AWARD NUMBER: W81XWH-20-1-0616

TITLE: Rational Targeting of Oncogenic Kras and Sos Interaction in JMML

PRINCIPAL INVESTIGATOR: Dr. Jing Zhang, PhD,

CONTRACTING ORGANIZATION: The University of Wisconsin System

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Fort Detrick, Maryland 21702-5012

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Rational Targeting of Oncogenic Kras and Sos Interaction in JMML

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5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

The Board of Regents of the University of Wisconsin System
Research and Sponsored Programs Office
21 N Park St, Suite 6301
Madison, WI 53715

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

KRAS mutations are particularly prevalent in childhood leukemia, including juvenile myelomonocytic leukemia, and in three major solid tumors, lung, pancreatic, and colon cancers. *KRAS* mutations often associate with resistance to chemotherapy/radiation therapy and significantly shorter survival. Therefore, how to selectively target oncogenic KRAS signaling becomes the primary focus of NCI RAS initiative and the "holy grail" in the RAS biology field. Our study identified a novel drug lead that targets oncogenic Kras and Sos interaction. Unlike previous FDA-approved drugs that target KRAS downstream proteins in both normal cells and cancer cells and thus have inherent toxicities, our compound only targets leukemia cells expressing the disease driver, oncogenic KRAS, while spares normal cells. In contrast to the recent development of KRAS inhibitors that only target one specific KRAS mutation and thus 13% of KRAS cancers, our Sos1 inhibitor approach inhibits the interaction between oncogenic Kras (regardless of oncogenic mutation residues) and Sos family members. In this application, we will further improve the potency of our compound by medicinal chemistry and test its usefulness in both mouse model and human patient leukemia cells.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	22	19b. TELEPHONE NUMBER *(include area code)*

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DOD PROGRESS REPORT OUTLINE

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Our goal is to apply the Sos1 allosteric site targeting strategy to preclinical applications of oncogenic KRAS-driven JMML treatment. We will determine whether the allosteric site of Sos1 is required for oncogenic Kras-driven JMML maintenance using mouse and human JMML cells. More importantly, we will define structure-activity relationship of NSC-70220, the lead Sos1 inhibitor, discover improved derivatives, and validate their functions in vitro and in vivo.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Oncogenic KRAS, Sos1 allosteric site, juvenile myelomonocytic leukemia (JMML), NSC-70220

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

   - **What were the major goals of the project?**

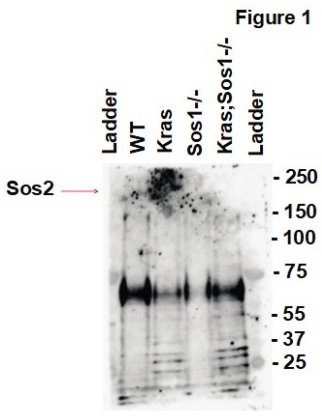
Goal 1: Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML. Goal 2: Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.

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

(1) Major Activities

Specific Aim 1. Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML.

Major Task 1: Determine that the allosteric site of Sos1 is required for the oncogenic Kras-driven JMML maintenance in a Sos1 gene floxed mouse model.

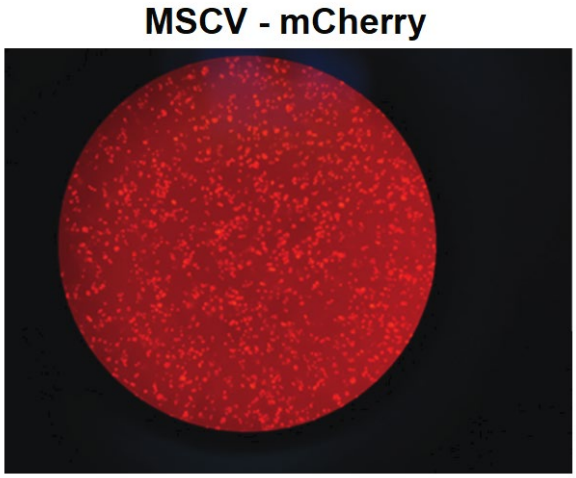


As we proposed last year, we wanted to screen shSos2 constructs and identify 1-2 that can sufficiently knockdown Sos2 expression using Kras; Sos1-/- HSPCs. However, we had trouble to detect Sos2 protein expression in these cells using the previous anti-Sos2 antibody. We purchased a new antibody from Abcam. Despite its strong online references, we found that Sos2 remained undetectable using this antibody (Fig. 1). We will try qRT-PCR to screen for anti-Sos2 in the future.

We tried to package the MSCV-mCherry retroviral constructs using a PEI based transfection reagent. Although we could observe the mCherry fluorescence from a CMV based vector, we could not detect any signal from our MSCV constructs using either a fluorescent microscope or flow

cytometry. After consulting with our colleagues, we learned that packaging large size viral constructs may need reagents with higher transfection efficiency. We then switched to Fugene based transfection reagents and could observe that ~70-80% 293T cells expressed mCherry 48 hours after transfection (Figure 2). We have collected the viral soups for the next step infection of primary mouse HSPCs.

