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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
Philadelphia, PA 19104

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

CHILDREN'S HOSPITAL OF PHILADELPHIA,
3401 CIVIC CENTER BLVD,
PHILADELPHIA PA 19104-4319

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Materiel Command
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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.

15. SUBJECT TERMS
bone marrow failure syndromes, stem cell transplant, Fanconi Anemia, DNA damage, DNA replication

16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Fanconi Anemia (FA) is one of the most common inherited bone marrow failure syndromes. Although initially identified over 85 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:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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. Mon 1-6

Major Task 2 for the first 12 months

Subtask 1 Subject hematopoietic stem/progenitor cells (HSPCs) from mice of different genotypes (double KO mice for different BMF models over Lnk deficient background, D2;Lnk, Fancc/g;Lnk, Fanca;Lnk, Brca2;Lnk, along with WT and single KO controls), to various replication stressors and measure fork stability using DNA combing assay, DNA damage using γH2AX/53BP1 immunofluorescence (IF) and COMET assay, and HSPC survival by colony assays (CFC).

Subtask 2 We found that WT HSPCs are sensitive to JAK signaling pathway inhibitors, thus we will subject HSPCs from different BMF models to various inhibitors to JAK pathways, and measure fork stability using DNA fiber labeling, DNA damage assays, and HSPC survival.
Subtask 3 To assess if cytokine signaling is important for replication fork protection, we will perform DNA fiber assays in splenic B cell cultures, which can be cultured in the presence and absence of cytokines.
Subtask 4 To investigate replication machinery in active and stalled replication forks affected by BMF genes, we will examine Rad51, Brca1, and Mre11 nuclear foci formation along with PCNA, rH2Ax an RPA, in the absence or presence of HU.
Subtask 5 To comprehensively explore replication machinery affected by BMF genes, we will perform iPOND in low and high HU stress for stalled and damaged fork, WB for RAD51 and MRE11. Mouse spleen cells will be used first, then BM cells
Specific Aim 2: Targeting LNK as a novel strategy to expand human HSPCs from BMF patients.
Major Task 3 for the first 12 months
Subtask 1 Knockout genes using lentiviral CRISPR/cas9 has been found difficult in our preliminary studies, thus we will use shRNA-mediated knockdowns (KDs). We found KD FANCD2 in human CD34+ cord blood stem cells reduces CFC progenitors. We will KD LNK in FA and other BMF genes-depleted CB progenitors and examine if it will restore cell growth *in vitro*.
Subtask 2 KD LNK in HSPCs depleted of different BMF genes and transplant them into sublethally-irradiated NOD/SCID-IL2Rg$^{-/-}$ (NSG) mice to assess of LNK inhibition will restore HSC repopulating ability *in vivo*.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. *major activities*

We are on target with our SOW plans and made significant progress in both aims.

For Specific Aim 1, we have obtained all necessary institutional IACUC and IRB approval, as well as HRPO/ACURO Approval (Major task 1). We investigated the mechanisms by which Lnk deficiency alleviates replication stress and ameliorates HSC defects associated with BMF.

For Specific Aim 2, we have begun to explore LNK inhibition to expand human FA-like HSPCs.

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.

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 planned in SOW (Major task 2-1), we tested if Lnk deficiency ameliorates ICL hypersensitivity of hematopoietic stem/progenitor cells (HSPCs) from mice of different genotypes (double KO mice for different BMF models over Lnk deficient background, D2;Lnk, Fancc/g;Lnk, Fanca;Lnk, Brca2;Lnk, along with WT and single KO controls). We found that LNK does not play an overt role in ICL DNA repair assessed by HSC survival assays and DNA damage assays.

FA proteins also play an important role in the tolerance of replication stress. As planned in SOW (Major task 2-2 and 2-3), we investigated the role of LNK in replication stress mitigation in both HSPCs and B cells of Lnk;FA deficient mice. We found that Lnk deficiency ameliorated replication stress by stabilizing replications forks in a manner dependent on cytokine-mediated JAK2 signaling. Lnk deficiency restored cell proliferation and survival of Fancd2-deficient HSCs to wildtype levels, while reducing replication stress and genomic instability associated with FA.

