute of City of Hope

*A Novel Therapeutic Strategy for MDS*

7/1/2019 - 6/30/2022

DNA methylation is a key epigenetic modification that regulates numerous chromatin activities such as gene transcription. However, aberrant DNA methylation is a hallmark of cancer development, such as myelodysplastic syndromes (MDS). Accumulated evidence has shown that hypermethylation at gene promoter region suppresses the expression of tumor suppressor genes, which in turn induces genomic instability and MDS.

DNA methylation is mainly catalyzed by DNA methyltransferases (DNMTs) that covalently link methyl group to the 5' position of cytosine in a CG dinucleotide context. It has been demonstrated that 5-aza-2'-deoxycytidine (DAC), a nucleoside analog, is a potent inhibitor to suppress the enzymatic activity of DNMTs, thus remarkably reduce the level of DNA methylation during DNA replication. The successful clinical trials allow DAC to be utilized for treating MDS patients. However, as a nucleoside analog, DAC has severe side-effects for MDS patients. Thus, it is important to identify alternative approach to target replication-dependent DNA methylation.

With the progress of understanding the molecular mechanism of DNMTs, it has been shown that activation of DNMT1 is regulated by a crucial ubiquitin E3 ligase UHRF1. UHRF1 contains multiple domains with the SRA domain specifically recognized hemimethylated DNA. It catalyzes histone ubiquitination which is recognized by the ubiquitin-binding motif of DNMT1. Thus, UHRF1 is required for mediating the recruitment of DNMT1 to the nascent daughter allele for methylation. In addition to DNMT1, UHRF1 could be an important target for modulating replication-dependent DNA methylation.

In our preliminary study, we have screened and identified a potent small molecule inhibitor that specifically targets the SRA domain of UHRF1. In this proposal, we plan to examine the molecular mechanism of UHRF1 in DNA methylation using this chemical probe. Moreover, we will examine the efficacy of this small molecule compound in MDS treatment.

### 2018 No Grants Cycle

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2019

### 2017 Awardee

**Iannis Aifantis, Ph.D.**

New York University School of Medicine

*Pharmacological Restoration of TET2 Function in MDS with High-Dose Vitamin C*

12/15/2017-12/14/2020

Vitamin C is an essential dietary requirement in humans, necessary for proper immune function and for healthy hair and skin. Vitamin C acts specifically as a cofactor of Fe<sup>2+</sup> and alpha-ketoglutarate (alpha-KG)-dependent dioxygenases, including Ten-Eleven-Translocation (TET1-3) proteins to maintain the full activity of these enzymes. TET family dioxygenases catalyze the hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), a key intermediate in the process of DNA demethylation. Vitamin C treatment has been shown to promote differentiation of embryonic stem cells and enhanced reprogramming of pluripotent stem cell formation in a TET-dependent manner by activating increased 5hmC levels and ultimately, DNA demethylation.

Recurrent somatic mutations in TET2, which lead to loss of 5hmC and DNA hypermethylation, are found in up to 30% of patients with myelodysplastic syndrome (MDS). Intriguingly, TET2 mutations are almost always heterozygous, raising the possibility that enhancing residual TET2 activity (encoded by the remaining wild-type TET2 allele) could represent a viable therapeutic strategy for the treatment of TET2-mutant MDS patients. Previous studies have shown that heterozygous deletion of the catalytic domain of Tet2 in mice leads to decreased 5hmC levels in HSCs, DNA hypermethylation, increased HSC self-renewal and the development of MDS, providing a powerful model system to test this concept.

We hypothesize that high dose vitamin C treatment will enhance the enzymatic activity of residual TET2, increase 5hmC levels and drive DNA demethylation in the MDS stem cell genome. Our preliminary data show that high-dose vitamin C blocks the aberrant self-renewal seen in Tet2-deficient mouse stem and progenitor cells. We will use mouse models of Tet2-deficiency and TET2 mutant patient-derived xenografts to study the effects of high-dose vitamin C treatment on MDS disease progression. Novel epigenetic therapies, such as high-dose vitamin C, could provide a safe and effective strategy to improve outcome in MDS patients with TET2 mutations.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2019

