AWARD NUMBER: W81XWH-20-1-0616

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

PRINCIPAL INVESTIGATOR: Jing Zhang

CONTRACTING ORGANIZATION: University of Wisconsin, Madison, WI

REPORT DATE: August 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2022	Annual	01Aug2021-31Jul2022

4. TITLE AND SUBTITLE

Rational Targeting of Oncogenic Kras and Sos Interaction in JMML

5a. CONTRACT NUMBER
W81XWH-20-1-0616

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, Ste 6401
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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

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
Unclassified	Unclassified	Unclassified	Unclassified	19	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:**

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:**

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

3. **ACCOMPLISHMENTS:**

- **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.

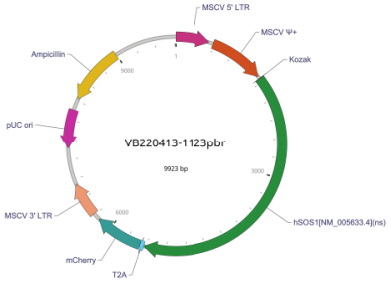
- **What was accomplished under these goals?**

(1) Major Activities

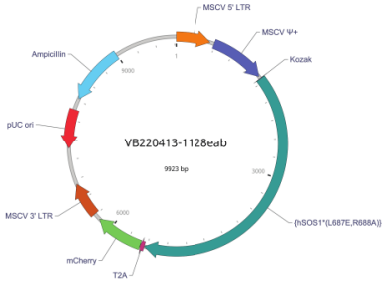
Specific Aim 1. Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML.

The IACUC protocol at Site 1 (Zhang Lab, UW-Madison) was renewed and approved by UW institutional committee on May 5, 2022. We submitted the renewed protocol for ACURO review and approval at the end of May 2022. The office acknowledged the receipt of our submission and informed us of a significant delay of the review process.

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.



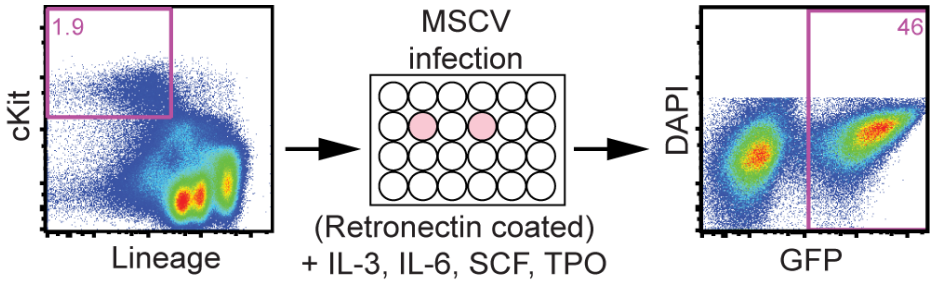
MSCV-WT Sos1-T2A-mCherry

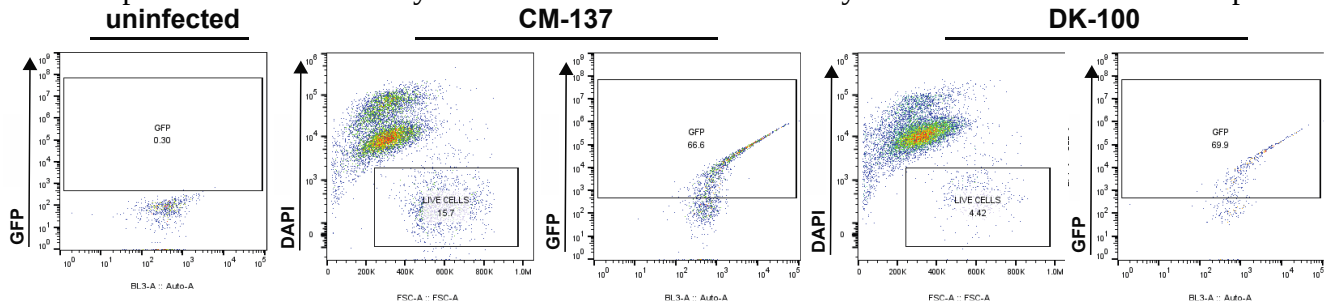


MSCV-mut Sos1-T2A-mCherry

As we proposed last year, we constructed MSCV-T2A-mCherry retroviral constructs to overexpress WT and mutant Sos1 in primary mouse hematopoietic stem and progenitor cells (HSPCs). The constructs were fully sequenced to ensure the sequence accuracy.

