FRI *to* SAT


APRIL 12 – 13 2019

ST. JUDE CHILDREN'S RESEARCH HOSPITAL

# PROGRAM

St. Jude Children's Research Hospital®

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# WELCOME

## TO THE 14TH MIDWEST BLOOD CLUB MEETING!

This meeting will feature exciting research on hematopoiesis, stem cell biology, gene therapy, the niche, and leukemia! The meeting will provide a forum for investigators to meet, share ideas, present recent findings, establish contacts and collaborations, and explore cutting edge research approaches.

## Conference Hosts

**Shannon McKinney-Freeman, PhD**
Associate Member
Department of Hematology
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**Wilson K. Clements, PhD**
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**Mitchell J. Weiss, MD, PhD**
Member, Chair
Department of Hematology
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# AGENDA

## REGISTRATION DESK AND CHECK-IN

The registration desk will be open in the Marlo Thomas Center for Global Education and Collaboration on Friday, April 12th beginning at 3 pm and on Saturday, April 13th at 7:45 am.

## SPEAKER PREPARATION ROOM

For your convenience, a Speaker Preparation Room will be available to review your presentation. Please inquire at the registration desk for the room location and details, if needed.

## POSTER EXHIBITION (FRIDAY, APRIL 12TH)

Poster viewing and reception will be from 5 pm – 6:30 pm. Posters will be on display through Saturday, April 13th.

The poster session and reception will be in the atrium of the Marlo Thomas Center for Global Education and Collaboration (MTC) on the St. Jude campus.

Posters may be displayed beginning at 3:30 pm on April 12th. Please take down your poster at the end of the meeting (6:15 pm, April 13th).

## EVALUATION

A link to the evaluation will be emailed to you after the conference. Your feedback is valuable to us, so please complete the evaluation.

## CELEBRATION AND DINNER AT THE COLUMNS

A celebration and dinner will occur at The Columns immediately following the conference.

## ADMINISTRATIVE SUPPORT

Pamela Franklin, Executive Assistant, can be reached by email (pamela.franklin@stjude.org) or by phone (office: 901-595-3760, or cell: 901-246-8312).

Heather Duncan, Administrative Specialist, can be reached by email (heather.duncan2@stjude.org) or by phone (office: 901-595-0944, or cell: 662-816-7080).

## FRIDAY, APRIL 12

**2:45 & 3:15 pm** Transportation from Peabody Hotel to St. Jude Campus

**3:30 pm** Registration, Check-in and Refreshments

**4:00 pm** **Welcome and Keynote Presentation**
*Ulrich Steidl, MD, PhD, Albert Einstein College of Medicine*

**5:00 pm** Poster Session and Reception

**6:45 pm** Transportation from St. Jude Campus to Peabody Hotel

## SATURDAY, APRIL 13

**7:00 & 7:30 am** Transportation from Peabody Hotel to St. Jude Campus

**8:00 am** Breakfast and Refreshments

**8:30 am** **Session 1: Developmental Hematopoiesis**
*Session Chairs: Wilson Clements and Owen Tamplin*

**8:30 am** Jeff Magee, MD, PhD, Washington University-St. Louis

**8:55 am** Emery Bresnick, PhD, University of Wisconsin-Madison

**9:20 am** Mark Althoff, BS, Cincinnati Children's Hospital Medical Center

**9:35 am** Erich Damm, PhD, St. Jude Children's Research Hospital

**9:50 am** Meghan Turnis, PhD, St. Jude Children's Research Hospital

**10:05 am** Break with Refreshments

**10:20 am** **Session 2: Malignant Hematopoiesis**
*Session Chairs: Mohammad Azam and Tanja Gruber*

**10:20 am** Charles Mullighan, MD, St. Jude Children's Research Hospital

**10:45 am** Daniel T. Starczynowski, PhD, Cincinnati Children's Hospital Medical Center

**11:10 am** Geoffrey Clark, PhD, University of Louisville

**11:35 am** Kimble Frazer, MD, PhD, University of Oklahoma Health Sciences Center

**11:50 am** Alison Meyer, PhD, Blood Research Institute

**11:55 am** Lunch

**1:00 pm** **Session 3: HSC Biology**
*Session Chairs: Shannon McKinney-Freeman and Marie-Dominique Filippi*

**1:00 pm** H. Lee Grimes, PhD, Cincinnati Children's Hospital Medical Center

**1:25 pm** Hal E. Broxmeyer, PhD, Indiana School of Medicine

**1:50 pm** Jose Javier, BS, Cincinnati Children's Hospital Medical Center

**2:05 pm** Maegan Capitano, PhD, Indiana School of Medicine

**2:20 pm** Antonio Morales-Hernandez, PhD, St. Jude Children's Research Hospital

**2:35 pm** Break with Refreshments

**2:50 pm** **Session 4: Cell and Gene Therapy**
*Session Chairs: Andrew M. Davidoff and Mitch Weiss*

**2:50 pm** Ewelina Mamcarz, MD, St. Jude Children's Research Hospital

**3:15 pm** Punam Malik, MD, Cincinnati, Children's Hospital Medical Center

**3:40 pm** Shailaja Hegde, PhD, Cincinnati, Children's Hospital Medical Center

**3:55 pm** Abishek Vaidya, MS, St. Jude Children's Research Hospital

**4:10 pm** Cuiping Zhang, MD, PhD, University of Kentucky

**4:25 pm** Break with Refreshments

**4:40 pm** **Session 5: HSC Niche**
*Session Chairs: Laura Schuettpelz and Jose Cancelas*

**4:40 pm** Robert Welner, PhD, University of Alabama-Birmingham

**5:05 pm** Damien Reynaud, PhD, Cincinnati Children's Hospital Medical Center

**5:35 pm** Mary Kraft , PhD, University of Illinois Urbana-Champaign

**5:50 pm** Safa Mohamad, MS, Indiana School of Medicine

**6:05 pm** Owen Tamplin, PhD, University of Illinois at Chicago

**6:15 pm** Concluding Remarks

**6:30 pm** Transportation to The Columns

**6:45 pm** After Conference Celebration and Dinner at The Columns

# KEYNOTE SPEAKER

## ULRICH G. STEIDL, MD, PHD

The Diane and Arthur B. Belfer Faculty Scholar in Cancer Research

Leader, Stem Cells, Differentiation and Cancer Program, Albert Einstein Cancer Center

Director, Stem Cell Isolation & Xenotransplantation Facility, Ruth L. and David S. Gottesman Institute for Stem Cell Research & Regenerative Medicine

Ulrich Steidl graduated from the University of Heidelberg Medical School and the German Cancer Research Center in Heidelberg, Germany.

He trained as a resident physician at the University of Duesseldorf Medical Center, Germany, and as a postdoctoral researcher at Harvard Medical School, Boston, MA. He joined the Albert Einstein College of Medicine – Montefiore Medical Center in the Bronx, New York, in 2008, where he currently holds appointments as a tenured Professor in the Departments of Cell Biology, and of Medicine (Oncology). Ulrich Steidl is the Program Leader of the 'Stem Cells, Differentiation and Cancer' program of the NCI-designated Albert Einstein Cancer Center, the Scientific Director of the Division of Hematology-Oncology within the Department of Oncology at Einstein/Montefiore Medical Center, and a faculty member of the Ruth L. and David S. Gottesman Institute for Stem Cell Research and Regenerative Medicine.

# Understanding and Targeting the Stem Cell Origins of Myeloid Malignancies

**Abstract:**

Relapse and malignant progression continue to be the most common causes of death in myelodysplastic syndromes (MDS) acute myeloid leukemia (AML) and many other cancers. Recent evidence has shown that a molecularly diverse pool of hematopoietic stem cells (HSCs) lead to the formation of pre-cancerous/pre-leukemic stem cells that play a pivotal role not only in disease origination, but also in relapse. While the existence and essentiality of such pre-cancerous cell states, including pre-MDS-SC and pre-AML-SC, has been demonstrated in mice and humans, still very little is known about the molecular mechanisms driving their formation and progression. We and others have performed molecular studies of pre-leukemic cell states in mouse genetic models as well as primary cells from patients, and discovered new molecular and stem cell biological mechanisms in pre-MDS-SC and pre-AML-SC, challenging current paradigms of bulk cell-focused “precision” oncology.

Such models and data sets at the stem cell level have provided novel tools for mechanistic study of pre-cancerous stem cells and their progression to overt MDS and AML, and for the development and testing of pharmacological approaches to therapeutically interfere with these processes. Together, recent studies have started to shed light on pre-cancerous stem cell states as the earliest origin of MDS and AML, and on cell-intrinsic as well as selection mechanisms driving their formation and progression. These advances provide a basis for the specific therapeutic targeting of pre-cancerous stem cells for the causative treatment and potential prevention of MDS and AML and other cancers developing from pre-cancerous conditions in the future.

# OTHER INVITED SPEAKERS

**EMERY BRESNICK, PHD**

University of Wisconsin-Madison

**HAL E. BROXMEYER, PHD**

Indiana School of Medicine

**H. LEE GRIMES, PHD**

Cincinnati Children's Hospital Medical Center

**EWELINA K. MAMCARZ, MD**

St. Jude Children's Research Hospital

**CHARLES MULLIGHAN, MD**

St. Jude Children's Research Hospital

**DAMIEN REYNAUD, PHD**

Cincinnati Children's Hospital Medical Center

**JEFF MAGEE, MD, PHD**

Washington University-St. Louis

**PUNAM MALIK, MD**

Cincinnati Children's Hospital Medical Center

**DANIEL T. STARCZYNOWSKI, PHD**

Cincinnati Children's Hospital Medical Center

**ROBERT WELNER, PHD**

University of Alabama-Birmingham

# ORAL ABSTRACTS

**OP-1**

**Developmental Programming and Re-Programming in Pediatric Leukemogenesis**

Jeff Magee

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Leukemias can arise at any stage of life, pediatric and adult leukemias have different genetic and epigenetic landscapes, different mechanisms of transformation and different clinical courses. This is true even for individual leukemia subtypes, such as acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The differences between pediatric and adult leukemias suggest that mutations may interface with normal developmental programs. Thus, the ability a given mutation to initiate leukemogenesis may change as a function of age and developmental stage.

Infant AML and ALL are particularly strong examples of leukemias that exhibit age-specific mutation profiles. Most infant ALL, and approximately half of infant AML, harbor an MLL rearrangement (e.g. MLL-ENL, MLL-AF9, MLL-AF4). In many cases, the MLL rearrangement arises prior to birth, and it can be the sole clonal driver mutation. This suggests that MLL fusion proteins may initiate leukemogenesis more efficiently in fetal or neonatal hematopoietic progenitors than in adult progenitors. To test this possibility, we used transgenic mice to induce MLL-ENL during fetal, neonatal, juvenile and adult stages of life. We found that MLL-ENL initiated AML most efficiently when it was induced in neonatal progenitors. At later stages of life, MLL-ENL failed to activate target genes when it was expressed at physiologic levels. Furthermore, during fetal development, the fetal regulator LIN28B antagonized AML initiation. This was surprising because LIN28B is often thought to be an oncogene rather than a tumor suppressor, but it may explain why human MLL-rearranged leukemias rarely occur before birth. Altogether, the data show that the efficiency of MLL-ENL-driven leukemia initiation changes with age and peaks during neonatal development.

In light of these findings, we sought to better understand the normal transcriptional and epigenetic changes that take place as neonatal hematopoietic stem cells (HSCs) and committed hematopoietic progenitors (HPCs) transition from fetal- to adult-like states. Using single cell RNA-seq, we found that murine HSCs and HPCs undergo a gradual, rather than precipitous, transition from fetal to adult transcriptional states. The transition begins just prior to birth, and it is accompanied by gradual epigenome remodeling that varies from cell to cell. The transition is driven, at least in part, by type I interferon signaling that spikes just before birth and persists at lower levels thereafter. Our results offer a mechanism to explain how gradual changes in cell identity can be timed. Heterochronic enhancer elements, and their associated transcripts, are activated independently of one another, stochastically, rather than as part of a robust network. This discordance allows neonatal HSCs and HPCs to transition to an adult-like state without guidance from precise, sequential temporal cues, but it also establishes epigenomic heterogeneity among progenitors that can potentially restrict their competence to give rise to leukemias.

**OP-2**

**From Genomes to Blood-Generating Mechanisms in Physiology and Pathology**

Emery Bresnick

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Our efforts to forge principles for how GATA factors control development and function of the hematopoietic system led to the discovery of conserved Gata2 enhancers (+9.5 and -77) essential for hematopoiesis, as well as other blood-regulatory enhancers. While genomic studies commonly predict enhancers, there were no reports of enhancers essential for stem cell genesis or progenitor fate decisions. Strikingly, +9.5 mutations resemble GATA2 coding mutations in causing human GATA2-deficiency syndrome involving immunodeficiency, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). -77 disruption also causes MDS/AML. This foundation led clinical centers to analyze -77 and +9.5 genetic variation. In the past few years, we discovered: 1) While +9.5 and -77 confer progenitor cell fate, only +9.5 triggers hematopoietic stem cell genesis (Johnson et al. J. Clin. Invest. 2012; Science Adv. 2015; Mehta et al. Cell Reports 2017). 2) GATA2 loss, resulting from -77 relocation, with EVI1 upregulation defines a novel leukemogenic mechanism (Yamazaki et al. Cancer Cell 2014; Johnson et al. Science Adv. 2015)). 3) An ensemble of “+9.5-like” enhancers exist, including one regulating a c-Kit facilitator that promotes erythrocyte regeneration and survival in severe anemia (Hewitt et al. Mol. Cell 2015; Dev. Cell 2017). 4) +9.5-regulated G-protein-coupled receptor circuits control hematopoiesis (Gao et al. J. Exp. Med. 2013; Stem Cell Rep. 2016). 5) Gain-of-function GATA2 disease mutants exemplify a paradigm-shift in the pathogenic mechanism (Katsumura et al. PNAS 2018). 6) +9.5 single-nucleotide disease mutation dissociates developmental and regenerative activities, creating a bone marrow failure predisposition (J. Clin. Invest. 2019). We are conducting multidisciplinary studies with human disease mutations, innovative mouse models, genetic rescue assays and multiomics to elucidate mechanisms underlying the function of disease-causing enhancers, GATA2 and components of GATA2 genetic networks and to forge unique translational/clinical strategies.

**OP-3**

**Scribble Controls HSC Self-Renewal through Polarity-Dependent Activation of Hippo Signaling**

Mark Althoff, Ramesh Nayak, Shailaja Hegde, Ashley Wellendorf, Fatima Mohmoud, Mei Xin, Q Richard Lu, Maria Diaz-Meco, Jorge Moscat and Jose Cancelas

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Hematopoietic stem cells (HSCs) represent a rare and highly quiescent population of cells residing within the adult bone marrow (BM) cavity that are responsible for the lifelong production of blood. HSC transplantation is commonly used in cancer therapy and often exemplifies novel ex vivo cell/ gene therapy for HSC driven diseases. Search of molecular targets to expand functional HSC for translational applications remains paramount. HSC respond to extrinsic cues and either self-renew or differentiate according to changes in cellular polarity (differential partitioning of protein or RNA determinants). The mechanisms underlying cellular polarity establishment and maintenance are well characterized in neuronal stem cells and epithelial cell populations, but the role that polarity regulators have on the function of HSC remains elusive. Scribble, a multi-modular cytoplasmic scaffolding protein, coordinates the spatial organization of fate determinant proteins. By using a combination of constitutive and inducible hematopoietic-specific Scribble-deficient animal models, hematopoietic reconstitution assays, structure-function mutants of Scribble and intracellular protein trafficking analysis, we identified a novel non-canonical function of Scribble in HSC activity. Scribble-deficient HSC maintain higher levels of hematopoietic self-renewal ability through paired-daughter clonal analyses, stromal-dependent cultures and serial transplantation. Mechanistically, Scribble polarizes Yap1 and its negative regulator Lats1 of the Hippo Signaling Pathway in HSC. Deletion of Scribble disrupts the Yap1/Lats1 complex and permits Yap1 nuclear translocation. Notably, cytoplasmic polarization of Yap1 is restored to wild-type levels in Scribble deficient HSC by reintroducing the full-length Scribble protein or a PDZ domain-containing mutant of Scribble. Furthermore, genetic deletion of Yap1 and its homologue Taz in HSC results in a loss of hematopoietic potential, suggesting a functional dependency of these transcriptional co-activators in HSC activity. Despite the observed nuclear accumulation of Yap1 in Scribble-deficient HSC, we failed to detect any corresponding canonical Yap1 transcriptional signatures. Similarly, inducible expression of a constitutively active (nuclear) Yap1 (Yap1S112A) fails to phenocopy the functional effects of Scribble deletion in vivo. Therefore, we hypothesize that the loss of cytoplasmic Yap1 holds more value than its nuclear accumulation. Along these lines, we show that cytosolic Scribble and Yap1 are required for cytokine-induced activation of Cdc42, a negative regulator of self-renewal. In summary, the Hippo-Yap1/Taz pathway, in conjunction with Scribble polarization, may play an essential role in regulating Cdc42 and HSC self-renewal. Understanding this connection will allow us to exploit novel targets in ex vivo HSC-based gene-therapy for treatment of cancer and hematologic diseases.

**OP-4**

## Neural Crest Cell Derived Epithelial Membrane Protein 3 is a Novel Regulator of Hematopoietic Stem Cell Specification

Erich Damm and Wilson Clements

St. Jude Children's Research Hospital, Department of Hematology, Memphis, TN

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Hematopoietic stem cells (HSCs) are the self-renewing population that generates all hematopoietic lineages over the lifetime of an animal. HSCs are highly biomedically relevant and are the basic units of bone marrow transplants used to treat a myriad of hematologic diseases. However, the availability of this therapy is limited by the supply of immunologically compatible HSCs. The in vitro directed differentiation of HSCs from induced pluripotent stem cells for use in autologous transplant is a major biomedical objective that has the potential to alleviate issues with HSC supply and would provide large numbers of genetically modifiable cells for gene therapy-based treatment of hematologic disease. However, it has not been possible to generate bona fide HSCs with high engraftment capability and full multi-lineage potential with current protocols. This is likely because we lack a full understanding of the required HSC specification signals and the cells generating them. In all vertebrate embryos, HSCs emerge from hemogenic endothelium (HE) lining the floor of the dorsal aorta (DA) during an endothelial to hematopoietic transition event. We have recently found that contact between HE and trunk neural crest cells (NCCs) is required for the specification of HSCs in the zebrafish embryo, suggesting that signaling from NCCs is required for HSC specification. To identify potential NC derived regulators of HSC specification, we performed a transcriptional profiling screen of NCCs and identified epithelial membrane protein 3b (emp3b) as gene with high expression in migrating NCCs and low expression in the DA. Emp3b is a poorly understood transmembrane protein, but is known to modulate Tgfβ,Egfr and integrin signaling. We found that knockdown of emp3b by morpholino resulted in a loss of runx1+ and cmyb+ HSCs in the DA and a loss of rag1+ T-cells in the paired thymi, suggesting a loss of HSC specification. We further found that markers of embryonic vasculature, arterial patterning and HE identity were unaffected in emp3b morphants, indicating that in the absence of emp3b the HE is competent to form HSCs. This phenotype recapitulates that of the loss of contact between HE and NC cells. However, we found that NC migration and NC/HE contact was unaffected in emp3b morphants, suggesting that the loss of HSC specification is likely due to defective signaling between trunk NCCs and HE. Interrogating the role of Emp3b in HSC specification and NC development will lead to novel insights into the signals regulating HSC specification in the vertebrate embryo.

