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TITLE: Preventing Blood Cancers and Other Malignancies in Military Personnel at Risk Due to Occupational Radiation Exposure

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CONTRACTING ORGANIZATION: The University of Chicago

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We are pursuing a project to examine the amino sugar N-acetylglucosamine, via its ability to increase protein O-GlcNAcylation, as a means to promote DNA double strand break repair and thereby prevent and mitigate the toxicity of radiation in the bone marrow and other rapidly proliferative tissues, toward reducing both the acute and delayed effects of radiation, including malignancy. During the second year of research, *in vitro* research has progressed on a reasonable pace, though *in vivo* studies continue to lag. Our work to date has set the stage for a productive third year of research.

15. SUBJECT TERMS

NONE LISTED

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1. Introduction

This project is directed at testing a non-toxic intervention as a means to protect individuals at risk of radiation exposures from damaging effects to the bone marrow and other proliferative tissues. Briefly, our work identified a core cellular metabolism pathway, the hexosamine biosynthetic pathway (HBP), as a determinant of radiation tolerance. The HBP incorporates metabolic inputs from glucose and glutamine to form UDP-GlcNAc. Then, O-GlcNAc transferase utilized the UDP-GlcNAc to transfer GlcNAc moieties to protein serines and threonines, leading to altered function, localization, stability and/or expression. Our data suggest that OGT substrates are activated upon O-GlcNAcylation to enhance repair of DNA double strand breaks, promote cell survival, and/or resist cellular senescence after radiation exposure. The impetus for this project is that simply feeding cells the precursor amino sugar N-acetyl glucosamine (GlcNAc) is sufficient to drive the HBP and overall O-GlcNAcylation, leading to enhanced radiation tolerance *in vitro*. Feeding GlcNAc to animals or humans similarly increases O-GlcNAcylation, suggesting that this may be sufficient to enhance radiation tolerance *in vivo*.

A complementary activity that similarly promotes DNA double strand break repair and cell survival and resists senescence is poly-ADP-ribose polymerase 1, PARP1. Upon activation by DNA breaks, PARP1 utilizes nicotinamide adenine dinucleotide (NAD+) as a subtrate to form poly-ADP-ribose (PAR), thereby depleting NAD+ pools. Like OGT with GlcNAc, PARP1 activity can be enhanced, leading to increased DNA damage tolerance, by treating cells with its substrate NAD+ or the precursor nicotinamide dinucleotide (NMN) or nicotinamide riboside (NR). NMN or NR, like GlcNAc, are orally available, suggesting the value of treating with both agents to further increase repair capacity to improve cellular survival after radiation.

Our focus is on bone marrow and decreasing morbidity and mortality due to the acute hematopoietic syndrome and then late effects such as bone marrow failure and hematopoietic malignancies after accidental, occupational or military radiation exposures. Using bone marrow cultures and mouse models, we hope to explore if increasing O-GlcNAcylation via feeding with GlcNAc, with or without NMN or NR, prior to and after otherwise lethal whole body irradiation may preserve hematopoiesis at the level of stem cell and precursor survival and proliferation.

2. Keywords

Radiation, bone marrow, metabolism, DNA repair, hematopoiesis, stem cells, precursors

3. Accomplishments

We have listed the tasks that were proposed in the SOW and progress on each:

Major Task 1: Establish bone marrow cultures

Subtask 1: Isolate bone marrow with high culture viability. In year 1, we established a simple workflow that allows us to culture bone marrow in the lab. Using a flow panel that detects hematopoietic stem and precursor cells (HSPC) to analyze the bone marrow, we detected 0.01-0.02% hematopoietic stem cells (HSCs) based on a marker pattern of CD150+, CD48-, Sca-1+, cKit+, Lin-. The stem cells displayed satisfactory viability upon isolation and then proliferated when grown in tissue culture.

Subtask 2: Optimize culture growth and feeding. In year 1, using mouse bone marrow cultures, we have examined culture conditions to obtain an optimal yield of HSCs. We started with Iscove Modified Dulbecco Media (IMDM) with stem cell factor (SCF) and interleukins 3 and 6 (IL3, IL6). Using the flow panel to detect HSPCs, the SCF+IL3+IL6 combination displayed the lowest proportion of HSCs compared to each factor used alone in IMDM. IMDM+SCF resulted in a sufficient proportion of HSCs and viable cells that it can be used for future cultures.

This year, we focused our efforts on isolating mesenchymal stem cells (MSCs) from bone marrow. MSCs appear to serve an important role in serving as a niche for hematopoietic stem and progenitor cell proliferation. Their senescence following irradiation may contribute to bone marrow failure. We found that culture in commercial MSC media was feasible and that the resulting cultures were depleted of hematopoietic cells and enriched in MSCs as hoped for. Over several passages, the potential to differentiate into adipocytes, characteristic of MSCs, was lost, reflecting heterogeneity in the cultures and outgrowth of other cells.

Major Task 2: Establish radiation response assays in cultures

Subtask 1: Establish assays of DNA damage and senescence In the first year, we developed a strategy to map γH2AX, a mark associated with DNA double strand break (DSB) detection and initiation of repair, across the genome over a time course following irradiation. Using K562 erythroleukemia cells as a model for bone marrow stem/progenitor cells and applying BLISS-seq to map DSBs across the genome and CUT&RUN to detect enrichment of γH2AX, we discovered that the initial distribution of γH2AX after irradiation is limited to

transcriptionally active euchromatin. We found this is very likely linked to detection of DSBs being the limiting factor. In euchromatin, DSBs appear to be rapidly recognized via collisions with RNA polymerases. In turn, γH2AX appears considerably later in other domains such as repressed regions and heterochromatin, presumably via a mechanism where DSBs persist and then are detected only when replication forks collide with them. An important observation was that many DSBs are co-enriched with the H3 K27me3 mark, which is abundant in domains where γH2AX is delayed. As a result, many of these DSBs may not be repaired until they are recognized as damage during S phase, when they are likely to be detected via collision with a replication fork. This new view of DNA damage detection depending on chromatin mobilization during transcription or replication suggests novel mechanisms that may link epigenetic marks to DNA repair.

Mechanism of OGT in DSB repair In complementary work, we examined the mechanism by which O-GlcNAcylation enhances DSB repair. Using cancer cell lines as an *in vitro* model revealed that O-GlcNAcylation and PARylation appear to be acting in the same pathway. GlcNac can compensate both for inhibition and knockdown of PARP1 to restore DSB repair. Rxamining the cell cycle stage dependence of the O-GlcNAcylation effect on DSB repair also recapitulated the cell cycle dependence of PARP1 inhibition. All of the impact of modulating O-GlcNAcylation or PARylation appeared to be in S phase and likely G2.

Notably, DSB repair in G1 is limited to end-joining repair. Several mechanisms for rejoining a break can be utilized, but the dominant mode of repair is conventional non-homologous end-joining (NHEJ), where the two ends are blunted off and then ligated. The presence of a sister chromatid in S phase and G2 permits some DSBs to be repaired by homologous recombination (HR), but this requires activation of a 3'-5' DNA endonuclease to expose a short stretch of sequence to bind recombination proteins and allow homology search on the sister strand. Notably, though, even in S/G2, most breaks are repaired by NHEJ or other end-joining mechanisms, meaning the ends are generally protected from 3' resection. So-called DSB repair choice reflects the dynamic balance between whether DSBs will be directed to NHEJ or HR repair.

Toward understanding the mechanism of radiation resistance, we examined the effect of O-GlcNAcylation on DSB repair at level of whether repair is favored for NHEJ and/or HR. Using the Traffic Light reporter system, we observed that blocking OGT or PARP yielded a decrease in NHEJ repair and *increase* in HR. This pattern suggests an overall shunting of S/G2 repair away from end-joining and toward HR.

Although uncertainty remains regarding the the role for PARP1 in DSB repair pathway choice, a strong argument has been made that this effect depends on PARylation stabilizing binding of the Ku NHEJ protein complex to protect DSB ends and thereby restricting onset of resection, a prerequisite for HR. Prior work from Kron lab post-doc Sera Averbek implicated CtIP as a gatekeeper for DSB repair choice that may be regulated by OGT, suggesting O-GlcNAcylation may function just downstream of PARP1 in repair choice. Either way, given the apparently overlapping effects of OGT and PARP1 in DSB repair we have observed, a reasonable hypothesis is that O-GlcNAcylation and PARylation have a similar effect on promoting NHEJ and thereby suppressing resection.