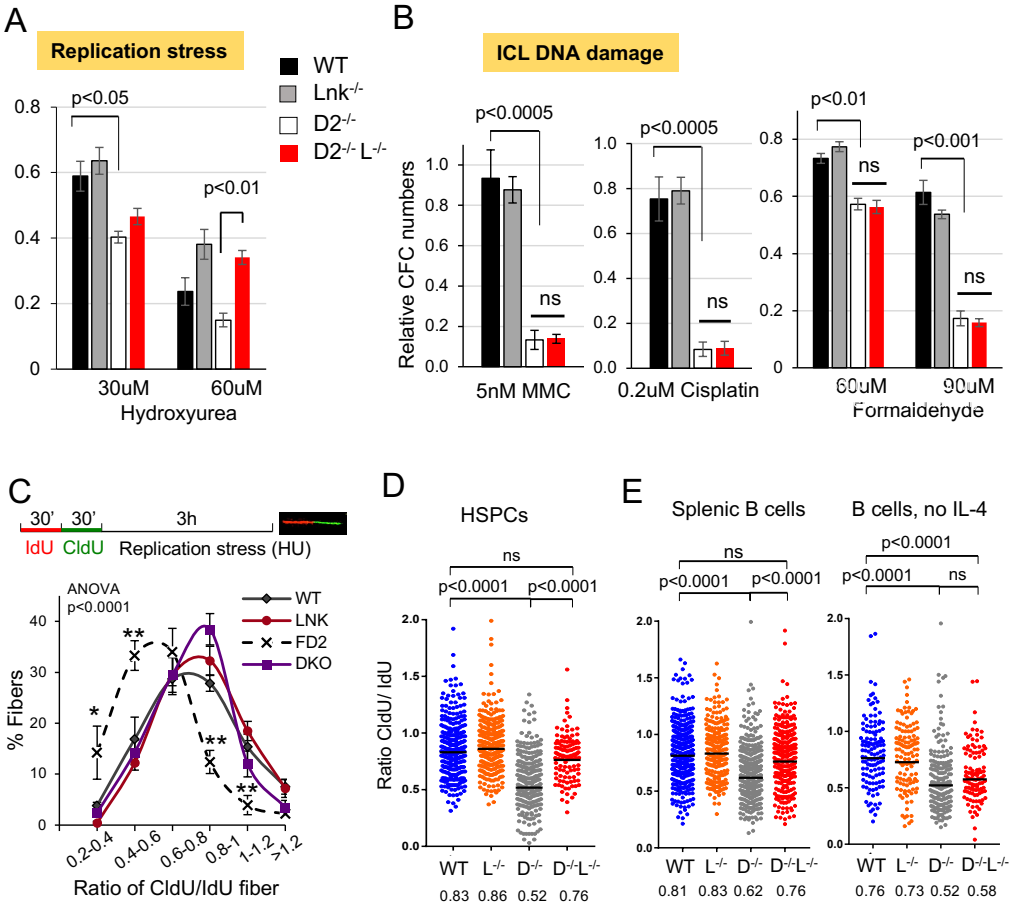

Figure 1: Lnk deficiency stabilizes stalled replication forks in *Fancd2*$^{-/-}$ HSPCs through cytokine-JAK2 signaling. (**A-B**) BM cells from WT, D2$^{-/-}$ and D2$^{-/-}$L$^{-/-}$ mice were plated in semi-solid methylcellulose media containing HU that induces replication stress (A) or indicated ICL DNA damage-inducing drugs, MMC, cisplatin, formaldehyde (B). Colony forming progenitor numbers relative to the vehicle-treated group (mean± SE) were enumerated and graphed. Representative of 3 independent experiments are shown. Statistics were calculated using two-tailed student's t-test. (**C**) The top panel shows the experimental overview of the fork protection assay in single molecule DNA fibers upon HU-mediated replication stalling. **(C-D)** Freshly-isolated HSPCs (LSKs) from WT, D2$^{-/-}$, Lnk$^{-/-}$, and D2$^{-/-}$Lnk$^{-/-}$ (D2$^{-/-}$L$^{-/-}$) mice were subjected to fork protection assay. The frequencies of different replication tract ratios are plotted in **(C)**. The distributions of CldU/IdU fiber ratios are shown in **(D)**. (**E**) Splenic B cells were cultured in RP-105 and LPS with (Left) or without IL-4 (Right), then subjected to fork protection assay. (**D-E**) The distribution of CldU/IdU fiber ratios is shown with the horizontal lines indicating geometric mean of fiber ratios, with corresponding number for each group shown at the bottom of the graph. (**A-C**) P values from two-tailed students' t-test are shown. (**D-E**) Statistical significance of each set of conditions was calculated using Kruskal-Wallis ANOVA test and comparisons between individual groups were calculated using Dunn's multiple comparison post test. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001; ns: not significant.

