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PRINCIPAL INVESTIGATOR: Jing Zhang

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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.

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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

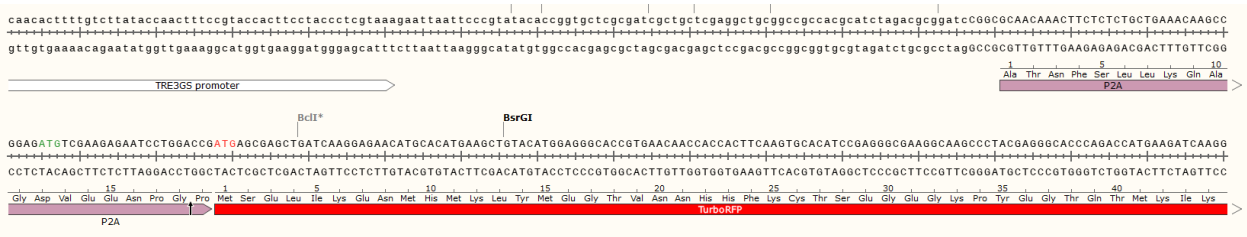
Specific Aim 1. Determination of the allosteric site of Sos1 as an oncogenic Kras-specific target in JMML.

We completed ACURO review and approval of IACUC protocol as well as HRPO review and approval of IRB protocol at Site 1 (Zhang Lab, UW-Madison).

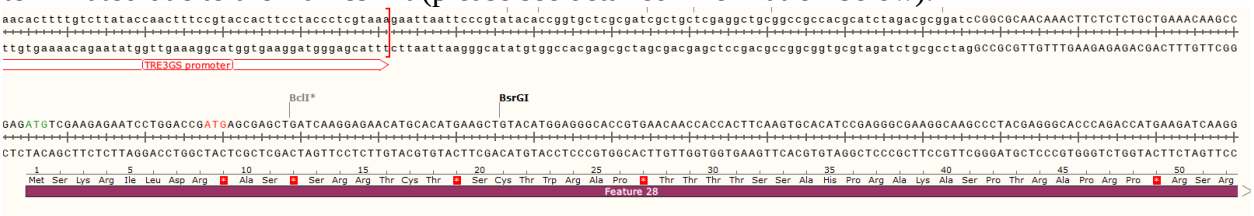
Major Task 1: Determine that the allosteric site of Sos1 is required for the oncogenic Kras-driven JMML maintenance in a Sos1 gene floxed mouse model.

We had an extensive discussion among Drs. Zhang, Zheng, and their colleagues regarding the choice of inducible system and the promoter that drives Tet3G expression. We finally decided to try the inducible Lenti-TRE3G-ORF-P2A-tRFP-PGK-Tet3G-puro system from Horizon Discovery for overexpressing WT SOS1 and L687E/R688A mutant SOS1. Because of the high identity between mouse and human SOS1 (>97%), we used human SOS1 sequence.

After receiving the plasmids, we tested their inducibility first in 293T cells using 1.0, 2.0, or 3.0 mg/ml doxycycline for 24 or 48 hours. The RFP+ cells was only ~20%-30% in empty vector-transfected cells and <1% in WT and mutant SOS1-transfected cells (Fig. 1A), much lower than what we expected. We first investigated why the transfection efficiency of the empty vector was so low. We checked the vector sequence and found that there is no ATG codon upstream of P2A but an ATG codon inside of P2A (marked green).



If the protein translation starts from this ATG codon, the RFP translation would be prematurely terminated due to the frameshift (please see detailed information below).



To enhance the RFP expression, we modified the control vector by inserting additional sequences that contain the Kozak sequence, which functions as the protein translation initiation site in most eukaryotic mRNA transcripts, FLAG tag, and AviTag, before P2A. We tested the modified empty vector in 293T cells again. Upon doxycycline induction, >95% of cells transfected with the modified control vector were RFP+ (Fig. 1B). We further packaged and concentrated the lentivirus particles. In a pilot infection experiment, ~15% of total WT BM cells were RFP+ (Fig. 1C).

