NEBRASKA

Good Life. Great Mission.

DEPT. OF HEALTH AND HUMAN SERVICES

Pete Ricketts, Governor

December 31, 2022

Mr. Patrick O'Donnell
Clerk of the Legislature
State Capitol Room 2018
Lincoln, NE 68509

Dear Mr. O'Donnell:

In accordance with Neb. Rev. Stat. § 81-638(3)(b), please find attached copies of two (2) reports provided to the Department reporting on activities related to the cancer and smoking disease research program. The Department of Health and Human Services holds contracts with Creighton University and the University of Nebraska Medical Center Fred & Pamela Buffet Cancer Center to conduct research in cancer and allied diseases. The reports provide an account of the activities completed under these contracts by Creighton University and the University of Nebraska Medical Center Fred & Pamela Buffet Cancer Center.

Sincerely,

Gary J. Anthone, MD

Gary J. Anthone, M.D.
Chief Medical Officer
Director, Division of Public Health
Department of Health and Human Services

Creighton UNIVERSITY
Research and Scholarship

September 27, 2022

Monica Pribil, MA
Program Manager
Nebraska Department of Health and Human Services
Division of Public Health
Cancer and Smoking Disease Research Program
301 Centennial Mall South
PO Box 94817
Lincoln, NE 68509-4817

Dear Ms. Pribil:

Enclosed please find the Creighton University Cancer and Smoking Disease Research Program Annual Progress Report for FY22. This has been a successful year for the program, and we are excited to share our progress with you.

We appreciate your assistance with the LB595 program and look forward to our continuing collaboration to address the important health concerns of Nebraska's citizens through Creighton's research efforts. Feel free to contact me or Beth Herr at (402) 280-5769 if you need additional information.

Sincerely yours,

DocuSigned by:
E5030E4C6FA54A9...

Juliane Strauss-Soukup, PhD
Principal Investigator
Creighton University
Cancer and Smoking Disease Research Program

Juliane Strauss-Soukup, PhD *Associate Vice Provost*
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Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

INTRODUCTION AND SUMMARY
Juliane K. Strauss-Soukup, PhD, Principal Investigator

Creighton University is pleased to submit this annual report to the State of Nebraska regarding the activities and advancement of its Cancer and Smoking Disease Research Program, funded by the State of Nebraska Cancer and Smoking Disease Research Program (LB595). This progress report provides details on the Administration and Planning Program, Development Program, and the continuing major research programs (Cellular Signaling and Molecular Trafficking in Cancer, Lynch Comprehensive Cancer Research Center, and Biorepository Infrastructure).

As documented in the program reports, the Cancer and Smoking Disease Research Program 2021-2022 has been productive for the investigators at Creighton University. Manuscripts were published in such journals as *PLoS One*, *Cell Reports*, *The Journal of Allergy and Clinical Immunology*, *ACS Applied Bio Materials*, and *European Journal of Pharmacology*.

Creighton University's Cancer and Smoking Disease Research Program has been extremely effective at leveraging the State of Nebraska's support into extramural funding over the past 27 years. The program has served as means to develop and expand important research projects. This support has provided Creighton the resources to develop investigators who then seek funding from other sources, such as the National Institutes of Health. During this period, the State has contributed $37,890,340 to Creighton University through LB595. This, coupled with Creighton's contribution of $16,861,201 through unrecovered indirect costs and $41,962,734 in internal seed grant funding, has led to $167,562,546 of extramural funding brought into Creighton University and the State of Nebraska. The return on the State of Nebraska's investment has therefore been exemplary, with each dollar of LB595 leading to nearly $4.4 in extramural funding for Creighton University. This return on the investment clearly demonstrates the effectiveness of Creighton faculty in leveraging the LB595 support.

Meeting and member details for the Executive, Internal Advisory, and External Advisory Committees are included in the Administration and Planning Program Progress Report. The Publications included in the program reports represent all those germane to the respective programs.

Total awards received by LB595 participants from inception of program (July 1, 1994 - June 30, 2022)

Participants	External Awards	Other Internal Awards				LB 595	Unrecovered Indirects on LB595	Total
		HFF	LB692	Haddix President's Award	Health Science Strategic Investment Fund/CURAS			
Adrian, Thomas	1,516,191					1,892,953	842,364	4,251,508
Abel, Peter	446,261		217,799			379,239	168,761	1,212,060
Arouni, Amy	365,824	19,385	75,000			119,999	53,400	633,608
Bagchi, Debasis	326,833	10,000				18,580	8,268	363,681
Bagchi, Manashi	5,000					175,942	78,294	259,236
Bergren, Dale	94,917				2,000	93,336	41,535	231,788
Bockman, Charles	120,944	30,000			8,978	80,000	35,600	275,522
Brauer, Philip	1,035,556	-				79,088	35,194	1,149,838
Brumback, Roger		410,758	534,363			330,500	147,073	1,422,694
Casale, Thomas	11,866,522	1,897,347	90,000			420,000	186,900	14,460,769
Chakkalakal, Dennis	43,600	9,921	33,251			80,000	35,600	202,372
Chen, Xian-Ming	7,509,421	390,827	614,256			1,030,000	458,350	10,002,854
Cornell, David						120,000	53,400	173,400
Cote, John						150,000	66,750	216,750
Cullen, Diane	3,459,396	351,552	75,000			1,450,873	645,638	5,982,459
Dash, Alekha	476,582		99,036	10,000	800	15,591	6,938	608,947
Deng, Hong-Wen	2,507,316	35,069	923,693			438,806	195,269	4,100,153
Dewan, Naresh	184,639					20,000	8,900	213,539
Dey, Bhakta	509,025	20,000	285,000			40,000	17,800	871,825
Dravid, Shashank	6,227,773	221,206	397,346	30,000	50,000	120,000	53,400	7,099,725
Drescher, Kristen	4,333,606	316,000	1,805,367	5,000		666,985	296,808	7,423,766
Edwards, John	43,294	316,647				19,953	8,879	388,773
Enarson, Cam	12,637,502	9,062,817	405,075			863,292	384,165	23,352,851
Farias-Eisner, Robin						591,449	263,195	854,644
Filipi, Charles	1,044,750	81,634				19,625	8,733	1,154,742
Foster, Jason		233,579				335,000	149,075	717,654
Fu, Yusi						60,000	26,700	86,700
Gatalica, Zoran						61,147	27,210	88,357
Gentry-Nielsen, Martha	721,421	5,100				80,000	35,600	842,121
Govindarajan, Venkatesh	1,887,395	40,000	319,798	15,000		642,622	285,967	3,190,782
Hagenkord, Jill		20,000	100,000			75,000	33,375	228,375
Hansen, Laura	5,115,630	79,897	1,311,100		15,000	2,030,000	903,350	9,454,977
Harrison, Christopher	738,723	16,485				61,977	27,580	844,765
Haynatzki, Gleb	85,741					107,135	47,675	240,551
Heaney, Robert	9,202,964	1,343,251	50,212			185,112	82,375	10,863,914
Hinder, Ronald						19,859	8,837	28,696
Hodgson, Clague	543,300					522,902	232,691	1,298,893
Hogenmiller, Jette						7,117	3,167	10,284
Jadhav, Gopal	414,770					150,000	66,750	631,520
Johnson, Mark		15,000				30,000	13,350	58,350
Khan, Manzoor	352,400					39,970	17,787	410,157
Knezetic, Joseph	76,000	395,100	1,253,148			761,420	338,832	2,824,500
Lefkowitz, David	108,271					20,000	8,900	137,171
Loggie, Brian			40,000			300,000	133,500	473,500
Lovas, Sandor	1,777,234	309,822	191,625			488,961	217,588	2,985,230
Lynch, Henry	18,057,746		100,000			5,937,344	2,642,118	26,737,208
Mackin, Robert	1,433,955	42,800			50,000	235,898	104,975	1,867,628
Mailliard, James	994,796					20,000	8,900	1,023,696
Mansky, Louis	92,176	10,000				108,182	48,141	258,499
Mohiuddin, Syed	4,109,847	3,584,120	2,126,460			241,531	107,481	10,169,439
Murphy, Richard	2,157,652	39,963				175,919	78,284	2,451,818
Murray, Thomas	5,126,706	32,811	682,941			2,898,708	1,289,925	10,031,091
Nairn, Roderick		1,087,647	116,450			551,432	245,387	2,000,916
Nawaz, Zafar	1,300,238		200,000			157,378	70,033	1,727,649
North, Brian	946,379		300,000		50,000	240,000	106,800	1,643,179
O'Brien, Richard	22,000	40,000				617,342	274,717	954,059
Oldenburg, Peter	714,028		60,935			450,000	200,250	1,425,213
Pisarri, Thomas	268,830	10,000				211,356	94,053	584,239
Recker, Robert	32,352,309	1,746,646	10,500			3,175,457	1,413,078	38,697,990
Roche, Victoria	59,215					19,435	8,649	87,299
Smith, Derek	525,589			5,000	10,000	775,201	344,964	1,660,754
Strauss-Soukup, Juliane	800,051		120,056		5,000	296,551	131,965	1,353,623
Stessman, Holly	966,822		523,492			1,176,848	523,697	3,190,859
Swanson, Patrick	5,973,485	237,481	1,575,171	15,000	50,000	1,280,000	569,600	9,700,737
Ternent, John						14,650	6,519	21,169
Terry, John		10,000				15,000	6,675	31,675
Townley, Robert	6,292,741	1,035,607				19,845	8,831	7,357,024
Tu, Yaping	5,819,062	20,000	218,538		50,000	2,020,000	898,900	9,026,500
Vanderhoof, Jon						19,170	8,531	27,701
Vollberg, Thomas	160,000					150,911	67,155	378,066
Wang, Zhaoyi	2,927,212	20,000	500,000			1,270,000	565,150	5,282,362
Watson, Patrice	303,561					44,058	19,606	367,225
Xiao, Gary	133,279		2,072,180			158,017	70,318	2,433,794
Xiao, Peng	64,575		473,719			213,000	94,785	846,079
Yan, Lin	146,896	34,595				66,568	29,623	277,682
Yee, John	34,595	10,000	96,378			16,106	7,167	164,246
Yilmazer-Hanke, Deniz						120,000	53,400	173,400
Totals	**$167,562,546**	**$23,593,067**	**$17,997,889**	**$80,000**	**$291,778**	**$37,890,340**	**$16,861,201**	**$264,276,821**

Creighton University Cancer & Smoking Disease Research Program FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

ADMINISTRATION AND PLANNING PROGRAM
Juliane K. Strauss-Soukup, PhD, Principal Investigator

Juliane K. Strauss-Soukup, PhD, Associate Vice Provost for Research and Scholarship, serves as the Principal Investigator (PI) of Creighton University's Cancer and Smoking Disease Research Program. Dr. Strauss-Soukup became the PI for the LB595 program at Creighton University on November 16, 2020. She has overall authority and responsibility for the direction and oversight of the program. Dr. Strauss-Soukup seeks and responds to input from the Executive, Internal Advisory, and External Advisory Committees, as well as from the Financial and Compliance Administrator. She ensures that the emphasis at Creighton University continues to be on the development of strong research programs that specialize in particular aspects of cancer and smoking diseases. Dr. Strauss-Soukup provides leadership for planning, implementing, and evaluating such programmatic development and communicates with the State of Nebraska and the appointed external reviewers.