To confirm effects on resection and HR, we have examined the kinetics of Rad51 foci formation after irradiation as a reporter for initiation of HR repair. We have also adopted a BrdU-based assay to directly measure 3' resection at DSBs. Our results remain preliminary, but are not able to implicate O-GlcNAcylation in resection or HR itself. This may favor a role for OGT in promoting NHEJ.

Given our current mechanistic studies, a reasonable model is that increased O-GlcNAcylation or PARylation may promote protection of DSB ends to enhance NHEJ in S/G2 cells and protect against resection. The net effect would be to increase the speed of DSB repair, insofar as NHEJ is several fold faster than HR, and to reduce the flow of DSBs so that it remains below the capacity of HR even at higher levels of radiation. The net effect would be higher cell survival at a given radiation dose.

Biomarkers of DNA damage response As an approach to evaluate the effects of radiation on bone marrow cells in culture and thereby detect cellular effects of modulation of OGT and/or PARP1 activity, we have applied a novel strategy to survey the secretome in tissue culture. Briefly, we use ProteoMiner beads (Bio-Rad), pools of particles coated with peptides that adsorb proteins from complex mixtures, for dynamic range compression (DRC) and collect a broad sample of protein present in conditioned culture media. After trypsinization and LC-MS/MS proteomics, we use informatic methods to identify proteins that derive from the cultured cells rather than the bovine serum and then apply systems biology methods to detect patterns of response. An advantage of the ProteoMiner DRC strategy is that it is non-destructive, allowing repeated analysis of bone marrow cultures over time. ProteoMiner DRC followed by LC-MS/MS proteomics or immunodetection is also amenable to analysis of plasma proteomes, potentially allowing individual protein and/or pathway-level biomarkers identified *in vitro* to be validated *in vivo*.

As a feasibility study, we used three 10 week old mice where one was an unirradiated control (NIR) and two were treated with 3 Gy X-ray (IR). The animals were sacrificed and bone marrow (BM) was harvested immediately following irradiation. BM cells were cultured in RPMI for 3 days and cell pellets and media

collected for proteomics. Secreted proteins were collected with ProteoMiner beads, digested with trypsin overnight at 37 °C and peptides cleaned up with C18 before LC-MS/MS analysis on a Q-Exactive 480 Orbitrap mass spectrometer. Data files were analyzed with MaxQuant and Perseus analysis software. Overall 1826 proteins were detected at 1% FDR. Protein label-free quantification intensities were log2 transformed and a ratio of IR/NIR was established Proteins were classified as IR only or NIR only if they were only detected in that secretome condition. Of the 1826 proteins, 1818 were unique, of which 305 were detected only in IR, 1437 detected in both IR and NIH, and 84 only in NIR. Using a 2.0 fold change cutoff (log2 ≥ 1.0) to select proteins "up-regulated after IR" or "down-regulated after IR", we classified 471 proteins as up-regulated, 200 down-regulated and 1155 proteins as no-change and performed pathway analysis via Kyoto Encyclopedia of Genes and Genomes KEGG (gProfiler) and Reactome.

KEGG analysis indicated enrichment of 8 major pathways in the secreted proteome after IR. Fatty acid metabolism (KEGG:01212, 12 proteins, Adj p-value 1.01e-05), Spliceosome (KEGG:03040, 16 proteins, Adj p-value 3.61e-05), Fatty acid degradation (KEGG:00071, 10 proteins, Adj p-value 1.49e-04), Carbon metabolism (KEGG:01200, 12 proteins, Adj p-value 1.28e-02), Thermogenesis (KEGG:04714, 17 proteins, Adj p-value 1.66e-02), Metabolic pathways (KEGG:0100, 66 proteins, Adj p-value 3.28e-02), Diabetic cardiomyopathy (KEGG:05415, 15 proteins, Adj p-value 3.80e-02), and mRNA surveillance pathway (KEGG:03015, 10 proteins, Adj p-value 4.22e-02). Reactome analysis identified Metabolism of RNA (R-HSA-8953854, 78 proteins, FDR 2.15e-12), Cell Cycle (R-HSA-1640170, 51 proteins, FDR 5.29e-04), Gene and protein expression by JAK-STAT signaling after IL-12 stimulation (R-HSA-8950505, 12 proteins, FDR 8.99e-04), Response of EIF2AK4 (GCN2) to amino acid deficiency (R-HSA-9633012, 15 proteins, FDR 1.10e-03), Cohesin Loading onto Chromatin (R-HSA-2470946, 5 proteins, FDR 1.459e-03), Cellular response to stress (R-HSA-2262752, 61 proteins, FDR 1.97e-03), Cellular response to starvation (R-HSA-9711097, 18 proteins, FDR 2.67e-03), Telomere Maintenance (R-HSA-157579, 11 proteins, FDR 3.58e-02), and SUMOylation of DNA damage response and repair proteins (R-HSA-3108214, 9 proteins, FDR 4.40e-02). These results point to multiple secretome proteins that might serve as biomarkers for mitigation of radiation stress.

In addition to profiling the secreted proteome, we prepared cytoplasmic and nuclear fractions of the cultured cells from each mouse and performed LC-MS/MS analysis, yielding 2445 cytoplasmic and 2609 nuclear proteins respectively at 1% FDR. A similar 2-fold cutoff yielded 750 up-regulated (i.e. IR-induced) proteins in the cytoplasmic fraction and 1253 up-regulated proteins in the nuclear fraction. Pathway analysis of the up-regulated cytoplasmic proteome pointed to a marked increase for immune response pathways (Adj P-value 5.75e-14) while the up-regulated nuclear proteome was enriched for DNA damage response pathways (Adj p-value 5.56e-09).

Subtask 2: Determine effects of radiation using flow cytometry. We have established a flow cytometry-based assay to track DNA damage and repair by immunostaining with anti-γH2AX antibody and HSPC markers. We have initially validated this assay as a tool to examine the kinetics of recovery from DNA damage.

As a simple test of feasibility, we examined γH2AX levels in bone marrow cells from mice provided with ad libitum water with or without 50 mg/ml N-acetyl-D-glucosamine for 7 days, collected 4 h after 3 Gy ^{60}Co irradiation. While a decrease in the percent of γH2AX positive cells was seen comparing the GlcNAc fed mouse to control, this effect would not be sufficient to predict increased radiation resistance per se. However, combining our methods to examine γH2AX in different cell subsets would identify the most responsive cells.

Subtask 3: Determine radiation effects on bone marrow cultures with single cell RNA-seq. Not initiated.

Major Task 3: Evaluate effects of increased O-GlcNAcylation on radiation response

Subtask 1: Develop methods to regulate O-GlcNAcylation in bone marrow culture. Using mesenchymal stem cell culture as a model, we have examined the effect of adding PUGNAc or GlcNAc to tissue culture media on total O-GlcNAcylation in MSC cultures. As expected, inhibiting OGA (PUGNAc) or increasing OGT activity (GlcNAc) lead to enhanced Og-GlcNAcylation on Western blot.

Subtask 2: Evaluate effects of O-GlcNAcylation on bone marrow growth and differentiation. Short term treatment to increase O-GlcNAcylation did not markedly impact MSC growth or differentiation potential.

Subtask 3: Confirm mechanisms of effects of increased O-GlcNAcylation on DSB repair, proliferation and senescence. Using MSC cultures, we were unable to observe signficant effects of O-GlcNAcylation inducers PUGNAc or GlcNAc or inhibitors OSMI-1 or ST060266 on the kinetics of γH2AX formation or resolution or on the formation of senescent cells. Based on these data, we do not anticipate continuing to evaluate MSC responses in vitro and refocus our efforts on HSCs and precursors.

Major Task 4: Develop bone marrow irradiation model to examine impacts of O-GlcNAcylation

Subtask 1: Identify radiation dose producing <20% mortality within 2 weeks due to H-ARS. Not initiated.