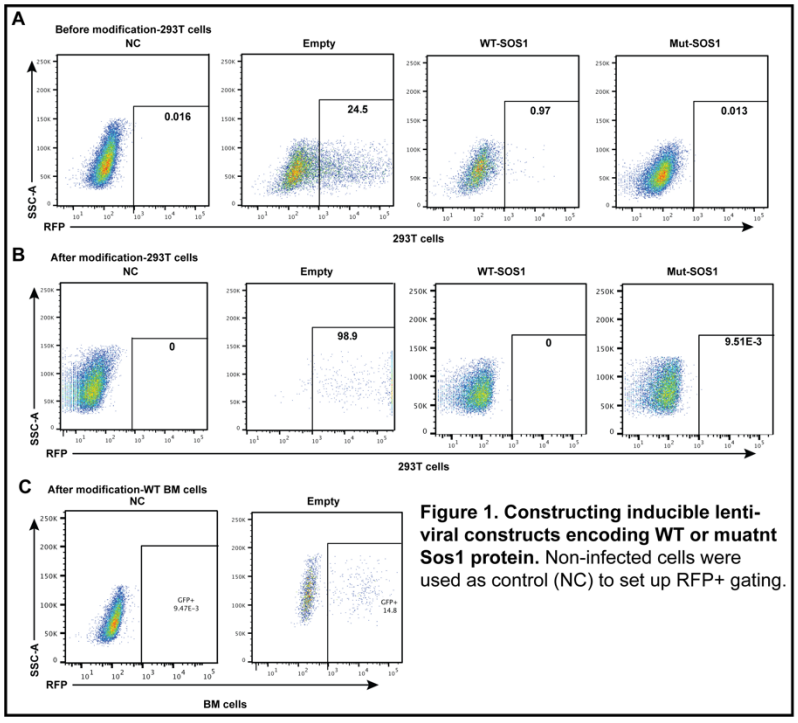


Figure 1. Constructing inducible lenti-viral constructs encoding WT or muatnt Sos1 protein. Non-infected cells were used as control (NC) to set up RFP+ gating.

We carefully examined the constructs encoding WT and mutant SOS1. Both of them have proper Kozak sequence followed by in-frame translation of SOS1-P2A-RFP fusion protein. Therefore, the mechanism underlying the low transfection efficiency of these two constructs was different from that of the empty vector. We postulated that hPGK promoter might not be strong enough to drive Tet3G expression, which in turn binds to the TRE3GS promoter upon doxycycline induction and turns on the expression of SOS1 fusion proteins. We thus replaced hPGK promoter with EF1a promoter following our colleague's suggestion. We repeated 293T transfection experiment and obtained comparable result as before (Fig. 1B). This negative result made us suspect that TRE3GS promoter is not strong enough to drive long gene expression. Dr. Wei Tong at CHOP, an expert in overexpressing different genes using viral vectors, confirmed our thought and

shared her experience with us. She suggested using retrovirus system (e.g. MSCV-IRES-GFP) to overexpress our SOS1 proteins.

In parallel, we re-visited our data from *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ mice. Clearly, Sos1 loss abolished oncogenic

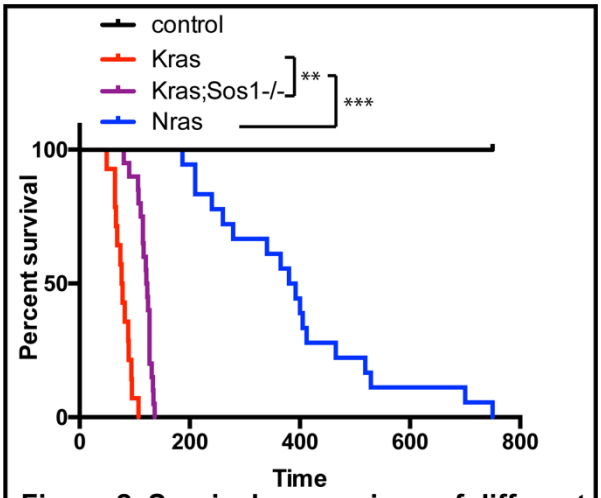


Figure 2. Survival comparison of different groups of animals. The Log-rank test was performed. **, P<0.01; ***, P<0.001.