The challenge of MSCV infection of primary mouse HSPCs is that the cells are not proliferative. The conventional approach is to use 5-FU injection and kill proliferative progenitor and precursor cells to force primitive quiescent HSPC into cell cycle. However, we were concerned that Kras;Sos1-/- mice may not tolerate 5-FU injections as they are anemic. We took two approaches to overcome this difficulty. First, we took a retroviral infection protocol established in Dr. Wei Tong's lab to culture flow sorted WT Lin- cKit+ HSPCs for 36 hours, load retroviral particles into retronectin-coated tissue culture wells by spin centrifugation for 2 hours, and incubate HSPCs in viral loaded wells for 24 hours. We could achieve ~50% infection rate with the MSCF-GFP vector. Second, we tried a couple of electroporation protocols provided by Lonza Nuclearfector-4 users. Again, flow sorted WT Lin- cKit+ HSPCs were cultured for 36 hours and electroporated using different protocols. Cell viability and GFP+ cells were flow analyzed 24-48 hours after electroporation.



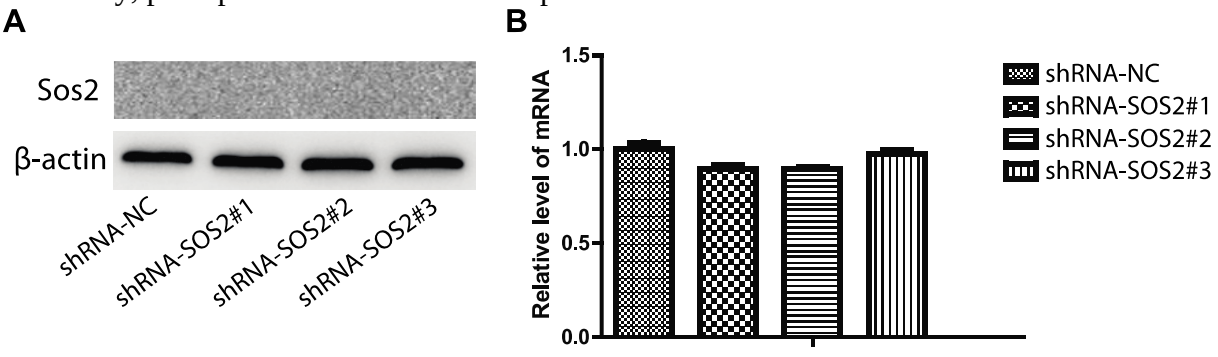


CM-137 program provided >15% survival and ~65% transduction efficiency.

To test if Sos2 is involved in the Kras-driven leukemia progression, we performed Sos2 Western blot analysis in BM cells isolated from moribund oncogenic Kras and Kras;Sos1-/- mice and age-matched

WT and Sos1-/- mice. Despite extremely low Sos2 expression in WT BM cells, we did observe upregulation of Sos2 expression in moribund Kras; Sos1-/- cells, suggesting that Sos2 upregulation may compensate for Sos1 loss and promote Kras-driven leukemia in Kras;Sos1-/- mice.

We ordered three Sos2 shRNA lentiviral constructs and evaluated their knockdown efficiency in NIH3T3 cells. Because Sos2 protein expression was not detectable in NIH3T3 cells, we did qRT-PCR using total RNA extracted from infected NIH3T3 cells. All three of them showed moderate knockdown efficiency, perhaps due to the low transcriptional level of Sos2 mRNA.