Figure 2

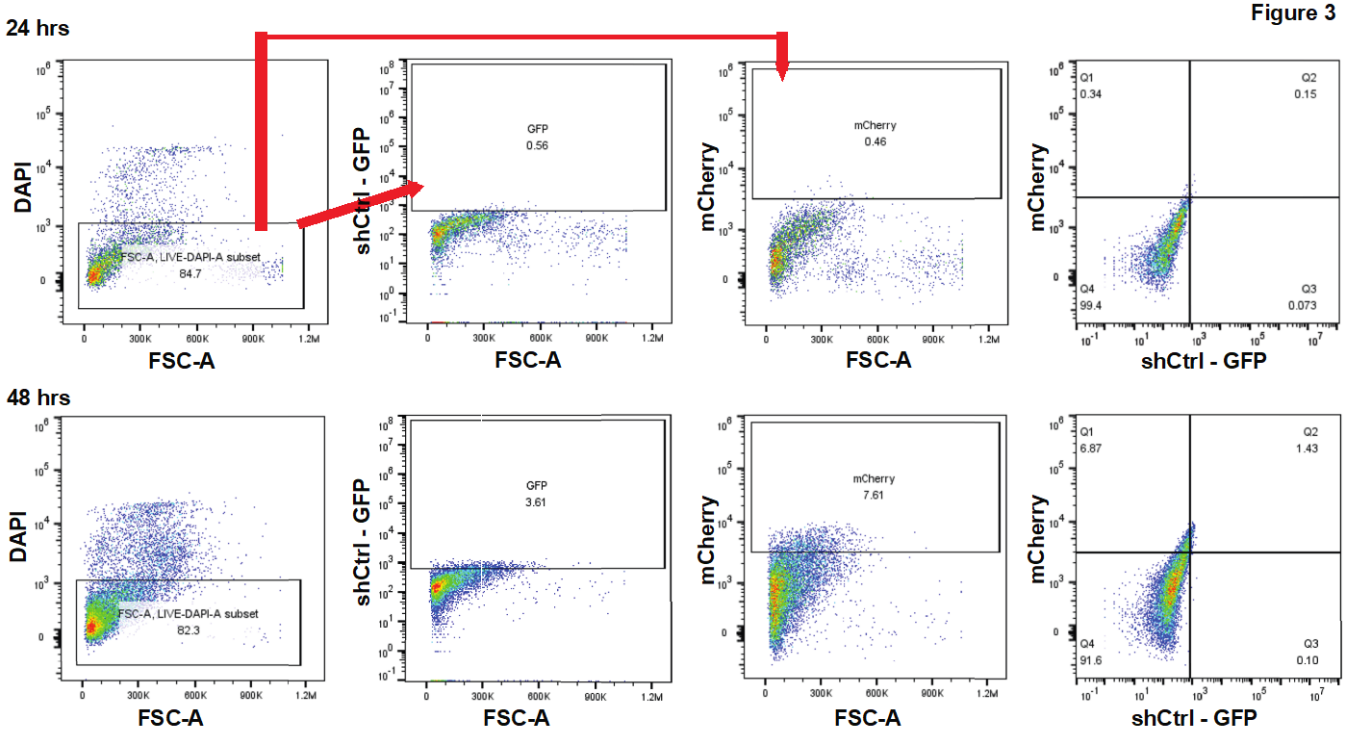


We have been trying to improve the cell viability using an electroporation approach. We cannot get the viability rate higher than 50-60%. Therefore, we will stick with our retroviral infection approach.

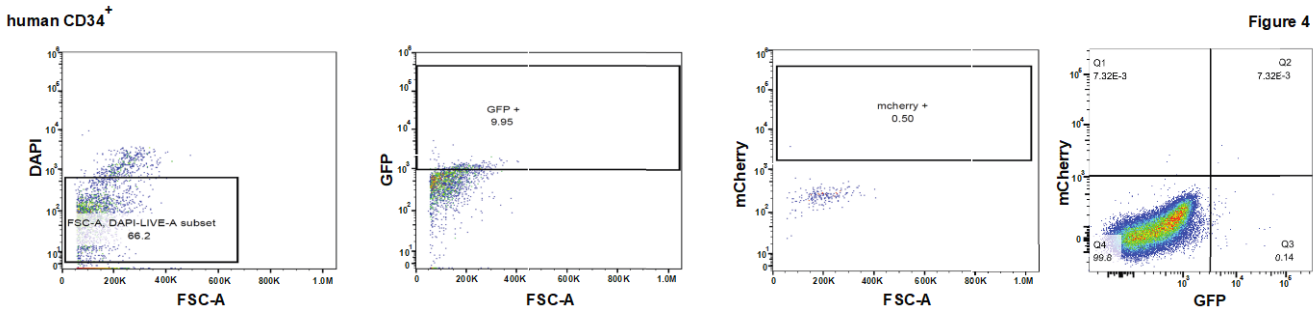
Work in Progress: The postdoc fellow who worked on the Sos1 project decided to take a job offer from a major research hospital in P. R. China and leave the Zhang lab prematurely. This project was thus transferred to a new fellow who joined the Zhang lab on June 15, 2023. We have resumed the animal breeding to generate new cohort of Kras; Sos1-/- mice and hope to complete the infection and rescue experiments within a few months.

Major task 2: Determine that the allosteric site of Sos1 is important for human JMML cell growth by shRNA and inducible "add-back" of allosteric-site specific mutant of Sos1, in human JMML cells.

Figure 3



We successfully packaged all the lentivral constructs. We first attempted to infect the cryopreserved PB cells collected from a BMT donor, which were enriched for CD34+ HSPCs and cultured overnight with full cytokines before infection. The infection rates of both GFP and mCherry control vectors were very low (Fig. 3). We troubleshooted a few times without any significant improvement. Our colleague suggested us to use her lab's well established protocol and infect purified CD34+ cells. Following her recommendation, we purchased all the necessary reagents and proceeded with purification of human CD34+ cells, culture in full cytokines for 24 hours, and sequential infection with GFP and mCheryy control vectors. The infection rates remained very low (Fig. 4).