As planned in SOW (Major task 2-4 and 2-5), 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 year 2 of this grant.

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 3-1), provided a proof-of-concept experiment showing that deletion of LNK in human FA-like hematopoietic stem and progenitor cells promoted clonogenic growth.

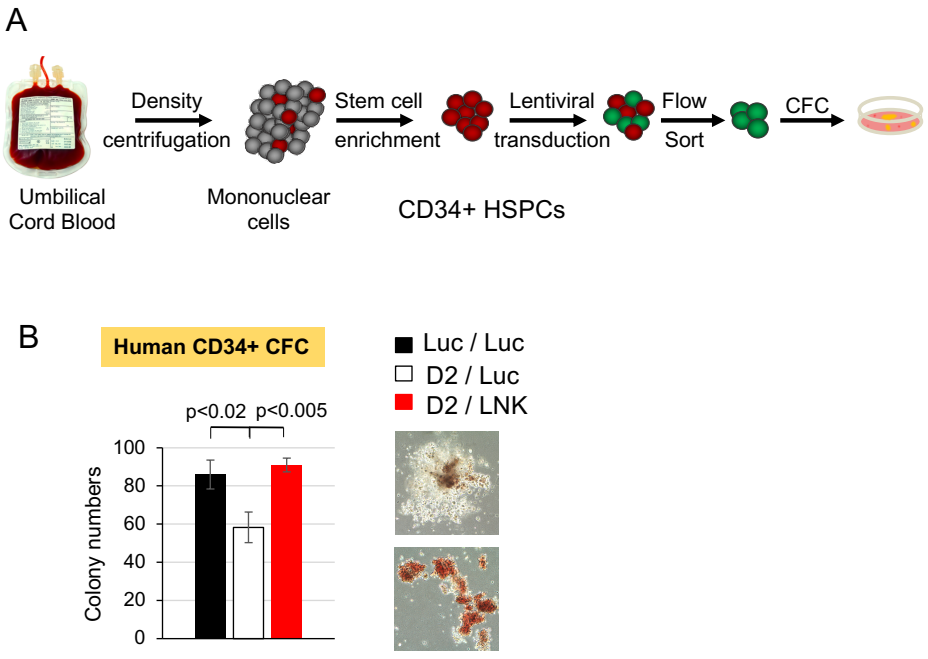

Figure 7. *LNK* depletion restores *FA*-like human progenitor cells. A, depicts a schematic overview of isolation of primary human HSPCs for lentiviral transduction followed by CFC assay. **B)** UCB-derived CD34+ cells were sequentially infected with lentiviruses expressing shRNA to Luciferase (Luc) or FANCD2 (D2) with GFP marker, followed by shRNA to Luc or LNK with mCherry marker. GFP+mCherry+ cells were then sorted and plated onto semi-solid methylcellulose media. Colony-forming progenitor numbers are shown with a two-tailed Students' t-test.

As planned in SOW (Major task 3-2), we are working on knocking down LNK in HSPCs depleted of FANCD2 and transplant them into sublethally-irradiated NOD/SCID-IL2Rg$^{-/-}$ (NSG) mice to assess of LNK inhibition will restore HSC repopulating ability *in vivo*. This is a challenging experiments as it requires optimizing protocol to breed and pre-condition NSG mice. We found that busulfan is a great alternative to irradiation, as NSG mice are sensitive to irradiation-caused lethality. We are now in the process doing transplantation with the new optimized protocol. These studies will be continued into year 2 of this grant.