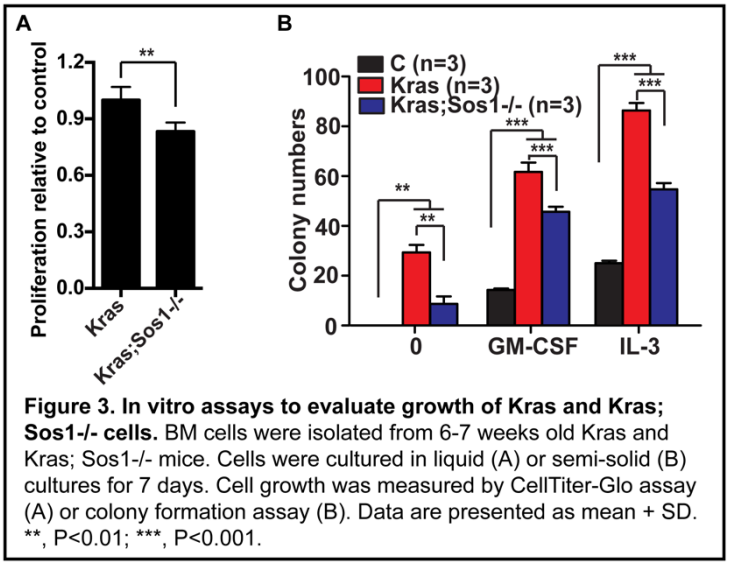


Figure 3. In vitro assays to evaluate growth of Kras and Kras; Sos1-/- cells. BM cells were isolated from 6-7 weeks old Kras and Kras; Sos1-/- mice. Cells were cultured in liquid (A) or semi-solid (B) cultures for 7 days. Cell growth was measured by CellTiter-Glo assay (A) or colony formation assay (B). Data are presented as mean + SD. **, P<0.01; ***, P<0.001.

Kras-induced WT Ras activation at the JMML initiation stage and significantly prolonged the survival of these mice {You, 2018 #3367}. However, when we compared the survival of *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ mice with that of *Nras*$^{G12D/+}$ mice, the difference remained huge (Fig. 2). This result made us wonder if Sos2, the close family member of Sos1, compensates for Sos1 loss during Kras-driven leukemia progression. If this is the case, the subsequent rescue experiment is unable to distinguish the function of WT SOS1 from that of mutant SOS1. Therefore, we are performing some experiments to test this hypothesis as detailed below.

Retroviral infection is notorious to preferentially insert into "hot spots" and drive overexpression of certain oncogenes. In particular, retroviral integration into the Evi1 promoter has been shown to cooperate with oncogenic Nras and drive AML formation {Li, 2011 #2874}. While we still plan to carry out the proposed rescue in vivo study using retroviral vectors as described above, we also worked on establishing in vitro assays as an independent approach to test our hypothesis. Towards this end, we set up two in vitro assays, growth assay in liquid culture and colony formation in semi-solid culture, to reveal the difference between *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ cells and *Kras*$^{G12D/+}$ cells (Fig. 3). In case we cannot draw meaningful conclusions from the in vivo study, these in vitro assays will be used to test if adding back WT Sos1 but not mutant Sos1 would rescue the growth disadvantage of *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ cells.

Work in Progress: Following Dr. Tong's suggestion, we ordered all the necessary reagents to construct retroviral vectors encoding WT or mutant Sos1, including primers and appropriate restriction enzymes. The sub-cloning is ongoing.

To test if Sos2 is involved in the Kras-driven leukemia progression, we will perform the Ras-GTP assay to determine if oncogenic Kras-induced WT Ras activation is restored in *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ cells, and the western blot analysis of Sos2 expression levels in *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ cells isolated from early and moribund stage of mice.

We also ordered Sos2 shRNA lentiviral constructs. Evaluation of their knockdown efficiency in NIH3T3 cells is ongoing.

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.

We discussed with our co-I, Dr. Elliot Stieglitz at UCSF, to select 3 representative JMML cases with oncogenic KRAS mutations and 3 with oncogenic NRAS mutations. In all these cases, RAS mutation is the only known leukemia-driver gene. For each case, two vials of cryopreserved cells were transferred to us.

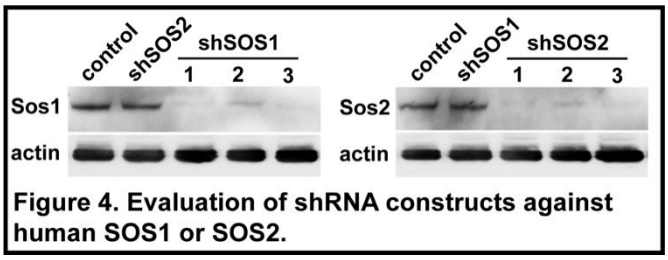


Figure 4. Evaluation of shRNA constructs against human SOS1 or SOS2.

We ordered shRNA lentiviral constructs against human SOS1 (hSOS1) or hSOS2 (Open Biosystem). After packaging in 293T cells and concentrating the viral particles, we infected 293T cells and identified multiple shRNA constructs that could specifically knockdown hSOS1 or hSOS2 (Fig. 4). We also established the protocol of infecting human mononuclear blood cells.

Work in Progress: After several rounds of negotiation with Open Biosystem, we eventually obtained the critical information of shRNAs against hSOS1. We designed the WT and mutant SOS1 that are resistant to these shRNAs. The sub-cloning work is ongoing.

Specific Aim 2. Optimization and validation of lead Sos1 allosteric site inhibitors in oncogenic Kras-driven JMML.
Major Task 3: Define structure-activity relationship (SAR) of NSC-70220 and discover improved derivatives.
Major Task 4: Validation of the mechanistic effects of NSC-70220 derivative inhibitors.