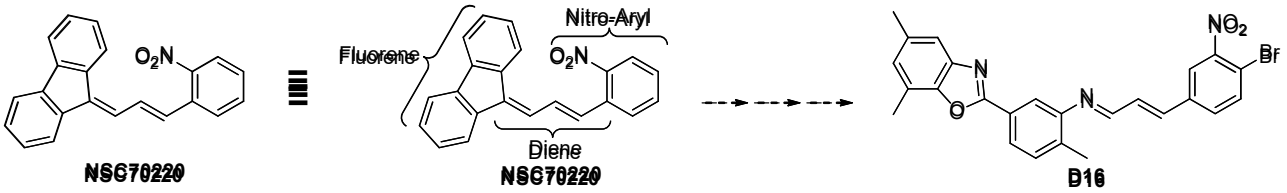
Work in Progress: We are currently tittering the lentiviral constructs using K562 cells in suspension. If the virus titers are low, we will use ultracentrifugation to concentrate the viral soup. Meanwhile, we learned that electroporation of CRISPR-Cas9 RNPs into human CD34+ cells has become a mature technology. We will give it a try as an alternative approach to deliver the shSOS and WT/mut Sos1 constructs.

Specific Aim 2. Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.

Sos1 allosteric site targeting is a promising therapeutic approach since it is one of few known oncogenic Kras selective targets, suppression of which could impinge upon oncogenic Kras transforming activity without affecting normal Ras physiology. In previous screening and characterizations, we have identified a lead Sos1 allosteric site inhibitor. NSC70220, that can selectively bind to the allosteric site (but not the catalytic site) on Sos1 to inhibit active Ras-GTP initiated feed forward activation of WT Ras. As reported last year, we have continued with the Structure-activity relationship studies of this first lead Sos1 inhibitor.

Major Task 3: Define structure-activity relationship (SAR) of NSC-70220 and discover improved derivatives.

We have completed the medicinal chemistry SAR analysis in development of upgraded "drug-like" lead to replace NSC70220 by identifying a more drug-like compound, D16, which has improved conformation to the Lipinski and Veber rules, improved polarity/hydrophilicity (see below scheme). We take D16 as our new lead drug for further validations.



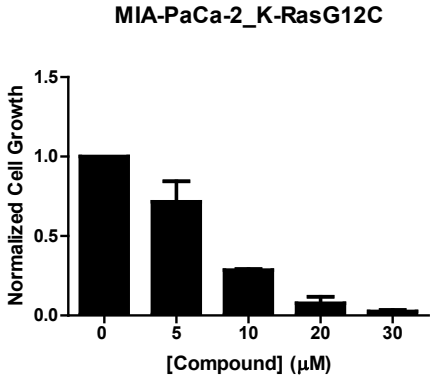
Major Task 4: Validation of the mechanistic effects of NSC-70220 derivative inhibitors.

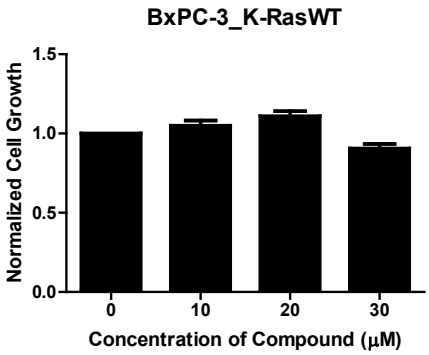
As proposed in the grant application, we have validated this new NSC70220 derivative, D16, in drug-protein interaction assay, oncogenic Kras mutant driven cell proliferation assay, and Kras-mediated cell signaling Western blotting.

We focused on D16 (UC312987) as shown in the figure above. In addition to its more drug-like chemical and structural properties, similar to NSC 70220, D16 could specific binding to SOS1-cat but not HRAS by a microscale thermophoresis (MST) assay. It selectively inhibited oncogenic Kras driven cell proliferation without affecting wild type Kras cells in proliferation (panel A).

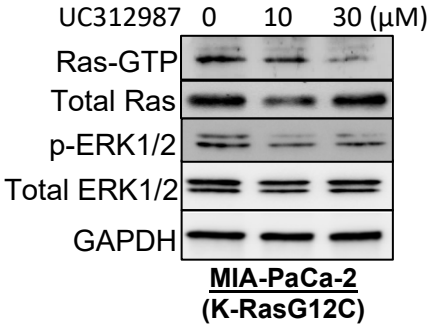
Its effects on Kras driven signaling, as manifested by Ras-GTP, p-ERK1/2 levels and total Ras-GTP, were selective toward oncogenic Kras cells, not on wild-type Kras cell line, BxPC3 (panel B). The estimated IC50 for oncogenic Kras driven cell growth is ~7.0 micromolar (panel C).