Dr. Strauss-Soukup leads the Administration and Planning Program and the Development Program and provides oversight of the three Research Program projects. She receives guidance and input from the Executive, External, and Internal Advisory Committees. Beth Herr, Director of Sponsored Programs Administration, provides financial and compliance guidance for the Cancer and Smoking Disease Research Program at Creighton University.

Rev. Daniel Hendrickson, SJ
President

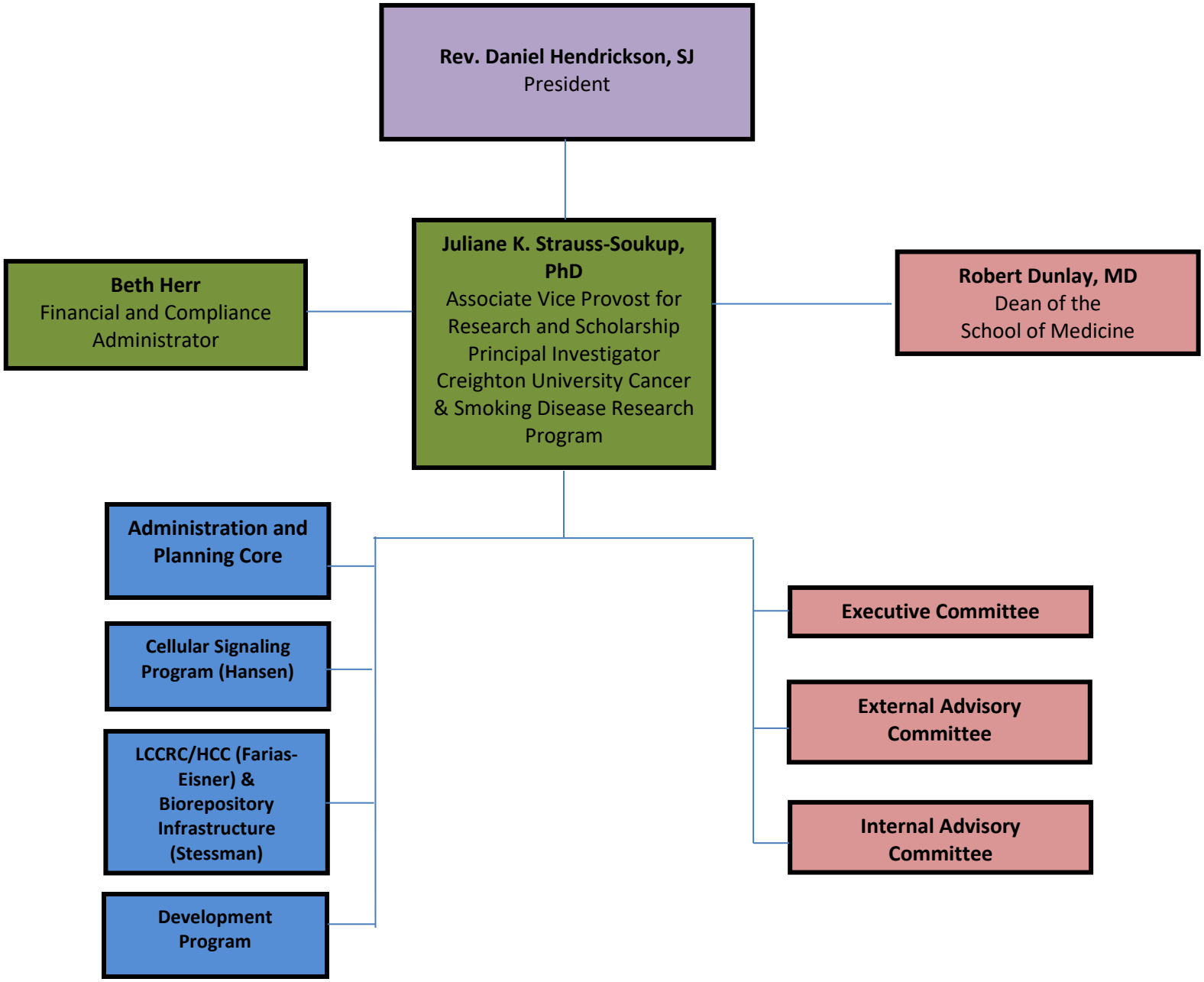
Juliane K. Strauss-Soukup, PhD
Associate Vice Provost for Research and Scholarship
Principal Investigator, Cancer and Smoking Disease Research Program

Beth Herr
Director, Sponsored Programs Administration; Financial and Compliance Administrator, Cancer and Smoking Disease Research Program

1. Cancer and Smoking Disease Research Program Administrative Structure

See the charts to the right and on the following page.

Creighton University Cancer and Smoking Disease Research Program Administrative Structure



The Executive Committee is responsible for overseeing and monitoring the Cancer and Smoking Disease Research Program at Creighton University. It receives all reports from the External Advisory Committee, minutes from all Internal Advisory Committee meetings, reports from the Program Directors, and updates on Development activities. The committee meets on an as-needed basis to assist the Principal Investigator with administrative decisions and to make recommendations regarding programmatic, financial, and compliance issues.

EXECUTIVE COMMITTEE

Juliane K. Strauss-Soukup, PhD
Associate Vice Provost for
Research and Scholarship
Principal Investigator, Cancer and Smoking
Disease Research Program

Robert Dunlay, MD
Dean, School of Medicine

Beth Herr
Director, Sponsored Programs
Administration
Financial and Compliance Administrator

2. Internal Advisory Committee

The Internal Advisory Committee reviews all program updates, as well as all committee and state reports. This committee assists with the implementation of recommendations from the State of Nebraska and the External Advisory Committee.

Members of the Internal Advisory Committee for this year are as follows:

- Richard Goering, PhD (Chair), Professor and Chair, Department of Medical Microbiology & Immunology, Creighton University School of Medicine
- Anthony Kincaid, PhD (Vice Chair), Professor of Pharmacy Sciences, Creighton University School of Pharmacy and Health Professions
- Juliane K. Strauss-Soukup, PhD, Associate Vice Provost for Research and Scholarship; Professor, Department of Chemistry, Creighton University College of Arts and Sciences

Ex officio members of the Internal Advisory Committee are:

- Beth Herr, Director, Sponsored Programs Administration
- Laura Hansen, PhD, Professor of Biomedical Sciences, Creighton University School of Medicine
- Robin Farias-Eisner, MD, PhD, Director, Hereditary Cancer Center, Professor and Chair, Obstetrics & Gynecology, Creighton University School of Medicine
- Holly Stessman, PhD, Assistant Professor of Pharmacology, Creighton University School of Medicine

3. External Advisory Committee

The External Advisory Committee assists the Principal Investigator with the annual on-site review of the Cancer and Smoking Disease Research Program at Creighton University and with review of applications for the Development Program. Dr. James P. Calvet is chair of the External Advisory Committee and participates in the State of Nebraska site visit. Additionally, Dr. Calvet provides guidance on an as-needed basis. The committee ensures the implementation of the State of the Nebraska

recommendations. The on-site review for the Cancer and Smoking Disease Research Program year 2021-2022 took place virtually on Zoom on August 29, 2022. See the External Advisory Committee report on the following page.

Donald Miller, MD, PhD: University of Louisville School of Medicine and James Graham Brown Cancer Center, notified us that he was retiring from the committee after the 2021 meeting. Several candidates to replace him were identified and two new members joined during the 2021-2022 year, as detail below.

Members of the External Advisory Committee are as follows:

- James P. Calvet, PhD (Chair): University of Kansas Medical Center
- Christine Eichen, PhD, Thomas Jefferson University
- Stephen Hecht, PhD, University of Minnesota
- Ralf Krahe, PhD: University of Texas MD Anderson Cancer Center
- Reynold Panettieri, Jr., MD: Rutgers, The State University of New Jersey

4. Lynch Comprehensive Cancer Research Center Reorganization

Dr. Robin Farias-Eisner, the former director of the Lynch Comprehensive Cancer Research Center (LCCRC) accepted a position at another institution in March 2022. As a result, the leadership of the LCCRC has changed.

The LCCRC is being reorganized with the goal of becoming a Center for Biomedical Research Excellence in the next two to three years. Dr. Lesley Conrad, a recently recruited gynecologic oncologist, and Dr. Laura Hansen co-chair the LCCRC oversight committee. Two talented basic scientists, Jun Xia and Yusi Fu, have recently joined Creighton University's faculty. Dr. Xia's research is focused on damageomes and Dr. Fu is an expert in genetic sequencing. We will soon announce the appointment of a new chair in surgery, a surgical oncologist from Georgetown University. He has approval from CommonSpirit to hire two additional surgical oncologists over the next three years. The Department of Medicine is currently recruiting a chief of hematology/oncology and has approval for three new faculty positions in the division over the next three years. The growth in oncology research will continue to be one of the School of Medicine's top strategic priorities.

5. Seminars

During the 2021-2022 year, support was again used to continue a seminar series focused on cancer and smoking-related diseases. This program was directed by Dr. Laura Hansen. Financial support was used to bring in speakers with outstanding research expertise in the area of cancer and smoking-related diseases to give a seminar at Creighton University. Scientists from premier institutions who are leaders in their fields were invited to present their cutting-edge research. The seminar series provides opportunities for CU faculty and trainees to meet the speakers, discuss their research, and establish or strengthen collaborations, which will enrich the research

environment at CU by facilitating interactions between CU SOM research faculty members and other scientists around the country and stimulate the progress of research projects supported by the LB595 program.