Our preliminary SAR exploration pulled compounds from both our internal (Univ Cincinnati) and NCI

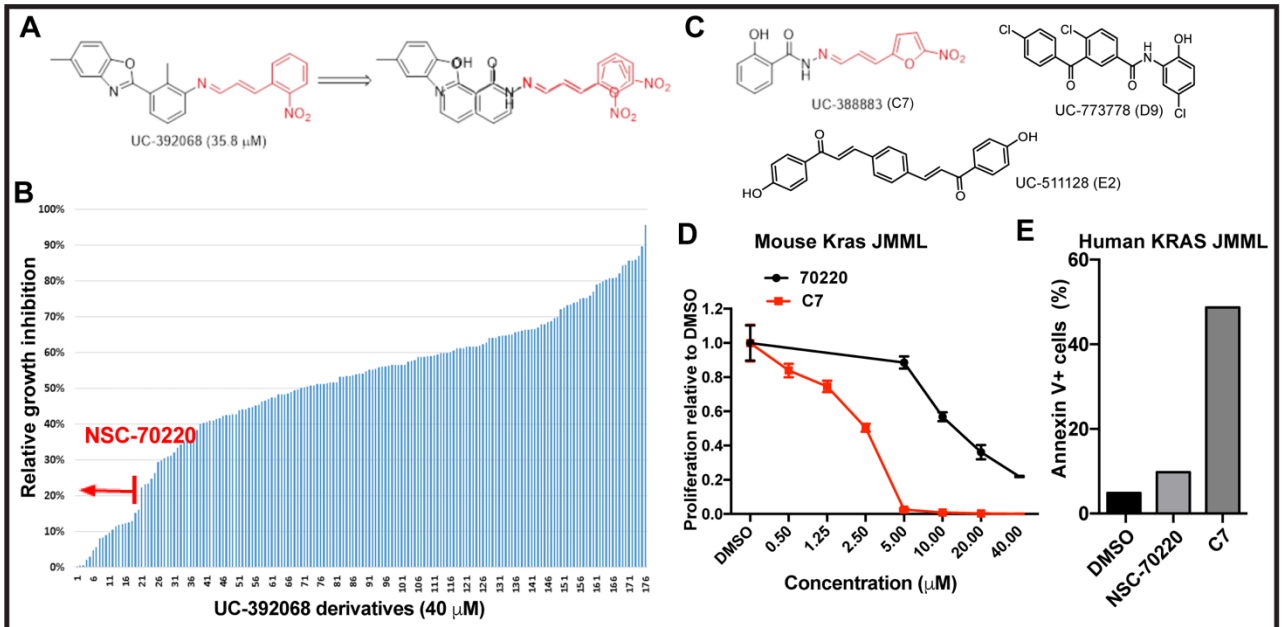


Figure 5. Screen and initial characterization of NSC-70220 derivatives for improved selective inhibition of Kras mutant JMML cell proliferation. (A) UC-392068 is a NSC-70220 analog with comparable binding activity to Sos1 allosteric site and is used for drivative vritual screen. (B) Screening for Kras G12D/+ selective inhibitors using muirne total BM cells. Mononuclear BM cells from Kras G12D/+ and Nras Q61R/+ mice were tested for proliferation in cell titerglo assay in the presence of 40 μM UC-392068 derivatives (176 total), and the red arrow points to the superior derivatives to NSC-70220 that are selective toward Kras G12D/+ cells. (C) Structures of a subset of improved UC-392068 derivatives. (D) Initial validation of the superior growth inhibitory activity of C7 over NSC-70220 in mouse Kras G12D JMML cells. (E) C7 (10 μM) is more potent than NSC-70220 (10 μM) in inducing apoptosis of human JMML cells after 72 hr incubation assayed by Annexin V staining.

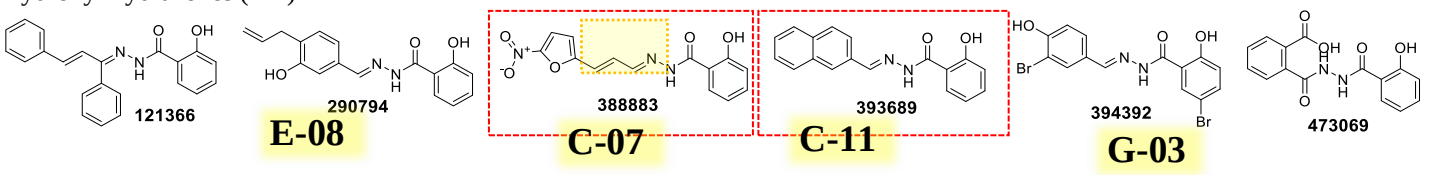
(NSC) libraries to systematically identify points where we might build in more polar functionality. Replacements with more complex functionality proved successful, leading to UC-392068 and NSC-636799, equipotent to NSC-70220 but with improved physical properties. We first focused on UC-392068, which is far less hydrocarbon in character - a two aryl system on the left connected to an aryl nitro/hydroxyl on the right via a 3-4 atom rigid tether (Fig. 5A). We used UC-392068 as a template for