Subtask 2: Obtain baseline data on H-ARS with respect to bone marrow function. Not initiated.

Subtask 3: Examine irradiation in Cux1 shRNA model. Not initiated.

Major Task 5: Examine impacts of O-GlcNAcylation on early bone marrow response

Subtask 1: Establish gavage treatment to increase bone marrow O-GlcNAc levels before and after irradiation. We have found that mice will accept water to which 50 mg/ml GlcNAc has been added. We have not explored whether O-GlcNAcylation levels in total bone marrow cell or relevant stem cell and precursor cell subsets are increased let alone whether this dose is sufficient to have a maximal effect.

Subtask 2: Examine effects of O-GlcNAcylation on blood counts after irradiation. Not initiated.

Major Task 6: Examine modulation by O-GlcNAcylation of changes in blood counts after irradiation.

Subtask 1: Optimize whole body irradiation dose to accelerate bone marrow failure and myeloid leukemia in Cux1 shRNA mice. Not initiated.

Subtask 2: Determine if altered O-GlcNAcylation modulates bone marrow failure or leukemia. Not initiated.

Major Task 7: Determine whether O-GlcNAcylation modulates the timeline for onset of malignancy in Cux1 sRNA model .

Subtask 1: Demonstrate long term maintenance of high O-GlcNAcylation in vivo. Not initiated.

Subtask 2: Obtain data on overall survival impact of high O-GlcNAcylation. Not initiated.

4. Impact

Nothing to report to date.

5. Changes/Problems

As of February, 2022, Sera Averbek PhD joined the laboratory as a postdoctoral fellow with a primary role in support of this project. Ms. Averbek was first author of the paper, "O-GlcNAcylation affects the pathway choice of DNA double-strand break repair", in *International Journal of Molecular Sciences*, https://doi.org/10.3390/ijms22115715. As she has gotten up to speed, she began by working primarily on *in vitro* studies and is now beginning *in vivo* studies.

Visiting scientist Woo Young Kim PhD departed as of July, 2022. However, we were able to complete sufficient studies with mesenchymal stem cells to determine that they are unlikely to be a primary target for intervention to enhance DSB repair during the radiation response.

6. Products

One paper has been published supported by this funding to date. Authors partially or fully supported by this funding are underlined.

Genomic studies controvert the existence of the CUX1 p75 isoform
M. Krishnan, M.D. Senagolage, J.T. Baeten, D.J. Wolfgeher, S. Khan, S.J. Kron and M.E. McNerney
Sci Rep 12(1):151, 2022 doi: 10.1038/s41598-021-03930-4.

Another was posted on bioRxiv

Global Epigenetic Analysis Reveals H3K27 Methylation as a Mediator of Double Strand Break Repair
J. Lutze, D. Wolfgeher, S.J. Kron
doi: https://doi.org/10.1101/2021.09.20.461136

Additional manuscripts partly or fully supported by this funding remain to be submitted.

7. Participants & Other Collaborating Organizations

Name: Stephen Kron, MD, PhD
Project Role: PI
Research Identifier: 0000-0003-1518-2436
Person Month Worked: 2 CME
Contribution to Project: Dr. Kron has been involved in planning and analysis of experiments and in coordinating activities of the research and technical staff.
Funding Support: See continuation pages.

Name: Megan McNerney, MD, PhD
Project Role: Co-Investigator
Research Identifier: 0000-0002-8260-3598
Person Month Worked: 0.5 CME
Contribution to Project: Dr. Dr. McNerney has participated in developing the strategy to track DNA damage response across the genome.
Funding Support: See continuation pages.

Name: Sandeep Gurbuxani, MBBS-PhD
Project Role: Co-Investigator
Research Identifier: 0000-0003-0716-8730
Person Month Worked: 0.5 CME
Contribution to Project: Dr. Gurbuxani has been available for consulation.
Funding Support: See continuation pages.

Name: Elena Efimova, PhD
Project Role: Senior research professional
Research Identifier: None
Person Month Worked: 6.0 CME
Contribution to Project: Dr. Efimova has been studying the mechanism of modulation of DNA damage response and repair by O-GlcNAcylation *in vitro*.
Funding Support: NA

Name: Julian Lutze, PhD
Project Role: Graduate Student
Research Identifier: 0000-0002-8062-7756
Person Month Worked: 3.96 CME (departed December, 2021)
Contribution to Project: Mr. Lutze developed proteomic and genomic methods to measure how radiation-mediated damage is detected and repaired, using K562 as a model for bone marrow cells.
Funding Support: NA

Name: Joanna Pagacz, MA
Project Role: Technician
Research Identifier: None
Person Month Worked: 6 CME (departed May, 2022)
Contribution to Project: Ms. Pagacz isolated mouse bone marrow and participated in analysis of radiation effects.
Funding Support: NA

Name: DeShawn Thompson
Project Role: Technician
Research Identifier: None
Person Month Worked: 2 CME
Contribution to Project: Mr. Thompson examined bone marrow proliferation and differentiation by flow cytometry.
Funding Support: NA

Name: Woo Young Kim, PhD
Project Role: Visiting Scientist
Research Identifier: 0000-0002-2895-4995
Person Month Worked: 4 CME (half-time, arrived in December, 2021, and departed July, 2022)
Contribution to Project: Dr. Kim established mesenchymal stem cell culture and examined GlcNAc in radiation response.
Funding Support: NA

Name: Sera Averbek, PhD
Project Role: Postdoctoral research scientist
Research Identifier: None

Person Month Work: 6 CME (arrived in February, 2022)
Contribution to Project: Dr. Averbeck has started her research by collaborating with Elena Efimova and Woo Young Kim on their projects.
Funding Support: NA

Other Support

Kron, Stephen

PREVIOUS:

Title: *Radiation response within the tumor microenvironment*
Supporting Agency: NIH/NCI, R01 CA164492
Project Goals: Examining determinants of radiation resistance and response mediated by both cancer and host tissue.
Specific Aims:
Aim 1. Image and model DSB kinetics after irradiation in relation to the microvascular tracking hypoxia and metabolism as potential determinants of repair
Aim 2. Examine the senescence response after irradiation in relation to the microenvironment
Aim 3. Examine angiogenesis and tumor regrowth after radiation in relation to tumor metabolism, hypoxia and senescence
Period of Performance: 9/21/11-7/31/17
Level of Effort: 32%; 3.8 CME
Contact: Debra Sowell
Debra.sowell@nih.gov
Overlap: None

Title: *PARP inhibition to enhance induction for head and neck cancer*
Supporting Agency: NIH/NCI, R01 CA176843
Project Goals: Examine PARP inhibitor veliparib as an immunogenic sensitizer for induction therapy.
Specific Aims:
Aim 1: Examine the interactions of veliparib with docetaxel, cisplatin and/or 5-fluorouracil in vitro and in xenograft and syngeneic tumors.
Aim 2: Pursue A) Proteomic analysis of the cell surface proteome and SASP and B) Gene expression analysis of signaling pathways to define the differential response of HNSCC cell lines to TPF plus or minus veliparib.
Aim 3: Characterize patient biopsy material in light of the molecular analyses performed with model systems.
Period of Performance: 9/1/13-8/30/17
Level of Effort: 10%, 1.2 CME
Contact: Mary Wolpert PhD
wolpertm@mail.nih.gov
Overlap: None

Title: *Environmentally-adaptive nanoparticles with focal irradiation for cancer therapy*
Supporting Agency: NIH/NIBIB, R01 EB017791 (Y. Yeo Purdue U., PI)
Project Goals: Studies directed at targeting novel nanoparticles to tumors.
Specific Aims:
Aim 1. To optimize synthesis of ENPs loaded with paclitaxel (PTX).
Aim 2. To optimize and validate IGRIP for delivery of NPs to prostate cancer tumors in mice.
Aim 3. To determine biodistribution and anti-tumor activity of PTX/ENPs.
Performance Period: 3/1/14-2/28/18
Level of Effort: 9%, 1.0 CME
Contact: Yoon Yeo PhD
yyeo@purdue.edu
Overlap: None