**OP-5**

**MCL1 is Required for Early Erythropoiesis**

Meghan Turnis, Ewa Kaminska, Peter Vogel, and Joseph Opferman

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Mature erythrocytes are under homeostatic control with the need for constant replacement from progenitors. This is regulated largely by erythropoietin (EPO), which in part induces the expression of anti-apoptotic BCLxL. Ablation of Bcl2l1 (which encodes BCLxL) results in embryonic lethality with a lack of mature erythrocytes, but does not perturb erythroid progenitors. Similarly, conditional Bcl2l1-deletion results in severe anemia with the death of late red blood cell progenitors and induction of extramedullary erythropoiesis. These data indicate that while BCLxL may be critical to the survival of mature erythrocytes, other anti-apoptotic molecules may mediate survival during earlier stages of erythropoiesis.

To explore whether other pro-survival molecules function in early erythropoiesis, we found MCL1 mRNA is expressed during early erythroid development, when BCLxL mRNA expression is almost absent. We tested MCL1's functional role in erythropoiesis by crossing Mcl1f/f and EpoRCre mice to yield animals in which Mcl1 is deleted in the erythroid lineage. Erythroid-specific MCL1-deletion results in embryonic lethality due to severe anemia caused by a lack of mature red blood cells. MCL1-deleted embryos exhibit stunted growth, necrosis, and decreased RBCs in the blood. Additionally, erythroid precursors in the circulation of MCL1-deleted embryos are large, nucleated, and often apoptotic. Erythroid-specific MCL1-deleted fetal liver cells are unable to form BFU-E in methylcellulose culture, but efficiently form monocytic and myeloid colonies. Flow cytometric analysis indicate an enrichment of erythroid progenitors in deleted fetal livers, suggesting a developmental arrest. These data clearly demonstrate a requirement for MCL-1 in promoting survival of early erythroid progenitors.

To address whether MCL1 plays a similar role in later stages of erythropoiesis, when BCLxL has been previously implicated, we developed an ex vivo system in which Mcl1 can be inducibly deleted at different times during differentiation. We isolated Mcl1f/f RosaERT2Cre embryos and purified precursors for differentiation in culture using established methods. Flow cytometric analysis indicates efficient differentiation in MCL1 replete cells. Tamoxifen treatment of Mcl1f/f RosaERT2Cre cells at the start of differentiation leads to apoptotic death and developmental blockade similar to Mcl1f/f EpoRCre embryos. In contrast, addition of tamoxifen 24 hours later does not affect the ability of erythroid cells to differentiate despite efficient Mcl1-deletion. These data indicate that MCL1 is essential in promoting survival only during early erythropoiesis, but at later stages is dispensable. We hypothesize that during the later stages of erythroid differentiation, BCLxL expression replaces the requirement for MCL1 in promoting survival.

**OP-6**

**The ALL Genome Across the Ages**

Charles Mullighan

St. Jude Children's Research Hospital, Department of Pathology, Memphis, TN

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Acute lymphoblastic leukemia remains a clinical challenge in children and adult, and identification of tumor drivers is critical to enable risk stratification and tailoring of therapy. However until recently, many patients have lacked a known driver, particularly in older children and adults. In this talk, I will provide an overview of a large, ongoing study seeking to provide a comprehensive portrait of the germline and somatic drivers that define ALL subgroups. Specifically, in a transcriptomic study of almost 2000 cases, we describe 23 ALL subtypes that classify almost all ALL cases with remarkable variation in subtype prevalence and outcome according to age. Many new subtypes were unrecognized due to the diversity or cryptic nature of driving alterations that define each subtype. Moreover, it is now recognized that sequence mutations drive the formation of multiple new subtypes that converge on key nodes in B lymphoid development. The implications of these findings for tumor formation, diagnosis and therapy will be described.

Key reference: Gu, Z. et al. PAX5 driven subtypes of B progenitor acute lymphoblastic leukemia. Nat Genet 51, 296,307, doi:10.1038/s41588,018,0315,5 (2019).

**OP-7**

**Dysregulation of Innate Immune Signaling in Hematologic Malignancies**

Daniel Starczynowski

Cincinnati Children's Hospital Medical Center, Cincinnati, OH

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Spliceosome mutations are common in MDS and AML, yet the oncogenic changes due to these mutations have not been identified. A global analysis of exon usage in AML samples revealed distinct molecular subsets containing alternative spliced isoforms of inflammatory and immune genes. IRAK4 was the dominant alternatively spliced isoform in MDS/AML and is characterized by a longer isoform that retains exon 4, encoding a protein, IRAK4-Long (L) that assembles with the Myddosome, results in maximal activation of NF-kB, and is essential for leukemic cell function. Expression of IRAK4-L is mediated by mutant U2AF1 and is associated with oncogenic signaling in MDS/AML. Inhibition of IRAK4-L abrogates leukemic growth, particularly in AML cells with higher expression of the IRAK4-L isoform. Collectively, mutations in U2AF1 induce expression of therapeutically targetable "active" IRAK4 isoforms and provide a genetic link to activation of chronic innate immune signaling in MDS and AML.

**OP-8**

**A Novel RAS Inhibitor to Suppress RAS Driven AML**

Geoffrey Clark, Howard Donninger, Grace Hovekamp, and Levi Beverley

University of Louisville, Louisville, KY

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AML is often driven by mutations in RAS oncogenes. There are currently no clinically effective inhibitors of RAS. We used an in silico approach to identify a novel RAS binding molecule that acts to inhibit the interaction of RAS with its oncogenic effectors at low uM levels. The compound has broad effects against RAS driven tumor cells and specifically inhibits the growth of RAS driven AML cells whilst having no effect on MLL-fusion driven AML tumor cells. The compound exhibits no in vivo toxicity and is active in vivo against RAS driven human tumor cell xenografts.

**OP-9**

**Multiple Forms of Zebrafish ALL Induced by a Single Transgenic Oncoprotein**

J. Kimble Frazer, Chiara Borga, Jessica Burroughs-Garcia, Clay Foster, Gilseung Park, Ameera Hasan, and Arpan Sinha

University of Oklahoma Health Sciences Center, Department of Pediatrics, Section of Pediatric Hematology-Oncology, Oklahoma City, OK

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Lymphoblast malignancies dominate pediatric oncology, representing ~1/3 of all childhood cancer. Most are acute lymphoblastic leukemia (ALL), and the rest lymphoblastic lymphoma (LBL). ALL and LBL can develop in either B or T lymphoblasts; B-lineage ALL is more common, while T-LBL exceeds B-LBL. Animal models have been useful to study all four diseases. The first cancer model in zebrafish (Danio rerio) was actually a transgenic line expressing murine Myc controlled by a zebrafish rag2 promoter (rag2:mMyc). 100% of fish developed highly-penetrant T-LBL/T-ALL. Soon after, other T-lymphoblast cancer lines were created, including human MYC-transgenic (rag2:hMYC) fish, which also acquire T-LBL/T-ALL. Until recently, no B-lymphoblast D. rerio cancer models had been described, but we discovered rag2:hMYC fish actually develop B-LBL/B-ALL as well as T-LBL/T-ALL; this is also true for rag2:mMyc fish. Unexpectedly, expression profiling revealed that seemingly-similar B-ALL of mMyc vs. hMYC fish are in fact quite different, arising in distinct B cell lineages. Gene expression in mMyc and hMYC T-ALL also has substantial differences. Finally, biphenotypic ALL expressing both B- and T-lineage genes was seen in one rag2:mMyc case, and we have new evidence of hMYC-driven biphenotypic ALL also. This means at least 6 different ALL types have now been described in these transgenic zebrafish lines, even though both use the same rag2 promoter and near-identical oncogenes. Possible explanations for these different ALL phenotypes include activation of different mMyc vs. hMYC target genes and downstream pathways, expression of rag2 (and thus MYC) in distinct stages of lymphocyte development, genetic differences in the D. rerio strain backgrounds used, or perhaps all of these. Overall, our findings demonstrate that MYC induces several forms of zebrafish ALL, and we hypothesize each may have a human counterpart. We believe deciphering the genetic and molecular details of these ALL types and how they foster lymphocyte transformation will be informative to ALL biology in general, and how it varies in distinct human ALL subtypes.

**OP-10**

**Combinatorial Genetics Uncovers Novel Targets for the Treatment of Npm1/Cohesin Mutated AML**

Alison Meyer, Cary Stelloh, Joseph Fisher, Kirthi Pulakanti, George S Vassiliou, Aaron Viny, Ross Levine, and Sridhar Rao

Blood Research Institute, Versiti,WI

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Adult acute myeloid leukemia (AML) is difficult to treat due to its genetic heterogeneity. Approximately 30 somatic mutations have been found to be recurrent, with an average of 5-15 mutations per patient (1). Therefore, combinatorial genetic approaches have the power to uncover novel gene targets that can be used to tailor therapies to an individual's unique mutational spectrum. The most common mutation in AML is referred to as NPM1cA (1; 2). While NPM1cA is a driver of AML development, mice with this mutation develop AML with a long latency and incomplete penetrance, suggesting additional mutations are required for transformation (3; 4).

Cohesin mutations are enriched in patients with NPM1 mutations (5). Mutations in any of the cohesin complex components (STAG2, SMC1A, SMC3, RAD21) results in haploinsufficiency (1). Although cohesin mutations alone are insufficient to generate AML in mice (6), they do result in increased hematopoietic stem and progenitor cell (HSPC) self-renewal. This is thought to be the result of changes in gene expression (6; 7; 8). We therefore hypothesized that cohesin mutations would cooperate with NPM1cA to alter gene expression, resulting in AML. We crossed the inducible NPM1cAflox/+ and SMC3flox/+ mouse models to examine this genetic interaction.

We found that the double mutant mice develop AML with increased penetrance compared to NPM1cA/+ mice. Double mutant HSPCs also exhibit increased self-renewal in vitro compared to the NPM1cA/+ or SMC3Î”/+ single mutants. We next performed RNA sequencing on pre-leukemic lineage-depleted bone marrow to identify transcriptome changes specific to the double mutant HSPCs. Consistent with our hypothesis, additive changes in gene expression were not observed in NPM1cA/+;SMC3 animals. Instead, a unique set of genes were found to be deregulated.

In our analysis, we found DOCK1 to be overexpressed in the double, but not the NPM1cA/+ single, cells. High expression of DOCK1 has been correlated with decreased overall and disease-free survival in AML patients (9). To determine if DOCK1 contributes to the enhanced cell growth observed in vitro in our leukemic lines, we used an inhibitor that targets Dock1 and shRNA-mediated knockdown of DOCK1. Both treatments resulted in decreased cell viability in NPM1cA/+;SMC3 leukemic lines, but not in NPM1cA/+ only lines. We thus hypothesize that Dock1 represents a unique target for the treatment of patients harboring the Npm1/Cohesin mutational combination. Our results provide validity to the concept that combinatorial genetics can be used to target the unique genetic landscape of an individual patient.

**OP-11**

## A Neutropenia-Associated Transcription Factor Mutation Differentially Impacts Target Genes in the Cell-states Traversed during Granulocyte Specification and Commitment

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Advances in genetics and sequencing have led to a deluge of disease-associated and disease-causing genetic alterations. Resolving causality between genetics and disease requires generating accurate models for molecular dissection; however, the rapid expansion of single-cell landscapes presents a major challenge to accurate comparisons between mutants and their wild type equivalents. Here, we generated mouse models of human severe congenital neutropenia (SCN) using patient-derived mutations in the Growth factor independent-1 (GFI1) transcription factor. To delineate the impact of SCN mutations, we first generated single-cell references for granulopoietic genomic states with linked epitopes, then developed a new computational approach to align mutant cells to their wild-type equivalent and derive differentially expressed genes. Surprisingly, the majority of differentially expressed GFI1-target genes are sequentially altered as cells traverse successive states. These cell-state-specific insights facilitated genetic rescue of granulocytic specification, but not post-commitment defects in the expression of innate-immune effectors, providing regulatory insights into granulocyte dysfunction.

**OP-12**

## Coordinated Means to Potentially Enhance Human Hematopoietic Cell Transplantation, Especially with Cord Blood Cells

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Cord Blood (CB) Hematopoietic Cell Transplantation (HCT) has been performed well over 40,000 times for clinical treatment of a multitude of malignant and non-malignant disorders. While there are a number of ways that CB HCT is as good as, or superior to that of bone marrow (BM)- and mobilized peripheral blood (mPB)-HCT, there are deficiencies that limit the full potential of CB for HCT, including slower time than BM and mPB for recovery of neutrophils, platelets, and immune cells. This presentation will discuss studies from the author's laboratory that are evaluating a number of different, but connected/coordinated, means to deal with this deficiency of CB HCT. This includes obtaining more hematopoietic stem cells (HSCs) via collection and processing of the CB cells in hypoxia (3% oxygen) rather than in ambient air (~21% oxygen), or in air in the presence of selected small molecules, the ex-vivo expansion of the HSCs, and especially that of enhancing the homing efficiency of the collected and/or cultured HSCs. Such studies may not only improve CB HCT, but also that of BM and mPB HCT when cells for these sources are in limiting numbers, and also offers a potential means for obtaining a more potent set of HSCs with increased functional capacity.

**OP-13**

## Up-Regulated TGFβ Signaling Drives MDS-Like Bone Marrow Failure Following Acute Inflammatory Stress

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Hematopoietic stem cells (HSCs) self-renew or differentiate to produce mature circulating blood cells after transplant or viral infection. However, how signaling pathways regulate these decisions is still unclear. Dysregulation causes either leukemia or bone marrow failure. Myelodysplastic syndromes (MDS) are a group of bone marrow failure syndromes defined by cytopenias in blood lineage, myeloid dysplasia and hematopoietic stem and progenitor cells (HSPCs) clonal expansion, with some sub-types more likely to transform into acute myeloid leukemia. Our lab has published that transplanted murine HSCs have a TGFβ signaling autocrine loop that accelerated bone marrow failure after transplant, reversible by using TGFβ pharmacological inhibitors. We decided to further explore the effect of TGFβ signaling during stress using a mouse model conditionally overexpressing transgenic a TGFβ in the hematopoietic system (Tg-Cre+). Preliminary data show that stress induced by polyinosinic:polycytidilic acid (pIC) to mimic viral infection causes persistent pancytopenia and increased progenitor dysplasia up to 9 months after stress in Tg-Cre+ mice compared to controls. Flow cytometry of Tg-Cre+ bone marrow revealed an expanded HSPC compartment, and RNA-seq of Tg-Cre+ HSCs revealed that pathways related to mitochondrial regulation and cell cycling were up-regulated compared to controls 3 months after stress. Tg-Cre+ pIC-stressed HSCs had higher mitochondrial membrane potential, and immunofluorescence analysis showed altered mitochondrial distribution in Tg-Cre+ mice 3 months after stress. Distribution of MAVS, a mitochondrial membrane protein that effects response to viral stress, was altered in Tg-Cre+ after a TGFβ overexpression, and remains after pIC stress. Results therefore suggest that up-regulated TGFβ signaling alters mitochondrial architecture, function and MAVS cellular distribution to pre-dispose towards MDS-like bone marrow failure triggered after acute inflammatory stress.

**OP-14**

## Re-Evaluation of Age-Related Dysregulation of Hematopoiesis Based on Oxygen Sensitivity of Hematopoietic Stem and Progenitor Cells

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Bone marrow (BM) of aged mice have increased hematopoietic stem cells (HSCs), but greatly decreased competitive engrafting capability with decreased lymphoid capability compared to young mice. HSCs and hematopoietic progenitors (HPCs) reside in vivo in a hypoxic (e.g. ~3% oxygen) environment compared to ambient air (~21% oxygen). Yet, until recently knowledge of HSCs and HPCs was based on cells collected in ambient air, thus underestimating numbers of HSCs. Within minutes of exposure to ambient air, mitochondrial-derived ROS levels are increased, inducing rapid differentiation, not death, of HSCs. We hypothesized that this functional status of BM HSCs and HPCs from aged mice (20-28 months old) does not reflect their true nature. Thus, we re-evaluated BM of young (6-10 weeks old) and old (20-28 months old) C57BL/6 mice collected and processed for HSC/HPC phenotype and function at 3% compared to 21% oxygen. BM collected from old mice under hypoxia had increased engrafting capability more closely matching that of young BM. The lymphoid/myeloid ratio of old BM collected under hypoxia matched that of young BM collected under air. Enhanced numbers/function of old BM HSCs/HPCs collected in hypoxia is associated with changes in expression of chemokine receptors (e.g. CCR2, CCR5, CXCR4), stress-induced proteins (e.g. HSP40) and ROS. Thus, the age related differences between the HSC/HPC populations are not as drastic as previously reported, but rather they may be more sensitive to ambient air oxygen tension stress.

**OP-15**

**GPRASP Family Members Function as Novel Negative Regulators of Murine Hematopoietic Stem Cell Transplantation by Controlling CXCR4 Degradation**

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Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many patients with leukemia and hematologic disease. Sadly, immunological complications and infections contribute to high morbidity and mortality post-transplant. Recent work suggests that transplanted HSC are subject to distinct molecular regulation compared with HSC at steady-state. Thus, we seek to identify novel molecular regulators of HSCT. Here, we report that multiple GPRASP (G-protein coupled Receptor (GPCR) associated Sorting Proteins) family members function as novel barriers to HSCT in mice. We found high expression of multiple GPRASPs (e.g. Gprasp1 and Gprasp2) in HSC, relative to downstream progenitors. We have also showed that both Gprasp1 and Gprasp2 act as negative regulators of HSCT. GPRASPs regulate the post-endosomal trafficking of GPCRs to the lysosome. GPRASP1 and GPRASP2 contain multiple GPRASP domains via which they bind the C-terminus of GPCRs. A search for GPCRs containing a GPRASP-binding motif revealed that CXCR4, a master regulator of HSC migration, niche retention, survival, and quiescence contains a close match in its C-terminus, implicating it as a putative target of GPRASP-mediated degradation. Cxcr4-/- HSC do not transplant efficiently. Conversely, ectopic Cxcr4 enhances HSCT, similarly to loss of Gprasp1 or Gprasp2. Cxcr4 also promotes HSC quiescence and suppresses apoptosis of both mouse and human hematopoietic progenitors. Interestingly, Gprasp1 and Gprasp2-deficient HSPCs display an increase in G0, a decrease in G2SM, and reduced apoptosis ex vivo and post-transplant; phenocopying the known effects of Cxcr4. These GPRASP-knockdown effects disappear upon deletion of Cxcr4. Further, Gprasp1 or Gprasp2 loss increased CXCR4 levels in HSPCs, modified CXCR4 cellular localization and increased migration toward SDF-1. Our preliminary studies also show that GPRASP2 physically interacts with CXCR4. In total, these data suggest that loss of GPRASP1 or GPRASP2 in HSPCs stabilizes CXCR4, resulting in increased engraftment, survival and quiescence acutely post-transplant. In sum, we report for the first time that multiple GPRASPs function as barriers to HSCT by effecting HSC survival, cell cycle status and engraftment via regulation of CXCR4 stability. These data illuminate a novel means to modulate CXCR4 and open up new avenues to developing therapies that improve HSCT.