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?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The trainee on this project, Dr. Brijendra Singh, is given various training opportunities on campus and off campus. We have joint lab meetings with Dr. Nancy speck's laboratory on stem cells that meet every Thursdays, when members of two groups rotate presenting work-in-progress and journal clubs. Brijendra has been presenting about once every quarter 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

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 Keystone Ubiquitin meeting in Jan. 2018. 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?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan to comprehensively investigate replication machinery affected by BMF genes and Lnk deficiency. We will explore if Lnk deficiency would restore HSC functions of various FA mouse models. 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 healthy subject or BMF patients, and examine if LNK inhibition will restore cell growth *in vitro* and *in vivo*.

4. **IMPACT:** D*escribe 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Not applicable.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Not applicable.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

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?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	*Wei Tong*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
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:	*11*
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 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Tong, Wei

Previously active grants that have closed:

R01DK119479 (Blobel) **08/05/2013 – 5/31/2018** **1.2 calendar**
NIH/NIDDK **$317,000 direct / yr ($40,000 to Dr. Tong)**
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. Email: tb232j@nih.gov, Phone 301-594-7726, Fax: 301-480-3510

R01HL100836-01A1 (Tong) **08/18/2014-07/31/2017(NCE)** **1.4 calendar**
NIH/NHLBI **$174,000 / yr**
Clonal hematopoiesis in Diamond Blackfan anemia
The goal of this project is to identify somatic mutations in stem cells or multipotent progenitors in Diamond Blackfan anemia that rescue cells from the effects of the inherited mutation, leading to clonal hematopoiesis and clinical remission.
Role: PI. Please note that Dr. Tong became the PI of this project upon the retirement of Dr. Philip Mason in July 2015.

The Single Aim of this proposal is to identify somatic candidate "rescue" mutations in hematopoietic cells of DBA patients.

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. Email: tb232j@nih.gov, Phone 301-594-7726, Fax: 301-480-3510

Leukemia Lymphoma Society (LLS) Scholar Award (Tong) 07/1/2013 – 06/30/2018
Leukemia Lymphoma Society $105,000 / yr
Investigation of Novel Signaling Pathways in Hematopoietic Stem Cells.
The goal of this project is to investigate the role of BRISC in hematopoietic stem cells
Role: PI

Aim 1: Investigate the physiological roles of BRISC in hematopoiesis, HSPC function, and cytokine signaling.
Aim 2: Investigate the mechanisms by which BRISC regulates cytokine signaling in HSPCs.
Aim 3: Determine the role of BRISC in $JAK2^{V617F}$-mediated proliferation, signaling, and MPN development.

Funding Agencies Grants Officer: Sammy Hattar, MSPH, Director, Research Administration, The Leukemia & Lymphoma Society, 1311 Mamaroneck Avenue, Suite 310, White Plains, NY 10605

ALSF Innovation Award (Tong) 07/1/2014 – 06/30/2017(NCE) 1.2 calendar
Alex's Lemonade Stand Foundation $125,000 / yr
The Tumor Suppressive Role of LNK in Acute Lymphoblastic Leukemia
The goal of this project is to investigate the role of Lnk and signaling mechanisms in normal and malignant B-progenitor cells.
Role: PI

Aim 1: Determine the impact of LNK deficiency on normal B cell development.
Aim 2: Investigate LNK-regulated signal transduction mechanisms in normal and malignant B progenitors.
Aim 3: Define the dependence of B-ALL self-renewal on cytokine signaling and devise therapeutic strategies using *Lnk*$^{-/-}$*Tp53*$^{-/-}$ mice using novel B-ALL models.

Funding Agencies Grants Officer: Mr. Jay Scott, Co-Executive Director, Alex's Lemonade Stand Foundation, 333 E. Lancaster Ave, #414, Wynnewood, PA 19096. Office 610-649-3034.