Title: *Probing the immune microenvironment to distinguish indolent from aggressive prostate cancer*
Supporting Agency: NIH/NCI/Chicago Prostate SPORE P50 CA180995 Pilot
Project Goals: Multiplex microscopy method to interrogate tumor immune microenvironment
Specific Aims:
Aim 1. Validate four-plex panels that interrogate the PCa immune microenvironment on individual FFPE blocks and tissue microarrays

Aim 2. Apply multiparameter imaging cytometry to A) Collect multiplex IF data on a training set of PCa biopsy TMAs and B) Identify candidate immune microenvironment biomarkers associated with indolence vs. aggressive behavior.
Aim 3. Evaluate candidate biomarkers on a test set of PCa biopsy TMAs and confirm feasibility with individual clinical samples.
Performance Period: 8/1/16-7/1/18
Level of Effort: 2%, 0.2 CME
Contact: Robyn Egan
regan@bsd.uchicago.edu
Role: Co-PI
Overlap: None

Title: *Radiation-enhanced delivery of checkpoint blockade antibodies*
Supporting Agency: Cancer Research Institute, CLIP Award
Project Goals: Targeted radiation-mediated delivery of antibodies for immunotherapy
Specific Aims:
Aim 1: Validate radiation-enhanced permeability and retention as a tool for targeted delivery of immunological checkpoint inhibitor antibodies to murine melanoma tumors.
Aim 2: Examine significance of enhanced delivery and tumor penetration on synergy of immune checkpoint inhibitor antibodies with image-guided radiation.
Performance Period: 7/1/17-6/30/19
Level of Effort: 10%, 1.2 CME
Role: PI
Contact: Ryan Godfrey
rgodfrey@cancerresearch.org
Overlap: None

Title: *Image-guided radiation-induced permeability (IGRIP) for IGDD*
Supporting Agency: NIH/NCI, R01 CA199663
Project Goals: We intend to leverage image-guided radiation as a means to target nanomedicines to tumors.
Specific Aims:
Aim 1. Optimize and validate image-guided radiation-induced permeability (IIGRIP) and establish IGRIP for image-guided drug delivery (IGDD) to prostate cancer tumors in mice
Aim 2. Towards establishing radiation-guided gene delivery, develop novel nucleic acid vectors for systemic injection and validate these nanocarriers by demonstrating efficient radiofection of mouse PCa tumor models with reporter genes
Aim 3. Toward translation of radiation-guided gene therapy, a) apply mouse models of PCa to demonstrate image-guided radiofection of CD-UPRT, evaluate CD-UPRT/5-FC enzyme prodrug therapy and examine synergy with radiation and b) target oncogenes by radiofection of siRNA for knockdowns and CRISPR for knock outs.
Performance Period: 7/1/15-6/30/2021
Level of Effort: 13%, 1.5 CME
Role: MPI with R. Weichselbaum
Contact: Pushpa Tandon
Tandonp@mail.nih.gov
Overlap: None

Title: *Nanoscale metal-organic frameworks for light triggered and X-ray induced photodynamic therapy of head and neck cancers*
Supporting Agency: NIH/NCI, U01 CA198989 (W. Lin, R. Weichselbaum MPIs)
Project Goals: Radiation-activated nanoparticle cancer therapy technology
Specific Aims:
Aim 1: Synthesis and characterization of nanoscale metal-organic frameworks (NMOFs) for NIR triggered and X-ray induced PDT.
Aim 2: Evaluation of NIR triggered PDT efficacy in experimental head and neck cancers.
Aim 3: Evaluation of X-ray induced PDT efficacy in experimental head and neck cancers.

Performance Period: 9/1/15-8/31/20
Level of Effort: 9%, 1.0 CME
Role: Co-I
Contact: Wenbin Lin PhD
wenbinlin@uchicago.edu
Overlap: None

Title: *Probing impact of cellular senescence on intestinal crypt function using organoid models*
Pilot & Feasibility grant; NIH, UChicago DDRCC Pilot
Project Goals: Examining aging in intestinal organoids using single cell methods.
Specific Aims:
Aim 1: Establish organoid formation defects associated with cellular senescence from models of intestinal epithelial aging including naturally aged mice, irradiated mice and mice lacking telomerase.
Aim 2: Examine effects of eliminating senescent cells on rescuing organoid formation by depleting senescent cells using genetic or chemical ablation.
Aim 3: Evaluate effects of genetic or chemical ablation of senescent cells on intestinal epithelial aging in vivo.
Period of Performance: 12/1/19-11/30/20
Level of Effort: 2%, 0.2 CME
Role: PI
Contact: Kailee Zingler
kzingler@bsd.uchicago.edu
Overlap: None

Title: *Tag-ChIP-MS for analysis of chromatin-level regulation of DNA repair*
Supporting Agency: NIH/NCI, R21 CA213247
Project Goals: We will leverage advanced tools for genome editing, protein tagging, chromatin enrichment and LC-MS/MS analysis to establish a new approach to chromatin proteomics
Specific Aims:
Aim 1. Establish split MPLUM tagging to visualize proteins involved in IRIF formation and resolution
Aim 2. Leverage split MPLUM tagging for TAG-CHIP-MS to dissect chromatin dynamics at IRF
Performance Period: 3/1/17-2/29/20 (NCE 2/28/21)
Level of Effort: 2%, 0.2 CME
Role: PI
Contact: John Knowlton
Jk339o@nih.gov
Overlap: None

Title: *Targeting Cancer Metabolism as a Novel Synthetic Lethality Strategy For BRCA Deficient Breast Cancers*
Funding Agency: NIH/NCI, F32 CA250347
Project Goals: NRSA to Tamica Collins PhD, a post-doctoral fellow in the Kron laboratory.
Specific Aims:
Aim 1. Assess effects of modulating O-GlcNAcylation in BRCA breast cancer in vitro and in vivo
Aim 2. Evaluate OGT inhibitors as PARPi sensitizers in BRCA breast cancer in vitro and in vivo
Aim 3. Define gene expression signatures for activation of O-GlcNAcylation in BRCA breast cancer
Period of Performance: 6/1/20-5/31/22 (Early Termination)
Level of effort: 0
Role: Mentor and Sponsor
Contact: Sonia B. Jakowlew PhD
jakowles@mail.nih.gov
Overlap: None

Title: *Veliparib interactions with genotoxic and immuno-therapy: Therapy-induced senescence and anti-tumor immunity*
Supporting Agency: AbbVie/UChicago collaboration grant
Project Goals: Exploring determinants of response to PARP inhibition including potentiating immunotherapy

Specific Aims:
Aim 1 Examine responses to Veliparib combined with a platinum agent or radiation in NSCLC PDX models in immunodeficient NSG mice.
Aim 2(A) Examine responses to Veliparib combined with genotoxic agents in NSCLC PDX models in CD34+ HSC humanized NSG mice and (B) Examine responses to Veliparib combined with genotoxic agents in i) CT26 tumor spheroids co-cultured with BALB/c mouse splenocytes and in ii) CT26 and 4T1 tumors in immunocompetent BALB/c mice.
Aim 3 Examine potentiation of Veliparib effects by PD-1/PD-L1 immune checkpoint blockade in i) CT26 tumor spheroids co-cultured with BALB/c mouse splenocytes and in ii) CT26 and 4T1 tumors in immunocompetent BALB/c mice.
Performance Period: 10/1/16-12/31/21
Level of Effort: 9%, 1.0 CME
Role: PI
Contact: Eric Johnson
Eric.f.johnson@abbvie.com
Overlap: None

Title: *Probing breast cancer immune infiltrates to monitor checkpoint blockade response*
Funding Agency: NCI, UCCCC Pilot
Project Goals: T3 and single cell analysis of TNBC biopsies to track response to immune checkpoint blockade.
Specific Aims:
Aim 1. Apply single cell RNA sequencing to discover biomarkers of response to immune checkpoint blockade (ICB) antibody therapy.
Aim 2. Establish workflow that applies T3 for rapid and reproducible 3D analysis in core needle biopsies from TNBC patients.
Aim 3. Validate candidate biomarkers of response to ICB therapy using T3 for analysis of TNBC core needle biopsy tissue
Period of Performance: 2/2/20-8/31/21
Level of effort: 2%, 0.2 CME
Role: PI
Contact: Toni Cipriano-Steffens
tciprian@medicine.bsd.uchicago.edu
Overlap: None

