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

PRINCIPAL INVESTIGATOR: Wei Tong, PhD

CONTRACTING ORGANIZATION: Children's Hospital of Philadelphia

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Wei Tong, PhD

E-Mail: tongw@chop.edu

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CHILDREN'S HOSPITAL OF PHILADELPHIA,
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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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TABLE OF CONTENTS

Page

1. Introduction…….... 2
2. Keywords……..... 2
3. Accomplishments ……..... 2
4. Impact ……..... 5
5. Changes and Problems…….. 5
6. Products ……..…… ……..... 6
7. Participants & other collaborating organizations ……. 7
8. Special reporting requirements ..…….. 10
9. Appendices……... 10

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 reports.

    Major Task 2 for the last 12 months (Month 13-24): Most of this part has been completed previously, and the new results shown in below sections.

    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.

    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 is being written up and we plan to submit it for publication 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 reports.

    Major Task 3 for the last 12 months (Month 13-24): Most of this part has been completed previously, and the new results shown in below sections.

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

    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 is being written up and we plan to submit it for publication in the coming year.

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

  1. *major activities*

We have completed most of our SOW plans and made significant progress in both aims in the NCE year of this grant.

For Specific Aim 1, we investigated how Lnk deficiency ameliorates DNA damage associated with replication stress in HSPCs specifically by reducing ssDNA (single strand DNA) formation.

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

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)*

Hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for a variety of hematological diseases and disorders caused by underlying dysfunction in hematopoietic stem cells (HSCs). One such disease is Fanconi Anemia (FA), one of the most common inherited bone marrow failure (iBMF) syndromes. FA is caused by mutations in one of the 23 FA genes responsible for DNA repair, DNA replication, and genome integrity. HSCT-based gene therapies show promise for curing FA, however, the efficiency is hindered by the reduced number and impaired growth of HSCs from FA patients, resulting in low engraftment and suboptimal restoration of blood production. We previously demonstrated in mouse models that the adaptor molecule LNK(SH2B3) attenuates the activation of tyrosine kinase JAK2 in hematopoietic stem and progenitor cells (HSPCs) in response to thrombopoietin (TPO) stimulation, a critical cytokine for HSC self-renewal. Consequently, *Lnk*-null mice exhibit enhanced JAK/STAT signaling in HSPCs resulting in expanded functional HSCs and increased blood counts (Bersenev et al., JCI 2008). Moreover, Lnk deficiency in a FA mouse model (FancD2-/-) restores HSC defects, in part, by improving DNA replication fork stability during stress **(Balcerek et al., Nat. Comm. 2018).** Based on these results, we studied how LNK deficiency reduces DNA damage in HSCs associated with FA, and tried to translate our studies to human HSCs.