### 2017 Awardee

**Benjamin Ebert, M.D., Ph.D.**

Brigham and Women's Hospital

*Targeting TP53-mutant MDS*

3/15/2018-3/14/2021

TP53-mutant MDS represents a distinct class of patients with an extremely poor prognosis, resistance to therapy, an extremely poor prognosis. New therapeutic options are needed for these patients. Recent studies have established the potential for major progress in this challenging subgroup of MDS. Among MDS patients with TP53 mutations who underwent allogeneic stem cell transplantation for MDS, TP53-mutant cases approximately 20% of cases had long-term survival. While this is low, if therapies could be developed to keep TP53-mutant MDS clones in remission long enough for the patient to benefit from a graft-vs-MDS immunologic effect of allogeneic transplantation, there is a potential to increase the number of TP53-mutant MDS patients who achieve long-term survival. A second promising finding was that TP53-mutant myeloid malignancies can achieve significant molecular responses to decitabine. The most common mutations in the TP53 gene are recurrent hotspot mutations that may alter the function of p53, rather than simply inactivating the gene. In this proposal, we leverage the recently developed approach of CRISPR-Cas9 homology-directed repair to generate isogenic cell lines bearing the recurrent missense and loss-of-function TP53 mutations. We will use these isogenic cells to determine the transcriptional and epigenetic consequences of the recurrent missense mutations and loss of p53 function in MDS, to test candidate therapeutic agents, and to identify novel synthetic lethal interactions. Finally, we will develop murine models of TP53-mutant MDS to test candidate therapeutic approaches. These studies have the potential to lead rapidly to clinical trials in TP53-mutant MDS given the extraordinarily poor survival of these patients.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2017 Awardee

**Coleman Lindsley, M.D., Ph.D.**

Dana-Farber Cancer Institute

*Understanding distinct mechanisms of U2AF1-S34 and Q157 mutations in MDS pathogenesis.*

1/15/2018-1/14/2021

Mutations in genes encoding RNA splicing factors are the most common class of molecular alterations in myelodysplastic syndrome (MDS). Spliceosome mutations tend to occur early in MDS development, are associated with distinct phenotypes, and have different patterns of co-mutations. Although they all cause aberrant RNA splicing, the molecular consequences of spliceosome mutations are distinct and no unifying mechanism in MDS pathogenesis has been identified. Our preliminary analysis of 263 patients with U2AF1 mutations from 2 independent cohorts strongly suggest that U2AF1S34 and U2AF1Q157 mutations have different mechanistic roles in MDS pathogenesis. We observed key features of U2AF1 mutated MDS: (1) U2AF1S34 mutations were significantly associated with co-occurring BCOR mutations, while U2AF1Q157 mutations were significantly associated with ASXL1 mutations, (2) BCOR and ASXL1 mutations were significantly anti-correlated in the context of U2AF1S34 mutations, (3) BCOR mutations typically arise in progression subclones of U2AF1 mutated MDS, and (4) U2AF1Q157 and U2AF1S34 mutations have distinct clinical characteristics. Our working hypothesis is that BCOR mutations cooperate with targets of U2AF1S34 missplicing to cause MDS progression via dysregulation of BCOR-dependent histone epigenetic marks that mediate repressive chromatin states. To test this hypothesis, we propose the following Aims: 1) Define the contribution of Bcor deficiency to myeloid transformation in primary mouse U2AF1S34/+ knock-in hematopoietic cells. We will use CRISPR/Cas9 in primary mouse U2AF1S34/+ cells using in vitro and in vivo analyses to define the functional impact of Bcor mutations on U2af1S34 hematopoietic stem cell self-renewal and lineage-specific differentiation. 2) Determine the influence of U2AF1S34F versus U2AF1Q157R context on epigenetic impact of BCOR inactivation using RNA-seq and ChIP-seq. 3) Define locus-specific changes in BCOR, H2AK119ub, and macroH2A occupancy based on U2AF1 mutation context. Collectively, the information learned in these studies will provide new insights into the biology of MDS and lay the groundwork for developing novel therapies.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2017 Awardee