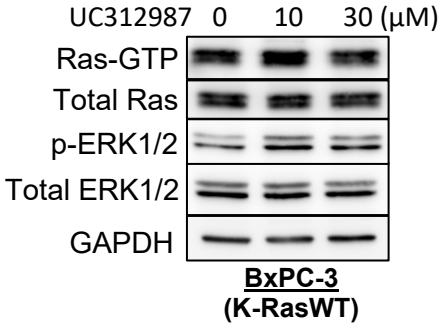
A


MIA-PaCa-2_K-RasG12C
Normalized Cell Growth
1.5
1.0
0.5
0.0
0
5
10
20
30
[Compound] (μM)

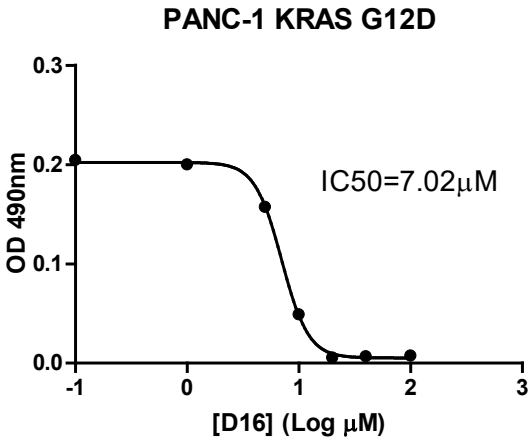

BxPC-3_K-RasWT
Normalized Cell Growth
1.5
1.0
0.5
0.0
0
10
20
30
Concentration of Compound (μM)

B


UC312987 0 10 30 (μM)
Ras-GTP
Total Ras
p-ERK1/2
Total ERK1/2
GAPDH
MIA-PaCa-2
(K-RasG12C)


UC312987 0 10 30 (μM)
Ras-GTP
Total Ras
p-ERK1/2
Total ERK1/2
GAPDH
BxPC-3
(K-RasWT)

C


PANC-1 KRAS G12D
OD 490nm
0.3
0.2
0.1
0.0
IC50=7.02μM
-1
0
1
2
3
[D16] (Log μM)

Major Task 5: Examine the PK/PD/efficacy/toxicity of NSC-70220 and derivatives in JMML mouse models.

Since the definitive lead compound remains to be identified, we do not think it is realistic to perform the previously proposed animal tests using JMML mouse models and primary JMML samples. We have submitted an official request to remove this major task from the SOW. We plan to seek separate funding to move those proposed experiments forward in the future.

Work in progress:
As proposed in the application, we are seeking to validate D16 in oncogenic Kras-driven JMML leukemia cells, in both oncogenic Kras+ and Kras- mouse JMML leukemia cells. Specifically, we are performing: (1) assays of the leukemia cell growth in liquid culture and semi-solid medium; (2) assays tracking cell survival over one and two days by AnnexinV flow; and (3) cell signaling assays of WT Ras-GTP level, and the p-ERK content that is an immediate effector of oncogenic Kras signaling activity.

In addition, we presented this project to the Wisconsin Alumni Research Foundation (WARF) Therapeutics Program in May 2021. Our project was unanimously accepted by the Scientific Advisory Board to be included into their portfolio (WT-015). CCHMC and WARF have completed the revenue sharing agreement. We are working with their medicinal chemistry team to set up a new screening assay.

(2) Specific Objectives: 1. Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML; 2. Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven cancers.

(3) Significant Results and Major Findings: (a) We successfully packaged MSCV-based retroviral constructs to overexpress WT and mutant Sos1 in mouse Kras;Sos1-/- HSPCs. (2) We packaged lentiviral constructs to overexpress WT and mutant Sos1 in human JMML CD34+ cells. (3) We have completed the SAR study and identified a new lead drug, D16, with improved drug features and activity to target oncogenic Kras and downstream ERK signaling in an oncogenic Kras-dependent manner. (4) A validation of D16 for Sos1 targeting at its allosteric site biochemical, and cellular validation in oncogenic Kras driven cell proliferation and signaling, has been done.

(4) Other Achievements: None.

The research activities were impeded by the personnel change in the Zhang lab as described in the Major Task #1.

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

  Dr. Meher Gayatri joined the Zhang lab in June 2023. She has become the new leader of this project.

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

  Nothing to report

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

(1) Determine if the re-expression of WT Sos1 but not the mutant Sos1 will rescue the growth defect of mouse *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ leukemia cells. *Sos2* may be simultaneously knocked down to maximize the rescue effects.

(2) Determine if the re-expression of WT Sos1 but not the mutant Sos1 will rescue the growth defect of human KRAS JMML cells that are deficient for SOS1. *SOS2* will be simultaneously knocked down to maximize the rescue effects.

(3) Complete the validation of our new lead, D16, in oncogenic Kras-driven JMML leukemia cells.

(4) We will seek separate funding to continue what was proposed for task 5, including monitoring the PK/PD/toxicity of D16 after administration to mice, with an established LC/MS /MS protocol to determine the serum level of the compound. As stated above, we expect that the proposed in vivo efficacy test on KrasG12D JMML model and primary human leukemia cells in xenograft models with KRAS mutations will be delayed and likely be performed in future studies.

4. **IMPACT:**

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

     Nothing to report

   - **What was the impact on other disciplines?**

     Nothing to report

   - **What was the impact on technology transfer?**

     After transferring the technology to identify allosteric site-specific inhibitors to the Wisconsin Alumni Research Foundation (WARF) Therapeutics Program in 2022, WARF contracted a biotechnology company to conduct a screen in their chemical library. They failed to identify any promising lead compounds. The possible reason could be that the library does not contain any compounds that share structural similarities with our initial lead compound. They now steered their way to develop Sos1 degraders.

   - **What was the impact on society beyond science and technology?**

     Nothing to report

5. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:*

   - **Changes in approach and reasons for change**

     Nothing to Report

   - **Actual or anticipated problems or delays and actions or plans to resolve them**

     Despite our hard work, we experienced a delay in adding-back experiments proposed in Task 1 and 2. We have generated all the necessary constructs and are implementing new approaches to overcome the problem as described above in SA1 Work in Progress.