the 2nd round of structure similarity search followed by a docking energy minimization at the predicted Sos1 allosteric site (Fig. 5A). The top 176 compounds from the analyses were assayed for their growth inhibitory activities on oncogenic Kras or Nras transformed murine BM cells and compared with that of NSC-70220. As shown in Fig. 5B, top 18 hits showed superiority to NSC-70220 in inhibiting growth of the *Kras*$^{G12D/+}$ murine BM cells vs. *Nras*$^{Q61R/+}$ cells. Amongst the top hits, compounds C7, D9, and E2 present improved drug-like structures with improved hydrophobicity and solubility (Fig. 5C). Further tests in mouse and human KRAS JMML cells reveal that C7 shows superior growth inhibition and apoptosis induction to NSC-70220 in preliminary tests (Fig. 5D, 5E, and data not shown).

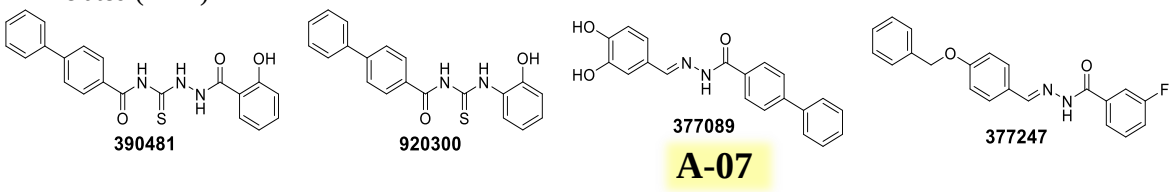
Based on our data from the 2nd round of SAR study, we first focused on the <20% compounds that show greater degree of growth inhibition than NSC-7022, as we proposed in the application.

From a structural standpoint, we selected a few representative structures of each of the Hydroxy-Hydrazones (HH), HH Related (HHR), 70220 Related (7R), and a few Miscellaneous, as grouped below in Fig. 6, for growth inhibition test.

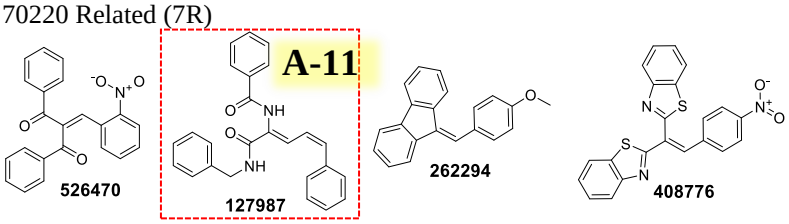
Hydroxy-Hydrazones (HH)



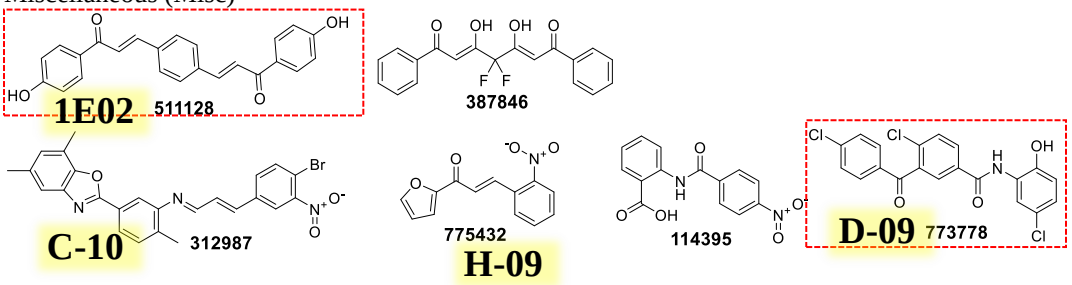
HH Related (HHR)