**Matthew Walter, M.D.**

Washington University School of Medicine in St. Louis

*The Role of U2AF1 Mutations in MDS Pathogenesis*

12/15/2017-12/14/2020

The long-term goal of this project is to understand how mutant U2AF1 contributes to myelodysplastic syndrome (MDS) development. Somatic heterozygous mutations in U2AF1, a spliceosome gene, occur in ~11% of MDS patients and are associated with altered pre-mRNA splicing. We reported that mice expressing mutant U2AF1(S34F) have altered pre-mRNA splicing and hematopoiesis, including leukopenia, apoptosis of maturing cells, and progenitor cell expansion -- all features of MDS. A second U2AF1 hotspot mutation codes for U2AF1(Q157P). The in vivo consequences of U2AF1(Q157P) expression on hematopoiesis and RNA splicing have not been studied. Only ~25-50% of splicing alterations induced by U2AF1(S34F) or U2AF1(Q157P) in cell lines are shared in common. We hypothesize that U2AF1-S34F and -Q157P mutant cells have distinct splicing targets that contribute to disease pathogenesis in vivo. We will prioritize mutation-specific targets identified in mutant mouse hematopoietic cells that are also alternatively spliced in MDS patient samples. Ultimately, modulating the expression levels of RNA isoforms that are induced by U2AF1 mutations could be a novel therapeutic approach to treat spliceosome mutant MDS.

Despite having a MDS-like phenotype, U2AF1(S34F) mutant mice do not develop full-blown MDS. We hypothesize that additional cooperating mutations are required for initiation of MDS in mutant U2AF1 expressing cells. ASXL1, an epigenetic modifier gene involved in chromatin remodeling, is the most significantly co-mutated gene in patients with U2AF1 mutations (e.g., up to 70% of patients with a U2AF1[Q157] mutation harbor an ASXL1 mutation). We will test the cooperation of U2AF1(Q157P) and Asxl1 mutations in vivo and determine if the order of ASXL1 and U2AF1 mutation acquisition is important for pathogenesis, as has been shown for other myeloid neoplasms. Identify how ASXL1 and U2AF1 gene mutations cooperate could nominate a novel mechanism contributing to MDS pathogenesis.



# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2016 Awardee

**Omar Abdel-Wahab, M.D.**

Memorial Sloan-Kettering Cancer Center

*Understanding and Manipulating Pathologic Splicing Preferences and Nonsense-mediated mRNA Decay (NMD) in Spliceosomal-mutant MDS*

1/15/2017-1/14/2020

Mutations in the spliceosomal proteins SF3B1, SRSF2, U2AF1, and ZRSR2 constitute the most common class of genetic alterations in patients with myelodysplastic syndromes (MDS). Since the discovery of these mutations, data from multiple groups have revealed that mutations in SRSF2 and U2AF1 alter the normal RNA binding and splicing preferences of these proteins in a sequence-specific manner. In addition, we and others have identified that cells bearing spliceosomal gene mutations are preferentially sensitive to further genetic or pharmacologic perturbations to RNA splicing, compared with spliceosomal wild-type (WT) cells. However, it is not yet clear whether global inhibition of splicing will be clinically viable. The frequency of spliceosomal gene mutations in MDS, combined with the specific manner by which these mutations alter RNA splicing, make efforts to therapeutically target spliceosomal mutant cells with greater specificity imperative. Here we aim to develop two novel mechanism-based approaches targeting spliceosomal-mutant MDS based on: (1) selectively targeting the pathologic RNA-binding preferences of mutant spliceosomal proteins; and (2) restoring the expression of key transcripts targeted for nonsense-mediated decay (NMD) due to aberrant splicing. Understanding the mechanistic link between NMD and spliceosomal gene mutations will be critical for this latter aim. We therefore propose the following Specific Aims:

Aim I. Determine the precise mechanism by which mutant SRSF2 promotes NMD.

Aim II. Utilize antisense techniques to restore expression of mRNAs pathologically degraded by NMD in spliceosomal-mutant MDS cells.