Following are the speaker and seminar topics for the 2021-2022 year:

- Rui Yi, PhD, Paul E. Steiner Research Professor, Feinberg School of Medicine, Northwestern University
  Seminar Topic: Specification, Maintenance and Aging of Hair Follicle Stem Cells
- 2022 Creighton University Cancer Research Symposium, Focus on Genomics and Sequencing
  - Angela Ruohao Wu, PhD, The Hong Kong University of Science and Technology
    Seminar Topic: scONE-seq: A One-Tube Single-Cell Multi-Omics Method Enables Dissection of Frozen Glioblastoma Heterogeneity
  - Ludovic Deriano, PhD, Institut Pasteur
    Seminar Topic: Origin of Somatic Mutations in Lymphoid Cancer: Role of V(D)J Recombinase
  - John Pierce. Wise, Sr., PhD, University of Louisville School of Medicine
    Seminar Topic: Mechanisms of Metal-Induced Chromosome Instability
  - Wenyi Wei, PhD, Beth Israel Deaconess Medical Center
    Seminar Topic: Targeting the Ubiquitin Pathways for Cancer Therapies
  - Gregg B. Fields, PhD, FNAI, Florida Atlantic University
    Seminar Topic: Application of a Novel Matrix Metalloproteinase Inhibitors in Cancer
  - Thomas W. Glover, PhD, FACMG, University of Michigan Medical School
    Seminar Topic: Mechanisms of Genomic Copy Number Alterations
  - Timothy Fleming, PhD, Norton Thoracic Institute, St. Joseph Hospital
    Seminar Topic: Targeted Therapy for Esophageal Cancer
  - Narenda Sandpal, PhD, Norton Thoracic Institute, St. Joseph Hospital
    Seminar Topic: Role of Membrane Proteins in Tumor Cell Growth and Metastasis

6. Cancer Journal Access at Library

During the 2021-2022 year, funds were used to provide access to the electronic full-text content of relevant cancer research journals. These journals include titles such as the *Journal of the National Cancer Institute* and *Current Opinion in Oncology.* Usage statistics continue to rise as more investigators access the electronic content of these journals.

EXTERNAL ADVISORY COMMITTEE REPORT
Cancer and Smoking Disease Research Program – LB595
Site Visit: Monday, August 29, 2022

External Advisory Committee: Dr. James Calvet, Ph.D. (Chair), University of Kansas Medical Center; Dr. Ralf Krahe, Ph.D., University of Texas MD Anderson Cancer Center; Dr. Reynold Panettieri, M.D., Rutgers, The State University of New Jersey. Dr. Christine Eischen, Ph.D., Thomas Jefferson University (added to the committee September 2021); Dr. Stephen Hecht, Ph.D., University of Minnesota (added to the committee March 2022). All attended the site visit by Zoom video conferencing.

Also attending the meeting were Julie Strauss-Soukup, Beth Herr, Barbara Bittner, and Karla Malesker, all of Creighton University.

Dr. Calvet announced that he will step off the committee this year, after serving for 15 years. It is recommended that at least one additional member and preferably two be appointed to bring the committee to full strength.

ADMINISTRATION & PROGRAM PLANNING – Dr. Juliane Strauss-Soukup

Dr. Juliane Strauss-Soukup, Ph.D. (Professor of Chemistry & Biochemistry, Associate Vice Provost for Research and Scholarship) became PI of the Creighton University LB595 Cancer and Smoking Disease Research Program in November 2020. She is well qualified to manage multi-investigator programs. The administrative aspects of the program are in very capable hands under her leadership, together with Beth Herr, SPA Director, who provides strong administrative support. Dr. Strauss-Soukup's emphasis on undergraduate education and building bridges across Creighton University adds an important new dimension to the LB595 program. She appears to be committed to the quality and success of the overall LB595 program and the mentorship of the individual PIs.

Dr. Strauss-Soukup reported that the research environment at Creighton continues to be outstanding and has excellent institutional support. Importantly, there is a desire to recruit cancer-related investigators and faculty at Creighton. Creighton's funding is improving with increasing NIH support over the last two years. The INBRE program has been refunded and is an important asset. This was another successful year scientifically for the LB595 program. All of the programs, including the infrastructure core, five individual projects and four development projects, presented significant progress. There were no major weaknesses or specific areas of major concern. Creighton continues to leverage LB595 into a successful seed program with an impressive overall return on investment. In FY21/22, seven of the LB595 investigators were awarded a total of 21 grants, including 11 NIH grants, for a total of $1,817,835. Brian North should be commended for his new R01.

To build on recent successes and to accelerate growth, it is suggested that the administration consider a webinar about the LB595 Development Program to advertise the program internally and increase grant submissions. To increase the awareness of the LB595 program across the Creighton community, the leaders may also want to consider promoting the program more directly with department chairs, as a means for the recruitment of new faculty and a mechanism to solicit high-risk/high-impact projects for which it would be difficult to obtain funding by more

established mechanisms or agencies. To increase program relevance and fit, a one-page LOI pre-review could be beneficial to assess competitiveness and amplify impact.

While seminars were on hold during the 2020-2021 year because of the COVID-19 pandemic, LB595 support was again used during the 2021-2022 year for a series of seminars, directed by Dr. Laura Hansen, focused on cancer and smoking-related diseases. This program included 8 seminars given by external speakers in the 2022 Creighton University Cancer Research Symposium, Focus on Genomics and Sequencing.

One area that should be noted was that the publication productivity of the LB595 investigators seemed somewhat modest this year, with unclear reasons as to why. Although they are still making progress, publishing appears to have slowed a bit. Only 5 publications originating from the LB595 program were reported (Swanson – *Cell Reports* and *PLoS One*; Tu – *European Journal of Pharmacology* and *ACS Applied Biomaterials*; Tu & Abel – *Journal of Allergy and Clinical Immunology*). Additionally, it will be important in the future to clearly define LB595 activities (publications, grant submissions, grant awards) as distinguished from other scientific interests of the LB595 investigators. It might also be helpful in the future to also count submitted manuscripts to get a better idea of ongoing publication activity.

Another area of concern is the Lynch Comprehensive Cancer Research Center (LCCRC). The recent departure of Dr. Farias-Eisner as Director of the LCCRC after only two years represents a major challenge. Because of this recent change, there was no report presented about the LCCRC. The committee was informed that the LCCRC will now have two co-Directors, Dr. Laura Hansen, a Ph.D. basic cancer scientist and Dr. Lesley Conrad, an M.D. obstetrician-gynecologist. Dr. Hansen is a permanent co-Director and Dr. Conrad is interim. Dr. Robert Dunlay, M.D, Dean of the School of Medicine provided the following information in a written statement: “The Lynch Comprehensive Cancer Research Center (LCCRC) is being reorganized with a goal of becoming a Center for Biomedical Research Excellence [COBRE] in the next two-three years. Dr. Conrad, a recently recruited gynecologic oncologist, along with Dr. Laura Hansen, [together] co-chair the oversight committee for the LCCRC.” “Our growth in oncology research will continue to be one of the top strategic priorities for the medical school in the years to come.” The Center’s plans will need clarification over the next year. Careful attention and focus by the co-Directors moving forward will be needed to define how the LCCRC and LB595 can mutually benefit each other, and to develop long-term plans to sustain the LCCRC to ensure its success. It is understood that the LCCRC LB595 funds originally awarded to Dr. Farias-Eisner will support the collaborative gynecologic oncology research projects of Drs. Fu, Conrad, and Stessman, and LB595 funds will continue to support the LCCRC Biorepository. This will need to be clarified.

BIOREPOSITORY INFRASTRUCTURE – Dr. Holly Stessman

As in the previous year, Dr. Stessman has continued to make significant progress with the conversion, update and restructuring of the existing LCCRC database and specimen collection and tracking system. The audit is focusing on the identification, organization and cataloguing of viable samples for future research as well as transition from paper records to a computerized database. The goals have largely remained unchanged. The previously noted IRB issues appear to have been resolved, resulting in retainment of ~80% of high value samples from the “legacy collection.” Under the leadership of a new IRB director, patient consents have been manually audited. Plans to re-consent existing patients with a new IRB protocol are on hold until

a plan can be developed and either a new PI is recruited to replace Dr. Farias-Eisner, or the co-Director model is permanently implemented.

It did not appear that there was a well thought out plan to use the biorepository internally and to market it to other institutions. Moving forward in the meantime, it may be worthwhile to consider reaching out to the NCI for guidance and support to market the unique resources of the LCCRC to other NCI-supported academic institutions. This biorepository is an amazing resource that should be used both within and outside of Creighton. The improvements, including the transition to computer records, are great and the Biorepository is a unique, valuable resource for Creighton. CreightonPORT (Participant Outreach Research Tool) has now been implemented as a digital tool for greater outreach to external investigators, but it is not known yet how it will be adopted and used by participants. A detailed business model would be helpful and would address sustainability. In response to questions, discussion elucidated that some attempts to EMR linkage is in progress. This is important to decrease transcription errors, etc. Newly acquired equipment and implemented technologies were briefly summarized and discussed. Plans for the future include increased cost sharing to offset expenses for laboratory staff by 20%. Also, it was unclear how much control the LB595 versus the LCCRC has on the Biorepository. Holly Stessman's contributions to modernizing the Biorepository have been exceedingly valuable and her continued involvement will be critical to its success. She has made extraordinary progress on a difficult project, but we continue to have concerns about the toll that this might have on her personal career. As such, it will continue to be important for her to be given adequate career support to ensure her success moving forward and to see that she is not spread too thin.

CELLULAR SIGNALING & MOLECULAR TRAFFICKING IN CANCER – Dr. Laura Hansen

The overall program under the leadership of Dr. Hansen consists of five projects. The program is well integrated and continues to be solid. All projects appear to be making good progress and there appear to be excellent inter-programmatic interactions among the investigators. Dr. Hanson provides strong leadership for the program.

Checkpoint Signaling and Cell Survival in Normal and Tumorigenic Skin Keratinocytes – Laura Hansen, Ph.D.

Dr. Hansen's project on Flower (FWE) isoforms in skin keratinocytes and squamous cell carcinoma (SSC) cell competition is multifaceted, quite novel, and potentially high impact. The effects of UV irradiation and serum starvation on human skin squamous cell carcinoma cell lines were studied. The project focuses on the roles of FWE protein isoforms that through cell-cell interaction promote a "loser" phenotype, a process leading to the survival of cells with higher fitness. The central hypothesis suggests that FWE isoforms determine the fate and carcinogenesis of SSC. Much of the murine studies have been confirmed in human SSC cell lines and have shown that that FWE overexpression alters S100A isoforms that modulate cell cycle progression. The project appears to be proceeding well but the results are quite complex. While overall progress has been substantial, the exact goals seem to be unclear and somewhat confusing. To study the FWE isoforms in vivo, a Crainbow mouse model for lineage tracing has been developed. Overall, productivity of the PI continues to be good, with an additional NE DHHS LB506 pilot grant awarded, originating from the LB595 project.

Cellular Pathways Targeting BubR1 to the Proteosome for Degradation: Implications for Skin Cancer – Brian North, Ph.D.

Dr. North reviewed the relationship between caloric restriction and longevity focusing on the mitotic checkpoint regulator BubR1, overexpression of which is associated with a 10-20% increase in longevity in mice. Because BubR1 is decreased in a number of cancers, he is investigating the dysregulation of the NAD+/SIRT2/β-TrCP/BubR1 pathway in chemical carcinogen and UV-induced skin cancer. This year's presentation focused on the role of over-the-counter NAD+ boosters, specifically nicotinamide mononucleotide (NMN), which, contrary to expectations, appeared to increase cell proliferation and tumor burden. The fact that NMN had the opposite results than expected for UV-induced skin cancer is interesting and has the possibility to impact patients taking these supplements. Young animals treated with NMN showed an increase in tumor burden, but overall tumor incidence was not affected. Further studies to elucidate the underlying mechanisms relative to the pathway are in progress. Portions of Specific Aim 3 of the grant have recently been funded by an NIH R01.

