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TITLE: Rescue Hematopoietic Stem and Progenitor Cell Functions in Bone Marrow Failure Syndromes

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14. ABSTRACT

Fanconi Anemia (FA) is one of the most common inherited bone marrow failure syndromes. If left untreated, 90% of children experience severe bone marrow failure or leukemia. There are few therapeutic options besides stem cell transplant (SCT), but the latter is associated with high risks of morbidity and mortality. The failure to appropriately deal with damaged genes especially hurts one type of cells in the body, called blood stem cells that are located in the bone marrow. These stem cells normally replenish blood supply for a lifetime but in the case of FA undergo attrition and finally complete exhaustion leading to a condition called bone marrow failure. Our work offers a new strategy by which the stem cell defect in FA might be overcome. Specifically, we discovered a gene, called SH2B3/LNK, which when disrupted leads to the expansion of blood stem cells in animal models including normal and FA animals. We identified the mechanisms by which SH2B3 deficiency improves FA HSCs, is not due to a correction of a particular type of DNA repair. Rather, SH2B3 deficiency enhances replication stress mitigation, decreases replication associated DNA damages, in part through cytokine/JAK signaling. In the past year, we have successfully explored the potential of LNK inhibition in expanding human hematopoietic stem cells in vitro and in vivo, and have begun to study LNK inhibition in restoring progenitor cells from FA patient cells.

15. SUBJECT TERMS

bone marrow failure syndromes, stem cell transplant, Fanconi Anemia, DNA damage, DNA replication

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1. **INTRODUCTION:**

Fanconi Anemia (FA) is one of the most common inherited bone marrow failure syndromes. Although initially identified over 90 years ago, FA remains a fatal genetic disease. If left untreated, 90% of children experience severe bone marrow failure or leukemia. There are few therapeutic options besides stem cell transplant (SCT), but the latter is associated with high risks of morbidity and mortality. Despite greater survival of children into adulthood as a result of SCT, the specter of the potential for solid tumors remains a serious problem. The defects underlying this disease spectrum impair the ability of affected individuals to repair damage to their genetic material as it occurs naturally or through exposure to environmental toxins. The failure to appropriately deal with damaged genes especially hurts one type of cells in the body, called blood stem cells that are located in the bone marrow. These stem cells normally replenish blood supply for a lifetime but in the case of FA undergo attrition and finally complete exhaustion leading to a condition called bone marrow failure. Our work offers a new strategy by which the stem cell defect in FA might be overcome. Specifically, we discovered a gene, called LNK, which when disrupted leads to the expansion of blood stem cells in animal models including normal and FA animals. We plan to follow up on what we believe to be a remarkable result by trying to better understand the mechanisms by which LNK functions in normal and FA blood stem cells, and devising means to perturb LNK as a novel approach to treat this devastating group of diseases.

2. **KEYWORDS:**

bone marrow failure syndromes, stem cell transplant, Fanconi Anemia, DNA damage, DNA replication

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - **Specific Aim 1: Elucidate the mechanisms by which Lnk deficiency alleviates replication stress and ameliorates HSC defects associated with BMF.**

    Major Task 1 Obtain HRPO/ACURO Approval. Month 3

    Major Task 2 for the first 12 months have been reported in last progress report.

    Major Task 2 for the last 12 months (Month 13-24):

    Subtask 6 Subject HSPCs from different BMF models over WT or Lnk deficient background to acetaldehyde (ICL damage), ATR inhibitors and PARP inhibitors (to induces replication stress), then measure DNA damage and cell survival.

    Subtask 7 Subject different BMF models over WT or Lnk deficient background to repeated pIpC injections, and measure DNA damage, progenitor cell survival, and HSC function by BMT.

    Subtask 8 To examine if Lnk deficiency reduces endogenous DNA damage, we will stain HSCs and MPPs with different markers, rH2Ax, 53BP1, and Rad51.

    Subtask 9 Determine if Lnk affects HSC reconstitution ability after transplant in vivo and cell growth/survival in ex vivo culture.

    Milestone(s) Achieved: Identify mechanisms by which Lnk deficiency alleviates replication stress and ameliorates HSC defects associated with BMF. Manuscript published in Nature Communications, 2019. Unpublished work will be written up in the coming year.