Aim III. Determine the therapeutic potential of oligonucleotide decoys that exploit the RNA-binding preferences of WT versus mutant SRSF2 in SRSF2-mutant malignant cells.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2016 Awardee

**Gang Huang, Ph.D.**

Cincinnati Children's Hospital Medical Center

*The Role of HIF-1 $\alpha$  As a Central Pathobiologic Mediator of Myelodysplastic Syndromes*

12/15/2016-12/14/2019

Myelodysplastic Syndromes (MDS) are characterized by multi-lineage dysplastic cytopenia. Every year, about 20,000 people are diagnosed with MDS in the United States. The leading causes of death are bleeding or infection resulting from refractory cytopenia. Generally, conventional chemotherapy is not effective for MDS. Transfusion dependence due to progressive anemia/thrombocytopenia also causes various complications. Further elucidation for the pathogenesis of MDS and establishment of novel therapeutic strategies are needed. To investigate the underlying mechanisms and preclinical targeting of MDS, we took an unbiased bioinformatic approach and found an elevated hypoxia inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) signature in the CD34+ cells from primary MDS patient samples of various genotypes. We further validated HIF-1 $\alpha$  protein (but not mRNA) upregulation with MDS patients' BM biopsies. We have also established novel MDS-like mouse genetic model. Most importantly, using these models, we validated the critical role of HIF-1 $\alpha$  in the disease development and MDS phenotypes. To molecularly dissect HIF-1 $\alpha$  mediated pathobiology, we generated hematopoietic cell-specific tetracycline-inducible HIF-1 $\alpha$  transgenic mice. Our preliminary data indicated that these mice developed MDS phenotypes which faithfully recapitulate a variety of human MDS phenotypes. Although MDS are heterogeneous diseases driven by a variety of mutations (genotypes), the key features of MDS (phenotypes) among patients are very similar. Thus, based on our preliminary data, we hypothesize that activated HIF-1 $\alpha$  signaling, driven by clonal hematopoietic and oncogenic mutations, is a central pathobiologic mediator of Myelodysplastic Syndromes. We propose to determine, 1) the mechanism of HIF-1 $\alpha$  activation by major MDS-associated mutations; 2) the essential and sufficient role of HIF-1 $\alpha$  in MDS with multiple mouse MDS models; 3) therapeutic potential of targeting HIF-1 $\alpha$  in human MDS xenograft models. Our study will not only provide stringent mechanistic tests of our hypotheses, but also lead to a better understanding the pathogenesis of MDS and to potential novel therapeutic targets.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2016 Awardee

**Daniel Link, M.D.**

Washington University in St. Louis  
*Stromal CXCL12 in MDS Pathogenesis*

12/15/2016-12/14/2019

There is evidence that alterations in the bone marrow microenvironment may contribute to the pathogenesis of myelodysplastic syndromes (MDS). CXCL12 expression in bone marrow stromal cells is increased in MDS. CXCL12 through interaction with its major receptor CXCR4 is a key regulator of hematopoiesis. It regulates neutrophil egress from the bone marrow and hematopoietic stem cell (HSC) quiescence and repopulating activity. Increased CXCR4 signaling in humans reproduces some features of MDS, including peripheral neutropenia despite an increased number of dysplastic neutrophils in the bone marrow. Increased CXCR4 signaling also decreases the competitive fitness of HSPCs. Our preliminary data suggest that pharmacologic or genetic attenuation of CXCR4 signaling in a murine model of MDS partially rescues some of the hematopoietic abnormalities. Based on these observations, we hypothesize that increased stromal expression of CXCL12 contributes to ineffective hematopoiesis and leukemic transformation in MDS.

Current evidence suggests that stromal cells are not part of the MDS malignant clone. It follows that alterations in the bone marrow microenvironment in MDS, including increased CXCL12 expression, must be secondary to changes in a hematopoietic cell population(s). Macrophages are a prime suspect. Bone marrow macrophages are a key determinant of stromal CXCL12 expression. They regulate erythropoiesis through formation of blood islands, and they support osteoblasts, thereby potentially affecting hematopoiesis through regulation of the osteoblastic niche. Based on these observations, we hypothesize that abnormal macrophage function in MDS contributes to the altered bone marrow microenvironment in MDS, including increased stromal CXCL12 expression. These studies may provide the foundation for a clinical trial of CXCR4 inhibitors in MDS. The following specific aims are proposed.

Aim 1. To characterize the contribution of increased stromal CXCL12 expression to ineffective hematopoiesis and leukemic transformation in MDS.