Localization of RAG1 Degradation and Implication of RAG1 Stabilization on Genome Instability and Cancer – Patrick Swanson, Ph.D.

Dr. Swanson's project explores the role of RAG1 and RAG1 turnover in genome instability with particular focus on aberrant V(D)J rearrangement in lymphoid neoplasia. The first aim is to identify the cellular localization of RAG1 degradation and factors involved. The second aim is to determine if impairing RAG1 turnover increases the frequency of apparent V(D)J rearrangement and lymphoid cell neoplasia. Using mass-spectrometry, RACK1 was identified as a novel RAG1-interacting protein possibly being recruited to the CRL4VprBP(DCAF1) E3 ubiquitin ligase complex by RAG1. RACK1 vs. Hsp90 competition is being investigated as a new model for RAG1 cellular degradation. A postulated model for RAG1 degradation was presented. Loss of RACK1 in a knock-out mouse model results in arrest of B cell development at the pro-B to pre-B transition and can be partially rescued by forced Bcl2 expression. However, the phenotype of RACK1-BKO mice is different than that of VprBP(DCAF1)-BKO mice in a Bcl2+ transgenic background, suggesting different roles for RACK1 and VprBP(DCAF1) in regulating RAG1 activity. Other targets of RACK1, including Bim will be investigated. It is a challenging project that once completed should be impactful for B cell biology and lymphoid malignancies. Overall productivity resulting from the project has been excellent, with 2 published papers (*PLoS One* and *Cell Reports*). He has two funded R21's based on these studies, which were originally supported by LB595 funding. He is a tremendous asset to the LB595 program.

Dysregulated Mitochondrial Dynamics and Cancer Metastasis – Yaping Tu, Ph.D.

Dr. Tu's project explores miR-133a upregulation, which increases Drp1-dependent mitochondrial fragmentation in cancer cells and is correlated with increased migration, invasion, and metastasis, and reduced overall survival in colorectal cancer (CRC). Parkin, a ubiquitin ligase, was identified as a modulator of Drp1 protein levels and a target for miR-133a-mediated repression, leading to enhanced mitochondrial fission and increased cell migration. Targeting miR-133a-dependent Drp1 upregulation with anti-miR133a and inhibitors to suppress CRC metastasis has excellent translational potential. Once again, overall productivity resulting from the project has been excellent, with 4 published papers, 3 grants awarded and 1 R01 submitted (which has received a 20th percentile score). Of note, however, while the mechanism of miR-133a regulation of Parkin was scientifically rigorous, it could have included an investigation of other genes in the same pathway since miRNAs do not typically regulate one mRNA. Two investigators (Drs. Tu and Abel) are studying miRNAs and their target genes. It should be noted

that miRNA studies are less scientifically exciting now unless RNA modifications and how they are regulated are also the focus.

Inhibition of Skin Cancer Growth with Highly Selective and Proteolytically Stable Peptide Analogs – Sandor Lovas, Ph.D.

Due to last year's awarded R01 and to avoid overlap, the focus of the current grant was changed last year from skin cancer to glioblastoma (GBM) brain tumors. GBM is the most common type of primary brain tumor, which is highly invasive with a 5-year survival rate of less than 10%. Breakdown of the extracellular matrix by matrix metalloproteinase (MMP-2) is critical in this disease. Chlorotoxin (CTX) is a 36 amino acid peptide that is known to specifically bind to MMP-2 and has high specificity for glioma and other cancer cells. The rationale of this study is to inhibit cancer growth with highly selective and proteolytically stable peptide analogs (P75 analogs) made from the C-terminal half of CTX. Dr. Lovas provided interesting new data indicating that MMP-2 can potentially serve as a select target for CTX peptides in GBM cells, based in part on molecular dynamics simulations. However, preliminary data on inhibition of GBM cell survival by the P75 analogues showed relatively modest high μM inhibitory activity. Solubility and bioavailability will be major challenges in the creation of this therapeutic approach. The concentrations of the molecules to inhibit GBM survival seem very high and not likely therapeutically achievable. This project has a long way to go and likely needs collaborators. Also, MMP inhibitors have historically failed in the clinic, so the reasoning to target MMP-2 is not strong and needs further justification. Unfortunately, recent experiments in the GBM cell line U-87 could not validate previous results. The PI should consider cell line authentication using ATCC recommended standard procedures (either through ATCC or another academic or commercial fee-for-service provider) to validate the identity of the cell lines used for both past and recent experiments to understand the nature of the discordant results. Overall, Dr. Lovas is an excellent team scientist, and he has generated a lot of data on the project. He is a Co-PI on several recent grant submissions and is a co-author on one published paper.

DEVELOPMENT PROGRAM – Dr. Juliane Strauss-Soukup

LB595 grant proposals require a statement of the project's relevancy to cancer or smoking disease as defined by Neb Rev Statute 81-637: "Cancer means all malignant neoplasm regardless of the tissue of origin, including malignant lymphoma and leukemia. Smoking disease means diseases whose causes are linked to smoking including, but not limited to, cardiovascular, pulmonary, and gastrointestinal diseases." The EAC also recommends that all LB595 investigators, where possible, link their research to diseases caused by smoking per se. The 2014 U.S. Surgeon General's Report entitled "The Health Consequences of Smoking – 50 Years of Progress" lists the following 12 types of cancer causally linked to smoking: oropharynx; larynx; esophagus; trachea, bronchus and lung; acute myeloid leukemia; stomach; liver; pancreas; kidney and ureter; cervix; bladder; and colorectal in addition to 16 other chronic diseases. Where possible, each PI could include a statement as to why their research project relates in some way, either basic or applied, to a type of cancer caused by smoking, or to one of the other diseases caused by smoking.

Development Awards

PI: Dr. Gopal Jadhav, Ph.D., Department of Pharmacology and Neuroscience
Title: Chemical Optimization of Small Chemical Molecules to Probe Triggering Receptors Expressed on Myeloid Cells 1 (TREM1): Novel Treatment for Hepatocellular Carcinoma

Dr. Jadhav provided an update on his efforts to identify small molecule inhibitors targeting triggering receptors expressed on myeloid cell 1 (TREM1) in the management of hepatocellular carcinoma (HCC), inflammatory disorders, and neurodegeneration using a medicinal chemistry approach. There remains a substantial significant unmet need for new therapies in HCC. Fifty-eight GPJ analogues have been synthesized. SPR sensograms of the analogues showed activity. Five underwent further in-depth analysis – 3 as positive binders/TREM1 antagonists and 2 as negative controls. Research on effective binders with sufficient half-lives is continuing. The approach is firmly based on traditional medicinal chemistry principles, although the current data suggest the molecules may not be potent. However, he needs to test the effects of his compounds on biological assays, and a lead compound will need significant optimization to go into in vivo studies. He needs collaborators. What will be the approach to improve bioavailability? One publication is reported as submitted, and he was successful in obtaining a P20 NIGMS pilot grant to investigate the role of TREM1 in neurodegeneration, which is outside of the primary focus of the LB595.

PI: Dr. John Cote, M.D., Department of Obstetrics and Gynecology
Title: Effects of 3D Ultrasonography and 3D Printed Images on Maternal-Fetal Attachment and its Correlation with Overall Smoking Within Pregnancy and Smoking Cessation

This is an interesting and provocative smoking cessation project and trial based on the premise that 3D-ultrasonography plus 3D fetal models vs. 3D-ultrasonography alone will more effectively reduce maternal smoking during pregnancy by increasing maternal-fetal attachment. This was indeed one of the few projects that directly addressed a clearly smoking-related problem. The concept is unique and original, and the project was clearly described. To achieve the desired statistical power, each cohort has a targeted minimum recruitment of n = 40. Although significant progress has been made over the last year in recruitment (n = 23 enrolled; results available for 19 and 4 awaiting results), it is unlikely that the targeted recruitment to achieve statistical power will be achieved during the funding period. Dr. Cote recognizes that recruiting the requisite number of subjects will be challenging considering the current low rate of smoking during pregnancy. As described, he is unlikely to recruit sufficient patients to obtain meaningful results. It is unclear if there is anything that can be done to help him at a program and/or department level. They may need to partner with other groups to achieve this goal. Preliminary analysis of the data suggests potentially provocative results. Therefore, if successful, impact on maternal-fetal health of smoking in expecting mothers could be significant.

New Development Awards

PI: Dr. Peter Abel, Ph.D., Department of Pharmacology and Neuroscience
Title: Identification of miRNA-146b as a Novel Antifibrotic Drug Target for Treatment of Idiopathic Pulmonary Fibrosis

This is a new project that explores the role of miR-146b as a novel antifibrotic drug target in idiopathic pulmonary fibrosis (IPF). The concept of studying molecular mechanisms regulating IPF is important and addresses an unmet need. IPF is strongly associated with cigarette smoking and thus is directly relevant to the LB595 program (the linkage to environmental tobacco smoke (ETS) exposure is somewhat tenuous). Significant progress has been made identifying miR-146b downstream targets and cellular signaling pathways, including EGFR, JUN, TGFBR1 and SMAD3. Repression of this miRNA caused enhanced response to stimulation in human lung fibroblasts from IPF and normal patients. Deletion of this miR caused increased proliferation and differentiation in mouse lung fibroblasts. The project is progressing

well, but is somewhat early, so its impact at this stage is somewhat uncertain to predict. As noted above, miRNAs are less scientifically exciting right now and harder to fund if the project is just to investigate the RNA they are targeting. RNA modifications and how they are regulated has a better chance of obtaining federal funding. Thus, it is a positive that they are investigating the miR-146b promoter and how it is regulated, and that they are planning to design therapies based on increasing miR-146b levels and activity.

PI: Dr. Yusi Fu, Ph.D., Research Assistant Professor, School of Medicine, LCCRC.
Title: Identify the Molecular Signatures of Pre-Cancerous Lesions and Endometrial Cancer with High-Throughput Single-Cell Analysis

This is a new project focused on the identification of molecular signatures of pre-cancerous lesions in endometrial cancer (EC). The PI was recently recruited from Baylor College of Medicine and has already made significant progress. Using cutting-edge novel omics technologies, the project uses single-cell analysis of uterine blood samples to identify transcriptomic changes and genomic mutations to study intra-tumor heterogeneity in EC tumorigenesis. The proposed research is cancer-related and highly significant, as it proposes to use high-throughput single-cell RNA and DNA sequencing to molecularly characterize early endometrial cancer profiles. Successful application of these highly sensitive diagnostic methods should enable a more accurate and less invasive assessment of endometrial cancer risk, which would provide a significant improvement over current approaches. There are two specific aims, one to carry out single-cell transcriptomics and the second to carry out single-cell genomics for endometrial cancer. The PI has extensive experience with both approaches and was instrumental in developing, validating, and publishing new single-cell approaches.