- **Specific Aim 2: Targeting LNK as a novel strategy to expand human HSPCs from BMF patients.**

  Major Task 3 for the first 12 months have been reported in last progress report.

  Major Task 3 for the last 12 months (Month 13-24): This part is still ongoing.

  Subtask 3 KD LNK in HSPCs from healthy subject or BMF patients, and examine if LNK inhibition will restore cell growth *in vitro*.

  Subtask 4 KD LNK in HSPCs from BMF patients and measure DNA damage upon ICL, chromosome aberrations, and replication fork stability upon stress, and CFC progenitor growth.

  Milestone(s) Achieved: Successfully inactivates LNK in primary human HSPCs, and expand HSPCs in both normal and BMF HSPCs. Manuscript published in Nature Communications, 2019. Unpublished work will be written up in the coming year.

- **What was accomplished under these goals?**

1. *major activities*

   We are on target with our SOW plans and made significant progress in both aims in the year 2 of this grant.

   For Specific Aim 1, we investigated the mechanisms by which Lnk deficiency alleviates replication stress and ameliorates DNA damage associated with BMF.

   For Specific Aim 2, we studied LNK inhibition to expand human FA-like HSPCs in vivo.

2. *specific objective*

   For aim 1, we set out to test the hypothesis that *Lnk* deficiency alleviates replication stress-induced DNA damage and genome instability, thereby rescuing the cell proliferation and survival defects of *Fancd2*$^{-/-}$ HSPCs ex vivo and HSC functions in vivo.

   For aim 2, we set out to test the hypothesis that *LNK* inhibition via shRNA-mediated knockdown would increase the growth and number of human FA-like HSPCs depleted of FANCD2 in xenotransplanted mice. Furthermore, we are testing if *LNK* inhibition via shRNA-mediated knockdown would restore the growth and blood forming ability of bone marrow HSPCs from FA patients.

3. *significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)*

   FA proteins are essential for repair of DNA inter-strand crosslinks (ICL) DNA damage as well as replication stress. As planned in SOW (Major task 2), we tested if Lnk deficiency restores HSC activities in multiple FA mouse lines. We found that Lnk deficiency rescued Fancc-/- and Fancg-/- HSC in transplantation assay (data not shown). Moreover, we found that Lnk deficiency rescued Fancd1-/- (also called Brca2) HSC in transplantation assay (data

not shown). These exciting data suggest that Lnk is a broad suppressor of FA, which can be further explored in therapeutic targeting.