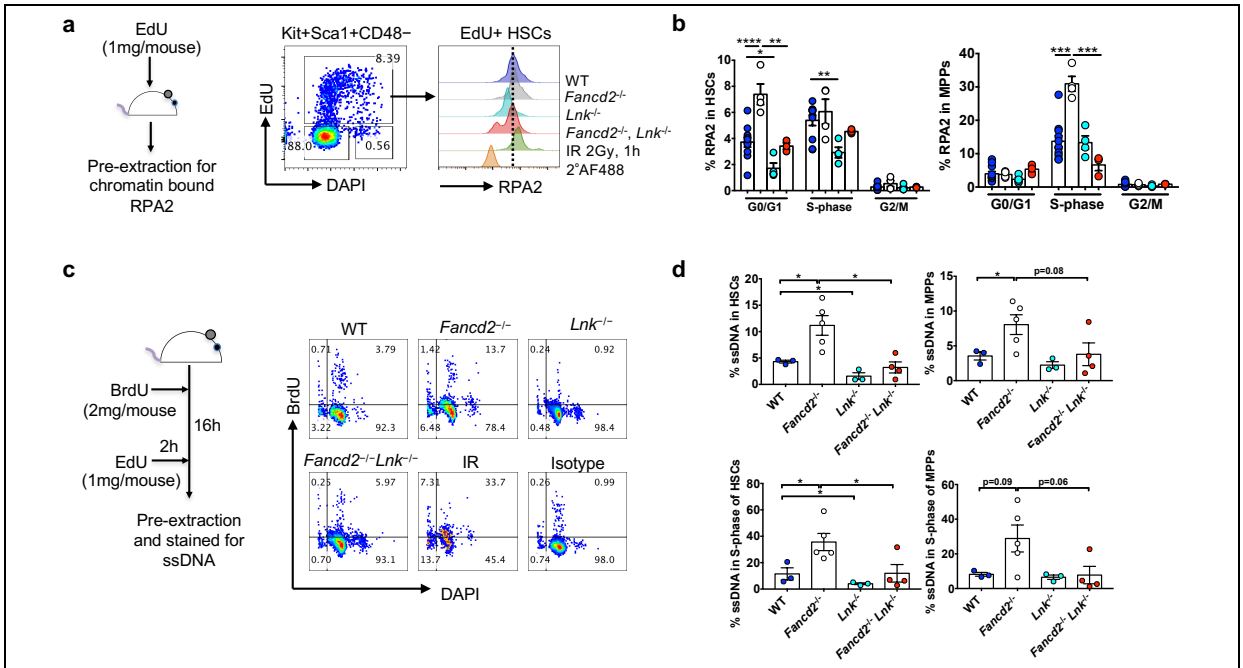

Figure 1. *Lnk* deficiency reduces ssDNA in proliferating FA cells. WT, Lnk-/- , FA single KO and FA;Lnk DKO mice were injected with DNA analogs and HSPCs were subjected to flow cytometry analysis of ssDNA **(c and d)** and RPA **(a and b)**, which is a ssDNA binding protein. Experimental schemes and representative flow cytometry plots are shown in **a and c**. **(b)** Percentage of chromatin bound RPA2^{+} cells in different cell cycle stages of HSCs and MPPs. **(d)** Quantification of total ssDNA within HSCs and MPPs (top), and percentage of ssDNA in S-phase of HSCs and MPPs (bottom). Two-tailed Student t-tests are shown. *; p<0.05; **: p<0.01; ***: p<0.001. ns: not significant.

For Specific Aim 1, we investigated how Lnk deficiency ameliorates DNA damage associated with replication stress in HSPCs specifically by reducing ssDNA (single strand DNA) formation.

FA proteins 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 stained HSCs and MPPs with DNA damage markers, rH2Ax, as well as p53 in ex vivo culture, and upon transplantation stress. We showed previously that Lnk deficiency mitigated these DNA damage and the reduced p53 activation in both Fancd2-/- and Brca2 ex27 (Fancd1) mutant background. In the past year, we showed that Lnk deficiency reduces the activation of ATR pathway, suggesting that it mitigates replication stress-activated ATR (data not shown). We then examined ssDNA in primary HSPCs. As shown in Figure 1, FA HSPCs accumulated ssDNA and chromatin-bound RPAs in the S-phase of the cell cycle. Importantly, Lnk deficiency markedly reduced ssDNA level in HSCs. These data suggest that Lnk deficiency ameliorates DNA damage associated with replication stress in HSPCs specifically by reducing ssDNA (single strand DNA) formation.

Work conducted here is in preparation for publication.

For Specific Aim 2, we investigated LNK inhibition to expand human FA-like HSPCs in xenotransplanted mice in vivo, in our previous report.

Based on these encouraging studies, and as planned in SOW (Major task 3), we tested LNK inhibition in HSPCs from FA patients in clonogenic assays *ex vivo*, due to the paucity of HSPC numbers associated with FA (Figure 2). Important to note, we obtained FA patient bone marrow cells from our collaborator, Dr. Tim Olson (co-I of this grant). Our data showed that targeting LNK in FA patient-derived HSPCs enhanced their colony forming potential *in vitro*. We were able to perform xenotransplantation of one patient sample and encouragingly, LNK inhibition enhanced blood, bone marrow and spleen reconstitution. Together, these results show abrogation of LNK expression augments HSPC function from healthy and FA patients *in vitro* and *in vivo*. Our results demonstrate the potential of targeting LNK to augment HSPC function and expand gene-corrected HSCs to improve HSCT-based gene therapy for FA. Strategies to inhibit LNK in FA gene therapy HSCTs should be further investigated to improve the engraftment rate of gene-corrected FA HSPCs to accelerate BMF stabilization and restore blood counts to normal levels. Work conducted here will be submitted for publication in November, 2020.