In this pilot proposal, the PI has IRB approval to obtain endometrial aspirates from patients in the CHI hospital system from 4 patients with true-positive endometrial cancer diagnoses and 4 patients with false-positive diagnoses. This is a reasonable number to obtain and to assess as a preliminary study. Cell samples will be prepared and subjected to single-cell analysis to determine their cellular phenotypes based on RNA expression, and the population frequencies of the cellular sub-types. DNA analyses will also be carried out to determine the somatic mutation load and the nature of the mutations. Aim 1 will specifically assess the immune cell population, in part to look for indicators of T-cell exhaustion. This aim will also identify rare cell types, based on transcriptome profiling, that might indicate pre-cancerous or cancerous phenotypes. Aim 2 will look for mutational fingerprints at a single-cell level to identify endometrial cancer drivers for future cancer risk assessment. The patient sample population should be large enough to identify expression profiles characteristic of pre-cancerous and cancerous phenotypes and to assess patient-to-patient and sample-to-sample reproducibility and variability and to determine if recurring mutations are associated with endometrial cancer. This is a new project with excellent promise. Dr. Fu's project is innovative, high impact, and highly fundable by the NCI. This approach could be used for many different cancers, once sufficiently verified. The graduate students and postdocs in the PI's lab will gain important knowledge and training as a part of this program. The molecular phenotyping of endometrial cancer is impressive. Is there any opportunity for securing intellectual property?

Additional New Development Award

Of note: Dr. Michael Belshan's project, "Connections Between SARS-CoV-2 Evolution, Patient Comorbidities, and COVID-19 Outcomes" was terminated because it was not sufficiently aligned with the goals of the LB595 program. As such, in mid-2022, another Development Award was announced. One (of 2 applications) was funded:

PI: Dr. Gajanan Shelkar, Ph.D., Department of Pharmacology and Neuroscience
Title: Glutamate Delta-1 Receptor in Cisplatin-Induced Neuropathic Pain and Anorexia.

There was no report on this project as it has just started. For informational purposes, the following provides a description of the project and the significance.

Description: This project utilizes a multidisciplinary approach including genetic mice, electrophysiology, immunohistochemistry, and confocal imaging to address the specific aims. The aims make use of cisplatin-induced models of pain and anorexia experienced in cancer chemotherapy. The first aim examines the effects of cisplatin on GluD1-Cbln1 signaling and neuroplasticity and will attempt a rescue approach involving injection of recombinant Cbln1 protein in WT and conditional GluD1 receptor KO mice with and without cisplatin treatment. These experiments will test if cisplatin affects glutamatergic mechanisms and whether these effects can be reversed and involve GluD1. The second aim will examine the effects of cisplatin on pain and anorexia and will attempt rescue approaches involving injection of recombinant Cbln1 protein and genetic conditional overexpression of the GluD1 receptor specifically in PKCδ+ neurons. The PI has preliminary data using complete Freund's adjuvant (CFA) and spinal nerve ligation (SNL) pain models. He has also shown injection of Cbln1 reduced mechanical hypersensitivity (pain) and increased body weight (anorexia) in a cisplatin model. Pain will be assessed with the von Frey filament test and the cold allodynia test. Anorexia will be assessed by bodyweight and food consumption.

Significance: Neuropathic pain and anorexia are major side effects of cisplatin treatment in cancer chemotherapy. The proposed studies are designed to identify the role of GluD1-Cbln1 signaling in chemotherapy-induced neuropathic pain and anorexia. Previous studies done in CFA-induced inflammatory pain and SNL-induced neuropathic pain have implicated the GluD1 receptor and Cbln1. However, a mechanistic understanding of the regulation of glutamatergic signaling in cisplatin-induced neuropathic pain and anorexia in PB-CeLC (parabrachial-central laterocapsular amygdala) synapses is lacking. This proposal will test the hypothesis that cisplatin will modulate GluD1-Cbln1 signaling at PB-CeLC synapses and thereby affect synaptic neurotransmission and behaviors. Identifying neural mechanisms regulating cisplatin-induced pain and anorexia should help to generate mechanism-based therapy to alleviate side effects.

Creighton University Cancer & Smoking Disease Research Program FY20/21 Progress Report

(July 1, 2021 – June 30, 2022)

Development Program Progress Report
Juliane K. Strauss-Soukup, PhD, Principal Investigator

The following investigators have completed the first year of their Development projects. This is the first report for the two-year Development projects that were awarded in 2021-2022:

PI: Peter Abel, PhD, Department of Pharmacology and Neuroscience
Title: Identification of miRNA-146b as a Novel Antifibrotic Drug Target for Treatment of Idiopathic Pulmonary Fibrosis

PI: Yusi Fu, PhD, Department of Obstetrics and Gynecology
Title: Identify the Molecular Signatures of Pre-Cancerous Lesions and Endometrial Cancer with High-Throughput Single-Cell Analysis

These are the year two reports for the Development projects that were awarded in 2020-2021.

PI: John Coté, MD, FACOG, Department of Obstetrics and Gynecology
Title: Effects of 3D Ultrasonography and 3D-Printed Images on Maternal-Fetal Attachment and Its Correlation with Overall Smoking within Pregnancy and Smoking Cessation

PI: Gopal Jadhav, PhD, Department of Pharmacology and Neuroscience
Title: Chemical Optimization of Small Chemical Molecules to Probe Triggering Receptors Expressed on Myeloid Cells 1 (TREM1): Novel Treatment for Hepato Cellular Carcinoma

The full reports follow this page.

Creighton University Cancer & Smoking Disease Research Program FY21/22 Progress Report (July 1, 2021 – June 30, 2022)

Development Program
Program Director: Juliane Strauss-Soukup, PhD

MiR-146b Repression and Pulmonary Fibrosis
Principal Investigator: Peter W. Abel, PhD

I. Progress Report Summary

A. Specific Aims

- Aim 1: To define the mechanisms of miR-146b regulation of pulmonary fibrosis progression.
- Aim 2: To determine the pathological importance of miR-146b repression in pulmonary fibrosis**.**

B. Studies and Results

Pulmonary fibrosis (PF) is a progressive interstitial lung disease characterized by lung scarring that causes irreversible loss of O_2/CO_2 exchange capacity. Most common is idiopathic pulmonary fibrosis (IPF), a fatal lung disease with more than 40,000 new cases each year in the USA. Cigarette smoke is the most strongly associated risk factor for IPF. Current smokers develop IPF at a younger age compared to non-smokers and ex-smokers, and IPF patients with a smoking history have a shorter survival than non-smokers. There is no *cure* for IPF. Nintedanib and pirfenidone have been approved to slow disease progression but the median *survival* time remains at only 2-3 years from diagnosis. Thus, a vast unmet treatment need exists for patients with IPF.

Though the cause of IPF is largely unknown, increases in profibrotic mediators, such as transforming growth factor (TGF) α and β1 due to lung injury, play a central role in IPF progression. TGFα binds epidermal growth factor receptors (EGFR) to stimulate fibroblast proliferation and TGFβ1 stimulates TGFβR to induce fibroblast differentiation into myofibroblasts that produce excessive extracellular matrix, resulting in lung remodeling and function deterioration. Although EGFR/TGFβR targeted therapies have been explored, a major hurdle is *in vivo* stability and tissue specificity of these agents. This proposal thus focuses on newly identified regulatory mechanisms of fibroblast proliferation and differentiation. MicroRNAs (miRNAs) are important post-transcriptional gene expression regulators that bind to the 3'-UTR of target mRNAs, leading to mRNA degradation or translational repression. Several miRNAs are dysregulated in IPF, but the mechanisms and pathological importance remain largely unknown. We recently identified miR-146b as a key anti-fibrotic factor that regulates EGFR/TGFβR-dependent fibroblast proliferation and differentiation, but its expression was markedly reduced in lung fibroblasts from IPF patients or mice with experimental pulmonary fibrosis. We propose to further utilize clinically relevant *ex vivo* and *in vivo* models and human lung fibroblasts (HLF) from IPF patients to define functions, mechanisms, and importance of miR-146b in IPF progression.

During the past year, we focused our efforts on the mechanisms of miR-146b regulation of pulmonary fibrosis progression. We also investigated the molecular mechanism underlying fibroblast miR-146b repression. The data we obtained form the basis for a new R01 application submission planned for October.

1. Repression of miR-146b and enhanced response to stimulation in HLF from IPF patients. Primary HLF (3-7 passages) from Drs. Moore and Huang labs were derived from lung tissues obtained from explanted lungs of IPF patients or from histologically normal lung regions of non-fibrotic patients. The protocol for cell isolation was approved by the University of Michigan IRB. IPF-derived HLF-F (n=5) had 2.7-fold higher collagen COL1A (**Fig.1a**) but 40-50% lower miR-146b expression than non-fibrotic HLF-N (n=6) with no significant difference in miR-218 expression (**Fig.1b**). Compared to non-fibrotic HLF-N, IPF-derived HLF-F expressed exhibited higher basal α-SMA protein and more robust response to TGFβ1 stimulation (**Fig.2a**) and greater cell proliferation (**Fig.2b**).

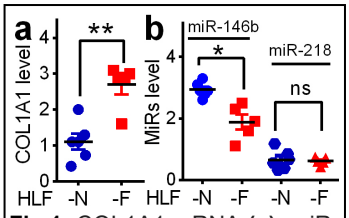


Fig.1: COL1A1 mRNA **(a)**, miR-146b and -218 **(b)** levels in HLF-N & HLF-F (*p<0.05; **p<0.01).

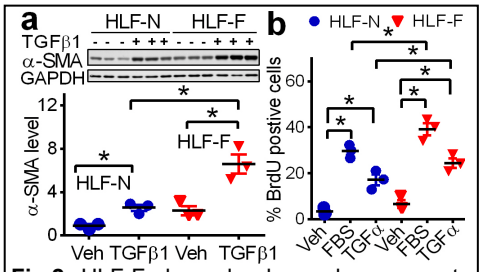


Fig.2: HLF-F showed enhanced response to stimulation. HLF-N and HLF-F (3 pairs) were treated with TGFβ1 (1ng/ml), TGFα (10ng/ml), FBS (2%) or vehicle for 48 h. **(a)** Western blot and quantification of α-SMA levels. **(b)** BrdU incorporation assays. *p<0.05.

2. miR-146b is highly expressed in mouse lung fibroblasts and deletion of miR-146b gene increased proliferation and differentiation. Expression of miR-146b is highest in lung compared to other organs [1]. We found that miR-146b expression in mouse lung fibroblasts is 10-fold higher than in mouse airway epithelial and smooth muscle cells (**Fig.3a**). Lung fibroblasts from miR-146b knockout (KO) mice showed more robust response to FBS, TGFα, and β1 (**3b, 3c**).