     We have completed a round of SAR study and identified D16 as a viable derivative of NSC70220 for SOS1 targeting in Task 3 and Task 4. While we have made solid progress pursuing the Task 4, the in vivo testing of our lead compound in JMML models of Task 5 as described in SA2 has been delayed due to the COVId-19 pandemic. As described, we intend to seek separate funding to pursue those unfinished experiments proposed in Aim 2 for task 5.

   - **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**

  Significant changes in use or care of human subjects

  None

  Significant changes in use or care of vertebrate animals.

  The proposed animal work in Task 3 will not be carried out in year 3 due to delays of the work by COVID-19. Therefore, we did not move forward with animal protocol approval at the Site 3.

  Significant changes in use of biohazards and/or select agents

  Nothing to report

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  - **Journal publications.**

    Nothing to report
  - **Books or other non-periodical, one-time publications.**

    Nothing to report
  - **Other publications, conference papers, and presentations.**

    Nothing to report
- **Website(s) or other Internet site(s)**
  Nothing to report
- **Technologies or techniques**
  Nothing to report
- **Inventions, patent applications, and/or licenses**
  Nothing to report
- **Other Products**
  Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

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

Name:	Jing Zhang
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-1194-0666

Nearest person month worked:	2
Contribution to Project:	The PI is responsible for the overall administration and scientific direction of the project.
Funding Support:	N/A

Name:	Yubin Feng (Jing Zhang lab)
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	11.5
Contribution to Project:	Dr. Feng has taken over the breeding of Kras and Kras;Sos1-/- mice. He generated most of the data presented in Task 1 and 2.
Funding Support:	N/A

Name:	Yun Zhou (Jing Zhang lab)
Project Role:	Associate Research Specialist
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3.0
Contribution to Project:	Ms. Zhou assists with maintaining mouse colonies, bleeding mice for complete blood count and flow to monitor leukemia development, and isolating cells from hematopoietic tissues (Aim 1). She is also responsible for ordering supplies and other general lab management duties.
Funding Support:	N/A

Name:	Meher Gayatri (Jing Zhang lab)
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.1

Contribution to Project:	Dr. Gayatri overlapped with Dr. Feng for one month and took over the Sos1 project. She has been breeding the mouse colony and troubleshooting for the problems of retroviral packaging and infection of human HSPCs.
Funding Support:	N/A

Name:	Yi Zheng
Project Role:	Subcontract PI
Researcher Identifier (e.g. ORCID ID):	0000-0001-7089-6074
Nearest person month worked:	1.2
Contribution to Project:	Led the medicinal chemistry studies in SA2 on NSC-70220 derivatives, testing in in vivtro assays, and data analyses and interpretations.
Funding Support:	N/A

Name:	William Seibel
Project Role:	Subcontract Co-Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.6
Contribution to Project:	Contributed to the medicinal chemistry studies of the NSC-70220 lead, and predicted by simulation and docking analyses the first round of NSC-070220 derivatives
Funding Support:	*N/A*

Name:	Xin Duan (Yi Zheng lab)
Project Role:	Subcontract Research Associate
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6.0
Contribution to Project:	Contributed to the discovery, testing and assays of the NSC-70220 derivative D16 in WT vs/ Kras mutant cells
Funding Support:	*N/A*

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
    - Active support changes follow (changes marked in red)
- **What other organizations were involved as partners?**
    - **Organization Name:** Cincinnati Children's Hospital Medical Center
    - **Location of Organization:** Cincinnati, Ohio
    - **Partner's contribution to the project**
        - Collaboration

8. **SPECIAL REPORTING REQUIREMENTS**
    - **COLLABORATIVE AWARDS:** N/A
    - **QUAD CHARTS:** N/A
9. **APPENDICES:** The Award Chart is submitted as an appendix, per Award Specific Research Terms and Conditions

PI PREVIOUS/CURRENT/PENDING SUPPORT – JING ZHANG

ACTIVE

R01 CA152108 (PI: Zhang) 04/01/2023 – 03/31/2028 1.8 calendar
NIH/NCI TC
Molecular and Cellular Mechanisms of Chronic Myelomonocytic Leukemia (CMML)
The aims of this project are: (1) to determine how *Nras*^{G12D} cooperates with mutations in epigenetic regulators to promote CMML development; and (2) to determine whether combined therapies effectively control CMML progression, transformation to AML, and/or AML progression in vivo.
Role: PI
Grant Officer: Yvonne Duglas Tabor / duglasy@mail.nih.gov / Ph: 240-276-6200
Overlap: None
(Competitive renewal has been funded)

CA190124 (Co-PIs: Zhang and Zheng) 08/01/2020 – 01/31/2024 1.2 calendar
DOD/ARMY TC
Rational Targeting Oncogenic Kras and Sos Interaction in JMML
The aims of this proposal are: (1) determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML; and (2) optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.
Role: Co-PI
Grant Officer: Jamie Shortall / jamie.a.shortall.civ@mail.mil
Overlap: None
(No cost extension)

R01 CA251595 (PI: Miyamoto) 07/01/2020 – 06/30/2025 0.36 calendar
NIH/NCI TC
New Multi-Drug Resistance Mechanism in Multiple Myeloma
The goals of this proposal are: (1) determine the pathologic role of HAPLN1 in MM patient cells and *in vivo*; (2) elucidate the mechanism of HAPLN1-mediated drug resistance in MM; and (3) immuno-target HAPLN1-mediated drug resistance in MM.
Role: Co-Investigator
Grant Officer: Morgan O'Hayre / ohayrem@mail.nih.gov / Ph: 240-276-7482
Overlap: None