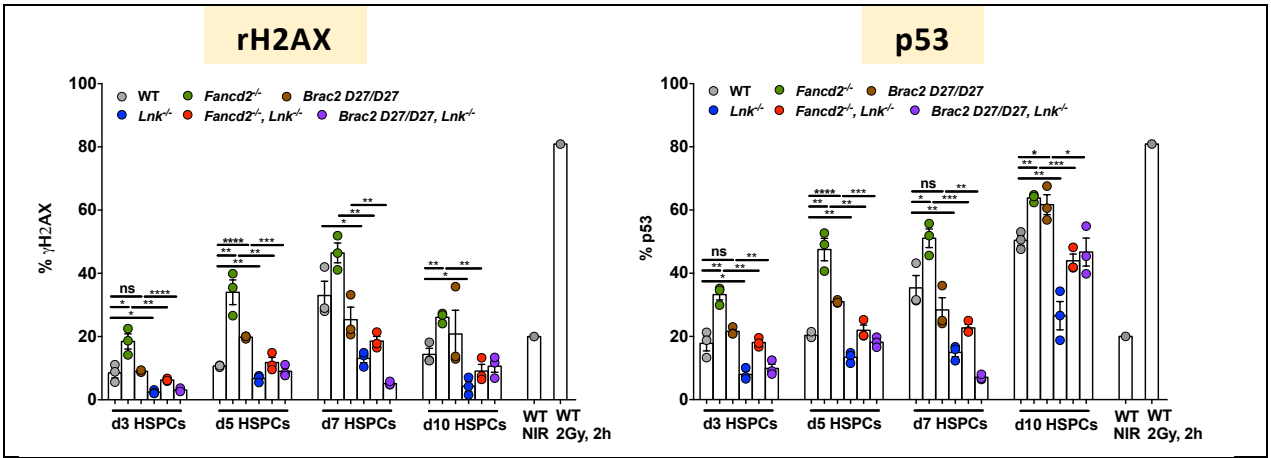

Figure 1. ***LNK/SH2B3*** **deficiency reduces DNA damage and p53 activation in FA cells.** HSPCs from WT, Lnk-/- , FA single KO and FA;Lnk DKO mice were cultured in cytokines. Different time points in culture, rH2Ax **(Left)** activation and p53 levels **(Right)** were quantified by intracellular flow cytometry and graphed. WT cells without (NIR) or with IR (2Gy) were used as controls. Two-tailed Student t-tests are shown. *; p<0.05; **: p<0.01. ns: not significant.

F

A proteins also play an important role in the tolerance of replication stress and mitigates DNA damage induced by endogenous stress. As planned in SOW (Major task 2), we tested if Lnk deficiency reduces endogenous DNA damage, we will stain HSCs and MPPs with different markers, rH2Ax, 53BP1, and Rad51 in ex vivo culture. As shown in Figure 1, FA HSPCs accumulated DNA damage and resulted in p53 activation that was increasing with culture time. Lnk deficiency mitigated these DNA damage and the reduced p53 activation in both Fancd2-/- and Brca2 ex27 (Fancd1) mutant background.

As planned in SOW (Major task 2), we set out to investigate replication machinery in active and stalled replication forks affected by FA and Lnk. We are in the process of optimizing the protocols and compare different method for enrichment of replication forks. These studies will be continued into the NCE stage this grant.

For Specific Aim 2, we investigated LNK inhibition to expand human FA-like HSPCs in xenotransplanted mice in vivo, based upon our encouraging in vitro data last year.

Mutations in FA genes severely compromise hematopoietic stem cell (HSC) capacity, culminating in bone marrow failure and cancer predisposition. Our findings in animal models suggest targeting LNK might be a therapeutic target to enhance HSC activities from BMF, in particular FA. As planned in SOW (Major task 3), provided a proof-of-concept experiment showing that deletion of LNK in human FA-like hematopoietic stem and progenitor cells promoted clonogenic growth (Figure 2).

As planned in SOW (Major task 3), we have knocked down LNK in umbilical cord blood (healthy donor) HSPCs and transplanted them into sublethally-irradiated NOD/SCID-IL2Rg$^{-/-}$ Kit ^{W41} (NBSGW) mice to assess of LNK inhibition will restore HSC repopulating ability *in vivo*. We are excited to report that we have performed 3 independent xenotransplantations showing that LNK inhibition marked enhanced blood reconstitution in all lineages as well as in HSPCs in the bone marrow (Figure 2 and data not shown). We are furthering our studies by knocking down LNK along with FANCD2 followed by transplantation to assess if LNK inhibition can restore FA-like HSPCs in vivo. Since each transplant takes 4 months and an addition 4 months in secondary transplants, we are waiting to obtaining results of these lone experiments. More importantly, we have obtained FA patient bone marrow cells from our

collaborator, Dr. Tim Olson (co-I of this grant). We are testing if LNK inhibition could enhances clonogenic growth in vitro and HSPC reconstitution ability in vivo. These studies will be continued into the NCE stage of this grant.