Previously pending grant are now active:
1R01HL133828 (Tong) 04/01/2017 – 01/30/2022 3.0 calendar
NIH/NHLBI $347,400 direct / yr
Regulation of protein ubiquitination in hematopoietic cytokine signaling
The goal of this project is to study the regulation of the ubiquitination of cytokine signaling molecules in hematopoietic stem and progenitor cells.
Role: PI

Aim 1: Investigate mechanisms by which CBL regulates JAK2 stability and signaling in hematopoietic cell lines and HSPCs.
Aim 2: Determine the influence of CBL on the stability of constitutively active JAK2 mutants and mutant JAK2-mediated MPN development.
Aim 3: Determine the role of CBL in regulating JAK2 level and signaling in primary human progenitors, and explore therapeutic potential of JAK inhibition in treating murine and human myeloid malignancies with *CBL* mutations.

Funding Agencies Grants Officer: John Thomas, PhD, Division of Blood Diseases and Resources, National Heart, Lung, and Blood Institute (NHLBI), Rockledge II Centre, Room 10154, 6701 Rockledge Drive, Bethesda, MD 20892. Email: thomasj@nhlbi.nih.gov Phone: (301) 435-0065 Fax: (301) 480-0868

LLS Translational Research Program (TRP)(Tong) 10/1/2017 – 09/30/2020 0.96 calendar
Leukemia Lymphoma Society (LLS) $180,000 direct / 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

Aim 1. Dissect CBL signaling network in hematopoietic stem/progenitor cells (HSPCs) and CMMLs.
Aim 2. Identify novel therapeutic targets of CBL and explore their therapeutic potentials in treating murine and human CMMLs.

Funding Agencies Grants Officer: Director of Research Administration, the Leukemia & Lymphoma Society, Inc., 3 International Drive, Suite 200, Rye Brook, New York 10573. Email: researchprograms@lls.org. Phone: 914.949.5213 Fax: 914.949.6691

5 T32 DK007780-19 (Tong, W) 12/01/2017-06/30/2019 0.6 calendar
Hematopoiesis Training grant **$0 to Dr. Tong / yr**
Role: PI

Active other supports do not significantly impact the effort on the project that is the subject of the project report.

Greenberg, Roger

Previously active grants that have closed:

1R21 CA194973 (Greenberg, Wellen)	04/01/2015-3/31/2017	1.2 CY, 10 %
NIH/NCI	$62,500 (average direct)	
Linking cancer cell metabolic reprogramming to DNA repair mechanism		
Goals: To determine how metabolic control of acetylCoA levels influences DNA repair.		

Aim 1: Test the hypothesis that acetyl-CoA availability is a critical determinant of the DNA repair mechanism.
Aim 2: Determine if specific oncogenic signaling alterations influence DNA repair mechanism by deregulating acetyl-CoA production or utilization.

Funding Agencies Program Officer: Richard Pelroy, National Institutes of Health (NIH) National Institutes of Health (NIH) 9000 Rockville Pike, Bethesda, Maryland 20892

Previously pending grant are now active:

P30 CA016520 (Vonderheide)	04/01/2015-3/31/2020	1.2 CY,
NIH/NCI	$18,126 (average direct)	
Abramson Cancer Center Core Grant		
Co- Leader for Abramson Cancer Center Breast Cancer Program		

Cancer Center Core grant, no aims requires.

Funding Agencies Program Officer: Henry Ciolino, National Institutes of Health (NIH)
9000 Rockville Pike, Bethesda, Maryland 20892

BRCA Team Convergence Award, The V Foundation (Greenberg and Nathanson)	02/01/2018 - 1/31/2021	1.5 CY 15 %
Th V Foundation	$619,000 direct ($225,000 direct to Dr. Greenberg)	
Identifying and Exploiting Heterogeneity in BRCA mutant cancers		
The major goal of this project is to identify therapeutic strategies to overcome resistance in BRCA mutant cancers that arises due to tumor heterogeneity		
Role: co-PI		

Target in Lupus Grant (Greenberg)	05/01/2017 - 04/30/2020	0.6 CY, 5 %
Lupus Research Alliance	$200,000 direct ($85,000 direct to Dr. Greenberg)	
BRISC DUB activity as a novel target for Lupus		
The major goal of this project is to develop BRISC deubiquitinating enzyme inhibitors to treat Lupus		
Role: PI		

Olson, Timothy

Previously active grants that have closed:

Title:
"*Studies of HSC Engraftment at the Osteoblastic Niche for Inherited BM Failure*"

Time commitment:
PI: Effort 75%, 9 Cal Mos.