ACTIVE:

Title: *Leveraging DNA repair to enhance CRISPR genome editing*
Funding Agency: Chicago Biomedical Consortium, Catalyst Award
Project Goals: Examining novel strategy to increase HDR by CRISPR for site-directed mutagenesis based on recruiting DNA repair activities.
Specific Aims:
Aim 1: Examine the ability of DNA repair enzymes linked to resolution of DNA-protein crosslinks to detect and resolve stable Cas9-DSB complexes in vitro.
Aim 2: Evaluate the efficiency of Cas9-mediated homology-directed repair using donor DNAs that incorporate specific forms of DNA damage (DNA-repair beacons) to target DNA repair to the Cas9-DSB complex
Period of Performance: 3/1/19-11/30/22 (NCE)
Level of effort: 2%, 0.2 CME
Role: MPI with L. Hanakahi, UIC
Contact: Karen R. Snapp, DDS, PhD
ksnapp@northwestern.edu
Overlap: None

Title: *Single cell analysis of tumor immune infiltrates to track breast cancer immunotherapy*
Supporting Agency: NCI, UCCCC Pilot
Project Goals: Evaluate scRNA-seq analysis of the immune repertoire and apply this approach to documenting

response to immune checkpoint blockade.
Specific Aims:
Aim 1 Modeling anti-PD-1/PD-L1 checkpoint blockade in mouse models of breast cancer to develop and validate scRNA-seq as an assay for anti-tumor immune activation
Aim 2 Applying scRNA-seq to analysis of on-treatment biopsies from breast cancer patients receiving anti-PD-1/PD-L1 therapy
Performance Period 12/1/17-11/30/18 (NCE 11/30/22)
Level of Effort: 2%, 0.2 CME
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title: *Mechanisms determining local and systemic anti-tumor immune response after metastasis-directed image-guided radiation combined with PD-1/PD-L1 checkpoint blockade*
Funding Agency: METAVivor, Research Award
Project Goals: Mouse model studies of radiation to potentiate immune checkpoint blockade in breast cancer.
Specific Aims:
Aim 1. Validate radiation-targeted delivery of anti-PD-L1 to both primary and metastatic murine mammary tumors.
Aim 2. Examine determinants of synergy of anti-PD-L1 with radiation
Aim 3. Explore requirements fo systemic anti-tumor immune response targeting distant metastases
Period of Performance: 2/1/20-12/31/22
Level of Effort: 9%, 1 CME
Role: PI
Contact: Sonya Negley
Sonya@metavivor.org
Overlap: None

Title: *Targets of Reactive Lipid Species regulating DNA damage response and cell senescence*
Supporting Agency: NIH/NCI, R01 CA217182
Project Goals: To establish a new mechanism of action for etoposide and related chemotherapy agents
Specific Aims:
Aim 1A) Examine the effects of lipid peroxidation and reactive lipid species on Top2 poisoning and cell senescence, and B) Examine modification of Top2 by 4-HNE and other reactive lipid species and mutate reactive sites to examine functional significance.
Aim 2 Directly test whether DNA damage is sufficient to induce accelerated senescence and examine if 4-HNE and DNA damage interact additively (same pathway) or synergistically (distinct pathways).
Aim 3A) Apply proteomics to identify targets of RLS in etoposide-treated cells, and B) Examine Top2 as a signal transducer mediating the adaptive response to oxidative stress and ionizing radiation.
Performance Period: 7/1/17-6/30/23 (NCE)
Level of Effort: 4%, 0.48 CME
Role: PI
Contact: Paul Okano PhD
Po8k@nih.gov
Overlap: None

Title: *Chemotherapy delivery with nanoparticles for targeted induction of immunogenic cell death*
Funding Agency: NIH/NCI, R01 CA232419 (Y. Yeo Purdue, PI)
Project Goals: Studies are directed at targeting nanoparticles bearing immune checkpoint antagonists to tumors.
Specific Aims:
Aim 1. To develop ICD-inducing NPs and evaluate their ability to promote an anti-tumor immune response.
Aim 2. To evaluate the anti-cancer effect of ICD-inducing NPs in combination with local ionizing radiation.
Aim 3. To investigate the contribution of ICD-inducing NPs to ICB therapy.

Period of Performance: 4/1/18-3/31/23
Level of effort: 4%, 0.48 CME
Role: Co-Investigator
Contact: Yoon Yeo PhD
yyeo@purdue.edu
Overlap: None

*Title: *Systemic delivery of short nucleic acid by anionic flexible carriers for cancer immunogene therapy*
Funding Agency: NIH (Yoon Yeo, Purdue)
Project Goals: *Studies of a soft nanocapsule as a gene carrier.*
Specific Aims:
Aim 1. To optimize design and production of Nanosac for multigene targeting
Aim 2. To define toxicity, pharmacokinetics (PK), biodistribution (BD), and pharmacodynamics of Nanosac
Aim 3. To leverage systemic delivery of Nanosac and tumor targeting by image-guided radiation
Period of Performance: 04/2021-03/2025
Level of effort: 10%; 0.96 CME
Role: Y. Yeo (PI), S. Kron (MPI)
Contact: Yoon Yeo PhD
yyeo@purdue.edu
Overlap: None

Title: *Bioinspired chemical probe approach targeting telomerase reverse transcriptase*
Funding Agency: NIH/NCI R01 CA254047
Project Goals: Subcontract for biological studies to further develop covalent TERT inhibitors to probe telomere and non-canonical roles of TERT.
Specific Aims:
Aim 1. Advance chrolactomycin analogs to inhibit htert in cells.
Aim 2. Explore selectivity and define extra-telomeric roles for htert catalytic activity with chrologs.
Aim 3. Investigate effects of chrologs on radiation sensitivity, tumor formation, recurrence and metastasis in the CT26 mouse colon carcinoma model.
Period of Performance: 7/1/20-6/30/25
Level of Effort: 8%, 0.96 CME
Role: MPI
Contact: Sharad Verma PhD
sharad.verma@nih.gov
Overlap: None

Title: *Lipid signaling in cellular senescence and tissue aging*
Funding Agency: NIH/NIA R01 AG069865
Project Goals: Study to examine role for lipid peroxidation and accelerated senescence in pulmonary fibrosis.
Specific Aims:
Aim 1. Characterize the lipidomic changes in pulmonary cell senescence in vitro and in vivo
Aim 2. Examine impacts of modulating sphingolipid, ceramide and other lipid pathways on senescence
Aim 3. Target lipid metabolism to block senescence and delay pulmonary fibrosis
Period of Performance: 9/1/20-8/31/25
Level of Effort: 8%, 0.96 CME
Role: PI
Contacts: Yih-Woei Fridell PhD
Yih-Woei.fridell@nih.gov
Overlap: None

Title: *Nanoscale Metal-Organic Frameworks Enable Radiotherapy-Radiodynamic Therapy and Deliver CpG Oligodeoxynucleotides to Generate Tumor Vaccines and Potentiate Immunotherapy of Head and Neck Cancers*
Funding Agency: NIH/NCI
Project Goals: This project examines cancer therapy with MOFs as nanomedicines for immunotherapy.