R01 (PI: Asimakopoulos) 07/01/2020 – 06/30/2025 0.24 calendar
NIH TC requested for Zhang subproject
Tumor Matrix Remodeling in Anti-Myeloma Immunity and Immunotherapy
Dr. Zhang will serve as a co-Investigator on this project. Together with her scientist, Dr. Zhi Wen, they will provide all the reagents related to VQ myeloma model and detailed experimental guidance and share their expertise on Ras signaling and MEK inhibitors.
Role: Subcontract PI
Grant Officer: Johanna Watson / watsonjo@mail.nih.gov / 240-276-6230
Overlap: None

PENDING

ICTR Pilot Award (PI: Nadiminti and Zhang) 0.36 calendar
UW ICTR 09/01/2023-08/31/2024

Role of tumor microenvironment in the progression of chronic myelomonocytic leukemia with NRAS and ASXL1 mutations.
Major Goals: (1) To characterize the dysregulated TME in NA-CMML and NA-sAML mice (2) To profile the TME landscape in NA-CMML and RAS;ASXL1-sAML patient samples.
Role: PI
Grant Officer: Maureen Smith
Overlap: None

ENDED

WT-015 (PI: Zhang and Zheng) 11/01/2021 – 10/31/2022 0.12 calendar
WARF Therapeutics Program
Rational Targeting Oncogenic Kras and Sos Interaction in KRAS-driven cancers
Major Goals: (1) screen lead Sos1 allosteric site inhibitors in vitro; and (2) optimization and validation of lead Sos1 allosteric site inhibitors in KRAS-driven cancers.
Role: PI
Grant Officer: Jon Young
Overlap: None

Im/Im Sp21-111-Pilot (PI: Zhang and Callendar) 10/01/2021 – 09/30/2022 0.12 calendar
UWCCC Immunotherapy program
Developing novel immunotherapies in a high-risk myeloma model via epigenetic modulation
Major Goals: (1) to assess effects of MEK and CARM1 inhibition on CD8 T cells and myeloma cells; and (2) to combine MEK and CARM1 inhibition with α-TIGIT checkpoint blockade.
Role: PI
Grant Officer: Meredith Luschen
Overlap: None

PI PREVIOUS/CURRENT/PENDING SUPPORT – YI ZHENG

ACTIVE

New:
Title: Testing Kurome compound in AML PDX models
Time Commitments: 0.12 Calendar Months
Supporting Agency: Kurome Therapeutics
Address: 3536 Edwards Rd #100
Cincinnati, OH 45208
Contracting/Grants Officer: Mark Munford
Performance Period: 06/2023 - 05/2024
Level of Funding:
Project Goal: Testing Kurome compound in AML PDX models
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

New:
Title: Assays for screening of the activity of a compound
Time Commitments: 0.12 Calendar Months
Supporting Agency: Mogling Bio INC.
Address: 3536 Edwards Road Suite 100
Cincinnati, OH 45208
Contracting/Grants Officer: Abram Gordon
Performance Period: 05/2023 - 05/2024
Level of funding:
Project Goals: Assays for screening of the activity of a compound
Specific Aims: To establish assays for compound screening
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

New:
Title: Novel Strategies to Improve Blood Transfusion Practice
Time Commitments: 1.2 Calendar Months
Supporting Agency: NHLBI
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: Ronald Caulder
Performance Period: 08/2022 - 07/2027
Level of Funding:
Project Goal: The goals of this project are to define the molecular mechanism of human platelet cold storage induced lesion and to improve means to prevent it.
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

Title: Small molecules targeting RhoA for platelet cold storage in cancer care
Time Commitments: 1.8 Calendar Months
Supporting Agency: NHLBI
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer:
Performance Period: 04/2019 - 02/2024
Level of Funding:

Project Goal: The goals of the grant are to define the molecular mechanism of inhibition of RhoA by G04 and derivatives and to demonstrate the therapeutic benefits of RhoA inhibitors for long-term cold storage of platelets.
Specific Aims: Aim 1. To define the molecular mechanism of inhibition of RhoA by G04 and derivatives. Aim 2. To demonstrate the therapeutic benefits of RhoA inhibitors for long-term cold storage of Platelets.
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

Title: The role of transcription elongation defects in immunotherapy resistance in cancers
Time Commitments: 1.2 Calendar Months
Supporting Agency: NCI
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: Dawn Mitchum
Performance Period: 05/2019 - 04/2024
Level of Funding:
Project Goal: The proposed studies will shed light on the mechanisms of TEdeff, its role in immune-evasion and identify new mechanisms of its targeting in the clinic.
Specific Aims: Aim 1. Mechanisms of TEdeff-mediated suppression of inflammatory pathway genes. Aim 2. Mechanisms of TEdeff-mediated immunotherapy resistance.
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

Title: Rational Targeting Oncogenic Kras and Sos Interaction in JMML
Time Commitments: 1.2 Calendar Months
Supporting Agency: Department of Defense Army/ University of Wisconsin
Address: 820 Chandler St.
Fort Detrick, MD 21702-5014
Contracting/Grants Officer: Jesus Ferrer
Performance Period: 08/2020 - 07/2023
Level of Funding:
Project Goal: The goal is to devise a novel small molecule-based approach targeting SOS1 as a therapeutic concept for JMML.
Specific Aims: 1. Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML; 2. Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