**OP-16**

**Lentiviral Gene Therapy with Low Dose Busulfan for Infants with X-SCID**

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X-linked Severe Combined Immunodeficiency (SCID-X1) is a rare primary immune disorder of infancy that results from mutations in IL2RG encoding the common γ-chain of several interleukin receptors. Affected patients lack T- and Natural Killer (NK) cells and humoral immunity. Previous gene therapy trials utilizing gamma retroviral (γRV) vector restored T-cell immunity, but did not correct B-cell function and resulted in leukemia in 30% of cases. The development of self-inactivating γRV vectors improved safety, but has not restored B-cell function to date. Our approach presented here utilizes a safety modified lentiviral (LV) vector (CL20-i4-EF1α-hGC-OPT) and reduced exposure busulfan (Bu) for newly diagnosed infants with SCID-X1.

We report interim results of the LVXSCID-ND trial; a dual center, phase I/II safety and efficacy study. Ten infants with SCID-X1 were treated at a median age of 4.3 months (range: 2.4-13.8), and the current median follow is 20.4 months (range: 2.8-30.1). Bone marrow (BM) CD34+ cells transduced with LV vector generated by a stable producer cell line were cryopreserved to ensure that release criteria were met prior to infusion of two single daily doses of Bu. An average Bu cumulative area-under-the-curve (cAUC) of 22.2 mg*hr/L (range: 20.0-23.0) was achieved, which was within 10% of the intended cAUC of 22 mg*hr/L. The median dose of transduced CD34+ cells was 9.12 x106/kg (range: 4.46-18.95) and the median vector copy number (VCN) of the graft was 0.44 VCN/cell (range: 0.16-1.13). No severe adverse events, other than hematologic, related to Bu were observed. All 10 patients had a robust hematopoietic recovery within 3-4 weeks without blood product support. Of these 9 are greater than 3 months post gene therapy and are currently off protective isolation and prophylactic antimicrobials with normal growth velocity. Eight achieved normal for age T-cell and NK-cell numbers within 3-4 months post gene therapy, and vector marking in T, B, NK, myeloid cells and marrow progenitor cells at 4 months post gene therapy. One patient required a gene therapy boost at 12 months post initial gene therapy, which resulted in functional immune reconstitution. All patients who presented with infections cleared them due to development of functional T-cell immunity manifested as normal for age in vitro responses to phytohemagglutinin (PHA). Five patients are now off IVIG of whom 3 responded to vaccines. Vector insertion site analysis of 7 analyzed cases showed polyclonal patterns without any clonal dominance with 27-4707 shared sites per case, indicating transduction of the pluripotent progenitors.

We conclude that lentiviral gene therapy with low exposure of Bu was well tolerated and resulted in successful engraftment of transduced long-term repopulating CD34+hematolpoiteic stem cells, development of a functional immune system without evidence of malignant transformation to date.

**OP-17**

## Gene Therapy for Sickle Cell Anemia Using a Modified Gamma Globin Lentivirus Vector (ARU-1801) and Reduced Intensity Conditioning (RIC; Arulite™) Transplant

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We generated a lentiviral vector encoding an antisickling modified g-globin gene ($sGbG^{M}$) for gene therapy for sickle cell anemia (SCA). Preclinical studies showed that gene transfer into autologous hematopoietic stem and progenitor cells (HSPC) in sickle mice (the Berkeley and Townes model of SCD) resulted in correction of the sickle cell disease phenotype mice following both myeloablative conditioning and reduced intensity conditioning, when the antisickling modified fetal hemoglobin ($HbF^{G16D}$) was greater than 10%. Based upon these preclinical data, we embarked upon a Reduced Intensity Conditioning (AruliteTM RIC conditioning) Phase I/II Pilot Study on Gene Transfer in Patients with SCA with ARU-1801 lentivector (NCT02186418), hypothesizing this approach will be safe, feasible and efficacious; Moreover, RIC will have significantly less toxicity, costs, and be implementable in many transplant centers, including those in some of the resource-poor countries, where supportive therapies for myeloablative transplants are unavailable and where majority of SCA patients exist. The study is open and enrolling adult patients with severe SCA. Two patients enrolled had CD34+ HSPC collected via bone marrow harvests and/or plerixafor mobilized leukopheresis. Patients received a single dose of Arulite conditioning (IV melphalan) followed by IV infusion of the gene modified-HSPC. Patients were monitored for adverse events, engraftment, vector copy number (VCN), $HbF^{G16D}$ expression and other parameters of SCA. Early results from the two SCA patients (for 1 and 1.5 years post-transplant) following RIC showed excellent feasibility with minimal post-transplant toxicity, rapid neutrophil and platelet count recovery within 7-9 days and 7-12 days, respectively, and detection of vector-derived fetal hemoglobin ($HbF^{G16D}$) expression which resulted in significant clinical amelioration of SCD phenotype. Early results are promising and will be presented. Longer follow up and additional patients will demonstrate whether thse levels of HbFG16D levels using a RIC approach will provide consistent clinical benefit to patients with severe SCA. If this RIC approach of delivering an antisickling globin gene is successful, it will be widely applicable.

**OP-18**

## Reversible Pharmacological Targeting of RHOA Prevents Refrigerated Platelet Storage Lesion and Restores Normal Platelet Survival

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The use of platelets in transfusion hematology, oncology and trauma therapy has increased dramatically in the last three decades. Cold temperature induces loss of galactosylation/Sialy/sialylation of GpIb with exposure of b-N-acetyl glucosamine of N-linked glycans, which clusters on the membrane. These clusters are recognized by macrophages (aMb2 integrin) and hepatocytes (Ashwell-Morell receptor) resulting in lectin-mediated platelet phagocytosis. Refrigerated platelets are being postulated as an excellent way to treat patients with acute hemorrhage, but refrigerated platelets do function normally but they cannot survive in circulation beyond a few minutes. As a result, current practice of platelet storage for transfusion uses 20-24ÂºC (RT) and associates to a relatively high risk of bacterial growth and infection in susceptible patients. Refrigerated storage lesion seems to depend on the activation of membrane-bound glycos yl- and syalyl-transferases, which are required for in situ removal of glycosyl residues of glycoproteins. We investigated whether RhoA, Rac and/or Cdc42, signal transducers of the family of Rho GTPases, which switch between active (GTP-bound) are inactive (GDP-bound) states are crucial regulators and can be targeted to prevent the cold storage lesion of platelets. Refrigeration activates RHOA and RAC, but not CDC42. RHOA inhibition with G04 prevents cold-induced platelet clearance after either long-term cold storage of human platelets. RHOA inhibition prevents impairment of normal extent of shape change and spreading on fibrinogen. Our results show that while untreated human platelets transfused into NSG mice continue to have a short lifespan due to macrophage independent phagocytosis; however, treatment with triple cocktail or G04 (RHOA) and NSC (RAC) inhibitors alone was sufficient to extend the survival of 7 day stored platelets in the levels of RT stored control platelets survival and normal function both in murine and human platelets. Our data also demonstrated that G04 mediated inhibition of RHOA maintains collagen induced shape change and aggregation of human platelets, restores bleeding time correction activity of cold stored platelets in mice. We elegantly demonstrated that activation of RHOA/RAC1 inhibition improve cold storage of platelets and help in many therapies.

**OP-19**

**Inducible Myd88/CD40 Costimulation to Enhance the Antitumor Activity of CD123-ENG T-Cells for the Immunotherapy of AML**

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Background: The outcome for pediatric acute myeloid leukemia (AML) remains poor and T-cell based immunotherapy has the potential to improve outcomes. CD123-ENG T-cells are cells genetically modified to secrete bispecific antibodies that recognize CD3+ T-cells and CD123+ AML. Preclinical studies show that these cells recruit bystander T-cells to kill CD123+ blasts in vitro and in vivo. However, CD123-ENGs are unable to maintain sequential killing capability of CD123+ targets. To overcome this limitation, we have devised an approach that provides inducible Myd88/CD40 costimulation activated by a chemical inducer of dimerization (CID). This strategy should increase the persistence, expansion and anti-AML activity of CD123-ENG T-cells.

Methods: We generated a retroviral vector encoding a CD20 safety switch, CD123-ENG, and the inducible costimulatory molecule MyD88.CD40 (iMC) linked by 2A sequences (CD20.2A.CD123-ENG.2A.iMC). We used a vector specific for CD19 (CD20.2A.CD19-ENG.2A.iMC) and non-transduced (NT) cells as controls for non-specific effects of the iMC construct. We genetically modified T-cells using a retroviral transduction protocol and evaluated their effector function +/- CID. We assessed the effector function of transduced T-cells using standard immunological assays and a flow cytometry based sequential killing assay.

Results: We successfully generated CD20.CD123-ENG.iMC T-cells, which maintained a transduction efficiency above 50% throughout our study period. We performed coculture and cytotoxicity assays using NT, CD20.CD123-ENG, CD20.CD19-ENG.iMC and CD20.CD123-ENG.iMC T-cells +/- CID as effectors and MOLM13 (CD123+), Kg1a (CD123+) and K562 (CD123-) as targets. Cocultures were performed +/- CID. CD20.CD123-ENG.iMC T-cells maintained CD123 antigen specificity, as evidenced by cytotoxicity and cytokine assays. CD20.CD123-ENG.iMC T-cells + CID secreted increased IL-2 and IFN-Î³ in the presence of CD123+ targets (MOLM13) when compared to baseline and to CD20.CD123-ENG T-cells. In addition, CD20.CD123-ENG.iMC T-cells + CID displayed enhanced sequential killing capabilities at a 1:1 ratio, compared to CD20.CD123-ENG T-cells. CD20.CD123-ENG.iMC T-cells + CID had potent anti-leukemia activity in an In vivo mouse xenograft model resulting in a significant survival advantage over CD20. CD123-ENG group (N=5 mice per group, p=0.0039).

Conclusion: CD20.CD123-ENG.iMC T-cells recognize and kill CD123+ AML blasts in an antigen dependent manner. In addition, control ENG. iMC T-cells have no antitumor activity, indicating that activation of Myd88/CD40 does not induce nonspecific AML blast killing. CD20.CD123-ENG.iMC T-cells have improved effector function in the presence of CID as judged by increased antigen-specific cytokine production and increased antitumor activity in vitro and in vivo.

**OP-20**

**Latexin Inhibition Mitigates Irradiation Induced Hematopoietic Injury**

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Radiation-associated bone marrow (BM) injury is one of the most serious limiting factors of radiotherapy. However, molecular mechanisms underlying radiation-induced HSC functional decline are largely unknown.

We previously demonstrated that HSCs in latexin (Lxn) knockout mice (Lxn-/-) had increased self-renewal and survival. In our new findings, we surprisingly found that Lxn-/- mice had the significant survival advantages under lethal dose of total body irradiation (TBI). We further found that HSCs and hematopoietic progenitor cells (HPCs) recovered much faster in Lxn-/- mice than wild-type mice (WT) within one month after sub-lethal dose of TBI. The better preserved HSC/HPC pool was due to the significantly decreased apoptosis of HSCs/HPCs cells in Lxn-/- mice than WT mice. These data suggest that Lxn inactivation protects HSCs and HPCs from radiation-induced cell death, thus mitigating acute hematopoietic suppression and conferring a survival advantage.

To determine the long-term effect of TBI on Lxn-/- HSCs, we performed limiting dilution competitive repopulation unit assay (CRU), and found that Lxn-/- CRU was significantly higher than WT CRU. Moreover, we performed serial transplantation experiment, and found that Lxn-/- HSC continuously regenerated blood and bone marrow cells even at the 4th round of transplantation, whereas WT HSCs were exhausted. Radiation can increase the risk of hematological malignancy later in life. We found that Lxn-/- mice did not spontaneously develop hematopoietic malignancies, their bone marrow HSCs/HPCs had normal population size, and bone marrow had normal histopathology at 20 months after the split low doses of TBI (2Gy daily for 6 days). These data suggest that Lxn inactivation mitigates radiation-induced short-term myelosuppression and long-term HSC functional impairment without induction of hematologic malignancy.

At the molecular level, we previously reported that Lxn sensitized leukemogenic cells to gamma-irradiation-induced cell-cycle arrest and cell death through Rps3 pathway, and Rps3 was a binding protein of Lxn. Rps3 has been shown to be involved in the NFkB pathway. We found that Rps3 bound Lxn in primary hematopoietic stem and progenitor cells (HSPCs) using Co-IP assay. Lxn-/- HSPCs had the increased expression of Rps3 and NFkB p65 before or post-irradiation. Knockdown of Rps3 in Lxn-/- HSPCs decreased NFkB p65 and increased radiation-induced apoptosis. Moreover, when Lxn-/- HSPCs were treated with NFkB p65 specific inhibitor, the similar phenotypes were also shown, suggesting that Lxn functions through Rps3-NFkB-mediated pro-survival pathway in primary HSPCs.

In conclusion, latexin inhibition mitigates irradiation induced hematopoietic injury via Rps3-NFkB-mediated pro-survival pathway.

**OP-21**

**Characterization of Stromal Cell Progenitors from Mouse Bone Marrow**

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The bone marrow microenvironment is composed of heterogeneous cell populations of non-hematopoietic cells with complex phenotypes and undefined trajectories of maturation. The mesenchymal cells maintain the production of stroma, bone, fat and cartilage cells, but resolving these unique cellular subsets within the bone marrow remains challenging. We have used an unbiased approach of single-cell RNA-sequencing for the non-hematopoietic cells to characterize specific subpopulations. By combining computational prediction of the cell state hierarchy with known expression of key transcription factors, we mapped differentiation paths from stromal cells to the osteoblast, chondrocyte, and adipocyte lineages. Our analysis reveals differentiation hierarchies for maturing non-hematopoietic cells, determines the key transcription factors along these trajectories, and provides an understanding of the complexity of the bone marrow microenvironment. Still, as compared to the hematopoietic system, mesenchymal stem and progenitor cells remain fairly unrefined. However, like hematopoietic stem and progenitor cells, stromal progenitor cell potential can be assessed through their colony-forming and multi-lineage differentiation. Using our newly discovered trajectories and knowing that mesenchymal stem/progenitor cells are defined in culture by their differentiation capacity, we validated the multipotentiality of the isolated cells. We combined limit-dilution assays for progenitors of adipocytes and osteoblast or chondrocyte associated cells with colony assays for progenitor potential. Progenitors with an absence in the expression of fate markers are remarkably enriched in clonal progenitors, and tri-lineage mesenchymal potential. We show the prospective identification, purification and characterization, using lineage specific markers and flow cytometry, of complementary progenitors that gives rise to each associated lineage in culture. Using this information, we have initiated and uncovered novel signaling pathways involved in stromal cell maintenance.

**OP-22**

**Adaptation of the HSC Compartment in Chronic Stress Condition: The Case of Obesity**

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Lifelong maintenance of the blood system requires the preservation of a healthy hematopoietic stem cells (HSC) pool. Integrity of the HSC compartment is disrupted by severe homeostatic perturbations such as acute infection, prolonged nutrient deprivation or irradiation, which can result in HSC loss and lead to dramatic hematological dysfunctions. HSC integrity is also affected by organismal pathophysiological conditions, which are associated with chronic low-grade stress. Although less acute, these conditions present, over time, a unique challenge to the maintenance of the long-lived HSCs. How the HSC pool adapts these conditions remains largely unknown. In our studies, we use obesity as a model of chronic low-level stress that combines inflammatory, oxidative and hormonal homeostatic perturbations. In this context, we describe the impact of chronic low-level stress on HSC activity and discuss several intrinsic adaptive mechanisms put in place to maintain the integrity of the HSC pool.

**OP-23**

## Noninvasively Screening Hematopoietic Stem and Progenitor Cell Responses to Extrinsic Cues in Microscale Screening Platforms

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Identification of the extrinsic cues that direct hematopoietic stem cells (HSCs) to self-renew, remain quiescent, or differentiate into distinct populations would facilitate efforts to expand specific hematopoietic cell populations for disease treatment. Due to the multitude of cellular and matrix cues that must be screened and the rarity of HSCs in the body, microscale biomaterial substrates with spatial variations in cellular and matrix cues have been developed to minimize the number of rare HSCs that are required for screening. These microscale screening platforms create a strong demand for noninvasive methods to accurately identify the fate decisions of individual hematopoietic cells at specific locations on the platform. Towards this aim, we have shown that a combination of Raman spectroscopy and multivariate spectral analysis enables noninvasively identifying the lineage-specific differentiation stages of individual hematopoietic cells in cultures with location specificity. In addition, the composition of the biomaterial substrate beneath each cell does not compromise our ability to accurately identify hematopoietic cell differentiation stage. We are now creating a microscale screening platform that combines a combinatorial microarray of biomaterial islands with single-cell Raman spectroscopy to enable noninvasively screening the effects of extrinsic cues on HSC fate decisions. The resulting platform is expected to facilitate correlating hematopoietic stem and progenitor cell fate decisions with the extrinsic cues that elicited them.

**OP-24**

## Parameters Contributing to the Role of Osteomacs in Regulating Stem Cell Function and the Hematopoietic Niche

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Networking between hematopoietic stem cells (HSC) and cells of the hematopoietic niche is critical for the maintenance of stem cell renewal and function. Among the cellular components of the niche participating in this function are a group of specialized bone-resident macrophages known as osteomacs (OM). Using multiple approaches such as single cell qPCR, flow cytometry, CyTOF and functional assays, we determined that calvarie-resident OM are phenotypically and functionally different from marrow-derived macrophages. Furthermore, we showed that OM are relatively radioresistant and survive several weeks post lethal radiation. However, they eventually deplete and are replenished by HSC derived OM, demonstrating that they are transplantable. Our studies revealed that, OM were required for the hematopoiesis enhancing activity of osteoblasts (OB) and this activity was augmented by megakaryocytes (MK). Serial transplantation demonstrated that HSC repopulating potential was best maintained by in vitro cultures containing OB, OM, and MK illustrating the importance of cross talk between these three cell types in maintaining HSC function. We performed single cell mRNAseq to assess changes in gene expression observed due to these interactions. Several genes such as lmo2, fli1 ikzf1, PF-4 and embigin were upregulated in OM cultured in the presence of MK. Further studies using CyTOF indicated the elevated expression of CD166 and embigin on OM cultured with MK. Next, we used clonogenic assays to demonstrate that CD166+ and embigin+ OM played a critical role in maintaining hematopoietic activity in vitro. These results were validated using recombinant CD166 and embigin protein to partially substitute for OM function thus validating our mRNAseq data. Interestingly, CD166 knockout OM were unable to mediate the same hematopoietic enhancing activity observed with WT OM regardless of the presence or abse nce of MK in culture. Similarly blocking embigin in neonatal calvarial cells reduced hematopoietic enhancing activity. These data demonstrate that CD166 and embigin are important mediators through which OM maintain HSC function in vitro and that MK augment the activity of OM through these mediators. Overall, our data demonstrate distinguishing phenotypic and functional characteristics between calvarie-resident OM and marrow-derived macrophages and demonstrate the importance of crosstalk between OM, OB and MK to enhance the expression of novel mediators such as CD166 and embigin to support HSC function.