Specific Aims:
Aim 1: Elucidate the cellular mechanisms of nMOF-mediated RT-RDT and CpG oligonucleotides.
Aim 2: Profile tumor microenvironment and extracellular matrix after treatment with RT-RDT.
Aim 3: Investigate the anticancer efficacy and adaptive immune response of nMOF/CpGmediated RT-RDT and immunotherapy
Aim 4: Determine novel immunotherapy combinations that are potentiated by RT-RDT in HNSCC models resistant to PD-1/PD-L1 blockade.
Period of Performance: 7/1/20-6/30/25
Level of Effort: 5%, 0.60 CME
Role: Co-Investigator
Contact: Jennifer Couch PhD
couchj@mail.nih.gov
Overlap: None

Title: *Enabling T3 imaging cytometry of the tumor immune microenvironment in formalin fixed paraffin embedded (FFPE) biopsy tissue*
Funding Agency: Duckworth Foundation; (University of Chicago Cancer Center)
Project Goals: Studies to accelerate translation of 3D imaging to its potential commercial applications.
Specific Aims:
Aim 1: Adapt T3 to enable 3D imaging cytometry in FFPE, using 20-50 μm thick macrosections cut from archival head and neck cancer tissue samples to examine immune microenvironment.
Aim 2: Validate 3D cytometry in FFPE macrosections by comparing T3 analysis of immune microenvironment on fresh tissue and fixed and embedded thick sections from individual head and neck cancers.
Aim 3: Demonstrate higher sensitivity, specificity and quantitative resolution of T3 3D cytometry in FFPE thick sections over conventional multiplex IHC in thin sections.
Performance Period: 5/20 – 12/22
Level of Effort: 2% 0.24 CME
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title: Roswell Park Ovarian Cancer SPORE (Kron RDP: *Targeting telomerase reverse transcriptase to improve treatment of advanced ovarian cancer)*
Funding Agency: NIH/NCI; Roswell Cancer Center; University of Chicago Comprehensive Cancer Center
Project Goals: Examine TERT as a target to enhance ovarian cancer treatment.
Specific Aims:
Aim 1: Confirm that NU-1 and NU-PROTAC-1 inhibit/degrade TERT and sensitize human and mouse OvCa cell lines as monolayers and spheroids to genotoxic chemotherapy and targeted agents in vitro.
Aim 2: Determine effectiveness of NU-1 and NU-PROTAC-1 in targeting TERT in xenograft, PDX and syngeneic models of OvCa peritoneal carcinomatosis.
Aim 3: Examine NU-1 and NU-PROTAC-1 as sensitizers A) to systemic and/or intraperitoneal chemotherapy in xenograft models and B) to chemotherapy and/or immunotherapy in the ID8 syngeneic OvCa model.
Performance Period: 2/1/2022-1/31/2023
Level of Effort: 2% 0.24 CME
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title: *Targeting TET2 to block adaptive resistance to radiation*
Funding Agency: The University of Chicago Comprehensive Cancer Center
Project Goals Targeting the ability of interferon gamma to induce expression of immunosuppressive factors by blocking demethylation and activation of promoters.
Specific Aims:
Aim 1: Validate TET2 as a critical mediator of adaptive resistance to radiation

Aim 2: Examine small molecule approaches to targeting TET2 to overcome adaptive resistance
Performance Period 6/1/2022-5/31/2023
Level of Effort: 2% 0.24 CME
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title: *ChicAgo Center for Health and EnvironmenT (CACHET)*
Funding Agency: NIH/NIEHS
Project Goals: CACHET will continue to promote multidisciplinary environmental health research among clinician, laboratory and population scientists from two Chicago area universities with complementary strengths and structure to understand, evaluate and ultimately reduce environmental health related disparities among residents of the region and beyond.
Performance Period 06/01/2022 – 03/31/2027
Level of Performance: 3% 0.36 CME
Role: Co-I, Co-leader, Career Development
Contact: H. Ahsan, The University of Chicago
habib@uchicago.edu
Overlap: None

PENDING:

Title: *Targeting adaptive resistance to radiotherapy in TNBC*
Funding Agency: NIH Prime; UC Breast Cancer Disparities (SPORE)
Project Goals: Examine veliparib to enhance immune response in TNBC.
Specific Aims:
Aim 1: A) Examine veliparib and radiation synergy in 4T1 triple negative mammary carcinoma and B) Confirm that veliparib promotes anti-tumor immune response after irradiation.
Aim 2: A) Characterize PARP1 dependent pathway upstream of PD-L1 to identify candidate mediators and B) Validate PARP1 and other targets that regulate the negative feedback loop.
Performance Period: 4/1/2022-3/31/2023
Level of Effort: 2% 0.24 CME
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title: *Targeting the radioadaptive response in head and neck cancer*
Funding Agency: The University of Chicago Comprehensive Cancer Center
Project Goals: To apply the PARP1 inhibitor veliparib as an immunogenic radiosensitizer, where we hope for radiation to recruit activated CTLs to tumors and veliparib to block IFNγ-dependent rebound immunosuppression.
Specific Aims:
Aim 1: Examine adaptive resistance in MOC1 syngeneic HNSCC model
Aim 2: Confirm veliparib and radiation synergy in MOC1 tumors
Aim 3: Define roles of PARP1 in inducible PD-L1 expression after irradiation
Performance Period: 5/1/2022-4/30/2023
Level of Effort: 2% 0.24 CME
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title: *Tumor Microenvironment in Adaptive Radioresistance (TMAR)*
Funding Agency: NIH/NCI

Project Goals: This project will work toward new insights into mechanisms of sensitivity and resistance to radiotherapy in non-small cell lung cancer and other epithelial tumors.
Specific Aims:
Aim 1: Characterize adaptive resistance in syngeneic mouse tumor models and lung cancer patients.
Aim 2: Develop approaches to disrupt adaptive resistance and release an effective anti-tumor immune response in syngeneic mouse tumor models.
Aim 3: Validate molecular targets and drug candidates that block the interferon-mediated negative feedback loop.
Aim 4: Identify non- or minimally invasive strategies to track adaptive resistance in radiotherapy treated patients.
Aim 5: Foster a highly interactive inter-institutional research program that brings together varied expertise for highly innovative approaches for increased translational impact.
Performance Period: 4/1/2023-3/31/2028
Level of Effort: 20% 2.40 CME
Role: Contact PI and Project Leader, Admin Core and Project 2
Contact: Anita Tandle
tandela@mail.nih.gov
Overlap: None

Title: *Enhancing radiotherapy as immunotherapy: Exploring epigenetic targets*
Funding Agency: University of Chicago Comprehensive Cancer Center
Project Goals: Team project to explore disrupting adaptive response to radiation at the level of epigenetic regulation
Specific Aims:
Aim 1: Dissect interferon signaling in adaptive resistance
Aim 2: Define mechanisms and targets of PARP1 in adaptive response.
Performance Period: 8/1/2022 – 7/31/2024
Level of Effort: None
Role: PI
Contact: Robyn Egan
regan@bsd.uchicago.edu
Overlap: None

Title*: Senescent cell vaccines to suppress breast cancer recurrence and metastasis*
Funding Agency: NIH
Project Goals: Apply immunogenic senescence as a means to enhance the efficacy of primary therapy for breast cancer.
Specific Aims:
Aim 1: Characterize the immune response to 4T1 senescent cells and senescent tumor cell-pulsed dendritic cell vaccines.
Aim 2: Develop 4T1 senescent cells and senescent tumor cell-pulsed dendritic cells as vaccines to eliminate orthotopic tumors, prevent recurrence and suppress spontaneous metastasis.
Aim 3: Evaluate feasibility of personalized senescent tumor cell-loaded autologous dendritic cell vaccines for breast cancer.
Performance Period: 4/1/2023-3/331/2025
Level of Effort: 5% 0.6 CME
Role: PI
Contact: William Timmer, PhD
William.timmer@nih.gov
Overlap: None

Megan McNerney

PREVIOUS

- Title: **Determining the role of CUX1 in myeloid neoplasia (McNerney)**
- Agency: NIH/NCI - 5K08CA181254-05
- Project Goals: The overall objective of the current application is to identify these biological functions and the molecular pathways regulated by CUX1, and how CUX1 haploinsufficiency alters these programs.
- Specific Aims: Aim 1: Identify the aberrant CUX1 transcription targets in acute myeloid leukemia. Aim 2: Identify the mechanisms of human CUX1 tumor suppressor activity.
- Performance Period: 9/16/14 - 8/31/19
- Effort: 50%
- Contracting/Grants Officer: Susan E Lim
    - Contact Information: Email: lims@mail.nih.gov Phone: 240-276-5630`1