Title:
Time Commitments: 2.4 Calendar Months
Supporting Agency: NIDDK
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: Norma DeGuzman
Performance Period: 08/2021 - 07/2026
Level of Funding:
Project Goal: The proposed studies will develop a center of excellence with three experimental cores studying blood diseases in CCHMC.
Specific Aims: Aim 1. To promote and sustain the growth of an established and vibrant non-malignant hematology research base, which will provide a cluster of top quality, cutting-edge research cores to center members and broader hematology researcher to study the molecular basis of hematology and related diseases in the local, national and international arena. Aim 2. To enhance the interdisciplinary and collaborative nature of

the center program and promote center research focus areas including bone marrow failure/myelodysplasia, red blood cell biology and sickle cell disease, hematopoietic stem cell biology and hematopoiesis, and immunohematology.
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

Title: Novel mechanism of intestinal stem cell aging
Time Commitments: 2.4 Calendar Months
Supporting Agency: NIA
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: Claire Cassard
Performance Period: 09/2020 - 05/2025
Level of Funding:
Project Goal: The goal is to unveil a new mechanism of changes in associating beta-catenin signaling and microbiota with the physiologic aging process of intestinal stem cells.
Specific Aims: Aim 1. To determine the role of the change in expression of Wnts for affecting beta-catenin signaling in ISCs and for the decline of ISCs function upon aging. Aim 2. To determine the contribution of various niche cells to the aging-associated changes in beta-catenin signaling in ISCs. Aim 3. To determine the role of microbiota in regulating beta-catenin signaling and function of ISCs upon aging.
Overlap: No scientific or budgetary overlap with the proposed PRMRP proposal.

Pending
Title: Pharmacological rejuvenation of human hematopoietic stem cells
Time Commitments: 1.2 Calendar Months
Supporting Agency: NIH/Mogling Bio Inc.
Address: 3536 Edwards Road Suite 100
Cincinnati, OH 45208
Contracting/Grants Officer: TBD
Performance Period: 12/2023 - 11/2024
Level of Funding:
Project Goal: The goal of this application is to further develop a first-in-class therapeutic regimen that attenuates aging of human hematopoietic stem cells (HSCs).
Specific Aims: N/A
Overlap: None

Title: Rational targeting of Cdc42 to benefit immunotherapy
Time Commitments: 3.0 Calendar Months
Supporting Agency: NIH
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: TBD
Performance Period: 12/2023 - 11/2028
Level of Funding:
Project Goal: Goal is to will establish a novel concept and present a useful approach for anti-cancer immunomodulation**.**
Specific Aims: Aim 1. To define the mechanism of action of CASIN inhibition of Cdc42 and to improve CASIN efficacy. Aim 2. To demonstrate a proof of concept of Cdc42 targeting to trigger an anti-tumor T cell immunity.
Overlap: None

Title: Rho GTPase inhibitor for refrigerated platelet storage
Time Commitments: 2.4 Calendar Months
Supporting Agency: NIH/Orange Grove
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: TBD
Performance Period: 07/2023 - 06/2024
Level of Funding:
Project Goal: The project will develop a method to prevent platelet damage upon refrigeration which is a dream in blood banking and it would revolutionize the current method of platelet storage. The proposal may lead to a method to store fully functional platelets by refrigeration and will move a novel regimen for platelet cold storage to FDA IND filing and commercialization.
Specific Aims: N/A
Overlap: None

ENDED
Title: Treating Fanconi Anemia Cancer with Proton Precision Therapy
Time Commitments: 0.6 Calendar Months
Supporting Agency: Fanconi Anemia Research Fund
Address: 360 E 10th Ave #201
Eugene, OR 97401
Contracting/Grants Officer:
Performance Period: 08/2019 - 03/2022
Level of Funding:
Project Goal: N/A
Specific Aims: Aim 1 of this project will define FA-dependent sensitivity, toxicity and biological response to proton vs. X-ray radiation in 3D cultured HNSCC cells and normal keratinocytes. Aim 2 will define the therapeutic efficacy of proton vs X-ray in human FA SCC xenografted immunodeficient and immunoproficient mice.
Overlap: None

Title: Leukemia stem cell polarity and differentiation therapy.
Time Commitments: 1.2 Calendar Months
Supporting Agency: NCI
Address: 9000 Rockville Pike
Bethesda, Maryland 20892
Contracting/Grants Officer: Kerry Gastley
Performance Period: 09/2017 - 07/2022
Level of Funding:
Project Goal: To maintain active homeostasis, leukemia stem cells (LICs), like normal hematopoietic stem cells, may undergo an asymmetric cell division whereby they segregate cell fate determinants into different daughter cells, maintaining their self-renewal potential.
Specific Aims: Aim 1. Determine the relationship of Cdc42 regulated cell polarity and division symmetry in LIC self-renewal and differentiation. Aim 2. Delineate the Cdc42-mediated signaling pathways that regulate LIC mode of division and differentiation. Aim 3. Target Cdc42 in human AML as a differentiation therapy in mouse xenograft models.
Overlap: None

PREVIOUS/CURRENT/PENDING SUPPORT – WILLIAM SEIBEL

ACTIVE

New:
Title: Decoding Innate Immune Signaling in Normal and Myelodysplastic Hematopoiesis
Time Commitments: 1.2 Calendar Months
Supporting Agency: NHLBI R35HL166430 (Starczynowski)
Address: NHLBI
Contracting/Grants Officer: Nahed El Kassar
Performance Period: 01/01/2023-12/31/2029
Level of Funding:
Project Goal: The NHLBI R35 Award provides support for my research program, rather than for a specific project. The programmatic theme is to investigate the genetic, molecular, and cellular underpinnings of MOS, to evaluate the developmental requirement of TLR signaling in normal HSC function and MOS, and to use the knowledge gained by our basic research to identify novel molecular targets for the treatment of MOS.
Specific Aims: none
Overlap: No scientific or budgetary overlap.