70220 Related (7R)

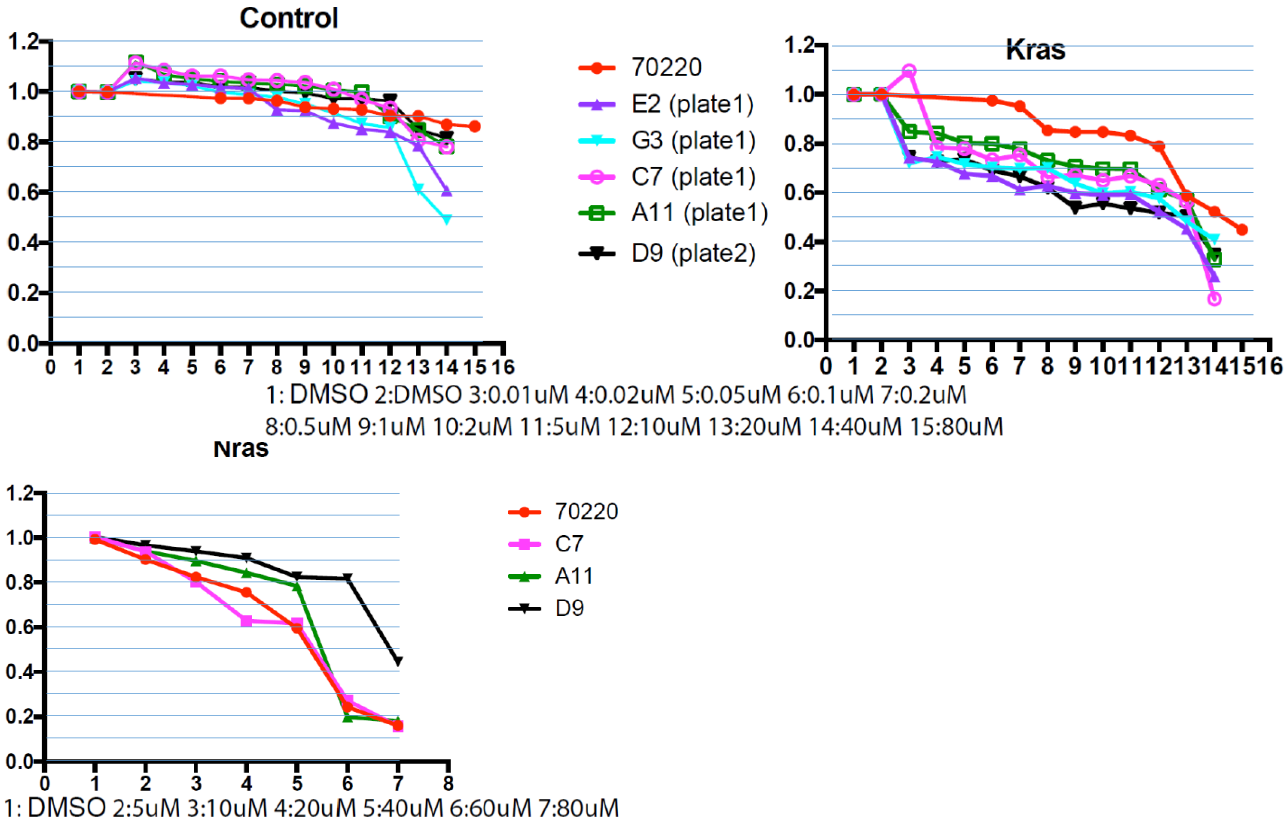


Miscellaneous (Misc)



Five Compounds (E2, G3, C7, A11 and D9; highlighted above) were selected for dose-response evaluation in Control, KRAS, and NRAS cell lines, which showed the following growth inhibitory curves (Fig. 7). In the Control cell line, there appears to be some toxicity at high dose for 1-E2 and 1-G3. In the KRAS cell line, all the non-blanks showed an initial drop (conc 3-4) and then all tail off around conc 13 (20 uM). Visually, it looks like all would be within statistical/experimental errors,

except there are no statistics for single point data.



The NRAS data looks similar, with D9 possibly 2-fold less potent. Importantly, when tested for their effects on p-ERK downstream of Sos1-Kras signaling using a Kras G12C mutant cancer cell MIA PaCa-2 vs. Kras WT cancer cell BxPC3, there were not detectable differences for these compounds E2 and D9 even though they were potent in inhibiting the Kras mutant cell growth (Fig. 8). These data suggest that our pursuit along this line of NSC-70220 derivatives does not yield useful information for Sos1-Kras signaling inhibitors.