**OP-25**

**Correlative Light Sheet and Electron Microscopy Defines the Ultrastructure of a Single Hematopoietic Stem Cell in the Endogenous Niche**

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The fetal bone marrow is colonized by circulating hematopoietic stem and progenitor cells (HSPCs), however this important event in niche establishment is poorly understood. The hematopoietic system of the transparent zebrafish model is highly conserved, and allows us to directly observe all stages of hematopoietic development. HSPC emergence from the dorsal aorta and colonization of the fetal liver-like caudal hematopoietic tissue (CHT) have been previously characterized. However, colonization of the larval kidney, which is the presumptive adult niche, has not been observed. To track HSPCs during kidney colonization, we used our previously developed HSPC reporter lines, Runx:GFP and Runx:mCherry. Using light sheet microscopy, we performed live imaging of the entire kidney niche during arrival of the earliest HSPCs. We tracked interactions between HSPCs and the perivascular niche. Similar to our previous studies of the CHT, we found that HSPCs lodge in a perivascular niche and are closely associated with a group of endothelial cells. To resolve the ultrastructure of a single HSPC in the endogenous niche, we performed correlative light sheet and electron microscopy (EM). Immediately after light sheet imaging, embryos were fixed and embedded for EM, followed by microCT as an intermediate step to orient and trim the sample. Next, serial block-face scanning EM was used to capture high resolution serial sections. To correlate the position of a single cell between different imaging modalities, we genetically labelled HSPCs with mCherry fluorescent protein for live tracking, and the engineered peroxidase APEX2 to deposit electron dense DAB stain for detection on electron micrographs. This approach has identified HSPCs in distinct niche structures made up of one HSPC, one mesenchymal stromal cell, and five endothelial cells. Our goal is to identify novel subcellular structures that form between an unperturbed HSPC and its endogenous perivascular niche.

# POSTER ABSTRACTS

**PP-26**

**Analysis on the Clonal Dynamics of Blood Precursors During Embryonic Development in a Non-Invasive Multi-Color Genetic Approach**

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In the mouse, the first Hematopoietic Stem Cell (HSC) is detected by transplantation at embryonic day E10.5. HSC emerge in the Aorta-Gonad-Mesonephros Region and migrate to the Fetal Liver (FL) where they are supposed to dramatically expand in number (by about 20-fold) between days E12.5-E15.5. HSC will finally migrate to the Bone Marrow (BM), where they will reside mainly during the adult life. Challenging previous dogma, we recently described that lifelong hematopoiesis is established by hundreds of blood precursors throughout mouse ontogeny instead of just a handful as previously assumed from transplantation studies. Our novel non-invasive approach based on a multi-color allele has allowed us to re-interrogate the clonal dynamics of blood precursors avoiding the dramatic stress of transplantation into conditioned recipients. In contrast to previous assumptions, we failed to detect a 20-fold expansion in the number of blood precursors during the FL expansion period. In contrast, we observed just a three-fold expansion from E11.5 to E15.5. These results are of critical importance as extensive efforts are being invested on uncovering critical components of the FL niche in an attempt to expand HSC ex vivo. Our work suggests that the FL might not constitute such an expansion niche. It is possible that in the FL, HSC are mainly undergoing asymmetric cell divisions and/or that blood precursors are maturing into HSC.

Further, we observed that the number of HSC expand about 5-fold from E15.5 to day 1 post-birth (P1). By about P9, all HSC have completed their migration into the BM, at this stage a 1.5-fold expansion was detected from P1 to P9. Additional analyses are on their way to unveil the cellular mechanisms supporting these clonal dynamics.

In summary, our study constitutes the first comprehensive and non-invasive quantification on the clonal dynamics of blood precursors during mouse embryonic development and early post-birth stages.

**PP-27**

**Somite Patterning and Its Role During HSC Niche Formation**

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Hematopoietic Stem Cells (HSCs) are born from endothelial cells in the floor of the primitive descending aorta, known as the dorsal aorta in zebrafish. A better understanding of the cellular specification niche that regulates the birth of these cells might inform attempts to instruct their specification in vitro. Recently, Wnt16, a non-canonical Wnt, was demonstrated to be required for HSC development in zebrafish. Wnt16 signals through a series of downstream relay signals, but the final signal(s) most proximal to HSC specification remain unknown. In Wnt16 loss of function animals, there is also an earlier defect in a compartment of the somite, the sclerotome, which may house vascular smooth muscle cells. These results suggest that sclerotome-derived cells might contribute to the regulatory environment, or "niche," that directs HSC specification, explaining the failure of HSC specification when this compartment develops incorrectly. We have found that loss of sclerotomal genes results in loss of HSCs and are further investigating the role of this somite compartment in HSC niche formation. Additionally, we are interested in understanding how the sclerotome is compartmentalized into its various fates and what proximal signals are required for its development. Our studies will provide a foundation to define the in vivo cellular specification environment and elucidate the complete set of signals involved in HSC specification, with the ultimate goal of informing clinical efforts at directed differentiation of pluripotent stem cells to an HSC fate.

Funding Sources: NHLBI: 5R00HL097150 and 1F32HL129819, March of Dimes #5-FY14-42

**PP-28**

**Regulation of Homeostatic and Malignant B Cell Development by the Tetraspanin CD53**

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Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy, most commonly originating from the transformation of progenitor cells of the B cell lineage (B cell precursor-ALL; BCP-ALL). Treatment of patients with high-risk or relapsed disease is difficult and prognosis remains poor in pediatric patients. Previous studies have shown an association of enhanced CD53 expression with many B cell malignancies, suggesting upregulation of CD53 may be implicated in carcinogenesis or maintenance of malignant cells. CD53 is a member of the tetraspanin family of transmembrane proteins, classically involved in cell adhesion, proliferation, and survival, and expressed exclusively on hematopoietic cells. While several studies have implicated a role for CD53 in regulating mature B cell proliferation, its role in early B cell development is not yet known. To elucidate the contribution of CD53 to normal and malignant B cell development, we have generated a CD53 knockout mouse.

In our CD53-/- mouse, we observe no differences in total white blood cell counts, yet the fraction of peripheral blood B cells is significantly reduced by 31% compared to wild-type (WT) controls (28.3% vs. 19.5%; $p<0.005$). During homeostatic B lymphopoiesis, CD53 increases through development, beginning at the pre-pro-B cell stage and reaching highest expression on mature B cells. Further investigation into the loss of B cells revealed that immature pre-B cells in the bone marrow and mature B cells in the spleen and lymph nodes are significantly diminished upon loss of CD53, resulting from decreased IL-7 receptor expression and increased apoptosis in CD53-/- mice. B cell differentiation of CD53-/- hematopoietic stem cells (HSCs) in vitro corroborates the dependence on CD53 for normal differentiation, as CD53-/- cultures have 26% fewer B cells than controls ($p=0.033$). Investigation into the signaling differences between WT and CD53-/- B cell progenitors by mass cytometry (C yTOF) shows that PI3K/Akt signaling is significantly decreased in CD53-/- early B cells. Additionally, downstream PI3K target gene expression is shifted from pro-survival/proliferation to pro-apoptosis in CD53-/- B cell progenitors.

With the observed loss of both B cell progenitors and mature B cells in CD53-deficient mice, CD53-/- mice were recently crossed to Emu-Myc transgenic mice, a model of B-lineage leukemia/lymphoma, to generate WT, CD53-/-, Emu-Myc+;CD53+/+, and Emu-Myc+;CD53-/- groups to assess whether loss of CD53 alters the pathology or survival of these mice. As observed in human patients, moribund Emu-Myc+ mice significantly upregulate CD53 on malignant cells, suggesting a potential role for CD53 during pathogenesis.

**PP-29**

**Flt3-ITD Target Enhancers Are Poised but Inaccessible in Fetal Hematopoietic Progenitors**

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The FLT3 Internal Tandem Duplication (FLT3ITD) mutation is common in adolescent and adult acute myeloid leukemia (AML), but it is rare in infant AML. One explanation for why infant leukemias rarely harbor FLT3ITD is it fails to convey a selective advantage to fetal or neonatal hematopoietic progenitors. Indeed, we have previously shown that FLT3ITD fails to induce changes in HSC self-renewal, myelopoiesis and leukemogenesis during fetal stages of life. FLT3ITD signal transduction pathways are hyperactivated in fetal progenitors, but FLT3ITD target genes are not. This suggests that postnatal-specific transcription factors may be required to help induce FLT3ITD target gene expression. Alternatively, repressive histone modifications may impose a barrier to FLT3ITD target gene activation in fetal HPCs that is relaxed during postnatal development. To resolve these possibilities, we used ATAC-seq, as well as H3K4me1 and H3K27ac ChIP-seq, to identify cis-elements that putatively control FLT3ITD target gene expression in fetal and adult hematopoietic progenitor cells (HPCs). We identified many enhancer elements (ATAC-seq peaks with H3K4me1) that exhibited increased chromatin accessibility in FLT3ITD adult HPCs relative to wild type adult HPCs. These elements were enriched near FLT3ITD target genes. FLT3ITD-dependent changes in chromatin accessibility were not observed in fetal HPCs, though the enhancers were poised early in development as evidenced by the presence of H3K4me1. HOMER analysis showed enrichment for STAT5, ETS, RUNX1 and IRF binding motifs within the FLT3ITD target enhancers, but motifs for temporally dynamic transcription factors were not identified. We considered that FLT3ITD target enhancers might harbor repressive histone marks that restrict target gene expression in the fetal stage. Indeed, ChIP-qPCR demonstrated enrichment for the PRC2 mark, H3K27me3, in fetal but not adult HPCs. Functional experiments are ongoing, but the data suggest that fetal specific H3K27me3 patterns may protect fetal HPCs from FLT3ITD-mediated changes in gene expression and transformation.

**PP-30**

**Understanding the Role of ULK1 and ULK2 in Hematopoietic Stem Cells**

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Hematopoietic stem cells (HSCs) are long lived and persist throughout the adult life of an organism, thus both quality control mechanisms and maintenance of cellular homeostasis are critical to their proper function. Regardless of whether hematopoietic cells are maintained at steady-state levels in the bone marrow or are rapidly mobilized to the spleen or liver during times of stress, autophagy is thought to be essential in promoting cellular homeostasis during HSC progression and aging. There have been several components of the autophagy pathway examined in the context of hematopoiesis, such as Atg7 and Fip200. Atg7-deficiency causes a severe defect upon bone marrow transplantation and a milder defect in fetal liver cell transplantation. In contrast, Fip200-deficient fetal liver cells from hematopoietic-specific conditional knockout (cKO) mice failed completely in the transplant setting. To further examine genetic determinan ts involved in regulating autophagy in HSCs, our lab is investigating the role of the autophagy-inducing kinases, ULK1 and ULK2, which together with other autophagy proteins (i.e. Atg13, Fip200 and Atg101) form the ULK/Atg1 initiation complex. As part of the initiation complex, ULK/Atg1 phosphorylates and activates downstream components of the autophagy pathway in response to nutrient and energy deprivation. Work from our laboratory revealed that in addition to their well-characterized roles in autophagy, ULK1 and ULK2 have a noncanonical function in ER-to-Golgi trafficking of specific under basal conditions. In addition, our work showed that ULK1 and ULK2 are not required for constitutive autophagy under basal physiologic conditions in the brain. To investigate ULK/Atg1's role in HSCs, we recently transplanted ULK1/2 cDKO (Vav Cre) bone marrow cells into lethally irradiated mice. The transplanted cells show a defect in short-term and long-term reconstitution of the bone marrow. We are in the process of characterizing the reconstitution defect to gain further insight into the role of ULK1/2 in stem cells. We are also examining the role of ULK1/2 in fetal liver cell reconstitution, given that autophagy is required for stem cell maintenance in fetal liver as well. The information gained by thoroughly investigating the requirement for ULK1/2 in HSCs may lead to new options for therapeutically manipulating HSCs for better bone marrow transplantations as there are already ULK agonists and inhibitors available.

**PP-31**

**Identifying the LSC Niche for Flt3 TKI Resistance in Flt3-ITD Acute Myeloid Leukemia**

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Acute myeloid leukemia (AML) is an aggressive cancer with a poor relative 5-year survival (~27%). One of the most common mutations in adult human AML (about 30% of patients) is a constitutively activating internal tandem duplication in FMS-like tyrosine kinase receptor 3 (Flt3-ITD), and patients with this mutation have a significantly worse prognosis than patients with wild-type Flt3. Flt3-ITD mutations often co-occur with loss-of-function mutations in DNA demethylation pathway genes (e.g. IDH1, IDH2, and TET2). We and others have shown that Flt3-ITD knock-in, conditional TET2 knock out mice develop AML and exhibit clinical features similar to those of human AML patients. There are Flt3-specific tyrosine kinase inhibitors (TKIs) which are FDA-approved for treating AML patients with Flt3-ITD mutations, but they frequently develop resistance to these treatments and relapse within months. There is mounting evidence that the bone marrow microenvironment plays an essential role in shielding leukemic stem cells (LSCs) from therapy, leading to relapse despite the elimination of the majority of the leukemic bulk during treatment. We have characterized a new Flt3-ITDki TET2-/- mouse model and its disease phenotype and identified the hematopoietic population that contains the LSC. In most models of AML driven by mutations other than Flt3-ITD, the LSC is contained in the committed granulocyte-macrophage progenitor (GMP) compartment, but we have shown that in this Flt3-ITD model the LSC is found in the more primitive compartment of the short-term hematopoietic stem cells (ST-HSC), and is very rare. We are also in the process of identifying the LSC-containing hematopoietic population in Flt3-ITD, TET2-mutated human AML by FACS sorting and transplanting candidate populations from patients into NSG mice. Others have identified an LSC population in some patients that phenotypically resembles a lymphoid-primed multipotent progenitor, but whether this is present in Flt3-ITD TET2-mutant AML has yet to be determined. We seek to further characterize this rare LSC population and identify its LSC niche using stromal lineage-specific CXCL12 KO mice. CXCL12/CXCR4 signaling has been extensively studied in AML, including clinical trials with CXCR4 inhibitors in combination with chemotherapy or TKI, and we propose that stromal lineage-specific knockout of CXCL12 can be a useful tool in identifying the stromal population(s) essential for TKI resistance in Flt3-ITD AML. Others have used a similar technique to identify stromal sources of CXCL12 that are essential for maintenance of various normal hematopoietic populations, including CXCL12 from mesenchymal stem cells and endothelial cells being essential for normal HSC and CXCL12 from osteoprogenitors being important for early B lymphoprogenitors. Our group has also used a similar method to identify the LSC niche in CML. We hypothesize that Flt3-ITD TET2-mutant AML LSC depend on an osteoprogenitor-rich niche for TKI resistance.

**PP-32**

**Genetic Dependency of Myeloproliferative Neoplasms (MPNs) on DUSP1**

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Myeloproliferative neoplasm (MPNs) are clonal stem cell disorders characterized by increased production of mature blood cells. Ph-negative MPNs (Ph- MPNs) encompass polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) and chronic neutrophilic leukemia (CNL), amongst others. The discovery of Jak2 kinase activating mutations and dependence upon constitutive JAK2 signaling in MPNs induced by MPLW515L, CALRfs and CSF3RT618I provided a strong rationale for Jak2 targeting. However, unlike imatinib (BCR-ABL inhibitor) treatment in chronic myeloid leukemia (CML), Jak2 targeting by small molecule kinase inhibitor (ruxolitinib) does not eliminate the mutant clone. Besides, inhibition or genetic deletion of Jak2 kinases causes substantial hematologic toxicity that includes moderate to severe thrombocytopenia and anemia, as well as non-hematological adverse events, including neurological toxicity. The later led to the discontinuation of clinical development of several JAK2 inhibitors. Furthermore, mouse knock-out studies suggest that prolonged inhibition of Jak2 will be detrimental for normal hematopoietic and adult tissue homeostasis. These observations warrant identifying additional therapeutic targets that must be safe and able to eliminate the mutant clone.

Results.

Recently we have shown that the expression of FOS and DUSP1 regulate apoptotic threshold in both hematopoietic stem cells (HSCs) and leukemic stem cells (LSCs), Kesarwani et. al, Nature Medicine 2017. We reasoned that MPN induced by activated Jak2 signaling, similar to LSCs in CML, might have elevated expression of c-Fos and Dusp1 resulting to a higher apoptotic threshold, and thus, abrogating the cytotoxic effect of Jak2 inhibitors. As envisioned, a whole genome RNA-seq analysis revealed higher expression of Dusp1 (4-10 fold in comparison to normal cells), but not the c-Fos. Likewise, human primary MPN patient samples showed higher expression of DUSP1, but not the c-FOS. Interestingly, mice transplanted with Jak2V617F expressing bone-marrow cells show dependence on Dusp1. Genetic deletion of Dusp1 is synthetic lethal to CSF3RT618I and Jak2V617F expressing hematopoietic cells. Mice transplanted with BM cells expressing CSF3RT618I and Jak2V617F lacking Dusp1 show gradual depletion and completely cleared from the bone marrow within five weeks of post-transplantation. In contrast, mice transplanted with cells expressing pMSCV-Ires-GFP (vector) show stable engraftment. These data provide evidence that CSF3RT618I and Jak2V617F uniquely addicted to Dusp1 and crucial for disease pathogenesis. These data provide a rationale for targeting the Dusp1 in MPNs to eliminate the mutant clones and cure the disease.

**PP-33**

**Alterations to Methionine/S-Adenosylmethionine Metabolism Regulate Dot1L Expression and Function; a Novel Vulnerability in MLL Fusion Leukemia**

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Dot1L, also known as DOT1-like (Disruptor of telomeric silencing 1-like) histone H3K79 methyltransferase, is a highly conserved histone methyltransferase (HMT), whose function has been implicated in regulation of crucial cellular processes including development, transcriptional and cell cycle control, DNA repair, and cancer/leukemia. Dot1L is the only known methyltransferase to catalyze the sequential methylation of H3K79 (mono-, di-, or tri-), a modification generally accepted as transcriptionally activating, and is enriched at transcribed promoters. Specifically, an aggressive subset of leukemia driven by translocations resulting in aberrant expression of oncogenic MLL fusion proteins (MLL-FPs), are uniquely dependent on the epigenetic function of Dot1L for maintenance of leukemic gene expression and survival. While transcriptional regulation mediated by Dot1L dependent H3K79 methylation of histones within target gene promoters is well studied, especially in the context of MLL-FP leukemia, regulatory mechanisms controlling expression of Dot1L itself remain relatively elusive and uninvestigated. Here, we show for the first time that alterations to methionine (Met)/S-adenosylmethionine (SAM) metabolism control expression and function of Dot1L through epigenetic regulation at the DOT1L promoter in MLL-FP driven leukemia. Moreover, targeted inhibition of this metabolic pathway induced apoptosis and DNA damage in MLL-FP expressing cell lines and patient samples, as well as extended the survival of mice xenografted with patient MLL-FP leukemia in combination with standard of care therapy. Inhibition or alteration to Met/SAM metabolism may provide a unique, targetable vulnerability in MLL-FP driven leukemia. Further investigation may reveal universal regulatory mechanisms controlling Dot1L expression to be intrinsically linked to Met/SAM metabolism.