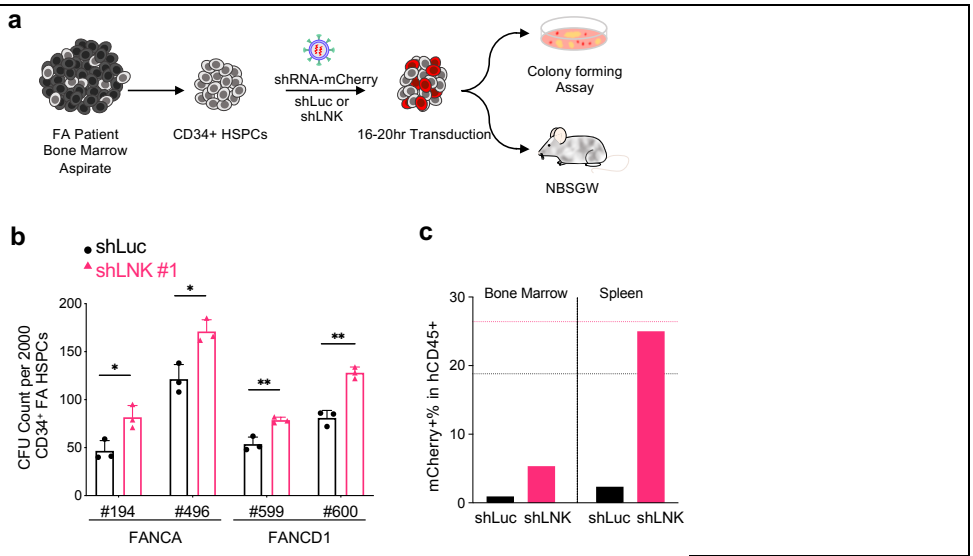

Figure 2. *LNK/SH2B3* inhibition enhances the function of primary patient-derived Fanconi Anemia HSPCs. a) Experimental scheme of lentiviral transduction followed by CFC (colony forming cell) or xenotransplantation assay. Primary CD34$^+$ HSPCs were isolated from BM aspirates of FA patients and transduced with lentivirus expressing shLuc or shLNK along with the mCherry marker. Transduced cells were either directly injected into NBSGW mice or plated for CFC assays after flow cytometric sorting of mCherry$^+$ cells. b) FA HSPCs were sorted for mCherry positivity 48 hours post-transduction and plated onto semi-solid methylcellulose culture media. Colony forming unit (CFU) progenitors were enumerated 14 days later. Each symbol represents an individual plate; bars indicate mean values; error bars indicate SD. *: $p<0.05$; **: $p<0.01$, as determined by two-tailed Student's *t*-tests. (n=4 patient samples). c) Transduced HSPCs from two FA patients (FA-D1 #599, 600) were transplanted into NBSGW mice and engraftment in the BM and spleen was assessed after 16 weeks. mCherry$^+$% in the engrafted hCD45$^+$ BM and spleen is shown. (n=1 patient FA-D1 #600, 1 mouse/per shRNA).

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4. *other achievements.*

   None.

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

  Since this is a NCE year, there was only enough funds to support Dr. Tong. A NRSA F31 fellowship trainee Nick Holdreith carried out studies in human HSCs. Nick is currently preparing a manuscript on this work for publication and plan to defend his PhD thesis next year. Nick and the trainee on this project in previous years, Dr. Brijendra Singh, have been given various training opportunities on campus and off campus. They present 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. They have been presenting about once every half a year in this joint meeting including presenting RCR trainings. 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 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 at international scientific meetings in the form of an oral presentations. We presented our results at the Fanconi Anemia Research Fund annual symposium in Sept. 2020. 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. 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?**

  Nothing to report.

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. With the support of this grant, 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.**

       None.

     - **Books or other non-periodical, one-time publications.**
       None.

     - **Other publications, conference papers, and presentations.**

     Nicholas Holdreith, Grace Lee, Peter Nicholas, Timothy S. Olson and Wei Tong: *LNK(SH2B3)* Inhibition Enhances Hematopoietic Stem and Progenitor Cell Function from Healthy Donor and Fanconi Anemia Patients. The 2020 Fanconi Anemia Research Fund (FARF) Scientific Symposium. Podium presentation (Virtual). September 2020.