Overlap with the Proposed Project: None

- Title: **Synergistic Role of the Microenvironment and MDS Stem Cells: A Model for the Pathogenesis and Treatment of MDS (LeBeau)**
- Agency: Edward P. Evans Foundation
- Project Goals: The overarching goal of our project is to integrate studies of the marrow microenvironment and hematopoietic stem cells (HSCs) to examine the novel hypothesis that deregulation of WNT signaling in the stroma is an early event that leads to the acquisition of mutations in HSCs, leading to myelodysplastic syndrome, and that mitigation of WNT signaling is a viable therapeutic target.
- Specific Aims: 1. To identify the molecular targets of altered WNT signaling in MSCs and HSCs derived from our MDS mouse model, and from MDS patients, particularly those with a del(5q); and 2. To extend our studies evaluating whether the microenvironment is a viable target in MDS for treatment or prevention of progression, and whether inhibition of aberrant WNT signaling in the niche in combination with standard or investigational therapy is a viable therapeutic approach using humanized mouse models, and by extending studies of our mouse models.
- Performance Period: 9/1/17 – 8/31/19
- Effort: 0.60 CM (5%)
- Contracting/Grants Officer: Michael Lewis, Ph.D.
    - Contact Information Email: grants@epefoundation.org

Overlap with the Proposed Project: None

- Title: **Establishing a genetically accurate preclinical model of high-risk myeloid malignancy (McNerney)**
- Agency: The Brinson Foundation
- Project Goals: The overall objective of the project funded by the Brinson Foundation is to determine the combined impact of Cux1 insufficiency and oncogenic Ras in cancer development.
- Specific Aims: Aims of Year 1 of the project were to: 1) determine the malignant myeloid phenotype in shCux1 x NrasG12D mice, and 2) identify the molecular mechanism(s) by which combinatorial loss drives disease. The specific Aims for Year 2 of this proposal are to 1) determine the role for oncogenic Nras and Cux1 knockdown in myeloid transformation through increased hematopoietic

stem cell survival and self- renewal; and 2) inhibit a pathway induced in Cux1lowxNrasG12D mice, such as PI3K, to block the malignant phenotype.
- Performance Period: 12/1/17 – 11/30/19
- Effort: .24 CM (2%)
- Contracting/Grants Officer: Jamie B. Bender
    - Contact Information: Email: Jamie.bender@brinsonfoundaiton.org

Overlap with the Proposed Project: None

- Title: **Molecular mechanisms of myeloid suppressor genes on chromosome 5 (LeBeau)**
- Agency: NIH/NCI - R01 CA190372-05
- Project Goals: The overall goal of this project is to identify cooperating mutations and genetic pathways leading to alkylating agent-induced t-MN with a del(5q).
- Specific Aims: Aim 1. To identify the molecular mechanisms of transformation by EGR1 by: a. Identifying the transcriptional targets of, and cellular pathways regulated by, EGR1 in normal hematopoietic stem cells (HSCs), and t-MNs with a del(5q); b. Examining the mechanism by which cell intrinsic loss of Egr1 and Apc cooperate with Tp53 loss in a mouse model of t-MN; and c. Examining the relationship of transcriptional regulatory pathways of the CUX1 transcription factor (a myeloid TSG on 7q22.1) and EGR1, and the mechanism by which lesions on 5q and 7q cooperate. Aim 2. To identify genetic mutations that cooperate with haploinsufficiency of EGR1 and/or APC in the pathogenesis of myeloid neoplasms by: a. Characterizing the genomic pattern of myeloid leukemias arising in mice with haploinsufficiency for Egr1, Apc, and Tp53; b. Expanding upon our studies showing that Egr1 haploinsufficiency cooperates with mutations induced by alkylating agents (ENU) to induce myeloid neoplasms by conducting genomic analysis of myeloid neoplasms arising in ENU-treated Egr1+/- mice; and c. Evaluating the cooperative role of candidate myeloid suppressor genes on 5q, e.g., CSNK1A1 (5q32, plays a critical role in hematopoiesis), SPRY4 (5q31.3, shown by S. Lowe to cooperate with Tp53 loss to promote AML), and a lysine-specific demethylase gene, KDM3B (5q31.2
- Performance Period: 2/1/15 - 1/31/20
- Effort: 0.36 CM (3%)
- Contracting/Grants Officer: Ian M Fingerman
    - Contact Information: Email: fingerma@mail.nih.gov

Overlap with the Proposed Project: None

- Title: **The role of CUX1 in human myelopoiesis (McNerney)**
- Agency: American Society of Hematology
- Project Goals: The objective of this proposal is to identify the role for CUX1 in human HSCs and the molecular pathways downstream of CUX1 haploinsufficiency that lead to malignant hematopoiesis.
- Specific Aims: Aim 1: Hypothesis —CUX1 transcriptionally regulates proliferation and differentiation genes in HSPCs. This will be tested by innovative functional genomic analyses including differential chromatin accessibility and gene expression due to CUX1 haploinsufficiency. Aim 2: Hypothesis —CUX1 suppresses human HSPC proliferation, blocks myelomonocytic differentiation, and is required for megakaryocyte and erythroid differentiation. This hypothesis will be tested by assays including cell-cycle, self-renewal, and myeloid/erythroid differentiation of cord-blood derived human HSCs with and without CUX1 haploinsufficiency.
- Performance Period: 7/1/18 – 6/30/20
- Effort: 0.6 CM (5%)
- Contracting/Grants Officer: Patricia Frustace
    - Contact Information: Email: awards@hematology.org

Overlap with the Proposed Project: None

- Title: **Tag-ChIP-MS for analysis of chromatin-level regulation of DNA repair (Kron)**
- Agency: NIH/NCI - R21CA213247-03
- Project Goals: The aim of this project is to establish Tag-ChIP-MS as an innovative technology for imaging-and-capture tagging to advance analysis of chromatin dynamics by microscopy and proteomics.
- Specific Aims: Aim 1. Establish split MPLUM tagging to visualize proteins involved in IRIF formation and resolution, Aim 2. Leverage split MPLUM tagging for TAG-CHIP-MS to dissect chromatin dynamics at IRF
- Performance Period: 3/1/17-2/29/21
- Effort: 0.24 CM (2%)
- Contracting/Grants Officer: John R Knowlton
    - Contact Information: Email: jk339o@nih.gov

Overlap with the Proposed Project: None

CURRENT

- Title: **Regulation of hematopoiesis by CUX1 (McNerney)**
- Agency: NIH/NCI - 5R01HL142782-05
- Project Goals: The overall objective is to determine the transcriptional role for CUX1 in normal HSPCs and erythroid progenitors and the pathways downstream of CUX1 haploinsufficiency that block erythroid differentiation.
- Specific Aims: Aim 1: Overall hypothesis – CUX1 is a transcriptional regulator of HSPC homeostasis conserved in mice and humans. Aim 2: Overall hypothesis – CUX1 promotes erythroblast cell cycle exit necessary for terminal differentiation by suppressing PI3K signaling.
- Performance Period: 7/15/18 – 6/30/23
- Level of Funding:
- Effort: 1.38 CM (11.5%)
- Contracting/Grants Officer: C Brian Bai
    - Contact Information: Email: brian.bai@nih.gov

Overlap with the Proposed Project: None

- Title: **The impact of chromosome 7q deletions in juvenile mylelomonocytic leukemia (McNerney)**
- Agency: NIH/NCI - 5R01CA231880-04
- Project Goals: The long-term goal of this proposal is to understand the molecular pathogenesis of -7/ del(7q) and to reveal new therapeutic targets for JMML patients.
- Specific Aims: Aim 1: Identify the cellular and molecular mechanisms by which Cux1 knockdown and Ras cooperate in JMML. Aim 2: Define the pathogenesis of combinatorial dosage imbalance of 7q genes in JMML.
- Performance Period: 9/20/18-8/31/2023
- Level of Funding:
- Effort: 1.38 CM (11.5%)
- Contracting/Grants Officer: Chamelli Jhappan
    - Contact Information: Email: jhappanc@mail.nih.gov