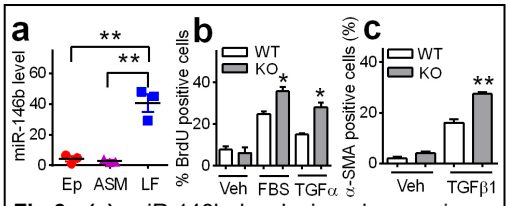


Fig.3: **(a)** miR-146b levels in primary airway epithelial (Ep), smooth muscle (ASM) and lung fibroblast (LF) cells from 3 mice **[30,44,45]**. LF from WT and miR-146b KO mice were treated with 2% FBS, TGFα (10ng/ml) or TGFβ1 (1 ng /ml). **(b)** BrdU-positive cells. **(c)** α-SMA-positive cells. Data are means ± SE, *p<0.05; **p<0.01.

3. MiR-146b targets EGFR-proliferative signaling pathways. TGFα binds the EGFR to stimulate fibroblast proliferation. It was reported that EGFR activation induces cyclin D1 by activation of the c-Jun transcription factor, leading to cell proliferation. We found that miR-146b blocks TGFα-induced c-Jun phosphorylation (**Fig.4a**) and cyclin D1 expression (**4b**) in HLF-F. EGFR and c-Jun proteins were also reduced (**4a**), suggesting that miR-146b can inhibit HLF proliferation by targeting EGFR/c-Jun signaling.

EGFR activates multiple signaling pathways, including the MEK/ERK and PI-3-kinase (PI3K)/AKT pathways. Treatment of HLF-F cells with TGFα induced a concentration-dependent phosphorylation (activation) of both ERK1/2 and AKT that was significantly reduced by miR-146b (**4c**).

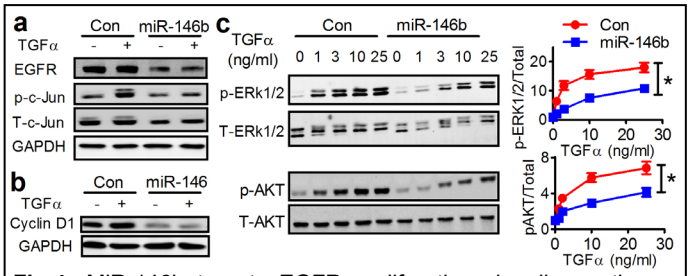


Fig.4: MiR-146b targets EGFR proliferative signaling pathway. HLF were transfected with miR-146b mimic or control. Serum-starved cells were stimulated without or with TGFα and subjected to western blot analysis. **(a)** 10 ng/ml TGFα for 10 min. **(b)** 10 ng/ml TGFα for 24h. **(c)** ERK1/2 and AKT phosphorylation. Images shown are representative of 3 separate experiments. *p<0.05.

4. Smad3 is a target of miR-146b. Phosphorylation of the transcription factor Smad3 by the activated TGFβR1/TGFβR2 complex is an essential step in TGFβ1 fibrogenic signaling. MiR146b blocked TGFβ1-induced Smad3 but not Smad2 phosphorylation in HLF-N (**Fig.5a**). Total Smad3 protein, but not Smad2/4, were also reduced (**Fig.5a**). Using two miRNA Target Prediction Tools (http://www.targetscan.org and http://www. microrna.org/microrna/home.do), we found that miR-146b is predicted to target genes *EGFR*, *Jun*, *TGFBR1*, and *SMAD3*, but not *ERK1/2*, *CCND1*, *ACTA2*, *CTGF*, *COL1A1*, *and TGFBR2*. The seed sequences of miR-146b are complementary to the 3'-UTR of mRNAs of these genes and are conserved in humans and mice (not shown). Thus, the 3'-UTR containing the putative miR-146b site of the *Smad3* gene was amplified and cloned into the pmirGLO dual-luciferase reporter vector, and mutations at the miR-146b seed region of the WT reporter plasmids was created as we described [2] (**Fig. 5b**). HEK293 cells were co-transfected with control or miR-146b mimic (50nM) and the reporter plasmids containing the miR-146b targeting site or its mutant. The effects of miR-146b on the luciferase activity were determined using a Dual-Glo® Luciferase Assay kit (Promega). As shown in **Fig. 5c**, miR-146b mimic inhibited luciferase activity, which was abolished by deletion of the seed region.

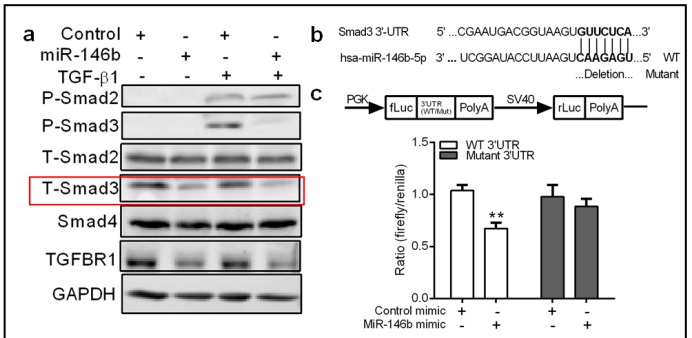


Fig.5: Smad3 is a target of miR-146b. **(a)** miR-146b reduced Smad3 protein expression and suppressed TGFβ1 (1 ng/ml)-induced Smad3 phosphorylation in HLF-N. WB images are representatives of 2 experiments. **(b)** The putative miR-424 binding site of the 3′-UTR of the *smad3* gene and its deletion mutant at the seeding region. **(c)** Firefly luciferase (fLuc) activity assays of the pmirGLO plasmids containing the smad3-3′-UTR WT or its mutant with Renilla luciferase (rLuc) activity as the internal control. N=4, $^{**}p<0.01$.

5. Identification of an essential promoter of miR-146b gene. The factors influencing miRNA transcription include methylation/demethylation and inhibited/activated regulatory factors. Hypermethylation and repression of miR-146b were reported in cancers. However, *5-aza*-2'-deoxycytidine (5-AZA-dC) treatment failed to induce miR-146b expression in HLF-F.

MiR-146b gene is located on human chromosome 10. The online program Neural Network Promoter Prediction predicts a transcription start site 832 bp upstream of pre-miR-146b and a poorly conserved TATA box upstream of the transcription start site (TSS) (**Fig.6a**), consistent with a previous report [3]. A 2333bp DNA fragment encompassing the region from -2270 to +63 was amplified and cloned into the luciferase reporter vector pGL3-Basic, designated as P(−2270/+63). This P(−2270/+63) construct displayed a 50-fold higher promoter activity over the pGL3-Basic in HLF-N. Following progressive deletion, the P(−275/+63) still retained promoter activity (**6b**), suggesting that this 338-bp fragment contains key elements of the miR-146b gene promoter. When the P(−275/+63) was transfected into six HLF cell lines, its promoter activity was 3-fold higher in HLF-N (n=3) than HLF-F (n=3) and activity positively correlated with their miR-146b levels (R = 0.878; Pearson's correlation, p = 0.006) (**6c**).

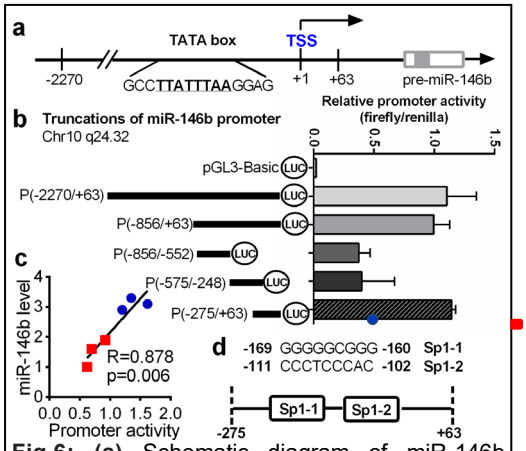


Fig.6: **(a)** Schematic diagram of miR-146b putative promoter. **(b)** Histogram shows luciferase activities driven by different regions of miR-146b promoter in HLF-N. **(c)** Pearson's correlation analysis of expression and promoter activities of miR-146b in HLF-N ( ) and HLF-F ( ). **(d)** Putative Sp1 sites in P(−275/+63).

C. Significance

Completion of this project will identify molecular mechanisms underlying miR-146b regulation of the signal pathways promoting lung fibroblast proliferation and differentiation. Establishing the critical role of miR-146b repression leading to exacerbated fibrogenic signaling will be a major step forward in understanding the pathobiology and molecular mechanisms underlying IPF progression. Success in these studies would be a major advance by providing target-directed therapy against both excessive fibroblast proliferation and differentiation, which would have significant long-term clinical impact by changing treatment paradigms for lethal IPF. A critical strength of this proposal is that the original data driving this project came from IPF patients and are consistent

with clinical observations. Moreover, the mechanisms unraveled here may also guide development of novel therapies for other fibrotic diseases with excessive EGFR/TGFβR activation.

References:

1. http://mirnamap.mbc.nctu.edu.tw/php/mirna_entry.php?acc=MI0003129
2. Wei P, Xie Y, Abel PW, Huang Y, Ma Q, Li L, Hao J, Wolff DW, Wei T, Tu Y. (2019) Transforming growth factor (TGF)-β1-induced miR-133a inhibits myofibroblast differentiation and pulmonary fibrosis. *Cell Death*. *Dis.* 10: 670. PMCID: PMC6739313.
3. Al-Khalaf HH, Mohideen P, Nallar SC, Kalvakolanu DV, Aboussekhra A. (2013) The cyclin-dependent kinase inhibitor p16INK4a physically interacts with transcription factor Sp1 and cyclin-dependent kinase 4 to transactivate microRNA-141 and microRNA-146b-5p spontaneously and in response to ultraviolet light induced DNA damage. *J. Biol. Chem*. 288: 35511–35525. PMCID: PMC3853297

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

1. Xie Y, Abel PW, Casale TB, Tu Y. (2022) T_H17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149(2):467-479. PMCID: PMC8821175.
2. Hulen J, Kenny D, Black R, Hallgren J, Hammond KG, Wickramasekara RN, Abel PW, Stessman HF. (2022) KMT5B is required for early motor development. Frontiers in Genetics. doi: 10.3389/fgene.2022.901228

III. List of extramural grants submitted from 7/1/2021–6/30/2022

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
R01 HL164593-01
Title: A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-Resistant (Refractory) Asthma
Dates: 7/2022 - 6/2027
Tu (PI): Role: Co-Investigator
Total funds requested: $ 2,049,764

National Institutes of Health – NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE
R01
Title: Neuroinflammation in global ischemia
Dates: 12/2022 - 11/2027
Hwang (PI): Role: Co-Investigator
Total funds requested: $ 2,244,963

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

None

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Development Program
Program Director: Juliane Strauss-Soukup, PhD

Identify the Molecular Signatures of Pre-Cancerous Lesions and Endometrial Cancer with High-Throughput Single-Cell Analysis

Principal Investigator: Yusi Fu, PhD

I. Progress Report Summary

A. Specific Aims

- Aim 1: Specify immune cell composition and expression changes for EC and pre-cancer stage patients.
- Aim 2: Identify EC-specific genomic mutations and their correlations with cancer risk.