Aim 2. To characterize the contribution of macrophages to the altered bone marrow microenvironment in MDS.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2016 Awardee

**Ulrich Steidl, M.D., Ph.D.**

Albert Einstein College of Medicine

*Mechanisms of MDS Initiation at the Stem Cell Level*

12/15/2016-12/14/2019

Relapse and progressive disease remain the most common causes of death in MDS. The development of fundamentally novel therapeutic approaches is urgently desired. Recent evidence has indicated that initially formed pre-cancerous stem cells (pre-MDS-SC) precede the formation of fully transformed MDS stem cells (MDS-SC), and play a pivotal role in disease origination and progression. While the existence and essentiality of such pre-cancerous cell states has been demonstrated in mice and humans, almost nothing is known about the molecular mechanisms driving pre-MDS-SC formation and progression. We have recently reported a new mouse model of induction of pre-cancerous stem cells and progression to MDS, molecularly driven by heterozygous PU.1 enhancer deletion (PU.1 function is frequently disrupted in human MDS) combined with Msh2 deficiency, leading to an increased frequency of mutation types occurring during aging and in MDS patients. This model is hallmarked by definable pre-cancerous stem cells and closely resembles human disease in many phenotypic and molecular features, and now permits for the first time the longitudinal identification and molecular study of mechanisms driving the formation and progression of pre-cancerous stem cells in MDS. Such pre-MDS-SC in this model are characterized by key functional alterations, including a myeloid bias of early 'uncommitted' stem cells, a numerical expansion of phenotypic "myeloid biased" stem cells, and reduced quiescence. We propose to: 1. Identify and study mechanisms/pathways which are critical for the formation and the functional alterations of pre-MDS-SC; and 2. Delineate molecular determinants of the progression of pre-cancerous pre-MDS-SC to overt MDS. The research plan will employ our novel pre-MDS-SC to MDS progression model and longitudinal analysis of transcriptional and genetic dysregulation at the stem cell level. Targets will be identified and tested for functional relevance in vitro and in vivo, and validated on sorted stem cells from patients with MDS.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2015 Awardee

**Lucy Godley, M.D., Ph.D.**

University of Chicago

*Identification Of Germline Predisposition Syndromes In Young MDS Patients*

11/1/2015-10/31/2018

Familial cases of adult myelodysplastic syndrome (MDS) are likely to be more common than currently appreciated. Because MDS is diagnosed at 71 years old on average, we hypothesize that those patients who present at much younger ages are an ideal population in which to identify individuals with germline predisposition. Therefore, this study will determine the frequency of mutations in predisposition genes (e.g., RUNX1, GATA2, CEBPA, ANKRD26, SRP72, TP53, ETV6, DDX41, and genes that comprise the inherited bone marrow failure syndromes) in young patients with MDS (Specific Aim 1). This study will take advantage of a cohort of 100 MDS patients diagnosed at less than 40 years of age assembled at The University of Chicago and its affiliates. We also seek to define new familial syndromes using our collection of more than 300 families, each of which contains at least two cases of hematopoietic malignancies within two generations, and test remaining young MDS patients for these syndromes (Specific Aim 2). We anticipate that our results will demonstrate that many young patients with MDS have a genetic predisposition to their disease, a finding that will prompt the examination of germline susceptibility as a routine step in the management of these patients. The recognition of the germline nature of these patients' disease is critical for proper clinical management. Because allogeneic stem cell transplant is the only curative treatment for MDS, and because young patients are candidates for allogeneic stem cell transplantation, determining those patients with a germline predisposition is critical to allow the selection of an appropriate allogeneic stem cell donor: either an unaffected HLA-matched sibling or an unrelated HLA-matched donor. Furthermore, the identification of genes that confer germline predisposition highlight critical cellular pathways that lead to MDS when disrupted and focus new research into areas previously unknown to contribute to MDS.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2015 Awardee