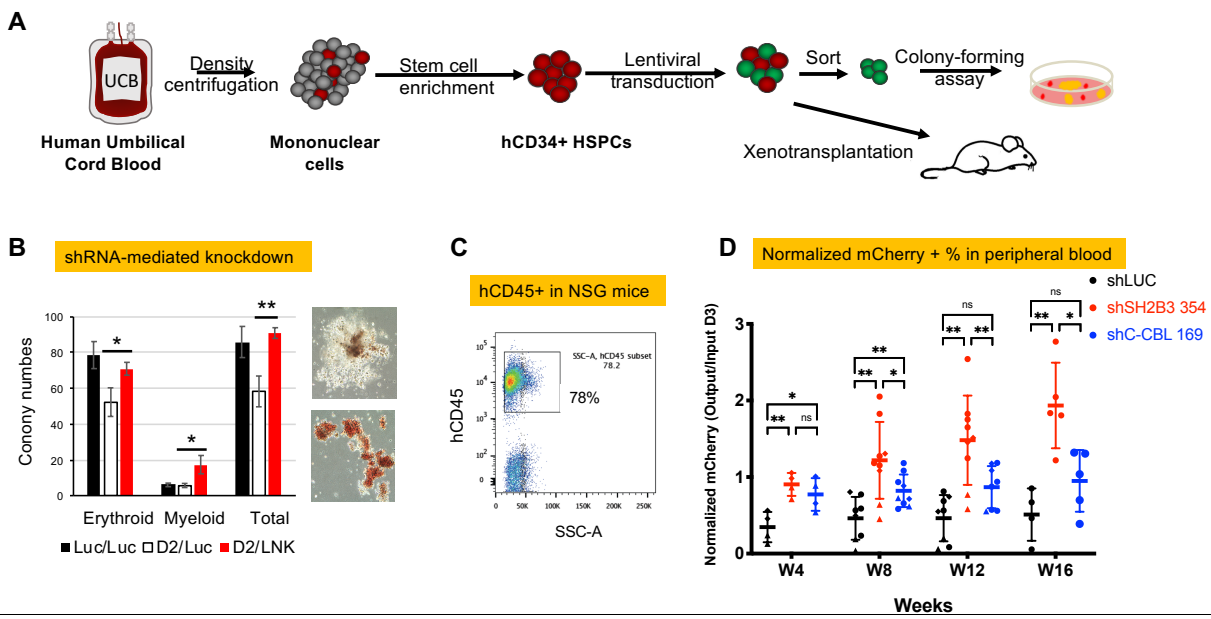

Figure 2. *LNK/SH2B3* depletion restores *FA*-like progenitor cells in vitro and expands HSCs in vivo. A) depicts a schematic overview of isolation of primary human HSPCs for lentiviral infection followed by colony-forming-cell (CFC assays or xenotransplantation. **B)** UCB-derived CD34+ cells were co-infected with lentiviruses expressing shRNA to Luciferase (Luc) or FANCD2 (D2) with GFP marker, along with shRNA to Luc or LNK with (LNK) mCherry marker. GFP+mCherry+ cells were then sorted and plated onto semi-solid methylcellulose cultures. Colony-forming progenitor numbers are shown with a two-tailed Students' t-test. Representative images of CFU-GM and B-CFU are shown. **C)** Fresh 100,000 CD34+ cells were transplanted into busulfan pre-conditioned NSG mice. Representative results of xenotransplantion is shown as hCD45+ cells in the bone marrow of NSG mice at 12 weeks. **D)** CD34+ cells were cultured and infected with shRNA to Luc, LNK (also called SH2B3), or c-CBL with mCherry as a marker, and subsequently 250,000 cells were injected into each NBSGW mice. The graph shows the normalized mCherry percentage (output/input) in the hCD45+ fraction of the peripheral blood of NBSGW mice every 4 weeks. Two-tailed Student t-tests are shown. *; $p<0.05$; **: $p<0.01$. ns: not significant.

Hence, our findings reveal the importance of communication between extracellular signals and replication associated genome maintenance, and highlight a new role for cytokine/JAK signaling in promoting replication fork stability and ameliorating replication stress. Our studies also illuminate replication stress as a major underlying origin of bone marrow failure in FA patients and have implications for therapeutic strategies to treat FA associated bone marrow failure.

4. *other achievements.*

None.

- **What opportunities for training and professional development has the project provided?**

The trainee on this project, Dr. Brijendra Singh, is given various training opportunities on campus and off campus. He presents quarterly in our weekly lab meeting. We have joint lab meetings with Drs. Nancy speck, Ivan Millard, Kathrin Bernt, and Vikram Paralker's laboratories on stem cells that meet every Thursdays, when members of groups rotate presenting work-in-progress and journal clubs. Brijendra has been presenting about once every half a year in this joint meeting. We have a bi-weekly Ubiquitin group meeting with multiple PIs from PENN including the co-I in this project, Dr. Roger Greenberg. Many of them, including our collaborator and co-I, Dr. Roger Greenberg, are experts in DNA repair and DNA replication.