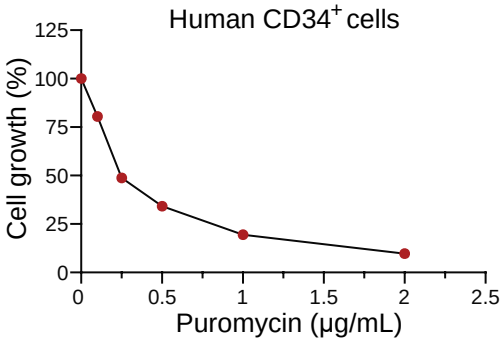
Work in Progress: We have communicated with Open Biosystem to obtain the shSos2 sequences. Once the sequence information is available, we will try to overexpress mouse Sos2 and then screen for shSos2 constructs if they target mouse Sos2 coding sequence. Alternatively, we will determine if these anti-mouse shSos2 could target human SOS2 as well. If so, we will screen them in human K652 cell line as we did before.

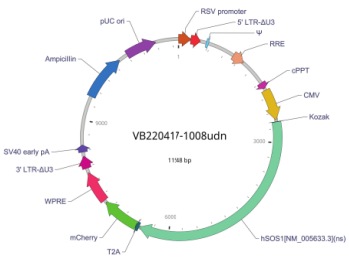
We are further optimizing the electroporation protocol using more gentle programs per Lonza's recommendation. This assay will provide us a straightforward platform to test if WT Sos1 but not mutant Sos1 would recue the reduced growth/colony formation in vitro after knocking down Sos2 in Kras;Sos1-/- cells.

For the in vivo rescue experiment, we will take the electroporation approach if cell viability is improved to >50-60% survival rate. If not, MSCV infection approach will be used.

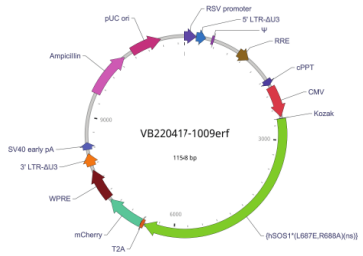
Major task 2: Determine that the allosteric site of Sos1 is important for human JMML cell growth by shRNA and inducible "add-back" of allosteric-site specific mutant of Sos1, in human JMML cells.

For the lentiviral constructs used for human primary JMML cell infection, we initially requested the published constructs from Dr. Bar-Sagi's lab at NYU. They sent us shSos1/2 with GFP and WT and mutant Sos1 with puromycin selection. We sequenced and validated the construct encoding the shRNA





pLV-WT Sos1-T2A-mCherry



pLV-mut Sos1-T2A-mCherry

against human Sos1 and Sos2. We cultured human CD34+ cells in the absence or presence of various concentrations of puromycin for 7 days and measured their viability using the CellTiter-Glo assay. We found that 2ug/ml of puromycin should be used for future selection of infected cells (see above, left). However, we had trouble to transform the lentiviral constructs encoding WT and mutant Sos1. After communication with the lab manager of the Bar-Sagi group, we changed E coli strain and culture medium per her suggestion. We were able to successfully transform the WT Sos1 construct. Neither the lab manager nor we could transform the mutant Sos1 construct despite many tries. Therefore, we had to make pLV-T2A-mCherry constructs ourselves. These constructs were fully sequenced and verified (see above, right).

Work in Progress: We are currently packaging the lentivirus. WT human CD34+ cells will be used to optimize an infection protocol.