**Ross Levine, M.D.**

Memorial Sloan-Kettering Cancer Center

*Role of Cohesin Complex in Myelodysplastic Syndrome*

11/1/2015-10/31/2018

Recent studies have identified loss-of-function mutations in the cohesin complex in a spectrum of malignancies, including most commonly in high risk MDS. Cohesin is a multi-protein complex which regulates sister chromatid alignment during mitosis; leading to the suggestion that cohesin mutations lead to chromosomal instability. However, in MDS, cohesin mutations are not associated with aneuploidy or complex cytogenetics, suggesting an alternate mechanism of transformation. To elucidate the pathophysiologic mechanism underlying cohesin mutations in MDS we have developed novel cohesin conditional mouse models. Our initial studies have shown that haploinsufficiency for Smc3 leads to increased stem cell self-renewal, and can cooperate with known leukemia disease alleles to induce myeloid transformation. Cohesin has been identified to have a role in regulating DNA loop formation, gene expression, and pluripotency. Young and colleagues discovered that a subset of transcriptional enhancers, which they denote as super-enhancers (SEs), is highly occupied by nuclear co-activator complexes including cohesin, mediator, and BRD4. This chromatin state is most common at genes that govern cell identity and differentiation status. In normal cells, cohesin maintains cell-type specific gene expression programs by facilitating chromatin looping of promoters and distal enhancers. Our preliminary data suggests that cohesin haploinsufficiency may promote aberrant chromatin architectures that promote tumorigenesis through the loss of innate nuclear topology, resulting in loss of cellular identity and in impaired hematopoietic differentiation. These data suggest that cohesin mutations reversibly alter the transcriptional landscape in MDS, which can be therapeutically targeted. Our proposal aims to use well characterized, genetically accurate models of MDS and patient samples to elucidate the role of cohesin alterations in MDS and to identify specific therapeutic targets in cohesin mutant MDS.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2015 Awardee

**Eirini Papapetrou, M.D., Ph.D.**

Icahn School of Medicine at Mount Sinai

*A Novel Genotype-to-Phenotype Platform to Study MDS Pathogenesis and Treatment*

11/1/2015-10/31/2018

With near-complete catalogues of the genetic alterations that drive MDS now available, the ushering of a new era of precision medicine in MDS care is within reach. Nearly all MDS patients have at least one genetic abnormality (mutation or chromosomal deletion) and genomics analyses are rapidly becoming routine. The next big challenge lies in the translation of this information into biomarkers to predict response to current disease-modifying therapies and into new therapies targeting specific genetic alterations. However, many of the recurrently mutated genes are not leading directly to obvious targets. A most promising avenue to uncover genotype-specific drug susceptibilities is the identification of common pathways and cellular processes in which different mutations converge. To this end we will develop MDS models based on patient-derived and genetically engineered induced pluripotent stem cells (iPSCs). This platform allows us for the first time to model each of a number of genetic abnormalities commonly found in MDS in a pure population of human cells in a controlled and defined isogenic setting and draw associations between genotypes and cellular phenotypes, transcriptome and methylome profiles of hematopoietic cells derived in vitro. We will then use these iPSC lines and this information to identify common drug susceptibilities and for in silico drug discovery approaches. This work will aid our ability to stratify MDS patients into genetically described subgroups towards more rational treatments. It can generate new hypotheses for follow up studies to discern the disease mechanisms and for clinical translation and potentially new therapeutic targets for drug development. This work is aligned with the mission of the Taub Foundation as it is high-impact, innovative translational research to understand the underlying causes of MDS and to advance its treatment and prevention.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2015 Awardee

**Joseph Scandura, M.D., Ph.D.**

Weill Medical College of Cornell University

*Curing MDS Using Hematopoietic Cells Reprogrammed From Autologous, Normal Endothelial Cells*

2/1/2016-1/31/2019

Currently, MDS can only be cured by transplanting normal hematopoietic stem cells (HSCs). The problem is that transplanting HSCs from other people (allogeneic) is very toxic and not always possible. Re-transplanting a recipient's own HSCs (autologous) just returns MDS. However, autologous transplants could treat and possibly cure patients with MDS if normal, autologous HSCs could be produced from these patients. The chance for cure would increase if the transplanted HSCs had specialized functions that allowed them to outcompete residual MDS cells. We've recently found that normal human endothelial cells (ECs) can be efficiently reprogrammed into hematopoietic stem and progenitor cells (HSPCs) that are potentially suitable for autologous transplantation. This is important because ECs and HSPCs reprogrammed from ECs (rEC-HSPCs) do not harbor the genetic/epigenetic lesion causing MDS. Our long-term goal is to use autologous, rEC-HSPCs to cure MDS. Our objective here is to provide an essential proof-of-concept demonstrating that rEC-HSPCs with customized functions can be manufactured and used for HSCT. The rationale for the proposed studies is that to get to MDS therapies we must define the optimal timing of EC reprogramming, identify a feasible tissue source for donor ECs and show that customized, transplantable rEC-HSPCs can be manufactured. Our objective will be met by completion of the following specific aims: Aim 1 - Define and optimize the kinetics with which engraftable human rEC-HSPCs emerge during cellular reprogramming of ECs; Aim 2 - Identify the extent to which the EC tissue of origin alters the reprogramming efficiency and long-term engraftment potential; Aim 3 - Design and manufacture human rEC-HSPCs with customized function that can reconstitute hematopoiesis in immune-deficient mice (NSG). The proposed studies provide critical steps towards translating this research into new, potentially superior, cellular therapies for MDS.



# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2014 Awardee

**Janis Abkowitz, M.D.**

University of Washington School of Medicine

*The Pathogenesis of Anemia in MDS*

12/1/2014-11/30/2017

Myelodysplastic syndrome (MDS) is characterized by ineffective hematopoiesis resulting in peripheral blood cytopenias. Anemia, and especially macrocytic anemia, is a prominent manifestation. At the time of diagnosis, over 80% patients with MDS are anemic, and 50% have a hemoglobin level < 10 g/dL.

Although this results from the apoptosis of early erythroid precursors, its pathophysiology is not known. The goal of this application is to characterize and discern the reason for erythroid marrow failure in MDS. Specifically, we will test the hypothesis that ineffective erythropoiesis occurs when heme exceeds globin. Intracellular free heme is toxic, leading to increased reactive oxygen species (ROS) and cell death. An in vitro culture system has been optimized that phenocopies in vivo differentiation, including the uptake and use of iron and the signaling and onsets of heme and globin synthesis as erythroid cells mature. In initial studies of marrow cells from two patients with MDS resulting from the deletion of chromosome 5q (characterized by ribosomal protein S14 (RPS14) haploinsufficiency) and three patients with Diamond Blackfan anemia (an inherited anemia characterized by the haploinsufficiency of RPS19 and other ribosomal proteins), we have shown that heme is in excess in early erythroid (CD36neg/GlyAneg) cells and apoptosis occurs. As only those cells that epigenetically down-regulate heme synthesis (have less ALAS2 by RT PCR) or upregulate heme export (have increased FLVCR) survive, we established that heme toxicity induces the erythroid marrow failure. Here we propose to test the relevance of this hypothesis to other MDS subtypes using marrow cells from early and intermediate grade MDS patients with anemia. These studies should provide novel insights into heme-globin coordination in MDS. Also, our hypothesis, if confirmed, would suggest that slowing heme synthesis (e.g., by aggressive iron chelation) or facilitating heme export could help ameliorate anemia in early stage MDS.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2014 Awardee

**Alan List, M.D.**

H. Lee Moffitt Cancer Center

*Targeting MDS Stem Cell with a Novel TLR Selective Therapeutic Conjugate*

1/15/2015-1/14/2018

Myelodysplastic syndrome (MDS) is an incurable hematopoietic malignant disease. Lenalidomide (LEN) has emerged as the only targeted therapeutic approved for the treatment of a small subpopulation of patients with chromosome 5q deletion (del5q). However, the majority of non-del(5q) patients do not benefit from LEN. We reported that LEN acts by inhibiting the activity of two haplodeficient phosphatases, PP2Ac<sup>#945</sup>; and Cdc25C, encoded within the 5q region. They account for the disease's karyotype selective specificity. Small interfering RNA suppression of these genes' expression recapitulates del(5q) susceptibility to LEN with induction of apoptosis in non-del(5q) MDS cells. In combination of our novel finding that MDS progenitors overexpress TLR9, which bind and internalize its native ligand unmethylated CpG oligonucleotides, we developed an innovative disease-specific targeting approach using a single molecule containing: CpG, siRNAs to Cdc25C-PP2A and LEN, which renders non-del5q progenitors sensitive to clonal suppression by the drug. In the first aim, we will exploit the novel concept of applied synthetic lethality in non-del5q progenitors using this TLR9 targeted siRNA payload drug delivery. In the second aim, we will verify the results of Aim 1 by characterizing the in vivo activity of the proposed CpG-siRNA-LEN conjugates in an MDS mouse model. Alternatively, CpG-linked lytic peptide that can only induce cell death upon receptor mediated internalization will be tested both in vivo and in vitro, permitting the combination of CpG-mediated specificity direct towards the malignant MDS clone with the internal delivery of cytotoxic agent. This strategy aims to have higher therapeutic efficiency with lower side effects as compared to current standard of care. The strategies proposed are innovative with strong clinical application potential and with broad applicability to other malignancies. Accomplishment of the goals proposed here will have a direct clinical impact on the improvement of MDS patient care.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2014 Awardee