   - **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:	

- **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:

LLS Translational Research Program (TRP) award (Tong) 10/1/2017 – 06/14/2020
1.8 calendar Leukemia Lymphoma Society (LLS) direct yr. | indirect/yr.
CBL Regulation of Ubiquitination and Cytokine Signaling in Myeloid Malignancies
The goal of this project is to investigate CBL signaling network in Chronic Myelomonocytic Leukemia.
Role: PI

Previously pending grant are now active:

W81XWH-19-1-0575 (Liu) 08/01/2019 - 07/31/2021
1.2 calendar DOD Idea Award direct /yr
Targeting Leukemia imitating cells to improve leukemia treatment
Role: co-Investigator

5 T32 DK007780-21 (Tong, W) 07/01/2020-06/30/2025 0.6 calendar
Hematopoiesis Training grant **to Dr. Tong / yr**
Role: PI

R01DK127738-01 (Tong) 09/18/2020 - 08/31/2025 2.4 calendar
NIH/NCI direct /yr
Regulation of Ribosome Biogenesis in Hematopoietic Stem Cells
The major goal of this project is to investigate the role of ribosome biogenesis proteins in regulating hematopoietic stem cell expansion.
Role: PI

HRFF grant (Tong) 06/1/2020 – 05/31/2023 0.6 calendar
Pennsylvania Department of Health total direct
Expand Hematopoietic Stem Cells to Improve the Treatment of Hematologic Disorders
The goal of this project is to identify regulators that control hematopoietic stem and progenitor cell expansion and to explore their therapeutic potential for the treatment of blood disorders.

Greenberg, Roger

Previously active grants that have closed:

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.

Target Identification in Lupus Grant (Greenberg) 05/01/2017 – 4/30/2020 0.5 Calendar Months Lupus Research Alliance Annual Direct:
BRISC DUB Activity as a Novel Target for Lupus
Goals: Identify BRISC substrates that are required for its activities in inflammatory signaling and develop BRISC DUB inhibitors with in vivo efficacy

Previously pending grant are now active:
1R01 CA138835 (Greenberg) 04/01/2020 – 03/31/2025 1.44 Calendar Months NIH/NCI Annual Direct:
The RAP80-BRCC36 Deubiquitinating complex in DNA repair
Goals: To determine how ubiquitin recognition and deubiquitinating enzyme activity within this complex contributes to BRCA1 dependent DNA repair.

002085-0001-14759 (Sung) 07/01/2019 – 0.48 Calendar Months 06/30/2023 Gray Foundation Annual Direct:
Dissection of BCRA-mediated Tumor Suppression Pathways
Goal: Project 2 Aim: Systemic investigation of BRCA-independent homology-direct DNA repair

The Mark Foundation for Cancer Research 6/1/2019-5/31/2024 0.48 Calendar Months (Minn) Annual Direct:
Goal: To determine DNA repair mechanisms that impact anti-tumor immune responses.

Olson, Timothy
Previously active grants that have closed:
Title: ***Defining the Landscape of Genetic Predisposition to Pediatric Myeloid Malignancies***
Role: PI
Time commitment: Effort 8.3%, 1 Cal Mo
Supporting agency: Canuso Family Foundation
Funding Agency Grant Officer: Kevin Kane, Director of Development, The Children's Hospital of Philadelphia, Phone 267-246-4545. Email: kanekj@email.chop.edu
Performance period: 7/31/17-6/30/19
Level of funding: total direct costs
Brief description of the project's goals: to use a groundbreaking genomic technology to closely examine the inherited, germline origins of pediatric MDS/AML susceptibility.
List of the specific aims:
Aim 1: To define the frequency and clinical characteristics of germline mutations in MDS/AML predisposition genes in a retrospective CHOP cohort of pediatric patients with BMF, MDS, and AML using a novel high throughput Next Generation Sequencing (NGS) Panel for Predisposition to Hematologic Malignancies.
Overlap: none