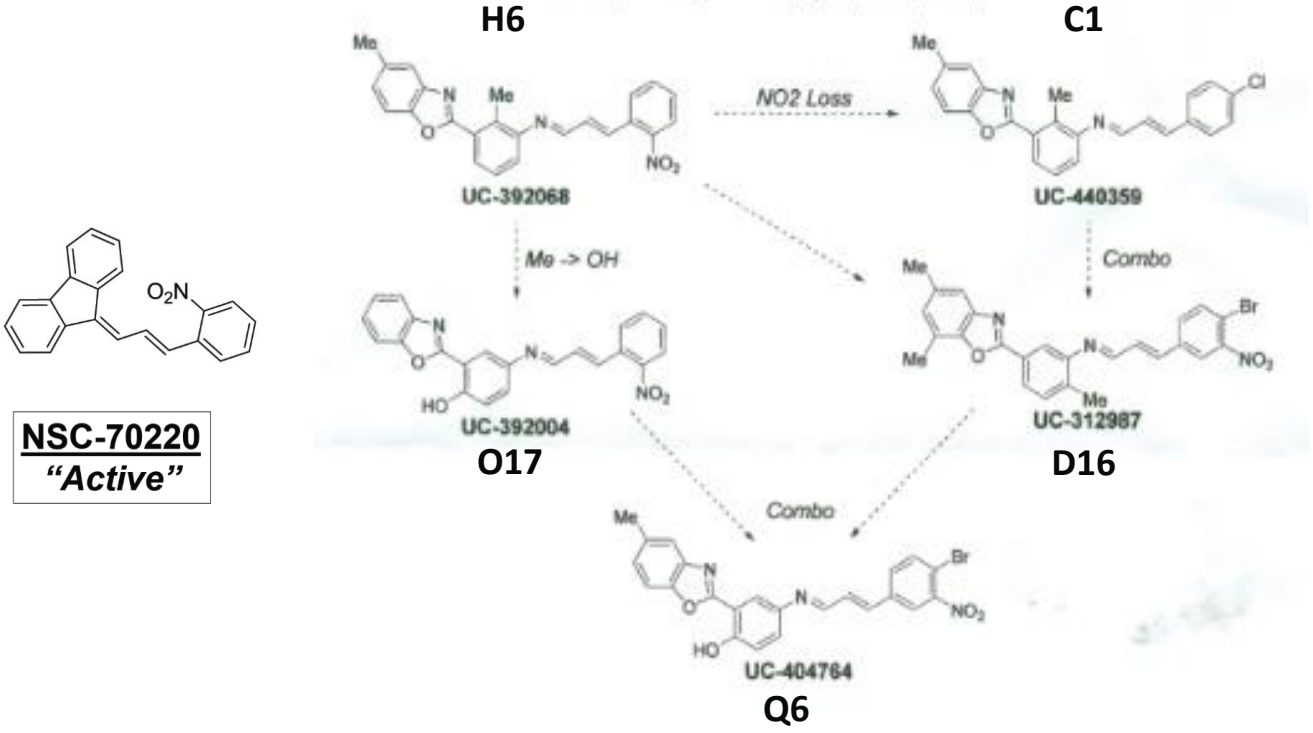
Specific Aim 2. Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.

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.

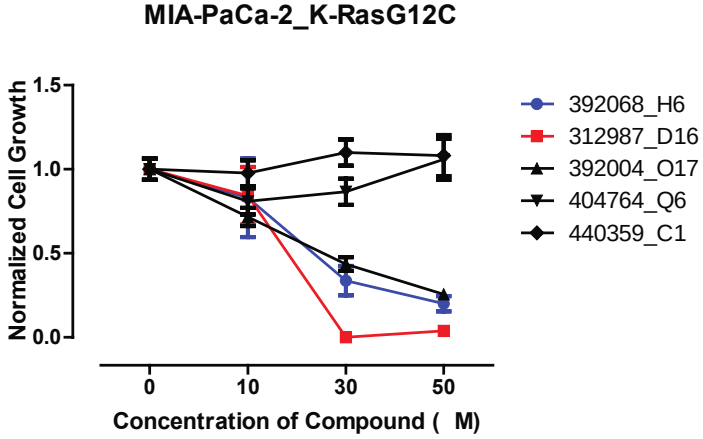
Major Task 3: Define structure-activity relationship (SAR) of NSC-70220 and discover

improved derivatives.

In the past year, we have carried out additional medicinal chemistry SAR analysis in development of upgraded "drug-like" lead to replace NSC70220, because this lead, although conforming to the Lipinski and Veber rules, is heavy in hydrocarbon, less in polarity/hydrophilicity, and does not conform to all classical drug properties. As shown in the figure below, among a series of a new class of NSC70220



analogs that contain improved hydrophilicity, UC312987, UC392068, UC392004, UC404764, and UC440359 have relative light hydrocarbon. We tested this class of derivatives in a cell proliferation assay of Kras oncogenic mutant driven cancer cells. As shown in the figure below, three compounds, D16 (UC312987), H6 (UC392068) and O17 (UC392004), were active in inhibiting MIA-PcCa-2 Kras mutant driven cell growth dose-dependently. We sought to further validate these lead derivatives.