**PP-34**

## Jarid2 Functions as a Tumor Suppressor in Myeloid Neoplasms by Repressing Self-Renewal in Hematopoietic Progenitor Cells

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The clonal evolution of myeloproliferative neoplasms (MPN) to secondary acute myeloid leukemia (sAML) is driven by sequential acquisition of co-operating genetic mutations. However, the contribution of specific genetic events that drive sAML transformation is not well understood. One such event is chromosomal deletion of the short arm of chromosome 6, which contains JARID2. These deletions are typically hemizygous, encompass only JARID2, and occur specifically upon progression to sAML. This implicates JARID2 as a tumor suppressor in chronic myeloid neoplasms.

To investigate the function of JARID2 as a tumor suppressor in MPN, we crossed Jarid2fl/fl mice to Mx1-Cre:Jak2V617F/+ (Jak2V617F) mice. Mx1-Cre expression that is induced by pIpC activates both mutations simultaneously. Jak2V617F mice developed a polycythemia vera-like MPN with a median survival of 104-days. However, either heterozygous or homozygous deletion of Jarid2 in Jak2V617F background accelerated disease progression and significantly reduced survival (median survival= 42-days and 19-days, respectively). Using potent shRNAs, we next inactivated JARID2 in CD34+ hematopoietic stem and progenitor cells (HSPCs) isolated from JAK2V617F-mutant myelofibrosis (MF) patients, and transplanted these cells into sublethally irradiated NSGS mice. Remarkably, JAK2V617F-mutant patient HSPCs, which normally fail to engraft NSGS mice, efficiently engrafted when JARID2 was inhibited. These engrafted recipients also produced hallmarks of MF, such as splenomegaly, polycythemia, thrombocytosis, and fibrosis in the BM. Collectively, these results establish Jarid2 as a bona fide MPN tumor suppressor. To investigate how loss of Jarid2 contributes to enhancement of the MPN phenotype, the mRNA expression profiles of hematopoietic stem cells (HSCs) with or without mutations in Jak2 and Jarid2 were compared. RNA sequencing revealed that Jarid2 acts as a tumor suppressor possibly by silencing pro-survival signaling (such as further elevation of JAK/STAT pathway) and self-renewal programs (such as HoxA cluster genes) in a Jak2V617F background.

We next sought to elucidate the role of Jarid2 in normal hematopoiesis. Competitive transplants suggested that loss of Jarid2 prevents phenotypically-defined HSCs to differentiate in vivo. Instead, Jarid2-KO multipotent progenitor populations (MPPs) gain self-renewal capacity to a similar extend compared to normal HSCs. ChIPmentation in combination with RNA sequencing revealed that the role of Jarid2 is to silence self-renewal networks in MPPs by recruitment of the polycomb repressive 2 (PRC2) complex to execute the repressive chromatin mark of H3K27me3. Cumulatively, our data suggest that the normal role of Jarid2 in hematopoiesis is to restrict self-renewal capacity to long-term HSCs, and that dysregulation of this process can lead to hematopoietic malignancies.

**PP-35**

**TNFAIP3/A20 is Required for MLL-Rearranged AML**

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Acute myeloid leukemia (AML) is characterized by a block in differentiation and acquired self-renewal properties of myeloid progenitor cells, leading to an accumulation of blasts in the bone marrow, which results in anemia and increased susceptibility to infection. Current treatment options include chemotherapy and/or bone marrow transplant, both of which can be taxing even for an otherwise healthy, young individual. A complex interplay of molecular, genetic, and epigenetic events contributes to the pathogenesis of AML. A20, or TNFAIP3, is a unique ubiquitin-editing enzyme with both E3 ligase and deubiquitinating activities that are essential for negative regulation of immune responses and necrosis by restricting NF-kB signaling and RIP1 signaling, respectively. Inactivating mutations of A20 are associated with lymphoid disorders, however, somewhat unexpectedly, we have determined that acquired A20 function may be involved in the initiation and progression of AML.

**PP-36**

**Leukemia Risk Dene Arid5b is a Crucial Regulator of B Cell Development**

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Among lymphoid malignancies, acute lymphoblastic leukemia (ALL) has a particular over-representation in children and there is growing evidence for inherited predisposition. Genome wide association studies by us and others have identified common non-coding germline variants at the ARID5B locus that are strongly associated with the risk of developing ALL during childhood, accounting for ~30% of all genetic susceptibility to this type of leukemia. However, the exact effects of the risk variants on ARID5B functions and the molecular mechanism linking ARID5B to leukemogenesis remain largely unknown. We first comprehensively sequenced the entire ARID5B locus in ~5,000 children with ALL and identified 123 variants significantly associated with leukemia susceptibility. In parallel, we engineered an ALL reporter cell line with fluorescently labeled ARID5B, in which we used to perform high throughput screening of all potential regulatory elements at this locus. In this assay, we used the CRISPR/dCas9 system to epigenetically silence 19 predicted enhancer sequences and defined 6 elements at which KRAB-mediated enhancer disruption led to significant downregulation of cellular ARID5B transcription. Strikingly, the top ALL risk variant rs7090445 is situated precisely within the strongest enhancer that is distally looped with the ARID5B promoter and this polymorphism results in the disruption of transcription factor MEF2C binding as confirmed by ChIP seq. Taken together, our data suggest that ALL risk variant impairs the activity of a cis-acting enhancer and thus negatively affects ARID5B transcription by compromising MEF2C binding in hematopoietic cells. Next we sought to characterize the role of Arid5b in normal and malignant hematopoiesis, using a variety of Arid5b mouse models. First using a vav specific Arid5b overexpression (AOE) mouse line, we systematically quantified the proportions of mature, as well as various stem and progenitor cell populations, during hematopoiesis at different ages. Overexpression of Arid5b resulted in a dramatic reduction in the proportion of peripheral blood B cells, all B cell fractions (Hardy factions) in the bone marrow, and specifically follicular B cells in the spleen. In vitro B cell activation assays also show significant defects of AOE B cells with IgM+ IL-4 stimulation. Older AOE mice exhibited anemia, hypergammaglobulinemia, and splenomegaly, with reduced white blood cells, red blood cells, and platelets and higher reticulocyte numbers and increased serum erythropoietin concentration. Arid5b is an important regulator of B lymphopoiesis; and enhancer polymorphisms in this gene may affect ALL pathogenesis by regulating ARID5B transcription and thus B cell development in patients.

**PP-37**

**Abnormal Lymphopoiesis Precedes the Onset of Leukemia in MYC-Transgenic Zebrafish**

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Hyperactivity of the myelocytomatosis (MYC) oncogene contributes to several human cancers, including leukemias, lymphomas, and many other carcinomas. Acute lymphoblastic leukemia (ALL) is an aggressive hematologic malignancy that afflicts both children and adults. ALL develops as a clonal expansion of an early precursor cell of either the B or T lineage, depending upon the specific genetic aberrations that occur, and which lymphocyte lineage they occur in. Over 60% of ALL overexpress MYC, causing global increases in transcription and translation, as well as activation of downstream pathways that drive malignant lymphocytes. Consequently, understanding the molecular mechanisms active in MYC-induced ALL may ultimately lead to the development of effective personalized treatments. Many experimental systems have been used to study MYC in cancers, but zebrafish provide a model organism where genetic manipulation is rapid and simple, and zebrafish develop highly penetrant MYC-driven ALL with short latency. The first zebrafish cancer model was created in 2003, using a zebrafish rag2 promoter to regulate a murine Myc (mMyc) transgene, and this induced T cell ALL (T-ALL) potently. Subsequently, multiple mMyc and human MYC (hMYC) transgenic zebrafish models have been used to study T-ALL. Recently, we discovered that rag2:hMYC zebrafish also develop B cell ALL (B-ALL), not only T-ALL. To identify zebrafish with ALL, and to purify lymphocytes from them, cell-specific fluorophores are often used, because monoclonal antibodies recognizing zebrafish proteins are scarce. One such ‘marker line’ is lck:eGFP. Originally, lymphocyte protein tyrosine kinase (lck) was thought to be only expressed by T lymphocytes. However, we recently found that although T cells express high levels of lck, a B cell subpopulation expresses lower levels of lck, and innate lymphoid (ILCs and NK cells) and even myeloid cells also exp ress lck. Thus, we can use lck:eGFP fish (with or without hMYC) to study several leukocyte populations, and to study lymphopoiesis that precedes the onset of MYC-induced ALL. In this regard, our preliminary data indicate that MYC, regulated by the rag2 promoter, alters the quantity, identity, and gene expression of multiple lymphocyte types, even before the onset of B- or T-ALL. Overall, our findings suggest lymphoid hyperplasia exists in all pre-leukemic hMYC fish, including thymic B cell hyperplasia, a rare and poorly-understood population in WT fish, and in humans. Our current efforts seek to identify the MYC-regulated genes that drive lymphoid hyperplasia, and pathways downstream of MYC that promote progression of lymphoid hyperplasia to B- or T-ALL.

**PP-38**

**Differential Effects of TLR1/2 and TLR2/6 Signaling on Normal and Premalignant Hematopoietic Stem and Progenitor Cells**

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Prior studies have demonstrated a role for Toll-like receptor 2 (TLR2) in normal and premalignant hematopoietic stem and progenitor cell (HSPC) regulation, however the contributions of its binding partners, TLR1 and TLR6, remain unknown. In CD34+ bone marrow cells of patients with myelodysplastic syndrome (MDS), increased TLR2 was associated with lower-risk disease, elevated rates of apoptosis, and enhanced survival. Conversely, increased levels of TLR6, but not TLR1, was associated with higher-risk disease and increased bone marrow blasts. These data suggest that there may be heterodimer-specific effects of TLR2 signaling on HSPCs influencing disease progression.

To elucidate the contributions of the heterodimers in MDS pathogenesis and leukemogenesis, we utilized the NUP98-HOXD13 (NHD13) mouse model of MDS. NHD13 mice were treated chronically with PAM2CSK4 (PAM2), a TLR2/6-specific agonist, or PAM3CSK4 (PAM3), a TLR1/2-specific agonist, to assess the effects on cytopenias and survival. After five months of treatment, a significant increase of total white blood cells was observed in mice treated with PAM2 ($p=0.007$), but not PAM3. Further, death was expedited in NHD13 mice treated with PAM2 compared to the PAM3-treated cohort ($p=0.009$), with a median survival of 269 days vs. 338 days. The most common cause of death was leukemia.

To investigate the mechanism of accelerated leukemogenesis and death in NHD13 mice, the HSPCs of premalignant mice treated with PAM2 or PAM3 were characterized by flow cytometry. The total number of KSL cells was increased in mice treated with PAM2 ($p=0.07$), but not PAM3, vs. controls. KSL frequency was also increased following PAM2 treatment compared to PAM3- or vehicle-treated controls ($p=0.004$ and $p=<0.0001$, respectively). Moreover, NHD13 mice treated with PAM2 demonstrated an increased percentage of cells in cycle ($p=0.0005$ vs. controls) and a decreased percentage of cells undergoing apoptosis ($p=0.02$ vs. PAM3). A microarray of bone marrow HSPCs revealed an activated c-Myc signature, suggesting enhanced TLR2/6 signaling may expedite leukemogenesis via Myc activation. The expression of six downstream targets of c-Myc was increased in WT and NHD13 mice following PAM2 treatment. In addition, evaluation of serum cytokines revealed elevated IL-6 levels in NHD13 mice treated with PAM2 compared to PAM3-treated mice ($p=0.002$). These data corroborate reports linking IL-6 to MDS pathogenesis and leukemic transformation. Ongoing studies aim to further elucidate the mechanism through which TLR2/6 activation specifically accelerates leukemogenesis and death in the NHD13 mouse model and hope to inform targeted therapeutics to delay MDS progression and reduce off-target effects.

**PP-39**

**Nuclear Vav3 Regulates Polycomb Repression Complex Activity and Nuclear Actin Microfilament System in Leukemic B-cell Progenitors**

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Relapse is common in Ph+ B-ALL due to resistance of leukemic progenitors to conventional therapies including tyrosine kinase inhibitors. BCR-ABL+ progenitors appear to develop additional epigenetic and genetic alterations that result in proliferative advantage frequently associated with lymphoid differentiation gene hotspot silencing. Earlier, our group identified activation of Rac2 and its upstream guanine nucleotide exchange factor Vav3 in BCR-ABL mediated leukemogenesis, acting as an oncogene regulating B-cell progenitor survival, proliferation and differentiation arrest (Chang KH et al., Blood 2012, Thomas EK et al., Cancer Cell 2007). Further, we identified Bmi1, a PRC1 complex protein, as a crucial repressor and re-programmer in leukemic B-cell progenitors (Sengupta A et al., Blood 2012). In this study, we found that Vav3 expression and activation are upregulated in leukemic B-progenitors with a predominant nuclear localization, and investigated mechanisms of Vav3 dependent epigenetic repression of factors involved in B cell leukemogenesis. Through proximity ligation and co-immunoprecipitation assays, we identified that nuclear Vav3 interacts with PRC complex proteins including Bmi1 and Ring ubiquitin ligases. Interestingly, Vav3 deficiency impairs the oncogenic effect of Bmi1 overexpression. Biochemically, the overexpression of Bmi1 results in increased nuclear Rac activity and H2AK119 monoubiquitination, which are dramatically impaired in Vav3 deficient cells. Mechanistically, the deficiency of Vav3 led to phosphorylation and inactivation of Bmi1 associated with nuclear activation of the Bmi1 kinase Akt and down regulation of the Akt specific phosphatase Phlpp2. The ectopic expression of Phlpp2 or treatment with an Akt inhibitor reversed the effect of Vav3 deficiency on nuclear Akt activation and Bmi1 phosphorylation. The expressions of Cdkn2a and Pax5, crucial regulators of B-ALL devel opment, are upregulated in Vav3 deficient leukemic B-progenitor cells. ChiP-q-PCR for H2AK119Ub and H3K27me3, another histone mark resulting from PRC activity, demonstrated that the repressive state of Cdkn2a and Pax5 loci is significantly reduced in Vav3 deficient leukemic progenitors in comparison to that of WT counterparts. Furthermore, the Chip-q-PCR analyses using anti-polymerase II (Pol-II) showed increased binding to Cdkn2a and Pax5 loci in Vav3 deficient leukemic B-cell progenitors. We found, both in vitro and in vivo, that Vav3 interacts with components of actin microfilaments system and the deficiency of Vav3 impaired the nuclear actin polymerization. These data indicate that nuclear Vav3 is leukemogenic by interacting with components of the PRC complex, and suggest that Vav3 controls nuclear actin microfilaments, and regulates the chromatin organization and epigenetic repression of key factors involved in B-cell differentiation, proliferation and survival.

**PP-40**

**Discovery of a MYC-Driven Pre-B Cell Acute Lymphoblastic Leukemia Zebrafish Model**

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Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Precursor B-cell ALL (pre-B ALL) represents ~85% of these cases. Despite its prevalence and clinical importance, zebrafish pre-B ALL models are lacking. Hyperactive MYC is known to induce D. rerio T-cell ALL (T-ALL). One example of this is rag2:hMYC transgenic fish, where human MYC (hMYC) is regulated by a zebrafish rag2 promoter. To detect T-ALL in rag2:hMYC animals, we added a transgenic fluorescent marker, lck:GFP. Unexpectedly, we discovered rag2:hMYC, lck:GFP fish also developed a second ALL type. Besides brightly-fluorescent T-ALL, we also found short latency, high incidence dimly-fluorescent GFP low cancers. Expression analyses of GFP low cancers using qRT-PCR, RNA in situ hybridization (RNA ISH), RNA-Seq, and other techniques confirm they express immature B cell genes, proving they are pre-B ALL. Zebrafish pre-B ALL resembles human pre-B ALL in several ways: (1) Histologic studies and RNA ISH show GFP low cancers invade lymphoid and non-lymphoid tissues aggressively; (2) Immunoglobulin V(D)J repertoire analyses demonstrate GFP low cancers are clonal; (3) Cancers are dexamethasone sensitive, like human pre-B ALL. Overall, our findings demonstrate that hMYC potently induces zebrafish pre-B ALL that is similar to the human disease, making this the first robust D. rerio model of the most important pediatric cancer.

**PP-41**

**Wnt16 and Ror1 are a Physiological Ligand-Receptor Pair**

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Hematopoietic Stem Cells (HSCs) are the self renewing cells that produce mature blood lineages and are used in transplantation therapies for leukemia and other diseases. We previously showed that wnt16 is required for HSC specification in zebrafish. Knockdown of zebrafish Wnt16 disrupts formation of runx1+ HSC precursors in the dorsal aorta. Our genetic interaction studies implicate the atypical Wnt receptor Ror1 (Receptor Tyrosine Kinase like Orphan Receptor) as a physiological Wnt16 receptor in this process. WNT16 was originally discovered as a human gene misexpressed in pre B-acute lymphoblastic leukemia (ALL) cases, and shown to be a direct transcriptional target of the TCF3-PBX1 (E2A-PBX1) fusion oncoprotein, which results from the defining t(1;19)(q23;p13) translocation. Strikingly, combined misexpression of WNT16 and ROR1 proteins is a shared feature of both chronic lymphocytic leukemia (CLL) and t(1;19)+ pre-B ALL cases. We observe a robust physical interaction between zebrafish Ror1 and Wnt16 proteins in embryos. In addition, we detect robust physical interaction between epitope-tagged overexpressed human proteins in HEK293T cells, in agreement with our genetic interaction data. To examine the oncogenic potential of combined expression of Wnt16 and Ror1 in B cells, we have generated transgenics and see low penetrance leukemic lesions. Our results support the conclusion that Wnt16 and Ror1 are a bona fide physiological ligand receptor pair with roles in developmental and malignant hematopoiesis.