New:
Title: Targeting the Dusp1 in Jak2 Dependent Myeloproliferative Neoplasm (MPN) for Curative Treatment
Time Commitments: 0.6 Calendar Months
Supporting Agency: NIH/NCI R21 CA280723 (Azam)
Address: NCI
Contracting/Grants Officer: Morgan O'Hayre
Performance Period: 04/01/2023-03/31/2025
Level of Funding:
Project Goal:
Specific Aims: AIM1 Determine the role of Dusp1 in Myeloproliferative neoplasm (MPN). Aim2: Define the mechanism of DUSP1 inhibition by BCI for improved efficacy
Overlap: No scientific or budgetary overlap.

Title: Small Molecule Modulators of Chromatin Remodeling for Myelin Repair
Time Commitments: 0.48 Calendar Months
Supporting Agency: National Multiple Sclerosis Society(Lu)
Address: 733 Third Avenue, New York, NY 10017-3288
Contracting/Grants Officer: Mark Allegretta
Performance Period: 05/01/2022-04/30/2025
Level of Funding:
Specific Aims: Aim 1 is to determine the critical time-window of HDAC3 inhibition in the oligodendrocyte lineage for remyelination in animal models of MS. Aim 2 is to define the mechanism of action of HDAC3-specific inhibitors and to improve their efficacy. Aim 3 is to determine the therapeutic benefits of HDAC3 inhibitors for myelin repair.
Overlap: No scientific or budgetary overlap.

Title: Targeted Inhibition in Leukemia
Time Commitments: 0.72 calendar months effort
Supporting Agency: NIH R01 CA237016 (Nassar)

Address: NIH, 9000 Rockville Pike, Bethesda, MD 20892
Contracting/Grants Officer: Amy Bartosch
Performance Period: 07/08/2020-06/30/2024
Level of funding:
Project Goals: To study the mechanism of action of the small molecule IODVA1 and to validate VAV3 as the target, to identify the binding site on VAV3 and to validate it, and to test the efficacy of IODVA1 in PDX models of Ph+ and Ph-like acute lymphoblastic leukemia.
Specific Aims: Aim 1. To validate Vav3 and downstream signaling pathways as IODVA1's target. Aim 2. To refine the molecular basis for Vav3/IODVA1 interaction. Aim 3. To test IODVA1's efficacy in PDX models of TKI-resistant pediatric ALL and AML.
Overlap: No scientific or budgetary overlap.

Title: Structure, Function, and Modulation of Claudin Cation Channels in the GI Tract
Time Commitments: 0.6 Calendar Months
Supporting Agency: University of Chicago/NIH R01DK131542
Address: NIDDK
Contracting/Grants Officer: Terez Shea-Donohue
Performance Period: 09/30/2021-07/31/2026
Level of Funding:
Project Goal: To identify inhibitors of CLDN2 and CLDN15 as tools to establish Claudin function and therapeutic potential for inhibitors thereof.
Overlap: No scientific or budgetary overlap

ENDED
Title: Rational Therapeutic Targeting of Oncogenic Immune Signaling States in Myeloid Malignancies
Status of Support: Completed
Project Number: 8021-20
Name of PD/PI: Starczynowski, Daniel
Source of Support: The Leukemia and Lymphoma Society
Primary Place of Performance: Children's Hospital Medical Center
Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/01/2020-06/30/2023
Total Award Amount (including Indirect Costs):
Major Goals: The goal of this sponsored award is to evaluate the effects of UBE2N inhibitors using in vitro and in vivo models of human AML
Overlap: No scientific or budgetary overlap.

Title: Targeting IRAK1/4 in Myelodysplastic Syndromes
Status of Support: Completed
Project Number: R01DK113639
Name of PD/PI: Starczynowski, D.
Source of Support: Ntl Inst of Diab & Digest & Kidney Dis
Primary Place of Performance: Cincinnati Childrens Hospital Medical Center
Project/Proposal Start and End Date: (MM/YYY) (if available): 08/2017 - 05/2022
Major Goals: The goal of this grant is to develop and evaluate dual IRAK1/4 small molecule inhibitors in MDS.
Overlap: No scientific or budgetary overlap.

CA190124: Rational Targeting of Oncogenic Kras and Sos Interaction in JMML

PI: Jing Zhang, University of Wisconsin-Madison, Wisconsin **Budget:** $1,248,959

Topic Area: Cancer Research **Mechanism:** FY19 Peer Reviewed Cancer Research Program; Impact Award (FO #W81XWH-19-PRCRP-IPA)

Research Area(s): SCS Coding **Award Status: 01-AUG-2020 to 31-JUL-2023**

Study Goals: In Aim 1, we will first determine whether the allosteric site of Sos1 is required for the oncogenic Kras-driven JMML maintenance through lentivirus-mediated re-expression of WT or allosteric-site specific mutant of Sos1 in *KrasG12D; Sos1-/-* leukemia cells *in vivo*. We will then determine whether the allosteric site of Sos1 is important for human JMML cell growth in vitro by shRNA knockdown and inducible "add-back" of allosteric-site specific mutant of Sos1, in human JMML cells. In Aim 2, we will define structure-activity relationship of NSC-70220 and discover improved derivatives. These derivatives will be validated in drugprotein interaction assays and their mechanistic effects will be validated in mouse and human Kras+ JMML leukemia cells. The top derivatives will be further validated in vivo using *KrasG12D* JMML mouse model and *KRAS+* JMML PDX models.

Specific Aims: 1. Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML; 2. Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.

Key Accomplishments and Outcomes:

Publications: none to date

Patents: none to date

Funding Obtained: none to date