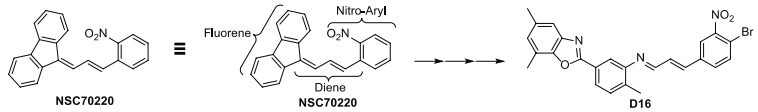


Major Task 4: Validation of the mechanistic effects of NSC-70220 derivative inhibitors.
As proposed in the grant application, we have validated this class of NSC70220 derivatives in drug-protein interaction assay, oncogenic Kras mutant driven cell proliferation assay, Kras-mediated cell signaling Western blotting, and drug-target simulation analysis.

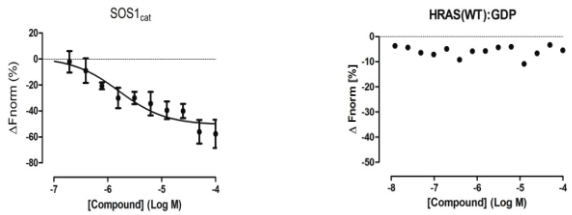
In the derivatives, we focused on D16 (UC312987) as shown in the figure below (panel A). In addition to its more drug-like chemical and structural properties, similar to NSC 70220, D16 could specific

binding to SOS1-cat but HRAS by a microscale thermophoresis (MST) assay (panel B). It showed a potent inhibition of Kras mutant cells without affecting wild type Kras cells in proliferation (panel C).

A

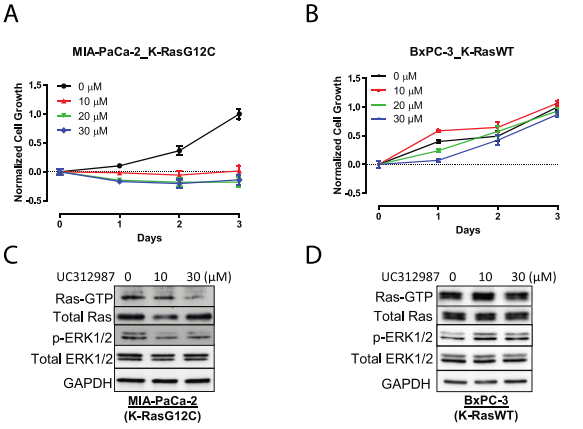


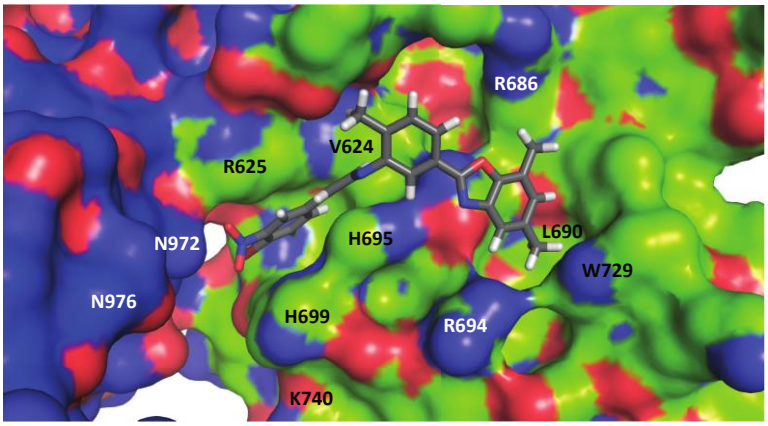
B C



K_D=1.6µmol/L

Its effects on Kras driven signaling, as manifested by p-ERK1/2 levels and total Ras-GTP, were selective toward oncogenic Kras cells, not on wild-type Kras pancreatic cancer cell line, BxPC3 (see the figure Below, left).





A computer simulation docking analysis revealed that D16 can interact with Sos1 at its allosteric site, in a groove surrounded by Sos1 residues V624, H699, L690, W729, and H695 (see the figure above, right). This mode of target binding is consistent with what we found for the original lead compound, NSC-70220, especially involving residues W729 and L690.

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

Due to the limitations imposed by the pandemic and additional round of SAR study carried out in year 2, we anticipate to complete part of the task 5 in year 3.