Brijendra has been presenting in this meeting twice a year. The trainees also have many opportunities to further their research training outside of my laboratory. My group participates and presents at the CHOP Hematology Research Group meeting in which we meet with the co-I on this project Dr. Tim Olson, the PENN Cancer center weekly seminars, monthly Benign and Malignant Hematopoiesis Research Affinity Group seminar series at CHOP and PENN, joint thrombosis and hemostasis group meeting, and the annual Cancer Center retreat. The trainees on this project have presented at least once a year in these meetings. They will also have the opportunity to attend one major meeting per year. In addition to these opportunities, they will have the opportunity to attend his choice of the myriad of seminars that occur at the University of Pennsylvania and take specific courses that will further his development. In addition, I have been involved in teaching summer students including minority students through various programs at PENN and CHOP.

- **How were the results disseminated to communities of interest?**

  Presentations of results arising from this grant have been made as the Annual meeting of the American Society of Hematology (ASH), in the form of an oral presentation whose abstract was published in the journal of Blood. We also plan to present our results at the Fanconi Anemia Research Fund annual symposium in Sept. 2019. In addition, results have been disseminated locally to the UPENN/CHOP joint Research-in-Progress Group and the CHOP Hematology Division as well as annual retreat, via internal seminars and discussion groups. Trainees on this grant also presented the work at our joint "ubiquitin and cytokine signaling" group meetings that consists of 6 laboratories from UPENN and CHOP campus. Results have also been disseminated by preparing articles for scientific journals.

- **What do you plan to do during the next reporting period to accomplish the goals?**

  We plan to comprehensively investigate replication machinery affected by BMF genes and Lnk deficiency. We will study if Lnk deficiency mitigate DNA damage, p53 induction and suppress genome instability associated with FA. Last and importantly, we will KD LNK in HSPCs from BMF patients, and examine if LNK inhibition will restore cell growth *in vitro* and *in vivo*.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

   - **What was the impact on the development of the principal discipline(s) of the project?**

     This work is based on our impactful finding that loss of a negative regulator of cytokine signaling, Lnk (also called Sh2b3) restores hematopoietic stem/progenitor cell (HSPC) functions in a mouse model of FA. We now show that LNK inhibition expands human HSPCs and ameliorates stem cell defects associated with FA patient HSPCs. Notably, loss of Lnk does not amplify the risk for leukemia transformation in this system. To our knowledge, this is one of the very few examples of in vivo genetic suppression of HSPC defects that are the defining feature of FA. If successful, our studies will deepen our mechanistic understanding of this disease and unveil new therapeutic strategies to treat this disease. A common thread among bone marrow failure syndromes is the attrition of hematopoietic stem cells. Therefore, through elucidation of signaling pathways that control stem cell homeostasis, the impact of our studies is expected to reach beyond FA. Finally, given our use of primary human samples, we anticipate that our results will be directly relevant to human FA biology.

- **What was the impact on other disciplines?**
  - Nothing to Report.
- **What was the impact on technology transfer?**
  - Nothing to Report.
- **What was the impact on society beyond science and technology?**
  - Nothing to Report.

5. **CHANGES/PROBLEMS:**
   - Nothing to Report.
   - **Changes in approach and reasons for change**
     - Nothing to Report.
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     - Nothing to Report.
   - **Changes that had a significant impact on expenditures**
     - Nothing to Report.
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
     - Nothing to Report.
   - **Significant changes in use or care of human subjects**
     - Nothing to Report.
   - **Significant changes in use or care of vertebrate animals.**
     - Nothing to Report.
   - **Significant changes in use of biohazards and/or select agents**
     - Nothing to Report.

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**
     - **Journal publications.**

     Balcerek J, Jiang J, Li Y, Singh B, Holdreith N, Chandra V, Jiang Q, Rozenova K, Greenberg RA, and **Tong W**. Lnk/Sh2b3 Deficiency restores Hematopoietic Stem Cell Function and Genome Integrity in Fancd2-deficienct Fanconi Anemia. *Nature Communications,* 2018 Sep 25;9(1):3915. [PMC6156422]

- **Books or other non-periodical, one-time publications.**
  None.
- **Other publications, conference papers, and presentations.**

Joanna Balcerek, Jing Jiang, Qinqin Jiang, Krasimira Rozenova, Roger A. Greenberg, and Wei Tong: Restoration of Hematopoietic Stem Cell Function and Genome Integrity in Fanconi Anemia The 2018 Fanconi Anemia Research Fund (FARF) Scientific Symposium, Newport Beach, CA. Podium presentation. September 2018.