Supporting agency
NIH/NHLBI 5 K08 HL 122306-02 (Olson)

Name and address of the Funding Agency's Procuring Contracting/Grants Officer
Jennifer J. Cho Grants Management Officer, National Heart, Lung, and Blood Institute
Email: jennifer.cho@nih.gov, phone: 301-402-6090

Performance period
4/10/2014-3/30/2018

Brief description of the project's goals:
The original purpose of this project is to define whether targeting the bone marrow stem cell niche can enhance engraftment outcomes in patients with bone marrow failure undergoing stem cell transplant. In 2015, a new aim was approved to investigate genetic clonal evolution in patients with acquired aplastic anemia.

Title:
"*Osteoblastic Niche Function During Stem Cell Transplantation for Inherited Bone Marrow Failure Syndromes"*
Time commitment:
PI: Effort 2.5%, 0.3 Cal Mos. (No direct salary support, effort subsumed by K08 per NIH guidelines)

Supporting agency
American Society of Hematology (ASH) Scholar Award (Olson)

Performance period
07/01/2014-06/30/2017

Level of funding:
$ 50,000/year (currently in no cost extension year)

Brief description of the project's goals:
This award is complementary to K08 HL 122306-01, with scientific overlap with the K08 proposal (overlap approved by NIH and ASH). ASH Scholar funds provide no PI salary support, and instead are used as funding for a technician and supply support for studies of osteoblastic niche function in mouse models of bone marrow failure

Title:
Toward Precision Medicine in Childhood Acquired Aplastic Anemia

Time commitment:
Co-Investigator: Effort 15%, 1.8 Cal Mos. (No direct salary support, effort subsumed by K08 per NIH guidelines)

Supporting agency
NIH/ NIDDK, 1R24 DK 103001-01 (Chou)

Name and address of the Funding Agency's Procuring Contracting/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
Email: tb232j@nih.gov, Phone 301-594-7726, Fax: 301-480-3510

Performance period
9/1/2014 – 7/31/2017

Level of funding:
$ 333,332/year

Brief description of the project's goals:
To investigate patterns of clonal hematopoiesis in patients with acquired aplastic anemia

Title:
"Clonal Hematopoiesis in Diamond Blackfan Anemia"

Time commitment:
Co-Investigator: Effort 5%, 0.6 Cal Mos. (No direct salary support, effort subsumed by K08 per NIH guidelines)

Supporting agency
NIH/ NIDDK, R01 DK 100836 (Tong)

Performance period
8/18/2014 – 7/31/2017

Level of funding:
$174,000/year (currently in no cost extension year)

Brief description of the project's goals:
The purpose of this project is to use genomics approaches to investigate the spectrum of clonal hematopoiesis that arises in patients with Diamond Blackfan Anemia

Previously pending grant are now active:

Canuso Foundation Pilot Grant. Olson (PI) 7/1/2017-6/30/2019
Title: Defining the Landscape of Genetic Predisposition to Pediatric Myeloid Malignancies
Description: Study of High Throughput Next Generation Sequencing as a method to define the frequency and characteristics of germline genetic predisposition to pediatric leukemia and myelodysplastic syndromes

NIH R34 HL133384 Williams/Pulsipher (Co-PI's) 4/10/2017-3/31/2020
Role: Co-Investigator (Multicenter Clinical Trial: Site Principal Investigator for CHOP)
Title: Unrelated Donor Transplant Versus Immune Therapy in Pediatric Severe Aplastic Anemia (TransIT)
Description: Pilot feasibility prospective HSCT trial conducted through the Pediatric Blood and Marrow Transplant Consortium and the North American Pediatric Aplastic Anemia Consortium

NIH U01 HL128568 Eapen/Krishnamurti (Co-PI's) 9/1/2015-7/31/2020
Role: Co-Investigator (Multicenter Clinical Trial: Site Principal Investigator for CHOP)
Title: BMT CTN 1503 (STRIDE2)
Description: Randomized prospective clinical trial of matched donor transplant versus supportive care in adolescents and young adults with sickle cell disease

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.