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 constructed MSCV-based retroviral constructs to overexpress WT and mutant Sos1. (2) We established two different approaches, retronectin-mediated retroviral infection and electroporation, to evaluate the effects of re-expressing WT and mutant SOS1 in *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ leukemia cells. (3) We showed that Sos2 protein was overexpressed in leukemia cells from moribund *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ mice, suggesting that Sos2 may compensate Sos1 loss and promote oncogenic Kras-driven leukemia in this context. (4) We carried out the 3rd round of SAR study and identified one of the derivatives, D16, with improved drug features and activity to enhance RAS-GTP level and downstream ERK signaling in an oncogenic Kras-dependent manner. (5) A computer simulation analysis suggests that D16 could interact with Sos1 at its allosteric site.

(4) Other Achievements: None.

The research activities were impeded by the pandemic. During the surge of new COVID-19 variant between December 2021 and February 2022, the research capacity was reduced in the research labs due to the infected personnel and taking care of their infected family members.

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

  Dr. Yubin Feng joined the Zhang lab in February 2022. He 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) Validate D16 in oncogenic Kras-driven JMML leukemia cells in vitro.

(4) We will monitor 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?**

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.

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

  Nothing to report

5. **CHANGES/PROBLEMS:**

- **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 carried out additional round of SAR study and observed some promising results in Task 3 and Task 4. We are actively pursuing the remaining Task 4 and part of Task 5 as described in SA2 Work in Progress.

- **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:	1.2
Contribution to Project:	The PI is responsible for the overall administration and scientific direction of the project.
Funding Support:	N/A

Name:	Xiaona You (Jing Zhang lab)
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1.5
Contribution to Project:	Dr. You helped the design of retroviral constructs and contacted the Bar Sagi lab for lentiviral constructs. She obtained shSos2 constructs and contributed to the Sos2 Western blot results before she left to establish her own lab.
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:	Yubin Feng (Jing Zhang lab)
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5.9
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:	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:	Ashley Davies (Yi Zheng lab)
Project Role:	Subcontract Research Assistant
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6.0
Contribution to Project:	Contributed to the in vitro testing and assays of the NSC-70220 derivatives 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 projec**t
        - 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

5 R01 CA152108-08 (PI: Zhang) 03/01/2017 – 02/28/2023 (no cost extension) 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:
Overlap: None

CA190124 (Co-PIs: Zhang and Zheng) 09/01/2020 – 08/31/2023 1.2 calendar
DOD/ARMY TC requested
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
Overlap: Yes

R01 CA251595 (PI: Miyamoto) 07/01/2020 – 06/30/2025 0.36 calendar
NIH/NCI TC requested
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:
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 /
Overlap: None

NEW:

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 / jyoung@warf.org / Ph:
Overlap: Yes

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 / Meredith.luschen@wisc.edu
Overlap: None

PREVIOUS/CURRENT/PENDING SUPPORT – YI ZHENG

ACTIVE

R01 HL147536 (Zheng/Cancelas) 04/01/19-02/28/23 1.8 calendar
NIH
Small molecules targeting RhoA for platelet cold storage in cancer care
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.
Grant officer: Laurel Kennedy

R01 CA234038 (Guo, Zheng) 5/15/19-4/30/24 1.2 calendar
NIH
The Role of Transcription Elongation Defects in Immunotherapy Resistance in Cancers
We have found that a subset of cancers is characterized by severe defects in the RNA Polymerase II – mediated transcription elongation, resulting in genome-wide deregulation of mRNA synthesis, splicing and processing (Transcription Elongation defect: TEdeff). TEdeff strongly affected immune-related pathways, and impaired tumor cell response to pro-inflammatory immune attacks in vitro and in vivo. As such, we found that TEdeff predicted poor response to immunotherapeutic agents in the clinic, including immune checkpoint inhibitors, in 4 different cohorts. Given that TEdeff is observed in >25% of all cancers, this proposal is of high clinical significance.
Role: Co-PI
Grant Officer: Susan McCarthy mccarths@mail.nih.gov