**PP-42**

**STAT3 Signaling Pathway Mediates Transient Drug Resistance in Chronic Myeloid Leukemia**

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Tyrosine kinase inhibitors (TKI) have been able to efficaciously control Chronic myeloid leukemia (CML) and yet many patients relapse upon treatment discontinuation due to the persistence of TKI-resistant leukemic stem cells. Moreover, being a single translocation disorder originating from a single cellular source, the LSCs, makes CML a good model to study drug resistance. There are two known evolutionary models for tumor drug resistance. First, a pre-existing sub-population is inherently resistant to treatment; and second, a sub-population acquires resistance by drug-induced transcriptional reprograming. Our preliminary data and previous studies have shown an increase in active STAT3 in drug resistant CML cells. Inhibition of STAT3 using a small molecule inhibitor, induced apoptosis of the K562-resistant CML cells, but had minimal effect on the TKI-sensitive lines. Drug withdrawal from the K562-resistant cells re-sensitized them to TKI with reduced susceptibility to STAT3 inhibition. Therefore, STAT3 activation seems to confer survival advantage to the resistant cells. Although, single cell RNA-sequencing show that the K562-resistant cells upon TKI discontinuation share a similar signature to the resistant cells, which is different from the sensitive line. This suggests that differences in chromatin accessibility could confer drug resistance. Furthermore, single cell RNA-sequencing of the stem and progenitor cells from CML mice reveals a unique transcriptional signature, off which Fos and Dusp1 have already been proven important for resistance. Similar to K562s, transcriptional reprogramming of the CML stem and progenitors was observed upon TKI treatment. Indeed, ChIP-seq using phosphorylated STAT3 revealed STAT3 localization to unique sites upon TKI treatment. Moreover, with treatment, phosphorylated STAT3 localized to a consensus site different from the known motif, possibly due to chromatin remodeling. We thus hypothesize that drug resistance in CML occurs through a combination of the above stated two mechanisms, regulated by the STAT3 signaling pathway. The goal of this study is to hence develop more effective therapies that can target drug-resistant LSCs in a BCR-ABL independent manner.

**PP-43**

**SAMD9 and SAMD9L Germline Disorders in Patients Enrolled in Studies of the European Working Group of MDS in Childhood; Prevalence, Outcome, Phenotype and Functional Characterization**

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Hereditary predisposition has been ever since implicated in the etiology of childhood myelodysplastic syndromes (MDS). In the past two years, we and others identified germline mutations in paralogue genes SAMD9 and SAMD9L on chromosome 7q21.2 as new systemic diseases with high propensity for MDS with monosomy 7 (-7). The initial mutations in SAMD9 and SAMD9L were associated with MIRAGE and Ataxia-Pancytopenia syndromes, respectively. Both genes act as negative regulators of cellular proliferation and mutations were shown to be activating. Their high evolutionary divergence makes in-silico prediction erratic, thereby establishing in-vitro testing as standard for pathogenicity evaluation. The objectives of this study were to define the prevalence of SAMD9/9L germline mutations in primary pediatric MDS, and to describe the clinical phenotype and outcome. In addition, we aimed to characterize the somatic mutational architecture and develop a functional scoring system.

Within the study-cohort of 548 cases with primary MDS in Germany, 43 patients (8%) carried SAMD9/9L germline mutations. MDS type refractory cytopenia of childhood was diagnosed in 91% (39/43), and MDS with excess blasts in 9% (4/43) of mutated cases. Karyotype at diagnosis was normal in 58%, and -7 in 37% of SAMD9/9L cohort. Notably, the demographics, familial disease, diagnostic blood and BM findings, overall survival (OS) and the outcome after HSCT were not influenced by mutational status. Next, we added 26 cases with SAMD9/9L mutations to the study-cohort. In the total cohort of 67 germline mutated patients, we found a total of 71 SAMD9/9L mutations, of which 65 were novel. Of those, 56/58 depicted inhibited proliferation by functional testing. We did not observe an association between germline mutation and phenotype. The physical anomalies were not mutually exclusive and were reported in ~50% in both genetic groups. The exploration of somatic mutational landscape in 58 SAM D9/9L patients identified recurrent hits in known oncogenes, earlier associated with pediatric MDS/-7 i.e. SETBP1(10%), RUNX1(7%), ASXL1(5%), EZH2(5%), CBL(3%). Finally, we utilized the in-vitro results as experimental cohort to develop combinatorial in-silico scoring. The rationale was to decrease the dependency on functional validation. Based on the results, we could concatenate a matrix of 5 algorithms to resolve the pathogenicity of >80% of variants.

In summary, SAMD9/9L genotype accounts for 8% of primary pediatric MDS and 22% of MDS/-7. The mutations pathogenic effect can be predicted using a combinatorial in-silico in-vitro approach. Finally, the clinical outcome and somatic mutational landscape are not influenced by the mutational status.

**PP-44**

**Chd1 - A Novel Epigenetic Regulator in Myeloid Malignancies with a Role in DNA Repair**

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Myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML) are clonal hematopoietic disorders caused by genetic alterations in hematopoietic stem cells or progenitors. These myeloid disorders are clinically heterogeneous and biologically complex. Despite advances in understanding the genetic landscape of MDS/AML and the introduction of new and targeted therapies, cure rates in childhood AML are still only ~60%, and markedly lower in adults. Exploiting the genetic tractability of the zebrafish (Danio rerio) vertebrate model, we are investigating a novel epigenetic regulator, Chromodomain helicase DNA binding protein-1 (CHD1) in hematopoiesis, and the role of CHD1 dysregulation in MDS and AML. CHD1 is located at chromosome 5q21, which lies within the most frequent breakpoints seen with the deletion of the long arm of chromosome 5 [del (5q)] in patients with MDS and AML. In addition, we found CHD1 levels are significantly lower in marrow cells of MDS cases lacking del (5q) relative to normal controls. To explore this further, we used CRISPR/Cas9 targeted mutagenesis in zebrafish to create homozygous chd1 mutant fish, and confirmed decreased chd1 expression in this mutant. Homozygous chd1 mutants have no significant developmental or hematopoietic phenotypes detected in embryogenesis, and are viable and fertile as adults. CHD1 acts as a tumor suppressor and is linked to the DNA damage response, so we hypothesized chd1 mutant fish might be more sensitive to DNA damage. Indeed, we found chd1 mutants have increased sensitivity to ionizing radiation, as shown by elevated cell death in the brain, increased rates of malformation and growth retardation, delayed hatching time, and decreased overall survival. To probe tumorigenesis, we also generated chd1het; tp53het zebrafish to test whether chd1 haploinsufficiency accelerated tumor rates in tp53 mutants. Single heterozygous chd1het or tp53het fish lacked tumors at one year of age, but chd1het; tp53het double-heterozygotes demonstrated substantial tumors by one year. Taken together, our data suggest CHD1 may promote genomic integrity, reconciling how diminished CHD1 levels could augment MDS and AML pathogenesis. This genetic interaction may be especially crucial in patients with combined del (5q) and TP53 alterations, and could explain the increased disease severity seen in this group. Extrapolating our findings further, CHD1-deficient AML may have attenuated DNA damage repair, allowing malignant cells to persist despite exposure to standard DNA damage-inducing chemotherapeutics

**PP-45**

**Abstract withdrawn.**

**PP-46**

**The Link Between Mitochondrial Morphology and Hematopoietic Stem Cell Function**

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For many hematological disorders, bone marrow transplantation is the only curative therapy. However, one-in-four patients will endure bone marrow failure. Maintenance of a functional hematopoietic stem cell (HSC) pool is crucial for successful transplantations. HSCs undergo stepwise differentiation to produce myeloid and lymphoid progenitors, as well as self-renewal to maintain a functional stem cell pool; however, HSCs become dysfunctional (i.e. unable to properly regulate their self-renewal and differentiation potential after transplantation). One cause is the deregulation of proper mitochondrial organization i.g. Transplanted HSCs acquire fused and polarized mitochondria while non-transplanted HSCs maintain healthy dispersed mitochondria. This deregulation of mitochondrial organization is due to the loss of function of the fission regulator Drp1 in transplanted HSCs. Here, we show this with single cell RNA seq data from transplanted and non-transplanted HSCs in conjunction with analysis of mitochondrial morphology of transplanted HSCs compared with Drp1 deficient HSCs. Transplanted donor HSCs are required to leave quiescence and undergo division to regenerate the recipients bone marrow, therefore, we wanted to analyze mitochondrial morphology of non-transplanted HSCs pre and post division. In order to do this, we used the widely established H2B mouse model where mice are treated with doxycycline for two weeks and then chased for 18 weeks and then sacked and analyzed. FACS analysis was used to sort HSCs that had not undergone division (i.e. GFP high expressing cells and HSCs that had undergone division) (i.e. GFP low expressing cells). Next, the mitochondrial morphology of these populations was analyzed. We were able to show that HSCs that underwent division had hyperfused mitochondria while cells that had not undergone division still had healthy dispersed mitochondria. In all, we were able to conclude that mitochondrial morphology is linked to the functional status of HSCs and that divisional history plays a role.

**PP-47**

**Modeling of an ALL-Associated Germline ETV6 Variant Reveals an Impairment in HSPC Function**

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Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and a leading cause of pediatric morbidity and mortality. Germline genetic variants can influence the risk of childhood ALL; however, the mechanisms by which these variants promote leukemia development remain unclear. Recently, we and others identified germline mutations in the transcription factor ETV6 in families with autosomal dominant thrombocytopenia and increased risk for hematologic malignancies, particularly ALL. Subsequent sequencing of 4,405 childhood ALL remission blood samples identified 30 potentially damaging ETV6 variants in 35 patients, further linking germline ETV6 mutations with B-ALL risk. To understand how B-ALL-associated germline ETV6 mutations impact hematopoiesis, we used a CRISPR-Cas9 strategy to create a C57BL/6 mouse model harboring a germline Etv6 mutation, R355X, which mimics a similar mutation in humans and introduces a stop codon within the DNA binding domain of ETV6. Etv6R355X/+ mice display variable degrees of thrombocytopenia consistent with patients harboring deleterious ETV6 variants. Furthermore, Etv6R355X/+ platelets exhibit abnormal morphology and impaired clot formation ex vivo. Flow cytometry of the bone marrow (BM) and peripheral lymphoid organs reveal normal frequencies and numbers of mature hematopoietic cells, but reveals a progressive decrease in hematopoietic stem and progenitor cells (HSPC), with mice 12 months of age or older showing reductions of up to 40% compared to WT. To evaluate HSPC function, we performed competitive repopulation assays by transplanting lineage-negative, cKit+, Sca1+ (LSK) cells from the BM of 8-12-week-old Etv6R355X/+ mice (CD45.2+) along with WT CD45.1+ LSKs at a 1:1 ratio into lethally irradiated CD45.1/2 recipients. We observed that Etv6R355X/+ HSPC are at a competitive disadvantage, as shown by significantly reduced peripheral blood chimerism and a significant loss of CD45.2+ HSPC populations in the BM of primary and secondary recipients. These results indicate that the presence of the ETV6 R355X mutation impairs HSPC fitness, a phenotype we are further evaluating. We have conducted RNA- and ATAC-sequencing from the LSK compartment of Etv6R355X/+ mice in order to identify the genes and pathways altered in the presence of this mutation. In summary, we have generated a mouse model of a germline ETV6 variant that phenocopies human thrombocytopenia and platelet dysfunction and exhibits impaired HSPC fitness as shown by decreased HSPC numbers with age and reduced engraftment of these cells. Using this Etv6R355X/+ mouse model, we will evaluate HSPC proliferation, survival, homing, and additional features to further investigate the underlying pathogenetic mechanisms of this variant in hematopoiesis.

**PP-48**

**Dynamins 2 and 3 Are Required for Human Megakaryocytes Directional Migration**

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Megakaryocytes (MKs) undergo directional migration from the proliferative osteoblastic niche within the bone marrow (BM) environment to the sinusoid-rich vascular niche for platelet production and release into the blood circulation. This process is regulated in part by dynamins, large GTPase proteins that regulate cellular functions such as endocytosis, vesicle transport and cell migration. Additional functions of dynamins include the formation of actin-rich structures, such as lamellipodia and dorsal membrane ruffles, invadopodia and podosomes. Previous studies have shown that mutations in Dynamin 2 (DNM2) are associated with low platelet counts in humans. To explore the function of dynamins in megakaryocyte migration and platelet production in more depth, we monitored the response of cells to chemotaxis SDF1Î± gradient signal by a microfluidic device-based approach. We observed an impaired directional migration by both human megakaryocytic cell lines and primary cells treated either with dynasore, a small molecule inhibitor of dynamins, or shRNA knockdown of Dynamin 2 and 3 (DNM2, DNM3). Since directional cell migration is tightly regulated by actin cytoskeletal rearrangements, we next measured actin polymerization and RhoA activity. We observed a profound decrease in the F-actin and Rho GTPase activity upon loss of DNM2 and DNM3 function. Next, since the response to chemoattractant signal is navigated by SDF1 through its receptor CXCR4, we explored the CXCR4 receptor response to ligand in dynamin defective megakaryocytes. Interestingly, we observed an increase in CXCR4 surface expression in the dynasore-treated primary human cells. Additionally, combined inhibition of DNM2 and DNM3 or overexpression of dominant negative Dnm2-K44A or GTPase-defective DNM3 decreased the active Î²1- integrin (ITGB1) activity, which indicates a decrease in the integrin mediated trafficking during cell migration. Finally, to understand the role of dynamin in endosome recycling, we assayed the distribution of Rab11, a marker of recycling endosomes. We noticed an abnormal clustered staining pattern of Rab11 in dynasore-treated MKs, which is indicative of a disruption in recycling pathways. This observation suggests decreased recruitment of the recycling pathway in dynasore-treated cells. Altogether, in this study we demonstrate that dynamins regulate MKs directional migration towards the SDF1Î± chemotaxis signal in the bone marrow and governs endocytosis and cell receptor trafficking.

**PP-49**

## Mll3 Promotes Differentiation, Activates Inflammatory Pathway and Limits Self-Renewal Capacity in Hematopoietic Stem Cells

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Hematopoietic stem cells (HSCs) undergo self-renewing divisions, and they can give rise to all blood lineages. The balance between HSC self-renewal and lineage commitment is tightly coordinated so that the HSC pool is maintained throughout life while, at the same time, providing adequate blood cell production during normal and stressed states. We have identified MLL3 as a transcriptional regulator that modulates the balance between self-renewal and commitment in HSCs. MLL3 encodes a histone methyltransferase that activates enhancer elements and promotes transcriptions. It is a tumor suppressor that is deleted in human myelodysplastic syndromes as part of larger chromosome 7q deletions, and loss of MLL3 has previously been associated with activation of self-renewal programs in hematopoietic progenitors. To better understand how Mll3 regulates HSC self-renewal, we developed novel germline and conditional Mll3 knockout mouse alleles. Mll3 deletion led to an increase in adult HSC numbers under non-stressed, homeostatic conditions. Serial transplantation assays showed that HSC self-renewal capacity was enhanced by deletion of one, or both, Mll3 alleles. In adult mice, homozygous Mll3 deletion led to a significant reduction in lineage-committed hematopoietic progenitor cells (HPCs). This suggests that MLL3 promotes differentiation of HSCs into HPCs. Interestingly, the enhanced self-renewal capacity of Mll3-deficient HSCs was not tied to changes in proliferation rate. We did not observe any increase, or decrease, in HSC proliferation following Mll3 deletion. Furthermore, when we conditionally deleted Mll3 in a small percentage of HSCs, the Mll3-deleted HSCs expanded in frequency over time, relative to undeleted HSCs, irrespective of whether the mice were maintained in a non-stressed state or treated with chemotherapy. Conditional Mll3 deletion led to a reduction in the expression of genes associated with innate immune signaling. The data suggest that MLL3 selectively regulates inflammatory pathways that promote HSC lineage commitment.

**PP-50**

**Homophilic Engagement of CD166 Enhances Function of Human and Murine Hematopoietic Progenitor Cells**

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The phenotype and function of murine and human hematopoietic stem cells (HSC) have been exhaustively investigated making HSC one of the most-characterized group of stem cells. However, not a single functional HSC marker has been identified that is common to both species while being also expressed on cellular components of the hematopoietic niche (HN), including osteoblasts (OB), mesenchymal stem cells (MSC), and endothelial cells (EC). We have shown previously that CD166 is a functional marker expressed both on human and murine HSC and on murine OB and MSC. We first examined phenotypically defined murine EC and detected a fraction of bone marrow (BM)-derived EC also express CD166. Next, we used inducible Tie2-Cre mice to examine whether cells expressing td Tomato under the control of Tie2 are also CD166+. We found that 67% of Tie2+ EC expressed CD166+. As CD166 is expressed on HSC and 3 cellular components of the BM niche, we hypothesized that homophilic engagement of CD166 between HSC and niche cells is critical for maintaining HSC function. CD166+LSK cells, cultured on either WT OB or recombinant murine CD166 (rmCD166) showed significantly increased colony forming units (CFU) compared to CD166-LSK cells cultured identically. WT HSC cultured on CD166 knockout (CD166-/-) OB showed significantly decreased CFU relative to WT HSC cultured over WT OB. Following transplantation, CD166+LSK cells cultured on rmCD166 showed increased engraftment in recipient mice compared to CD166+LSK cells cultured on BSA. To determine if CD166-CD166 interactions also enhance function of human HSC, we cultured cord blood-derived CD34+CD166+ and CD34+CD166- cells with recombinant human CD166 (rhCD166). CD34+CD166+ cells cultured with rhCD166 showed significant increase in CFU compared to CD34+CD166- cells. Collectively, these data demonstrate the enhancing effect of CD166 interactions on human and murine progenitor cell function. To elucidate the underlying signaling mechanism of CD166-CD166 mediated hematopoiesis enhancing activity, we cultured WT and CD166-/- HSC with rmCD166 and performed single-cell RNA seq. We identified 148 pathways regulated by genes showing increased expression in CD166-/- HSC. Most of these pathways were associated with loss of HSC activity, include cell cycle, translational regulation, and mitochondrial signaling. Downregulated genes in CD166-/- HSC also affected pathways associated with stemness, including oxidative stress response, and metabolism. Overall, our studies suggest that homophilic CD166 interactions involving HSC are required for maintenance of essential pathways that sustain HSC functions, including stemness, mitochondrial function, metabolism, cell cycle and growth factor signaling.

**PP-51**

**Pharmacological Activation of Nitric Oxide Signaling Promotes Human Hematopoietic Stem Cell Homing and Engraftment**

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The successful clinical outcome of hematopoietic stem cell (HSC) transplantation relies on adequate homing and long-term engraftment of HSC into the bone marrow (BM). The free radical nitric oxide (NO) is a gaseous molecule that plays important roles in a variety of physiological regulations. NO can freely diffuse across cellular membranes and activate an enzyme, soluble guanylyl cyclase, to produce cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). However, the roles of NO and cGMP in regulating HSC function still remain poorly understood.

To explore the importance of NO signaling in HSC, we first evaluated the effects of NO on human cord blood (CB) HSC chemotaxis in an in vitro transwell migration assay. We found that treatment of human CB CD34+ cells 16 hours with NO donor compound sodium nitroprusside (SNP) resulted in 55% more migration toward CXCL12 compared to vehicle control. NO activates soluble guanylyl cyclase in target cells, so next we explored the relationship between HSC migration and soluble guanylyl cyclase. By treating human CB CD34+ cells with riociguat, a soluble guanylyl cyclase stimulator, we found that riociguat treatment also resulted in 78% more HSC migration toward CXCL12. Inside the cell cGMP is degraded by phosphodiesterase 5 (PDE5), so a PDE5 inhibitor would suppress cGMP breakdown and activate cGMP signaling. We found that CD34+ cells with PDE5 inhibitor avanafil or sildenafil treatment showed increased HSC chemotaxis compared with vehicle control. To directly evaluate in vivo homing, vehicle, SNP or riociguat treated CB CD34+ cells were injected into sublethally irradiated NSG (NOD.Cg-PrkdcscidIL2rgtm1Wjl/Sz) mice, and human cell homing to mouse BM, as indicated by human CD45 percentage, was analyzed 24 hours after transplantation. Consistently, SNP or riociguat treatment resulted in significant increase of human cell homing in NSG mice respectively compared with vehicle control treatment. Next, we performed a limiting dilution assay to compare the frequency of SCID-repopulating cells (SRCs) in vehicle and riociguat treated CB CD34+ cells. Poisson distribution analysis revealed an SRC frequency of 1/2977 in vehicle control treated group and 1/512 in Riociguat treatment.