Nicholas Holdreith, Joanna Balcerek, Yang Li, and Wei Tong: Enhancing Gene Therapy Efficacy by Increasing Hematopoietic Stem Cell Expansion. Gene Therapy meeting of the Fanconi Anemia Research Fund, San Francisco, CA. Podium presentation April 2019.

- **Website(s) or other Internet site(s)**
  None.
- **Technologies or techniques**
  None.
- **Inventions, patent applications, and/or licenses**
  None.
- **Other Products**
  Plasmid DNA: Mammalian expression constructs for shRNA or CRISPR for LNK will be available for distribution upon publication.

  Cell lines: Cell lines with stable expression of knockdon/knockout constructs for LNK and FANCD2, were published and are available upon request.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Wei Tong*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0001-9951-2273
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Tong supervised the entire project, involving in the design, execution and interpretation of all data.*
Funding Support:	

Name:	*Brijendra Singh*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g.	

ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Singh has performed work in the specific aims proposed, which is to investigate the mechanisms by which LNK regulates replication stress and mitigate DNA damage in BMF HSCs.*
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Tong, Wei
Previously active grants that have closed:
R01DK119479 (Blobel) 08/05/2013 – 5/31/2018 1.2 calendar
NIH/NIDDK direct / yr
Functions, mechanisms, and therapeutic potential of chromatin looping
The goal of this research is to investigate the mechanisms by which chromatin loops regulate gene expression and provide therapeutic strategies for treatment of sickle cell disease.
Role: Co-I.

Aim 1: Mechanisms of Ldb1-mediated chromatin looping.
Aim 2: Enhance the applicability of forced chromatin looping.
Aim 3: Forced chromatin looping to reprogram the ß-globin locus.
Aim 4: Reactivation of gamma-globin expression in a humanized mouse model of sickle cell anemia (SCA).

Funding Agencies Grants Officer: Dr. Terri Bishop, Division Kidney, Urologic & Hematologic Diseases NIDDK, National Institute of Health, Building 2DEM, Room 619, 6707 Democracy Blvd., Bethesda, MD 20892.

Previously pending grant are now active:
BRCA Collaborative Grants (Greenberg) 02/15/2019 - 02/14/2020 0.12 calendar
V Foundation for Cancer Research direct to Dr. Tong / yr
Understanding and Exploiting the Heterogeneity of Cell Intrinsic and Extrinsic Responses to DNA Damage in BRCA mutant Cancers
Role: PI of a subaward

SBF Research grant (Tong) 07/1/2019 – 06/30/2020 0.6 calendar
St. Baldrick's Foundation (SBF) direct / yr
Genome-wide CRISPR/Cas9 Screens in Precursor B Acute Lymphoblastic Leukemia
The goal of this project is to preform genome-wide CRISPR/Cas9 screens in precursor B acute lymphoblastic leukemia in vitro
Role: PI

ALSF Innovation grant (Tong) 10/1/2019 – 09/30/2021 1.2 calendar
Alex's Lemonade Stand Foundation for Cancer research direct / yr *Identify Novel Druggable Targets to Treat Philadelphia chromosome-like B-Acute Lymphoblastic Leukemia*

The goal of this project is to explore therapeutic targets in treating Philadelphia chromosome-like Acute Lymphoblastic Leukemia
Role: PI

Greenberg, Roger
Previously pending grant are now active:
1U54 CA193417 (Discher) 5/1/2016-4/30/2020 0.00 Calendar
NCI (average direct)
Liver Cancer: Pre-Malignant Stiffening, Membrane Transduction, & Nuclear Rheology
Goals: To understand how nuclear structure affects DNA damage responses and genome stability.