B. Studies and Results

Aim 1: Specify immune cell composition and expression changes for EC and pre-cancer stage patients

- Sample collection in progress. For patients meeting our inclusion criteria, we collected uterine blood with vacutainer EDTA tubes and endometrial tissues with tissue collection tubes. We have obtained matching samples from four patients. According to International Federation of Gynecology and Obstetrics (FIGO) staging, two patients are FIGO stage 0 and two are FIGO stage 1.
- We have established a standard operating procedure for the sample process and archive. The blood and tissue samples are transferred on ice and processed within 1 hour to retain the high quality and integrity of RNA and DNA. For uterine blood, we did red blood cell lysis and created single-cell suspension without further treatment to keep the original cell population and the composition ratio. For long-term storage of the cell suspension, we use methanol to fix the cells, followed by storage at -20°C; for tissues, we immerse them in serum-free cell freezing medium for gradual cooling down and cryopreserve them at -80°C for future access.
- We performed high-throughput single-cell RNA sequencing (RNA-seq) with fresh single cell suspensions from the uterine blood samples. We used the Chromium Single Cell Gene Expression kit to isolate single cells into separated water-in-oil droplets and did reverse transcriptome and cDNA amplification according to the standard protocol provided. Sequencing-ready libraries were built for each sample and sequenced to saturation on Illumina Nextseq 2000.
- We obtained the transcriptome of 7,394 single cells and identified their cell types. We recovered the transcriptome of 1114, 930, 2721, and 2629 cells from four patients' uterine blood samples after mapping to a human reference genome and genomic annotations. We then integrated these cells with published endometrium RNA-seq datasets and used the known cell markers to annotate the cells. We identified lymphocytes, smooth muscle cells, endometrial cells, endothelia, macrophages, stromal fibroblasts, etc., in our datasets.

- We found that subpopulations of monocytes change with cancer progression. After comparing the cell type compositions between different individuals, we found three distinct subpopulations of monocytes among different patients (Figure 1). In normal individuals, monocytes express a high level of CD14 and do not express CD16 ($CD14^+$ $CD16^-$ monocytes). At the early stage of endometrial cancer, we find the monocyte population is dominated by $CD14^+$ $CD16^+$ monocytes. With the progression of cancer, a population of $CD14^-$ $CD16^+$ monocytes emerge and become the major monocyte type.

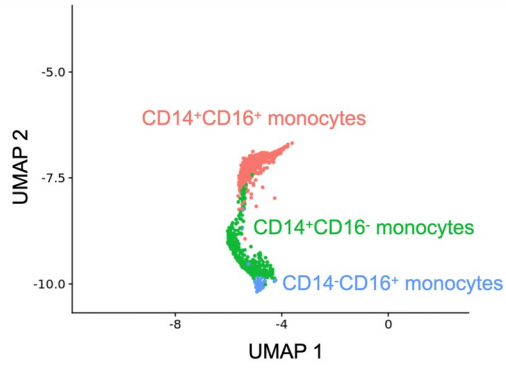


Figure 1. Subpopulations of monocytes

- We identified the gene expression difference between subpopulations of monocytes. We compared the transcriptome of the three subpopulations of monocytes and used the log2 fold change larger than 2 as a cut-off to find differentially expressed genes. The endometrial cancer early stage-associated $CD14^+CD16^+$ monocytes have higher expressions of metabolism, proliferation-related genes, and cancer-related genes, such as PIK3CD, GSK3B, TGFA, etc., when compared with normal $CD14^+$ $CD16^-$ monocytes. Higher expression of those genes can grant growth advantage to the cancer cells. For late-stage enriched $CD14^-CD16^+$ monocytes, extracellular matrix-related genes that inhibit cell migration are downregulated (Figure 2), thus facilitating the dissemination of cancer cells.

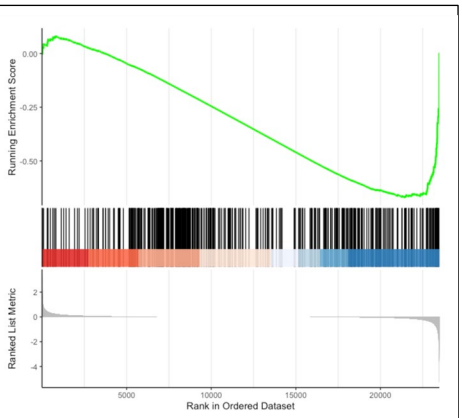


Figure 2. Gene set enrichment analysis of extracellular matrix related genes for differentially expressed genes between $CD14^-CD16^+$monocytes and $CD14^+$ $CD16^-$ monocytes

Aim 2: Identify EC-specific genomic mutations and their correlations with cancer risk

- We applied the protocol of Tn5-transposase-assisted single-cell whole-genome sequencing (Tasc-WGS) to both single-cell suspension and single-nucleus suspension. The results

showed a lower efficiency when starting with single-nucleus suspension. Thus, we tested three kinds of Tn5 transposons to improve the tagmentation efficiency for nuclei and assessed different amplification conditions to get enough DNA for sequencing.

C. Significance

- Our findings show that the types of cells in endometrium tissues can be sampled from uterine blood. With high-throughput single-cell technologies, we can profile the cell-type composition and transcriptome of immune cells existing in endometrium tissues through non-invasive uterine blood. Uterine blood allows regular monitoring of cancer patients and provides a non-invasive, sensitive, and molecular way to assess cancer risk, helping to overcome the inadequate sampling and analysis issues plaguing diagnostic methods such as dilation and curettage.
- The result shows that there is a subpopulation change for the monocytes during endometrial cancer progression, which could be used as a diagnostic and treatment target.
- Our analysis identified several key gene changes of monocytes during cancer progression, which could be used as microenvironment molecular signatures, as well as a reference for cancer classification. Our findings will have a direct effect on treatment decisions for patients and provide opportunities for genome-guided clinical trials and drug development.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

Submitted to *Frontiers in Cellular Neuroscience*: Profiling mouse cochlear cell maturation using 10x Genomics single-cell transcriptomics. Authors: Zhenhang Xu, Shu Tu, Caroline Pass, Yan Zhang, Huizhan Liu, Yusi Fu, David ZZ He and Jian Zuo.

III. List of extramural grants submitted from 7/1/2021–6/30/2022

- Nebraska Stem Cell Research Project 2022
  Title: ALDH1A1^{+} cancer stem cells abundance in uterine blood as a potential diagnostic marker for endometrial cancer
  PI: Yusi Fu

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

None

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Development Program
Program Director: Juliane Strauss-Soukup, PhD

Effects of 3D Ultrasonography and 3D-Printed Images on Maternal-Fetal Attachment and Its Correlation with Overall Smoking within Pregnancy and Smoking Cessation

Principal Investigator: John Coté, MD, FACOG

I. Progress Report Summary

A. Specific Aims

Our central hypothesis for this proposal was that 3D ultrasonography and 3D-printed models increase baseline maternal-fetal attachment scores. We hypothesized that we would then see a decrease in the number of cigarettes smoked in pregnancy and an increase in smoking cessation. We tested our central hypothesis by pursing two specific aims:

- **Aim #1:** Review global maternal-attachment scores in pregnant smokers and correlate total number of cigarettes smoked and salivary cotinine levels over the course of the pregnancy.
- **Aim #2:** Determine the effect 3D ultrasonography and 3D-printed models have on the overall amount of smoking in pregnancy.

B. Studies and Results

Our study included pregnant women who were admitted smokers. Before the completion of any interventions, the study coordinator obtained written consent. The eligibility criteria included singleton pregnancy, gestational age 26-31 weeks, current smoker, participant age between ages 19 and 45, and fluent in English. After consent, participants completed demographics questions and the MAAS and TLFB interview and supplied salivary cotinine. After the questionnaires were completed and saliva was collected, an ultrasonographer performed a 20-minute 3D/4D ultrasound examination. Computer-generated block randomization with equal allocation and block size of four was used to randomly assign participants to 3D ultrasonography and 3D print versus 3D ultrasonography and 3D-printed model. Patients received their model or print one week after their enrollment. Two weeks after the initial ultrasound, all participants continued the TLFB interview, collected salivary cotinine, and answered the MAAS questionnaire again. Every participant continued to have the TLFB interview administered every week until 6 weeks postpartum. Salivary cotinine was collected at 2 and 6 weeks postpartum.

We had a rolling enrollment and as of this report, we have a total of 19 out of 96 patients. COVID-19 restrictions have significantly restricted enrollment for about 1.5 years, yet we are continuing to enroll patients. One participant within the 3D print arm dropped out prior to the second MAAS questionnaire, and one participant within the 3D print arm did not finish the second MAAS questionnaire completely. We had attempted to adjust our strategy of recruitment

and have just started to increase the rate of our enrollment, mostly due to the decreased burden of COVID-19 and the increased ability of patients to able to be seen within the clinic environment. We currently have 6 more patients for possible enrollment and will continue to seek funding going forward until we can meet power. While our interdisciplinary research team was well prepared and qualified to undertake this clinical trial, there have been a multitude of roadblocks along the way. The following interim results, although encouraging, should be viewed with scientific skepticism as we have not recruited enough patients to meet power.

Table 1 Baseline demographic characteristics

	3D Picture (*n* = 10)	3D Model (*n* = 9)	*p*
Age	25 ± 5.3	28.8 ± 4.9	0.126
Race			
White	70	77.8	
Black	20	22.2	
Native	10	0	
Marital Status			
Single	90	77.8	
Married	10	22.2	
Gestational Age	29.2 ±1.3	29 ±1.1	0.709
Primigravida	30	44.4	
Multigravida	70	55.6	
Nulliparous	40	66.7	
Multiparous	60	33.3	
Education	40	11	
Some High school	50	66	
Grad High school			
College	10	22	
Insurance	100	90	
Medicaid			
Commercial	0	10	

Note Data presented as mean ± SD, or percent

Table 2. Attachment stratified by intervention

	3D Picture-Pre (*n* =10)	3D Model-Pre (*n* = 9)	3D Picture-Post (*n* = 8)	3D Model-Post (*n* = 9)	*p*-value
MAAS					
Global					
Total	81.1 ± 9.1	79.7 ± 9.1	85.9 ± 7.2	81 ± 4.9	
Average Likert	4.16 ± 1.0	4.19 ± 1.0	4.52 ± 0.8	4.3 ± 0.9	
3D picture pre vs 3D model pre					
Total					0.736
Average Likert					0.119
3D picture post vs 3D model post Global					
Total					0.750
Average Likert					**0.009**
3D picture pre vs 3D picture post (*n*=8) Global					
Total					**0.016**
Average Likert					**0.001**
3D model pre vs 3D model post (*n*=9) Global					
Total					0.5
Average Likert					0.367