Taken together, our study suggests that human HSC homing and engraftment can be enhanced by modulating the NO/cGMP signaling pathway. Some compounds tested in our study, such as SNP, riociguat, avanafil are FDA approved medications. So, utilization of these drugs in HSC transplantation should be practical. Our work offers a new and simple approach to bolster the effectiveness of HSC transplantation.

**PP-52**

**GASP Family Members Are Novel Regulators of GPCRs in Hematopoietic Progenitors**

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Understanding the molecular regulation of G-protein coupled receptors (GPCRs) in hematopoietic stem and progenitor cells (HSPCs) is essential for advancing treatment of hematological disorders and malignancies, including HSPC transplantation (HSCT). Our laboratory recently discovered that the efficacy of HSCT can be improved through knockdown of the GASP (GPCR Associated Sorting Protein) family members GPRASP1 and GPRASP2. Interestingly, both GPRASP1 and GPRASP2 are highly expressed in HSPCs as compared to downstream progenitors. GASPs are well-known regulators of GPCR signaling in the nervous system, but their roles in HSPCs are currently unexplored. The importance of understanding the molecular role of GASPs in HSPCs is highlighted by our finding that they impact HSCT, as well as by the fact that GPCRs are a major class of therapeutic targets (comprising approximately 30-40% of all approved drug targets). GPRASP1 and GPRASP 2 interact with GPCRs that contain a putative GASP binding motif, predominantly targeting these receptors to the lysosome for degradation. Bioinformatic analysis of GPCRs revealed that multiple chemokine, somatostatin and prostaglandin receptors expressed by HSPCs contain GASP binding motifs. CXCR4, which is recognized as a master regulator of HSPC biology, is among the chemokine receptors harboring a putative GASP binding motif. We are currently employing co-immunoprecipitation, immunofluorescence, and biotin proximity labeling approaches to determine the full complement of GPCRs regulated by GASPs in HSPCs. Preliminary data shows that endogenous CXCR4 and GPRASP1 co-immunoprecipitate in the K562 human CML cell line. Additional prospective GASP targets will be investigated by endogenous and overexpression co-immunoprecipitation studies in K562 and HPC5 (mouse hematopoietic multipotent progenitor) cell lines, with all identified interactions being validated by immunofluorescence in primary murine HSPCs. To complement these approaches and provide an unbiased analysis of the GPCRs regulated by GASPs in hematopoietic cells, we will perform proximity labeling using GPRASP1- and GPRASP2-APEX fusion proteins in K562 cells followed by streptavidin pull-down and protein identification by mass spectrometry. This work will begin to elucidate an as yet unexplored layer of GPCR regulation in HSPCs with broad clinical relevance.

**PP-53**

**Hematopoietic Connexin 43 is Required for Bone Marrow Mesenchymal Regeneration and Mitochondrial Transfer from Hematopoietic Stem Cells/Progenitors to Mesenchymal Progenitors through AMPK**

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Hematopoietic stem cell/progenitor (HSCP) transplantation (HSCT) is routinely used for the treatment of germinal mutations in the lympho-hematopoietic system. The bone marrow (BM) microenvironment (ME) is a major regulator of hematopoietic function and fate. Clinical data supports that bone mesenchymal lineage diseases may be ameliorated by HSCT despite the inability of BM mesenchymal stem cells/progenitors (MSC/P) to engraft robustly in the recipient bone tissues. Therefore, understanding the hematopoietic-dependent mechanisms controlling BM mesenchymal ME regeneration is expected to provide molecular targets for intervention in the context of HSCT. Previously, we identified the physical interaction between HSCP and BM mesenchymal ME, where hematopoietic Connexin-43 (H-Cx43) mediates HSCP survival and efficient blood formation by transfer of damaging excess reactive oxygen species (ROS) to BM mesenchymal stromal cells after chemotherapy, preventing lethal hematopoietic failure. We hypothesized that H-Cx43 mediated ROS transfer upon stress depends on mitochondria transfer from HSCP to BM-MSC/P. We created chimeric mice by transplanting Vav1-CreTg/-, Cox8 mitochondrial localization signal-Dendra2Tg/- wild-type (mDendra2/WT) or Cx43fl/fl (mDendra2/Cx43fl/fl HSCP to lethally irradiated, congenic WT mice and assessed the recovery of stromal cell regeneration via transfer of mitochondria to BM-MSC/P. H-Cx43fl/fl chimeric mice exhibit decreased (~60-80%) colony-forming-unit-fibroblast (CFU-F) and osteoblast (CFU-Ob) regeneration and hematopoietic recovery after irradiation. The delayed hematopoietic response in H- Cx43fl/fl chimeras associated with ~40% reduction in mitochondrial transfer from HSCP to Lin-/CD45-/PDGFRÎ±+/Sca1- BM-MSC/P. Impaired mitochondrial transfer in H- Cx43fl/fl chimeras associated with ~30-40% decreased mitochondrial ROS, membrane potential and proliferation (assessed by in vivo BrdU uptake) of recipient MSC/P containing donor derived mitochondria, suggesting that the transferred mitochondria reprogram the recipient mesenchymal progenitor metabolism. The loss of benefits of H-Cx43 expression was recapitulated in in vitro co-cultures. Interestingly, intracellular [ATP] was upregulated (~2 fold) in MSC/P from chimeric H- Cx43fl/fl BM that received donor derived mitochondria, as compared to donor mitochondria containing MSC/P from WT chimeras. Forced glycolysis-dependent restoration of [ATP] in MSC/P but not in HSCP enhance the transfer of mitochondria from HSCP to MSC/P, suggesting that BM stromal [ATP] is an irradiation-responsive positive regulator of mitochondria transfer. We found that MSC/P recipient of H- Cx43fl/fl mitochondria have increased AMP-activated protein kinase (AMPK) activity, and in vivo inhibition of AMPK by the BML-275 dramatically increases the transfer of mitochondria from HSCP to BM-MSC/P and BM microenvironment regeneration (CFU-F and CFU-Ob) in both WT and H- Cx43fl/fl chimeras. Collectively, our data suggests that H-Cx43 is indispensable for hematopoietic and BM-ME regeneration, and control both mitochondria transfer and BM-MSC/P energetic balance after myeloablative irradiation.

**PP-54**

**Identifying Putative Targets of Nuclear Factor I-X in Hematopoietic Cells**

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Hematopoietic stem and progenitor cells (HSPC) are routinely exploited in the clinic to treat patients with cancers or other hematologic diseases. We recently identified the transcription factor nuclear factor I-X (NFIX) as a novel regulator of HSPC survival post-transplant. Nfix-deficient HSPC display a loss of in vivo hematopoietic repopulating potential and increased apoptosis 10 days post-transplant. Further, ectopic expression of NFIX protects HSPC from stress-induced apoptosis during ex vivo culture. We recently demonstrated that this protective effect depends on TPO and upregulation its receptor, c-Mpl on HSPC. Dual reporter luciferase assays and chromatin immunoprecipitation revealed c-Mpl as a direct transcriptional target of NFIX. C-Mpl represents the first transcriptional target of NFIX identified in hematopoietic cells. To comprehensively identify direct transcriptional targets of NFIX in hematopoietic cells, we are currently performing chromatin immunoprecipitation followed by sequencing (ChIP-seq). Our lab has recently validated an anti-NFIX monoclonal antibody that will be used to isolate NFIX-bound DNA fragments in a surrogate mouse bone marrow-derived cell line, HPC5 cells. As one control, we are transducing FLAG-NFIX into HPC5 cells. CRISPR-mediated deletion of NFIX in HPC5 cells will provide another key control for ChIP-seq. Based on its proven role in neural stem cell populations, we expect NFIX may directly regulate genes involved in cell death, cell cycle control and adhesion in primitive hematopoietic cells. This study will illuminate putative transcriptional targets of NFIX and provide further insight towards genes involved in HSPC regulation.

**PP-55**

**Transfer, Transcription, and Translation of Adeno-Associated Virus Producer Plasmid Contaminants**

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Adeno-Associated Virus (AAV) is the one of the most commonly used clinical gene therapy vectors, and has been successfully implemented in clinical trials for many genetic diseases, including the coagulation disorders Hemophilia A and B. It is known that during the AAV production process, AAV particles containing contaminating DNA sequences are packaged. We sought to characterize these contaminants in detail and examine whether they might have any implications post-infection.

Analysis of AAV particles for FVIII and FIX preparations showed abundant DNA contamination from the AAV production plasmids. qPCR and Next Generation Sequencing analysis of AAV particles showed that contaminant sequences were in greatest abundance at three distinct regions: from the vector genome plasmid at regions proximal to the 5' ITR, and to the 3' ITR; and in the REP/CAP plasmid, upstream of the P5 promoter. Sequences proximal to these regions are in high abundance, whereas sequences remote from ITRs and the P5 promoter sequence were undetectable.

We examined the in vivo transfer and retention of these contaminant sequences post AAV infection in C57BL/6 mice at both a short time post infection (1-2 weeks) and long term (4 months). Mouse hepatocyte DNA was analyzed by qPCR and Next Generation Sequencing, and contaminant AAV sequences were identified within AAV-infected hepatocytes. Further analysis of post infection hepatocytes by RT-PCR and RNA-seq revealed abundant transcription of all three regions of detected plasmid backbone contaminant sequences, at both the short and long term timepoints. We subsequently developed a fluorescent contaminant reporter model to test whether contaminant sequences in AAV have the potential to be translated. A GFP cassette was placed in the contaminant incorporation region, upstream of the P5 promoter in the REP/CAP plasmid. This GFP cassette was packaged as a DNA contaminant of a FVIII expression cassette into AAV8. Infection of 293T cells with this viral prep resulted in 4% of the treated cells expressing GFP protein 72 hours after AAV infection.

This is the first demonstration that producer plasmid contaminants packaged into AAV can be transcribed and translated by infected cells. Given that AAV vectors are currently being implemented in several clinical gene therapy trials for blood related disorders, these findings highlight the need for rigorous examination of contaminating DNA levels in AAV vectors destined for the clinic.

**PP-56**

**Pharmaceutical Inhibition of Latexin for Protection of Padiation-induced Hematopoietic Damage**

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Radiation therapy as applied in the treatment of solid and liquid tumors and for bone marrow (BM) transplantation preconditioning commonly results in not only acute hematopoietic suppression, but also long-term BM injury. This late effect predisposes patients undergoing radiotherapy to the development of secondary malignancies associated with significant mortality. To date, no effective treatment has been developed to protect patients against radiation-induced acute and long-term BM injury. Hematopoietic stem cells (HSC) and their capacity for self-renewal and regeneration of blood cells are crucial to reduce radiation-induced BM toxicity. Latexin (Lxn), a 222 amino acid protein in both human and mouse, is the only known naturally occurring carboxypeptidase (CPA) inhibitor in mammals. We have reported that latexin acts endogenously in HSCs to negatively regulate their population size by enhancing apoptosis and decreasing self-renewal. We further found that Lxn knockout mice are resistant to radiation by protecting HSCs from radiation-induced functional decline, thus enhancing hematopoietic recovery and animal survival. We reasoned that pharmaceutical inhibition of Lxn could protect radiation therapy-induced hematopoietic damage. We therefore performed a high-throughput screening of small molecule compounds based on its inhibitory activity on CPA. We found that 60 compounds showing sigmoidal dose-response curve, among which 8 compounds had the half maximal effective concentration ($EC_{50}$) ≤50Um. We next performed molecular docking to predict the potential binding of these compounds to Lxn protein, and identified one compound with the highest likelihood of binding. Its CID number in NIH PubChem library is "5483801" (https://pubchem.ncbi.nlm.nih.gov/ compound/5483801). Molecular docking shows that Lxn inhibitor 5483801 specifically binds to Lxn in the binding pocket between Lxn and CPA3, thus blocking their interaction. We next evaluated the functional and molecular effects of 5483801 on HSCs. The result showed that 5483801 increases LSK number and CPA3 level in Lxn-dependent manner. Moreover, it protects HSCs from radiation-induced loss in vitro. These phenotypes are similar to those in Lxn-/- mice, suggesting that 5483801 inhibits Lxn activity, and increases HSC function and radiation resistance. In future, we will determine the molecular mechanism by which Lxn inhibitor 5483801 protects and mitigates radiation-induced HSC toxicity by regulating Lxn-CPA3 pathway. We will also further optimize 5483801 for drug development purpose.

**PP-57**

**Genome Editing of Therapeutic Loci Results in Acute Chromosomal Crisis**

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The postnatal expression of fetal hemoglobin (HbF) in red blood cells alleviates the disease severity of sickle cell disease (SCD) and beta-thalassemia. Previously, we and others employed CRISPR-Cas9-mediated non-homologous end joining to disrupt regulatory elements involved in the repression of the gamma-globin genes (HBG1 and HBG2) in primary CD34+ hematopoietic stem and progenitor cells, which raised HbF to potentially therapeutic levels. The clinical application of these strategies is limited by the efficacy of the treatment while balancing unintended on- and off-target effects from CRISPR-Cas9. To that end, we investigated whether CRISPR-Cas9 caused unintended on-target consequences of genome editing at two therapeutically-relevant loci: 1) the duplicated distal CCAAT box in the promoters of HBG1/HBG2 and 2) an erythroid-specific enhancer regulating the Î³-globin repressor, BCL11A. We hypothesized DNA double-strand breaks induced by CRISPR-Cas9 result in on-target chromosome missegregation, leading to micronucleus formation, and subsequent genomic rearrangement. Genome editing was performed using ribonucleoprotein complexes (RNPs) consisting of Cas9 protein conjugated with chemically-modified gRNAs and electroporated into primary CD34+ cells. By day 4 post-electroporation, the overall editing frequency of the target loci ranged from 68-95%. The degree of editing was sufficient to raise HbF levels in erythroid differentiated CD34+ cells 2-3-fold by high-performance liquid chromatography. We monitored the formation and rupture of micronuclei through immunofluorescence and changes in chromosomal copy number by fluorescence in situ hybridization (FISH) at days 1 and 5 post-electroporation. Editing of the BCL11A enhancer resulted in a 2-fold increase in the percentage of micronuclei compared to background. However, the micronuclei did not frequently display hallmarks of DNA damage nor nuclear envelope rupture commonly associated with micronuclei. Interestingly, we did not observe micronuclei formation in CD34+ cells when targeting the HBG1/HBG2 loci. FISH was performed 1-day post-electroporation on CD34+ cells for positions telomeric and centromeric to the on-target loci. In agreement with the immunofluorescence results, only cells edited at the BCL11A enhancer displayed micronuclei. Moreover, 21/400 (5.25%) of analyzed cells contained duplicated foci of the telomeric portion of the on-target BCL11A locus. Together, our data indicate that targeting of the erythroid-specific BCL11A enhancer, but not the Î³-globin gene promoters, results in micronucleus formation and copy number changes, which may lead to chromosomal abnormalities. These results emphasize the need to carefully assess the safety of genome editing strategies pertinent to clinical application.

**PP-58**

**Antigen Discovery for the Immunotherapy of Acute T-cell Lymphoblastic Leukemia**

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T-cell acute lymphoblastic leukemia (T-ALL) continues to be a high-risk disease due to treatment related complications and poor overall survival of relapsed disease cases. Immunotherapy with monoclonal antibodies (MAbs) and/or chimeric antigen receptor (CAR) T-cells for T-ALL is limited by identification of target antigens, which are expressed only on tumor cells. Differential expression is necessary to prevent on-target/off-tumor toxicities and fratricide of activated T-cells. This project establishes an antigen discovery pipeline that can be used to generate single or multiple antigens specific for T-ALL. Its use will be valuable for addressing immune escape and can be expanded to other tumor types. The goal of this project is to identify unique surface antigens expressed in T-ALL using proteomic and transcriptomic analyses. These antigens could then serve as targets for immunotherapies including MAbs and/or CAR T-cell therapies. We generated an Illumina total stranded RNAseq library from the myeloid and lymphoid cells of the bone marrow, peripheral blood and cord blood (N= 147) from healthy donors. Data was compared to 185 St. Jude pediatric T-ALL samples and against 53 normal tissue expression data from the GTEx (Genotype-Tissue Expression) project. To analyze the T-cell surface proteome, we isolated plasma membrane fractions from 11 samples including healthy lymphocytes and T-ALL cell lines (MOLT3, Jurkat, RPMI8402) using a differential centrifugation-based method. Resulting samples were processed using LC/MS/MS. 778 proteins were quantified from the membrane fraction, of which 458 had significant differential expression. Preliminary analysis of proteomic and transcriptomic data yielded a panel of candidate antigens including CD26, an antigen overexpressed in 30 out of the 265 T-ALL samples analyzed. Further proteomic analysis including primary T-ALL samples is ongoing. In conclusion, we have set up an unbiased platform to identify differentially expressed antigens on the cell surface of T-ALL blasts. Preliminary results are encouraging, warranting further active exploration of our approach.

**PP-59**

**The St. Jude Cloud Sickle Cell Disease Portal; Data Access and Interactive Visualization of Whole Genome Sequences from 807 Subjects with Sickle Cell Disease (SCD)**

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Sickle cell disease (SCD) is a monogenic disorder that causes multiorgan disfunction, debilitation and early death. However, specific organ involvement and severity vary greatly between patients, largely due to genetic factors. Identifying genetic variants that influence SCD phenotypes is of great scientific interest and should also inform therapeutic decisions according to the principles of precision medicine. Most SCD modifier genes are unknown and their identification is hampered by low statistical power due to small cohort sizes. Hence, improved platforms for data sharing, harmonization and analysis will accelerate scientific discovery of genetic variants that influence SCD pathophysiology. We developed the Sickle Cell Disease Portal (SCDP) (https://sickle-cell.stjude.org), a user-friendly system that provides facile data access and computing through a Cloud-based interface existing within the St. Jude Cloud (https://stjude.cloud), a web-based resource with over 5,000 whole genome sequences (WGS) from pediatric cancer patients, developed in collaboration with DNANexus and Microsoft. The newly created SCDP extends the St. Jude Cloud by adding numerous capabilities to advance genetic epidemiology studies of SCD:

1. Access to currently unpublished raw WGS data and clinical information on 807 pediatric SCD subjects from St. Jude (n=503) and The Baylor College of Medicine (BCM) (n=304). Current clinical data includes red blood cell (RBC) traits including fetal hemoglobin level, serum bilirubin, renal function and pain events.