**Christopher Park, M.D., Ph.D.**

New York University School of Medicine

*The Contribution of Aging to the Pathogenesis of the Myelodysplastic Syndromes*

11/1/2014-10/31/2017

Aging is associated with changes in the hematopoietic system including anemia, reduced cellular immunity, and increased innate immune system activation. Recent data indicate that many of these changes may be due to alterations in hematopoietic stem cells (HSCs), as aged HSCs exhibit a myeloid-lineage bias as well as decreased long-term reconstitution potential and self-renewal. Aging is also associated with increased risk of developing malignancies originating from HSCs including the myelodysplastic syndromes (MDS). While the presence of somatic mutations and karyotypic alterations in MDS indicate a significant cell-intrinsic contribution to MDS pathogenesis, it is likely that cell-extrinsic factors modify MDS biology. Indeed, the hematologic changes observed in MDS patients are similar to those seen in the normal elderly (e.g. anemia, decreased lymphoid output), and thus, we hypothesize that MDS HSC function is modified by cell-intrinsic and cell-extrinsic factors present in the normal aging background.

Given that MDS arises from HSCs and elimination of MDS HSCs is required for cure, it is important to identify all the factors that regulate MDS HSC function. Thus, we will investigate the contribution of the physiologic context in which MDS arises - aging - to the development and severity of MDS phenotypes. We will use a combination of our recently described MDS HSC xenograft model and our genetically faithful MDS mouse model, the Tet2/Asxl1 double knockout, in order to: (1) determine the role of aging on cell-intrinsic alterations in MDS HSC function, (2) determine the role of the aging niche on MDS HSCs, and (3) determine whether anti-aging interventions that reverse/inhibit normal HSC aging can improve MDS HSC phenotypes. We hope this approach will elucidate the age-related factors that contribute to MDS pathogenesis and will allow us to develop novel strategies to inhibit disease progression, improve hematologic parameters, or even prevent disease.

# The Taub Foundation Grants Program for MDS Research

## Awardee List 2014 - 2017

### 2014 Awardee

**Leonard Zon, M.D.**

Boston Children's Hospital

*Ribosome Protein Deficiency and Modulation of p53 as a Therapeutic Target*

11/1/2014-10/31/2017

Mutations in genes for ribosomal proteins (RPs) or for processing factors are found in patients with Diamond Blackfan anemia (DBA), 5q- syndrome, a subtype of myelodysplastic syndrome (MDS), and Shwachman-Diamond syndrome (SBDS), among others. Major complications of these diseases include defective erythropoiesis, craniofacial abnormalities and the increased cancer risk. RP mutations cause activation of p53, through accumulation of free RPs that bind and sequester MDM2, a negative regulator of p53. A large percentage of mortalities in DBA are treatment-related. To find more effective therapies, our lab performed an in vivo chemical screen in a zebrafish model of DBA, and found that inhibitors of calmodulin (CaM), and of the CaM-dependent kinase CHK2, block p53 activity and rescue the erythroid defect. However, although many CaM inhibitors are already FDA-approved as antipsychotics, they cross the blood brain barrier (BBB) and their use is associated with dyskinesia, making them too risky for treating children. Our goal is to find CaM or CHK2 inhibitors that do not cross the BBB, and thus have the potential to be used in clinical trials for patients with DBA. We will leverage a novel chemical scaffold approach, developed by Dr. James Bradner, to generate 400 new CaM inhibitors, and will test these molecules for BBB permeability and hemoglobin rescue in zebrafish. Compounds will then be further characterized in a human and mammalian DBA model. The technology we propose to use - rapid generation and screening of compounds excluded from the BBB - may also be applicable to other diseases in which treatment toxicity related to BBB permeability is a problem. Overall, this work will identify potential compounds to test in clinical trials for DBA, MDS and for other RP and bone marrow failure disorders.