BRCA Team Convergence Award (Greenberg PI) 03/1/2018-2/28/2021
The V Foundation average direct Understanding and Exploiting the Heterogeneity of Cell Intrinsic and Extrinsic Responses to DNA Damage in BRCA Mutant Cancers
Goals: To define the molecular basis for differential responses to PARP inhibitors in BRCA mutant cancers and devise strategies to activate immune responses that eradicate cells regardless of their sensitivity to DNA damaging therapies.

BRCA Team Science Grant (Greenberg Co-PI) 09/1/2019-8/31/2023
The Gray Foundation
(average direct) Dissection of BRCA-mediated Tumor Suppression Pathways
Goals: To define the molecular basis for noncanonical DNA repair networks that become active in BRCA mutant cancers.

Olson, Timothy
Previously pending grant are now active:
Title: ***Immune Pathogenesis of Acquired Aplastic Anemia***
Role: Co-PI
Status of application: Active
Time commitment: 1% effort (0.12 calendar months)
Supporting agency: University of Pennsylvania Institute for Translational Medicine and Therapeutics' Transdisciplinary Program in Translational Medicine and Therapeutics (supported by NCATS/NIH Award number UL1TR001878
Funding Agency Grant Officer: Garret A. FitzGerald, MD. Agency email: aalbelda@pennmedicine.upenn.edu
Performance period: proposed: 2/1/2019-1/31/2021
Level of funding: $150,000 total direct costs
Brief description of the project's goals: to use knowledge gained regarding specific HLA Class I risk alleles in acquired aplastic anemia to identify autoantigens that drive this disease
List of the specific aims:
Aim 1: To identify the shared structural characteristics of AA HLA class I risk alleles using a multi-institutional AA patient cohort.
Aim 2: To identify candidate autoantigens in AA.
Aim 3: To screen candidate autoantigens for reactivity with T cells from patients with AA.
Overlap: none

Title: ***Improving Stem Cell Niche Function During Cellular Therapy for Leukemia Predisposition Syndromes***

Role: PI
Status of application: Active
Time commitment: 15% effort (1.8 calendar months)
Supporting agency: Hyundai Hope on Wheels Scholar Hope Award
Funding Agency Grant Officer: Zafar Brooks. Agency email:
Performance period: 12/31/2018-12/30/2020
Level of funding: total costs
Brief description of the project's goals: to identify novel pathways within bone marrow stem cell niche cells that are defective in animal models of leukemia predisposition syndromes that can then be targeted to improve engraftment after hematopoietic stem cell transplantation
List of the specific aims:
Aim 1: To determine whether BM niche dysfunction drives hematopoietic failure pre- and post-SCT in SAMD9 and SAMD9L-associated leukemia predisposition syndromes (LPS).
Aim 2: To define molecular signatures that predict BM niche dysfunction during SCT by study-ing niche composition and gene expression across multiple LPS models.
Aim 3: To develop a therapeutic approach targeting insulin-like growth factor (IGF)-1 signaling to overcome engraftment barriers caused by BM niche dysfunction.
Overlap: None.

Title: ***Bone Marrow Niche Targets To Prevent Cancer in Shwachman Diamond Syndrome***
Role: PI
Time commitment: 5% effort (0.6 Calendar Months)
Supporting agency: Cure Childhood Cancer Foundation
Funding Agency Grant Officer: Kristin Conner, Executive Director. Email: kristin@curechildhoodcancer.org
Performance period: 7/1/2019-12/31/2020
Level of funding: total costs
Brief description of the project's goals: Using an animal model of Shwachman Diamond Syndrome identify molecular targets to improve niche function and develop a gene therapy approach targeting bone marrow niche cells
List of the specific aims:
Aim 1: To define the impact of SBDS deficiency on HSCT recipient BM niche cellular composition and molecular signaling.
Aim 2: Determine whether conditional Sbds deletion in BM niche cells causes poor engraftment of healthy donor HSC due to defects in hematopoietic stem and progenitor cell homing during HSCT
Aim 3: To develop a BM niche-specific gene correction approach to rescue engraftment deficits after HSCT caused by SBDS deficiency.
Overlap: None.

- **What other organizations were involved as partners?**
  - Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**
   - Nothing to report.

9. **APPENDICES:**
   - None.