2. Visualization tools for subject demographics including sex, race, age and clinical diagnosis (HbSS/HbSC/Hb S--thalassemia, other); interactive PCA plots for studies of genetic ancestry; interactive fitted regression plots to explore relationships between age and relevant RBC traits; interactive heatmaps and an interactive genome browser that allow users to study genome wide associations between genetic variants and phenotypes.

3. Using our WGS data and computational algorithms, we have validated several established genetic modifiers of SCD phenotypes including variants that regulate red blood cell size and hemoglobin content, red cell fetal hemoglobin levels, serum bilirubin and urine albumin concentration.

The SCDP represents one of the first WGS datasets from primarily African American SCD patients to be made available to clinicians and researchers worldwide and the first SCD-centric data portal to offer restricted access to raw genomic data and open data access to aggregated genotype calls with graphical tools for visual analysis. Overall, the genomic data and bioinformatic tools provided by the SCDP provide a valuable resource for data sharing and analysis that will advance genomic studies of SCD.

**PP-60**

**Obesity-Primed HSCs Display an Unique Response to Oxidative Stress**

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Obesity is a chronic organismal stress that disrupts multiple systemic and tissue-specific functions. We recently described the impact of obesity on the activity of the hematopoietic stem cell (HSC) compartment (Lee et al, JEM, 2018). We showed that obesity alters the composition of the HSC-SLAM (Lin- ckit+ Sca1+ CD48+ CD150-) compartment and its activity in response to hematopoietic stress. Mechanistically, we established that the oxidative stress induced by obesity dysregulates the expression of the transcription factor Gfi1 and that increased Gfi1 expression is required for the long-term alteration of the HSC functions. Following this work, we confirmed that obesity mainly affects the short- and long-term stress response of the most primitive HSC compartment (HSC-SLAM CD34-"CD49b-"), but not of its immediate downstream short-term progeny (HSC-SLAM CD34- CD49b+ and HSC-SLAM CD34+ CD49b+). In addition, we are investigating the molecular characteristics that regulate HSCs activity in obesity. Genome-wide gene expression analysis shows that HSCs isolated from obese mice up-regulate multiples genes involved in the phosphatidylinositol signaling pathway (e.g. Pik3c2a, Pik3c2b, Pi3kap1, Pi3kip1). Consistent with this gene expression pattern, phosphoflow cytometry analyses indicate the constitutive activation of the protein kinase AKT in these cells. While AKT activation has been previously linked to rapid functional HSC exhaustion, obesity-primed HSCs remained quiescent at steady state, potentially revealing the existence of compensatory mechanisms that protect the integrity of the HSCs in an obese environment. Consistent with this idea, we found that HSCs isolated from obese mice display an aberrant response to oxidative stress in vitro, as they are able to maintain nuclear localization and activity of FOXO proteins upon oxidative challenge. We are currently exploring the mechanisms underlying this abnormal activity of the AKT-FOXO axis in obesity-primed HSCs and their potential functional consequences. Our present results suggest that chronic stress such as obesity induces a profound rewiring of the HSC molecular properties, allowing for the maintenance of their function at steady state, but potentially contributing to a dysregulated stress response.

**PP-61**

**Contribution of Sclerotome to the Hematopoietic Stem Cell Niche and Vascular Smooth Muscle**

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Hematopoietic stem cells (HSCs) are a self-renewing population that ultimately sustains production of blood lineages throughout adult life. In vertebrates, these HSCs are initially specified in the early embryo, arising from hemogenic endothelium in the ventral wall of the dorsal aorta (DA). The cell types within this three-dimensional specification environment and the molecular signals they provide to promote HSC development are not well defined. A better understanding of the native HSC specification niche could inform efficient directed differentiation of true HSCs, which is not currently possible, from induced pluripotent stem cells for clinical and research applications. We previously showed that wnt16 is required for the development of zebrafish HSCs, as well as normal patterning of the ventral somite, the sclerotome. We hypothesized that normal sclerotome patterning is required for HSC specification by contributing to the cellular environment that presents inductive signals. To follow sclerotome development in living embryos, we have developed transgenic reporter zebrafish where expression of the mNeonGreen fluorophore is driven by regulatory elements from the paradigmatic sclerotome gene, pax1a. We show that sclerotome-derived cells migrate to and make contact with hemogenic endothelium of the DA shortly before initiation of the definitive hematopoietic program, demonstrating that they are present at the right time and place to present specification signals. By live imaging and profiling of sorted cells, we show these cells also give rise to arterial vascular smooth muscle cells. Loss-of-function analyses demonstrate a requirement for multiple sclerotome genes, including pax1a, for both proper patterning of the sclerotome itself, and subsequent specification of HSCs and downstream definitive hematopoietic lineages. Our data indicate that sclerotomal cells contribute to the HSC specification niche of the early embryo, and form a basis for defining the niche architecture and signals that drive definitive hematopoietic programming.

**PP-62**

**Identification of the Differentiation Stages of Individual, Living Hematopoietic Stem Cells on Fibronectin-Functionalized Substrates Using Raman Micro-Spectroscopy and Multivariate Analysis**

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The Leukemia and Lymphoma Society projected that approximately 170,000 new cases of blood cancer (i.e., myeloma, lymphoma and leukemia), accounting for 10% of the total number of new cancer cases, would be diagnosed in 2018. Blood cancers result from anomalies during hematopoiesis, which is the process of producing all the blood and immune cells in the body via hematopoietic stem cell (HSC) differentiation. Bone marrow transplantation is used to replace the mutated HSCs in the cancer patient with healthy HSCs from a living donor. To enable expanding HSCs outside the body for transplantation, the effects of various environmental cues on HSC fate decisions to self-renew or differentiate must be determined. Microscale platforms have been developed to minimize the number of rare HSCs needed to screen the effects of extrinsic cues on HSC fate decisions. However, conventional techniques cannot accurately and noninvasively identify differentiation stage on the single cell level. To address this obstacle, we are developing an integrated microscale screening platform for accurately identifying the fate decisions of individual, living cells from the rarest and most immature hematopoietic cell populations in response to environmental cues. Our strategy combines Raman microspectroscopy and a multivariate analysis technique, partial least square-discriminant analysis (PLS-DA), to noninvasively identify cell differentiation stages. We have demonstrated that this approach can accurately identify the lineages of fully differentiated cells from laboratory lines, and the differentiation stages of individual, chemically fixed cells from distinct hematopoietic cells populations. To extend this approach to screening the fate decisions of individual, living HSCs and their progeny within microscale screening platforms, a microscale artificial bone marrow niche that is compatible with Raman microspectrsocopy analysis of living cells had been fabricated and characterized. Progress in optimizing the single cell Raman microspectroscopy and PLS-DA approach to accurately identify the lineage-specific differentiation stages of living hematopoietic stem and progenitor cells harvested from mouse bone marrow will also be reported. Successful development of this microscale screening platform will facilitate efforts to identify the effects of matrix stiffness, cytokines, ligands, and niche cells on early HSC fate decisions. This knowledge may accelerate progress towards the ultimate goal of in-vitro HSC expansion.

**PP-63**

**15-PGDH Inhibition Mitigates Aplastic Anemia Severity**

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Acquired aplastic anemia (AA) is a primary bone marrow (BM) failure syndrome in which hematopoietic stem cells (HSC) are depleted in an immune-mediated fashion. AA is rare, with an estimated 900 new cases per year in the U.S., and onset is typically in childhood or adolescence. Although the precise etiology is not known, incidence is 4.2 times higher among children of East/South-east Asian and East Indian descent than that of white/mixed ethnic descent, likely due to a combination of genetic and environmental factors(1). AA is fatal if untreated and poses a high burden of frequent, long-term transfusions and associated complications. The current standard of care for AA is allogeneic stem cell transplantation for young patients who have a matched related donor or immunosuppressive therapy (IST) for those who do not. Neither of these therapies are well-tolerated, however, and prolonged neutropenia and immune suppression remain a clinical problem even in IST-treated AA patients. Importantly, this disorder is also associated with clonal evolution of myeloid progenitor cells, and the development of myelodysplastic syndrome and acute myeloid leukemia in 10-20% of cases(2). Therefore, additional therapies to either block immune-mediated BM destruction, or to accelerate hematologic recovery in patients receiving IST, are needed.

Inhibiting 15-hydroxyprostaglandin dehydrogenase (15-PGDH) using the small molecule SW033291 (abbreviated PGDHi) in mice was recently shown by Zhang et al. to increase BM prostaglandin E2 (PGE2) levels, expand HSC numbers, accelerate hematologic recovery from BM transplantation, and synergize with G-CSF, the standard of care for neutropenia(3). To determine whether PGDHi is capable of improving hematopoiesis in acquired AA, we induced immune-mediated BM failure in mice4 and treated with SW033291. PGDHi mitigated both neutropenia and anemia, and increased BM HSC and progenitor cells compared to untreated controls. These data suggest that PGDHi protects hematopoietic stem cell function in acquired AA. Experiments are underway to determine if PGDHi impairs T cell activation and inflammatory cytokine production that drive disease, and if PGDHi has the capacity to augment IST efficacy, which could allow lower, better tolerated cyclosporine dosing and/or accelerate recovery and reduce hospitalization of AA patients.

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**PP-64**

**Title Analysis of Leukemia Stem Cells in a MYC-Driven ALL Zebrafish Model**

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Acute lymphoblastic leukemia (ALL), the most common childhood cancer, is characterized by the malignant transformation and proliferation of B- or T-lymphoid progenitors. Precursor-B (pre-B) ALL and T-ALL are the two principal subtypes, together representing over 25% of all pediatric malignancies. Although current therapies are often curative, 10-20% of ALL patients relapse. Relapsed ALL has a much worse prognosis than de novo ALL and requires more aggressive treatment. One cause of relapse is thought to be a failure of initial therapy to eliminate leukemia stem cells (LSC). LSC are rare cells that are treatment resistant, quiescent, and epigenetically plastic. Despite their relative quiescence, LSC harbor self-renewal capacity that can, ultimately, re-grow ALL from a single cell. Complicating their study, LSC fates are at least partly governed by the microenvironment. An additional challenge is that LSC are defined by their functional ability to regrow ALL, making identifying and isolating LSC cumbersome.

Despite the key role of LSC in ALL, little is known about LSC gene expression and how it differs from that of normal stem cells or bulk ALL cells. To tackle this, we have developed strategies to enrich B- and T-ALL LSC from a transgenic rag2:hMYC zebrafish (Danio rerio) ALL model. We found that dexamethasone (DXM) or gamma irradiation (IR) depletes most D. rerio B- or T- ALL ALL cells, and markedly increasing LSC frequencies. In parallel studies, we identified putative B- and T-ALL LSC using a flow cytometric side population assay. This method exploits the ability of stem cells to extrude chemotherapeutics and fluorescent dyes (e.g., Hoechst 33342) via ATP-binding cassette (ABC) transporters. After dye exposure, Hoechst-positive cells are enriched for stem cells or, in other systems, cancer stem cells. In this assay, we found side population cells are increased in relapsed ALL from IR- or DXM-treated zebrafish. Moreover, allo-transplants using side population cells have higher engraftment rates, indicating LSC enrichment. Overall, our results suggest that ALL side population cells from rag2:hMYC zebrafish are significantly enriched for LSC by DXM or IR treatment. Using these LSC-enriching methodologies, we next aim to define the expression profiles of LSC vs. bulk ALL, in order to find LSC-specific markers to detect them, and LSC-specific pathway to target therapeutically.

**PP-65**

**Towards Understanding & Uncovering New Key Players in T Cell Development Upon Aging**

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Background: The decline of the immune system, which is referred to as aging-associated immune remodeling (AAIR), that occurs upon aging is due to many factors acting in concert. AAIR leads to an impaired ability to respond to vaccination and combat infections and therefore impacts negatively on the quality of life of the elderly. In contrast to other blood lineages, which develop primarily in the bone marrow (BM), the T-cell lineage develops in the thymus by continuous replenishment of thymic seeding progenitors (TSPs) from the BM. Several studies have linked AAIR to thymic involution. However, there is novel and mounting evidence that also aging of hematopoietic stem cells (HSCs) and lymphoid-primed multipotent progenitors (LMPPs) are immune system intrinsic players in AAIR. Very little, though, is known on mechanisms by which aging of HSCs and LMPPs contribute to AAIR.

Hypothesis: A central aim in understanding the AAIR is identifying how aged HSCs and LMPPs could possibly drive this phenomenon. Few possibilities could individually or in combination explain such a defect in an intrinsic fashion: aged HSCs and LMPPs could give rise to a defective TSP population associated with either: 1- size reduction, and/or, 2- lineage potential bias, and/or, 3- homing defect, already in the BM, that would fail to engage in normal T-cell differentiation.

Results: Our findings demonstrate that both aged HSCs and aged LMPPs retain the T-lineage potential in ex-vivo at the single cell level and in vivo upon transplantation assays. However, the detailed analysis of transplanted young recipients by aged HSCs shows significant decrease in the pool size of LMPPs in the BM and early thymic progenitors (ETPs) in the thymus, confirming that the old BM and thymic microenvironment could not be the sole reason behind this defect. Importantly, we were able to show that aged LMPPs are associated with a dramatic disadvantage in the development of early thymic T-cell populations as well as generating mature T-cell populations when intravenously transplanted along young LMPPs into young recipients in competitive settings. Whereas, similar competitive transplantation assays when injected intra-thymically led mainly to a dramatic disadvantage towards generating more matures T-cell stages, suggesting that aged LMPPs are associated with thymic developmental defect and a potential homing defect that occurs with age.

Conclusion: AAIR is a consequence of multiple immune parameters at play and we present here new data demonstrating a role for aging of LMPPs and changes in early thymic differentiation events in driving AAIR. Understanding how aged HSCs and LMPPs could possibly drive this AAIR phenomenon at the cellular and the molecular level is of crucial importance for developing new therapies to attenuate AAIR.

**PP-66**

**Altered Differentiation of Mesenchymal Stromal Cells during Acute Inflammation**

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The bone marrow microenvironment (BMM) is a complex network of blood and non-hematopoietic cells. These cells form the stem cell niche to aid in regulation of hematopoietic stem cells (HSCs) self-renewal, quiescence and differentiation. Within the non-hematopoietic compartment, subpopulations of mesenchymal stromal cells (MSCs) give rise to osteoblasts and adipocytes. These cells communicate with the hematopoietic system through adhesion molecules, cytokines, and chemokines to maintain homeostasis. Inflammation from infection or disease is an insult to hematopoietic homeostasis. Inflammationâ€™s disruptive impact on the hematopoietic system has been greatly studied; but how inflammation impacts the BMM is poorly understood. To grasp this impact, two well-characterized forms of inflammation will be studied: polyinosinic-polycytidylic acid (Poly(I:C)), a double-stranded RNA that stimulates IFNa, and Lipopolysaccharide (LPS), a lipoglycan that works through TLR4 to stimulate IFNg. During inflammatory conditions bone loss has been noted; therefore, we hypothesize an increase in the MSC population to compensate for the block in bone differentiation. Our initial experiments use flow cytometry to assess phenotypic changes in BMM subpopulations. Additionally, we use lineage-tracing models for an unbiased view of altered stromal lineages. The lineage specific Cre models mark stroma (Prx1), adipocytes (AdipoQ), and early (Osx) and late (OCN) stages of osteoblasts. Our data shows an increase in the MSC compartment with a decrease in osteoblasts and adipocytes 2 days after stimulation similar to when HSCs respond to inflammatory cues. One week later, the osteoblast compartment is still decreased, while the MSCs are unchanged. However, there are differences in maturation potential between in vivo and in vitro stimulation when stromal cells are differentiated into their respective lineages. In vivo stimulate d cells show increased osteoblasts differentiation while cells stimulated in vitro have the opposite impact on bone formation. During adipocyte differentiation there is no change in the amount of adipocytes present; however, the adipocytes are more mature from in vivo stimulation and less mature in in vitro. Consequently, the hematopoietic system likely impacts the stromal compartment leading to these alterations in differentiation bias. Indeed, TLR4 knockout hematopoietic cells are able to clarify these biases. Understanding changes in the BMM during inflammation will allow for therapeutic intervention in inflammatory diseases such as arthristis, osteomyelitis, and leukemia.

**PP-67**

**Distinct Tissue-Specific Functions for Bone Marrow Regulatory T Cells**

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Regulatory T cells within the bone marrow (BM-Tregs) represent a specialized population that has just recently been characterized. In this study, we establish that BM-Tregs regulate hematopoiesis and are necessary for the maintenance of stromal cells. Using RNA sequencing, we found that Tregs isolated from BM as compared to other secondary lymphoid tissues are enriched for genes associated with activation as well as express a unique chemokine and cytokine receptor profile. BM-Tregs exhibited enhanced expression of CD49a, a marker of mature, circulating Tregs, as well as S1PR1, involved in trafficking of cells through the lymphatics. These data are corroborated using parabiosis experiments and suggest that BM-Tregs are a migratory population equipped for circulation and immuno-surveillance. Remarkably, although circulation and T cell exchange in the BM is restricted compared to lymph node or spleen, the BM had the highest lev el of Treg chimerism and Tregs were exchanged at a higher frequency. Furthermore, depletion of Tregs via administration of anti-CD25 has widespread effects across several BM populations. Loss of BM-Tregs results in increased hematopoietic stem cell (HSC) cycling and proliferation, while fostering myeloid-skewed differentiation. Additionally, supportive stromal populations are also altered, as evidenced by increased cycling and proliferation. Non-genotoxic conditioning and transplantation of HSCs reveals that the Treg-depleted microenvironment is less supportive thus establishing a novel role for Tregs in maintaining niche function. Specifically, we found that BM-Tregs are high producers of IL-10, an immunosuppressive cytokine that limits inflammatory response. In fact, these cells are the primary source of this cytokine in the BM. Here, we show that the loss of IL-10 production by Tregs disrupts hematopoiesis and alters BM microenvironment. This includes an increase in myelo id cell production, and perturbation to multipotent stem and progenitor cells. More importantly, loss of Treg-produced IL-10 significantly alters stromal cells. These changes include a decrease to mesenchymal stromal cells, PDGFRÎ±+ progenitor cells, and bone forming precursors. Interestingly, in context of chronic myeloid leukemia, Treg numbers are dramatically reduced, and the functional and phenotypic characteristics of these cells are acutely disrupted, including IL-10 production. This is the first evidence that IL-10 from BM-Tregs impacts stromal cell maintenance, function, and differentiation. Collectively, our observations provide a novel mechanism by which BM-Tregs regulate hematopoiesis. These findings provide a better understanding of the specific mechanisms of IL-10 function on bone marrow stromal cells and it could be exploited during transplantation or hematopoietic disease.

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# ACKNOWLEDGMENTS


## SPECIAL THANKS FOR THE 14TH MIDWEST BLOOD CLUB MEETING ARE DUE TO:

St. Jude Children's Research Hospital for their support.

Pamela Franklin, Deanna Walls, Heather Duncan, and
Terri Davis for administrative and organizational support.


BMC 3/20 (AO76000)