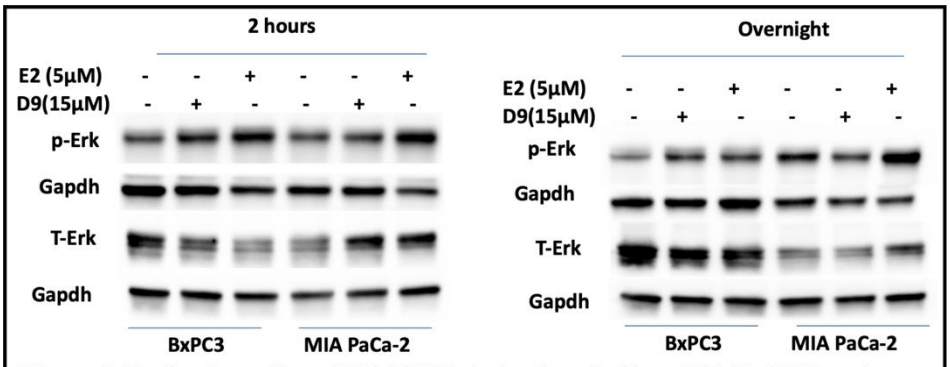


Figure 8. Evaluation of two NSC-70220 derivatives in Kras WT (BxPC3) and mutant (MIA PaCa-2) cell lines.

Although there is not the slightest correlation between docking scores and the activities seen, we can propose an operating hypothesis. Dockings into Sos1 crystal structures 3ksy look more interesting than the 1xd2. At the key binding pocket, it might be that these compounds inhibit Allo RAS binding by helping stabilize the autoinhibitory DH/PH conformation blocking the allosteric RAS.

Work in progress: We will attempt to conduct the 3rd round of SAR study using NSC-636799, another compound identified from our preliminary SAR study, as a template. NSC-636799 is important in illustrating benefits from NO_2 to OH substitutions as well as fluorene to a diphenylacetoyl moiety, both key concerns for NSC-70220 from a physical property and toxicological perspective. This transition from what is fundamentally a nitro hydrocarbon to more complex structures with a range of heteroatom functions gives us confidence we can initiate a medicinal chemistry program to optimize potency, selectivity and physical properties from these leads.

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 are working on a revenue sharing agreement. Once completed, we will work with their medicinal chemistry team to validate the lead compounds they may identify and improve.

(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) The inducible lentiviral system did not work as expected. It is possible that the TRE3Gs promoter is insufficient to drive long gene expression. (2) We established in vitro assays to evaluate the effects of re-expressing WT and mutant SOS1 in *Kras*$^{G12D/+}$*; Sos1*$^{-/-}$ leukemia cells. (3) We validated several shRNA constructs to specifically knockdown human SOS1 or SOS2 expression. (4) We followed the promising result from our preliminary SAR study and screened 176 NSC-70220 derivative compounds using oncogenic Kras vs oncogenic Nras leukemia cells. (5) We validated several compounds that showed more potent killing activities in oncogenic Kras cells. However, further characterization demonstrated that they could not distinguish between WT Kras and mutant Kras, suggesting that they may not act through interrupting Kras and Sos1 interaction.

(4) Other Achievements: None.

The research activities were impeded by the pandemic. The research capacity was reduced in the research labs in August-December 2020 to maintain social distancing.

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

  Dr. Xiaona You presented her project at the Wisconsin Blood Cancer Research Institute Colloquium. She applied for jobs and accepted a professorship at Shangdong University, China. She will leave my lab in September 2021.

- **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* may be simultaneously knocked down to maximize the rescue effects.

(3) Conduct 3rd round of the SAR study using NSC636799 as a template.

(4) Validate the mechanistic effects of NSC-70220 derivative inhibitors.

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 are working on a 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**

  The inducible lentiviral system did not work as expected. It is possible that the TRE3Gs promoter is insufficient to drive long gene expression. Therefore, we decided to switch to the retroviral system in mouse cells. For human cells, we will test both regular lentiviral vector and retroviral vector.

- **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. The inducible lentiviral system did not work as expected. We have designed the new approaches to overcome the problem as described above in SA1 Work in Progress.

  We completed Task 3 and part of task 4 on schedule. However, the results are negative. We are actively seeking other options 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.

  Because of the delay in Task 1, the proposed animal work will occur in the next funding cycle.

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:	Xiaona You (Jing Zhang lab)
Project Role:	Research Associate

Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	7
Contribution to Project:	Dr. You has been working on Task 1 and 2 and helped part of Task 3 (screen 176 NSC-70220 derivatives using Kras vs Nras leukemia cells). She generated the preliminary data presented in Fig. 1-4 and part of the Fig. 5.
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:	Xin Gao (Jing Zhang lab)
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Gao has taken over the breeding of Kras and Kras;Sos1-/- mice. She will test shRNAs against mouse Sos2 and construct retro- and lenti-viral constructs proposed in Aim 1.
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 – 02/28/2022 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

CHANGES FOLLOW:

ACTIVE

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: None

690641 (PI: Zhang) 07/01/2020 – 06/30/2023 0.6 calendar
AACR/Bristol-Myers Squibb TC
Developing Novel Immunotherapies in the First Ras-driven Myeloma Model
The aims of this proposal are: (1) characterize the immune landscape in the aggressive VQ MM model; and (2) use VQ model to evaluate and optimize the efficacy of targeted therapies and cancer immunotherapies.
Role: PI
Grant Contact: aacr@aacr.org
Overlap: None

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
Grants Officer: Johanna Watson / watsonjo@mail.nih.gov /
Overlap: None
ENDED

MSN227098 (PI: Zhang) 04/01/2019 – 03/31/2020
0.12 calendar
UWCCC Pilot Project TC
Optimizing Targeted Therapy and Immunotherapy Approaches in the First Ras-driven Myeloma Model
Role: PI
Grants Officer: Hasnaa Shafik / shafikh@mail.nih.gov /
Overlap: None

Overlap:
None

PREVIOUS/CURRENT/PENDING SUPPORT – YI ZHENG

ACTIVE

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 240-276-6294

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 240-276-7589

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 301-827-4777

CHANGES FOLLOW:

ACTIVE

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 2.4 admin core, 1.2 animal
Cincinnati 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
R01 CA193350 (Zheng) 05/01/15-04/30/20 1.8 calendar
NIH
Targeting CDC42 for Bone Marrow Transplant Therapies
The aims of the proposed studies are (1) to define the mechanism of action of lead Cdc42 inhibitor and improve the lead efficacy by medicinal chemistry, and (2) to establish a proof of principle of Cdc42 targeting as a non-myeloablative conditioning regimen for HSC engraftment in mouse models.
Grant Officer: Roger Gross 240-276-7589

R01DK104814-01A1 (Zheng) 12/15/2015-11/30/2020 0.6 calendar
NIH NCE
Cdc42, hematopoietic stem cell polarity and cell fate
We will examine the potentially causal relationship between cell polarity and the mode of HSC division by a series of in vitro and in vivo cell polarization and division symmetry analysis at single cell resolution. Second, we will define the role of Cdc42 in regulating the ratio between polar and apolar HSCs and consequently thecell fate. Third, we will determine the signaling pathway from HSC polarity into divisional asymmetry by examining non-canonical Wnt5a regulated Cdc42 activity and subsequent actomyosin andJNK/p38 signaling in polarity and division symmetry of HSCs.
Grant Officer: Diana Ly 301-594-9249

NIH R01 HL134617 (PIs: Zheng; Geiger) 8/1/16 – 5/31/20 1.8 calendar
NHLBI
Blood stem cell aging and biomarker studies
The goal of this grant is to define Cdc42 in murine hematopoietic stem cells as an aging biomarker.
Grant officer: Tracee Foster 301-827-8030

No number assigned (Zheng) 06/01/19-05/31/21 0.6 calendar
FARF
Treating Fanconi Anemia Cancer with Proton Precision Therapy
Goals are 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.
Grants officer: research@fanconi.org 541-687-4658

Academic Research (MPIs: Wells, Zheng) 3/1/17-2/28/21
Committee Fund (CHMC)
Proton radiation biology and therapy

Overlap:
None

PREVIOUS/CURRENT/PENDING SUPPORT – WILLIAM SEIBEL

ACTIVE

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 240-276-6294

CHANGES FOLLOW:

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

R01 CA193350 (Zheng) 05/01/15-04/30/20 0.6 Cal
NIH
Targeting CDC42 for Bone Marrow Transplant Therapies
The aims of the proposed studies are (1) to define the mechanism of action of lead Cdc42 inhibitor and improve the lead efficacy by medicinal chemistry, and (2) to establish a proof of principle of Cdc42 targeting asa non-myeloablative conditioning regimen for HSC engraftment in mouse models.
Grant Officer: Roger Gross 240-276-7589

R01 DK113639 (Starczynowski) 08/07/17-05/31/21 0.6 CalNIH

Targeting IRAK1/4 in Myelodysplastic Syndromes
The objective of this proposal is to optimize and evaluate our candidate dual IRAK1/4 inhibitors in human MDS cells in vitro (Aim 1), and in mouse and human MDS models in vivo (Aim 2).
Grant officer: Carolyn Kofa 301-594-7687

R21CA229930 (Nassar) 08/01/18-07/30/20 0.24Cal
NIH
Targeted Inhibition in Triple Negative Breast Cancer
Aim 1. Investigate the mechanism of action of compound H9. Aim 2. Test the ability of compound H9 to inhibitgrowth of TNBC tumors in vivo.
Grant Officer: Roger Gross 240-276-7589

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