Title: ***Targeting the Marrow Niche to Improve Stem Cell Transplantation***
Role: PI
Status of application: Active
Time commitment: No direct salary support currently, but past salary support with 10% research effort (1.2 calendar months) and (currently in no cost extension)
Supporting agency: W.W. Smith Charitable Trust
Funding Agency Grant Officer: Ed Flood, flooded@email.chop.edu The Children's Hospital of Philadelphia Foundation 100 Penn Square East - 8th Floor, Suite 8050 Philadelphia PA 19107
Performance period: 1/1/2018-12/31/2019
Level of funding: total costs
Brief description of the project's goals: Studies using animal models investigating how SBDS deficiency and IGF-1 impact stem cell transplantation
List of the specific aims:
Aim 1: To define how reduction in BM IGF-1 levels enhances donor HSC engraftment following SCT.
Aim 2: To define how SBDS deletion within the BM niche impairs donor HSC engraftment after SCT.
Overlap: none

Previously pending grant are now active:
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

Title: ***Pre-malignant Clonal Evolution in Telomere Biology Disorders (TBD)***
Role: Co-PI (Babushok PI)
Status of application: Active
Time commitment: 1%
Supporting agency: MILLION DOLLAR BIKE RIDE GRANT PROGRAM, Orphan Disease Center
University of Pennsylvania
Funding Agency Grant Officer: Nico Meyering. Agency email: nmeyeri@upenn.edu
Performance period: 2/01/2020 - 1/31/2021
Level of funding: total direct costs
Brief description of the project's goals: to evaluate drivers of pre-malignant clonal evolution in telomere biology disorders
List of the specific aims:
Aim 1: To determine genetic drivers of pre-malignant clonal evolution in TBD.
Aim 2: To evaluate transcriptional alterations in cell senescence and DDR pathways in TBD
patients.
Overlap: None

Title: ***Risk stratification for Genetic Causes of Severe Aplastic Anemia***
Role: Subcontracted Co-Investigator (Furutani, Boston Children's Hospital, primary PI)
Status of application: Active
Time commitment: No salary support (supports lab personnel time)
Supporting agency: Aplastic Anemia and MDS International Foundation
Funding Agency Grant Officer: Dr. Ellen J. Salkeld, PhD; Senior Director of Research, Aplastic Anemia and MDS International Foundation, 4330 East West Highway, Suite 230, Bethesda, MD 20814, Office: (301)279-7202 x123
Performance period: 1/01/2020 – 6/30/2021
Level of funding: (spread over 3 study sites)
Brief description of the project's goals: To determine how frequently germline genetic testing identifies an actionable mutation in patients presenting with newly diagnosed aplastic anemia.
List of Specific aims:
Primary Aim: To estimate the incidence of iBMFS in patients presenting with SAA who have had a negative chromosomal breakage test and normal telomere lengths
Secondary Aims: To describe the spectrum and frequency of pathogenic germline mutations consistent with diagnosis of iBMFS in patients presenting with SAA. To describe the clinical features of patients with idiopathic SAA and those with iBMFS. To describe the frequency of response to IST in patients with idiopathic SAA and those with iBMFS. To estimate the frequency of iBMFS in patients with idiopathic SAA or HAA who fail to respond to IST or relapse following IST
Overlap: None

Title: ***Comprehensive Center for the Cure of Sickle Cell Disease and Other Red Cell Disorders***
Role: Co-Investigator, Co-Clinical Director (Rivella, PI)
Status of application: Active
Time commitment: 20% effort (2.4 Calendar Months). (Only 50% of this effort was received until 7/1/2020)
Supporting agency: Children's Hospital of Philadelphia Frontier Programs
Funding Agency Grant Officer: Robert DeNight. denight@chop.edu
Performance period: 7/01/2019 – 6/30/2022
Level of funding: (entire program funding)
Brief description of the project's goals: To develop a comprehensive clinical and research center dedicated to the development and implementation of curative cellular therapies for patients with sickle cell disease, thalassemia major, and other severe red blood cell disorders.
List of Specific aims:

Clinical Aim: To establish multidisciplinary clinics for consultation and patient management for patients with severe red cell disorders seeking curative therapy
Scientific Aim: To develop novel stem-cell based gene therapy platforms for patients with sickle cell disease and thalassemia major.
Overlap: None

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

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

9. **APPENDICES:**
   - None.