R01 AG063967(Zheng/Geiger) 04/01/20-03/31/25 1.8 calendar
NIH
Novel Mechanism of Intestinal stem cell aging
The proposed studies will unveil a new mechanism of changes in associating beta-catenin signaling and microbiota with the physiologic aging process of intestinal stem cells and alterations in tissue homeostasis. The findings of the proposal may lead to future therapeutic interventions preventing or reversing tissue aging.
Role: PI
Grant Officer: Candace Kerr candace.kerr@nih.gov

CA190124 (Co-PIs: Zhang and Zheng) 09/01/2020 – 08/31/2023 1.2 calendar
DOD/ARMY
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

U54 DK126108 (Zheng) 07/01/20-06/30/25 1.2 Bio core; 1.2 Gene Core
NIH Cincinnati 2.4 admin core, 1.2 animal
Cooperative Center of Excellence in Hematology (CCCEH) 1.2 Single cell core; 1.2enrichment
The long-term goal of the Cincinnati Cooperative Center of Excellence in Hematology (CCCEH) is to understand and correct, at the molecular level, hematological diseases of various lineages.
Grant Officer: Daniel Gossett daniel.gossett@nih.gov

ENDED

R01CA211614 (Chen) 1/1/17-12/31/21 0.6 calendar
NIH/City of Hope

Targeting TET1 signaling to treat acute myeloid leukemia
The major goals of this project are: i) To determine the definitive role of TET1 in both development and maintenance of t(8;21) AMLs, and identify critical target genes of TET1 in t(8;21) AMLs; ii) To decipher the molecular mechanism by which the lead compound (NSC-370284) inhibits TET1 signaling and exhibits an anti-leukemia activity; and iii) To develop novel therapies targeting TET1 signaling to treat *MLL*-rearranged AMLs and t(8;21) AMLs.
Grant officer: Barbara Hodgkins

R01 CA204895-01 (Zheng/Mulloy) 09/01/17-07/31/22 1.2 calendar
NIH, NCI
Leukemia stem cell polarity and differentiation therapy
Goals are: 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.
Role: Co-Principal Investigator
Grant Officer: Roger Gross

Pending:
P01 HL158688 (PI: Hongbo Luo; project 3 co-leaders: Jose Cancelas and Yi Zheng)
NIH, NHLBI 09/01/2022 – 08/31/2027 1.2 calendar month
"MECHANISM OF A NOVEL APPROACH FOR PLATELET COLD STORAGE"
The studies will provide the mechanism and a stringent proof-of-principle of a novel approach to refrigerated platelet storage
Role: Co-Principal Investigator, project 3
Grant Officer: Laurel Kennedy

R01 CA234038 (MPI: Yi Zheng & Fukun Guo)
NIH, NCI 04/01/2023 – 03/31/2028 2.4 calendar month
"Small molecules targeting Cdc42 for immunotherapy modulation"
The proposed studies will define the role of Cdc42 in Treg mediated immunosurveillance..
Role: Contact Principal Investigator
Grant Officer: Roger Gross

Overlap:
None

PREVIOUS/CURRENT/PENDING SUPPORT – WILLIAM SEIBEL

ACTIVE

R01 CA237016 (Nassar) 12/01/19-11/30/23 0.36 Cal
NIH
Targeted Inhibition in Leukemia
No number assigned 09/01/20-08/31/23 0.6 Cal
DOD/WISC
Rational targeting oncogenic Kras and Sos interaction in JMML
Grant Officer: Sharad Verma sharad.verma@nih.gov

ENDED

R01CA211614 (Chen) 1/1/17-12/31/21 0.6 Cal
NIH/City of Hope
Targeting TET1 signaling to treat acute myeloid leukemia
The major goals of this project are: i) To determine the definitive role of TET1 in both development and maintenance of t(8;21) AMLs, and identify critical target genes of TET1 in t(8;21) AMLs; ii) To decipher the molecular mechanism by which the lead compound (NSC-370284) inhibits TET1 signaling and exhibits an anti-leukemia activity; and iii) To develop novel therapies targeting TET1 signaling to treat *MLL*-rearranged AMLs and t(8;21) AMLs
Grant officer: Barbara Hodgkins

Overlap:
None

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