Overlap with the Proposed Project: None

- Title: **The genetic and environmental etiology of therapy-related myeloid neoplasms (McNerney)**
- Agency: American Cancer Society - #132457-RSG-18-171-01-LIB
- Project Goals: The overall objective of the current application is to identify the mechanism by which CUX1 deficiency drives t-MN.
- Specific Aims: Aim 1: Hypothesis – PI3K inhibition blocks the genetic interaction of CUX1-loss and RAS signaling in myeloid transformation. Aim 2: Hypothesis – Insufficient CUX1 causes increased HSC 'fitness' in response chemotherapy due to increased PI3K activity leading to clonal expansion and t-MN.
- Performance Period: 01/01/19 - 12/31/22
- Level of Funding:
- Effort: 0.6 CM (5%)
- Contracting/Grants Officer: Janet Meadows-Harriss
    - Contact Information: Email: janet.harris@cancer.org;

Overlap with the Proposed Project: None

- Title: *Cancer Center Support Grant - Molecular Mechanisms of Cancer Core*
- Agency: NIH/NCI 4P30CA014599-46 (PI: Odunsi)
- Project Goals The overall goal of the University of Chicago Medicine Comprehensive Cancer Center is to discover and translate new cancer-specific knowledge to prevent, detect, and treat cancer.
- Performance period: 5/22/18 - 3/31/23
- Level of Funding:
- Time Commitments: 0.6 CM, (5%)
- Contracting/Grants Officer Contact: David G Ransom

Overlap with the Proposed Project: None

- Title: **Genomic interrogation of high-risk myeloid neoplasms to identify new therapies**
- Supporting Agency: Leukemia & Lymphoma Society
- Performance period: 7/1/22 – 6/30/27
- Level of Funding:
- Time Commitments: 4.56 CM, (38%)
- Project Goals: Over 50,000 people are diagnosed with a myeloid neoplasm every year in the U.S. alone. A high-risk subset of patients is unresponsive to treatment and their survival is less than a year. The long-term goal of my lab is to improve the outcome for these patients. To this end, our research focuses on understanding the underlying genomic abnormalities in high-risk myeloid neoplasms, to identify new treatment avenues.
- Specific Aims:
- Contracting/Grants Officer Contact: researchprograms@lls.org

Overlap with the Proposed Project: None

SUBMITTED/PENDING

- Title: Establishing CUX1 as a determinant of hematopoietic stem cell fate
- Supporting Agency: NIH, 1 R01HL166184-01
- Performance period: 9/1/22 – 8/31/27
- Level of Funding:
- Time Commitments: 2.4 Calendar (20%)
- Project Goals: We aim to understand the mechanism by which CUX1 levels regulate hematopoietic development with the goal of informing new interventions to improve the quality of life for patients with these genetic changes.
- Specific Aims: **Aim 1*:*** To quantify CUX1 protein at the single-cell level, we have generated a novel CUX1mCherry-reporter mouse with endogenous CUX1 tagged with an in-frame mCherry fluorochrome. **Aim 2:** To determine how CUX1 regulates chromatin remodeling and enhancer poising and activation, we will leverage cutting-edge functional genomics approaches and single-cell methodologies in primary HSCs using our CUX1-reporter and -knockdown mice.
- Contracting/Grants Officer Contact: Brian D. Bai
  301-435-0080
  Brian.bai@nih.gov

Overlap with the Proposed Project: None

- Title: **Differentiation therapy of high-risk childhood myeloid malignancies**
- Supporting Agency: Alex Lemonade Stand Fnd
- Performance period: 2/1/23 – 1/31/27
- Level of Funding:
- Time Commitments: .6 calendar (5%)
- Project Goals: The proposed studies will allow us to achieve our long-term goal of identifying urgently needed therapies to improve the outcome for high-risk myeloid malignancies of childhood.
- Specific Aims: **Aim 1**: Define the molecular mechanisms by which CUX1 levels are regulated. **Aim 2:** Drive normal differentiation of -7/del(7q) myeloid disease through therapeutic restoration of CUX1.
- Contracting/Grants Officer Contact: grants@alexslemonade.org
  610-649-3034

Overlap with the Proposed Project: None

- Title: **CDK6 haploinsufficiency as a therapeutic vulnerability in AML**
- Supporting Agency: When Everybody Survives
- Performance period: 10/1/22 – 9/30/23
- Level of Funding:
- Time Commitments: .6 calendar (5%)
- Project Goals: The overall objective of this proposal is to determine if -7/del(7q) is a biomarker for sensitivity to drugs targeting CDK6.
- Specific Aims: Aim: Inhibit -7/del(7q) AML growth through therapeutic targeting of CDK6.
- Contracting/Grants Officer Contact: grants@wesfoundation.org
  770-595-3573

Overlap with the Proposed Project: None

Sandeep Gurbuxani

Previous, Current, and Pending Support

PREVIOUS
None

CURRENT

- Title: **Regulation of hematopoiesis by CUX1 (McNerney)**
- Agency: NIH/NCI - 5R01HL142782-05
- Project Goals: The overall objective is to determine the transcriptional role for CUX1 in normal HSPCs and erythroid progenitors and the pathways downstream of CUX1 haploinsufficiency that block erythroid differentiation.
- Specific Aims: Aim 1: Overall hypothesis – CUX1 is a transcriptional regulator of HSPC homeostasis conserved in mice and humans. Aim 2: Overall hypothesis – CUX1 promotes erythroblast cell cycle exit necessary for terminal differentiation by suppressing PI3K signaling.
- Performance Period: 7/15/18 – 6/30/23
- Level of Funding:
- Effort: 0.24 CM (2%)
- Contracting/Grants Officer: C Brian Bai
    - Contact Information: Email: brian.bai@nih.gov Phone: 301-435-0080

Overlap with the Proposed Project: None

- Title: **The impact of chromosome 7q deletions in juvenile mylelomonocytic leukemia (McNerney)**
- Agency: NIH/NCI - 5R01 CA231880-04
- Project Goals: The long-term goal of this proposal is to understand the molecular pathogenesis of -7/ del(7q) and to reveal new therapeutic targets for JMML patients.
- Specific Aims: Aim 1: Identify the cellular and molecular mechanisms by which Cux1 knockdown and Ras cooperate in JMML. Aim 2: Define the pathogenesis of combinatorial dosage imbalance of 7q genes in JMML.
- Performance Period: 9/20/18-8/31/2023
- Level of Funding:
- Effort: 0.24 CM (2%)
- Contracting/Grants Officer: Chamelli Jhappan
    - Contact Information: Email: jhappanc@mail.nih.gov

Overlap with the Proposed Project: None

SUBMITTED/PENDING
None

W81XWH2010556: Preventing Blood Cancers and Other Malignancies in Military Personnel at Risk Due to Occupational Radiation Exposure

PI: Stephen Kron, U. of Chicago, IL

Budget: $1,608,718.00

Topic Area: Blood Cancers

Mechanism: FY19 PRCRP Impact Award

Research Area(s): 0200, Genetics and Molecular Biology, 0600, Primary Prevention, 0800 Clinical and Experimental Therapeutics

Award Status: 01-JUL-2020 to 30-JUN-2023

Study Goals:

The primary objective of this study is to evaluate whether increasing protein O-GlcNAcylation can protect and/or mitigate late effects of radiation including bone marrow failure and myeloid neoplasms, using mouse bone marrow culture and wildtype and leukemia-prone mice treated with radiation as models. By validating N-acetyl glucosamine as a radioprotector and radiation countermeasure, we will be poised to translate these findings to protect military and other personnel from the acute and delayed effects of accidental, occupational or military radiation exposures.

Specific Aims:

Aim 1 Examine impacts of hexosamine biosynthetic pathway modulation on repair of double strand breaks, survival, proliferation, senescence and differentiation in bone marrow cultures.
Aim 2 Examine effects of modulation of the hexosamine biosynthetic pathway on repair of double strand breaks and bone marrow integrity and function after total body irradiation.
Aim 3 Examine whether modulation of the hexosamine biosynthetic pathway can alter kinetics of bone marrow failure and myeloid neoplasms in Cux1 knockdown mice.

Key Accomplishments and Outcomes:

Publications:

Krishnan *et al*. "Genomic studies controvert the existence of the CUX1 p75 isoform", *Sci Rep,* 12:151, 2022

Lutze *et al*. "Global epigenetic analysis reveals H3K27 methylation as a mediator of double strand break repair", *bioRxiv*, 2021

Patents: none to date

Funding Obtained: none to date