Note Data presented as mean ± SD, median [IQR], or percent

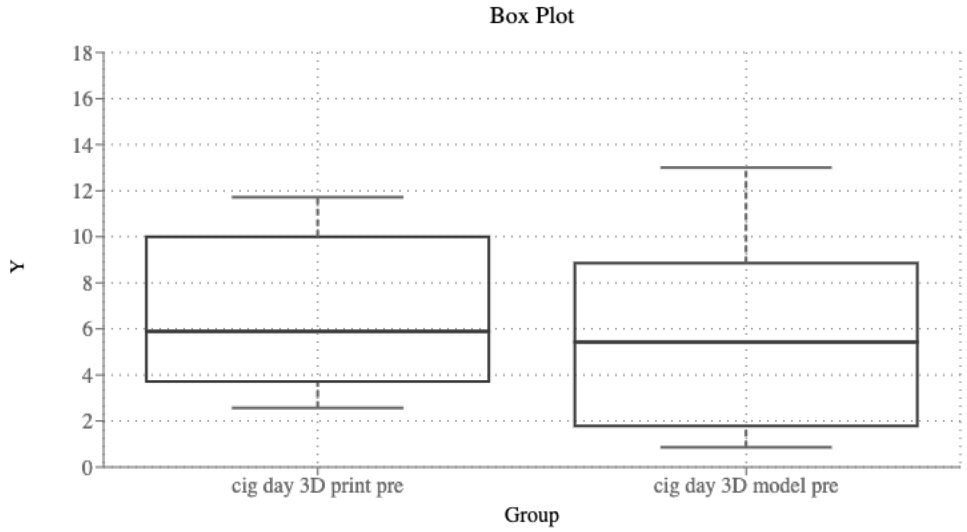
Table 3. Timeline follow back (TLFB) and psychological characteristics stratified by intervention

	Pre		Post	
	3D Picture (*n* = 8)	3D Model (*n* = 9)	3D Picture (*n* = 8)	3D Model (*n* = 9)
TLFB				
Cig/day				
Total	6.69 ± 3.5	7.25 ± 7.5	5.64 ± 3.9	6.42 ± 7.1

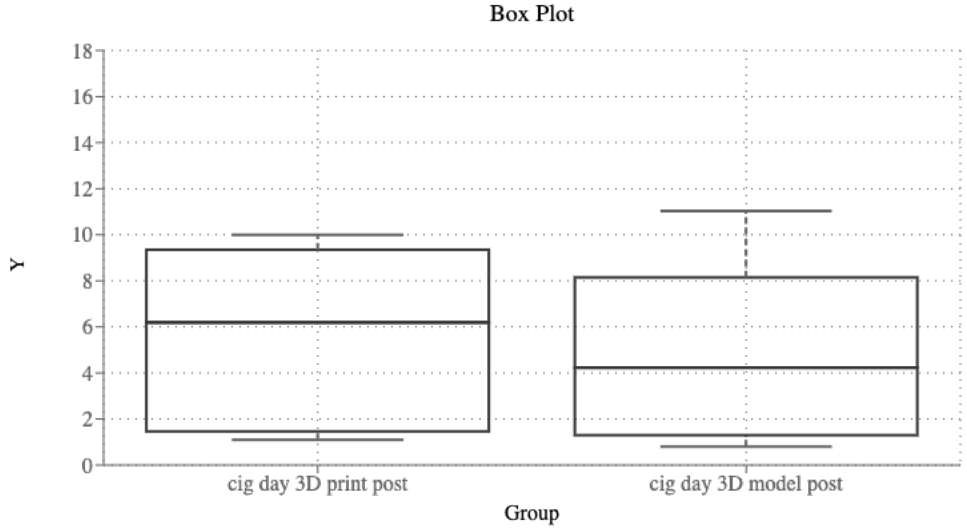
Note Data presented as mean ± SD, median [IQR], or percent

	3D Picture (*n* =10)	3D Model (*n* = 9)	*p*
Weight of Baby (g)	3094.7±198	3189.2±578	0.6509
EGA at Delivery	39.2±1.0	38.1±1.6	0.0755
Weight %	34.2±12.9	46.5±31	0.2911
Z-score	-0.43±0.37	-0.17±1.0	0.4916
Hypertension	40	44.4	

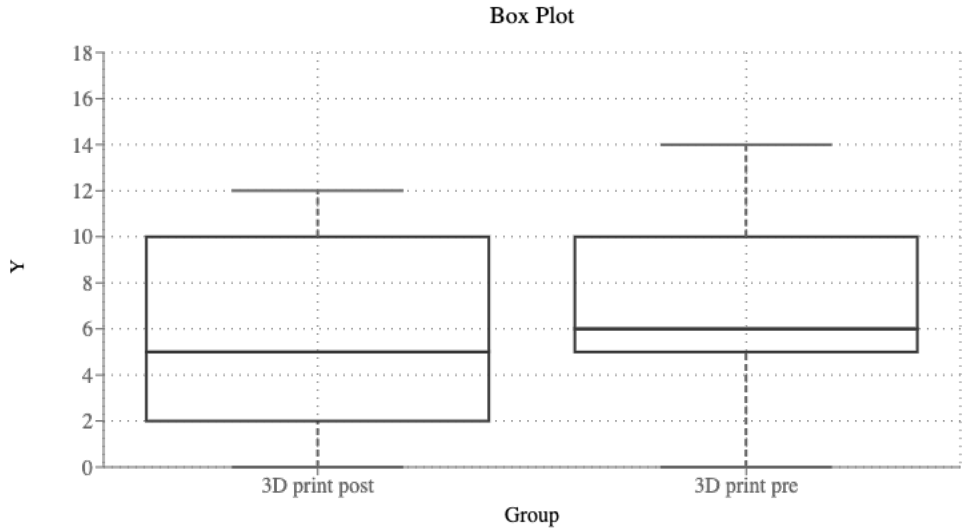
Note Data presented as mean ± SD, median [IQR], or percent



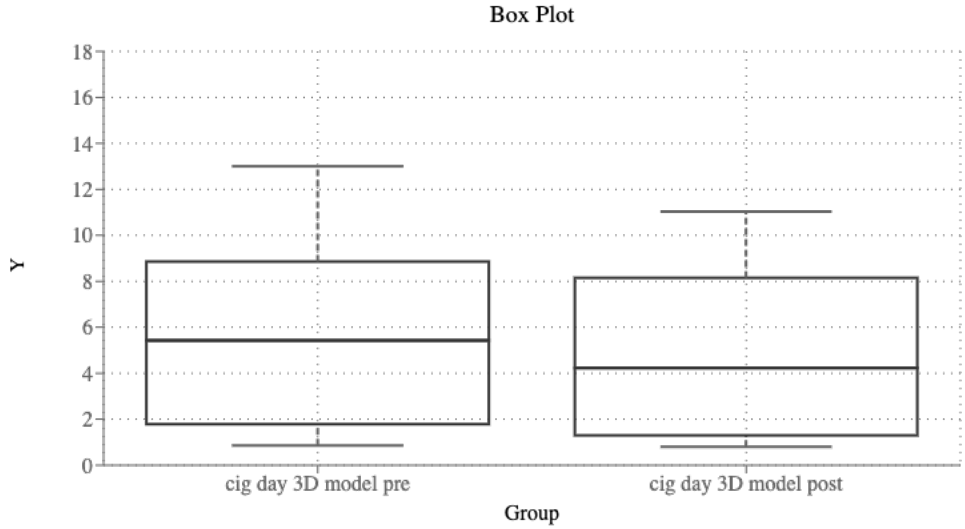
p-value	0.848



p-value	0.7861



p-value	**0.0002**



p-value	**0.0064**

C. Significance

There are multiple significant observations.

Attachment scores

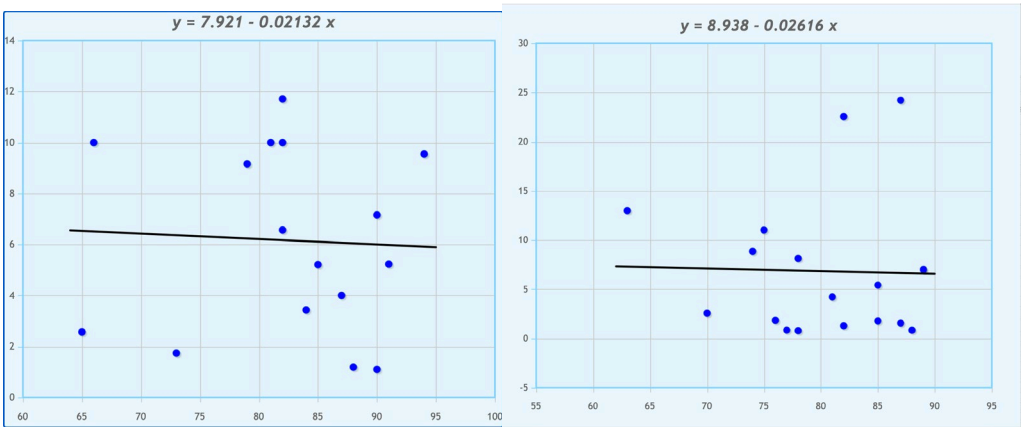
First, the MAAS global scores pre-intervention are not statistically different when comparing the two groups (3D model 79.7 versus 3D print 81.1). Second, the MAAS global scores post-intervention are not statistically different when comparing the two groups (3D model 81 versus 3D print 85.9). Third, **the MAAS global score pre- versus post-intervention in the 3D print group is statistically significantly different** (p=0.016). Fourth, while the MAAS global score pre- versus post-intervention in the 3D model group is non-statistically significantly different, the **trend is positive as the MAAS global scores on average are higher post-intervention compared to pre-intervention.** These findings are in line with previous research suggesting that either intervention increases attachment scores equally.

Cigarettes smoked per day

First, the cigarettes smoked per day pre-intervention are not statistically different when comparing the two groups (3D model 7.25 vs 3D print 6.69). Second, the cigarettes smoked per day post-intervention are not statistically different when comparing the two groups (3D model 6.42 vs 3D print 5.64). Third, the **cigarettes smoked per day pre- versus post-intervention in the 3D print group was statistically significantly different** (p=0.0002). Fourth, the **cigarettes smoked per day pre- versus post-intervention in the 3D model group was statistically significantly different** (p=*0*.0064).

Attachment versus cigarettes smoked per day

First, in the 3D print group there is a negative correlation between the Global MAAS attachment scores and the cigarettes smoked per day (r=-0.0499) such that **the higher the Global attachment score the fewer cigarettes smoked per day** after the intervention. Second, in the 3D model group there is also a negative correlation between the Global MAAS attachment scores and the cigarettes smoked per day (r=-0.0263) such that the higher the Global attachment score, the fewer cigarettes smoked per day after the intervention. Additionally, two participants quit smoking within one month of delivery and one quit within a week of delivery for a **quit rate of 18% (2/8-25% 3D print group; 1/9-11% 3D model group)**.



High quality interventions to help pregnant women quit smoking produce an absolute difference of 8.1% in validated late-pregnancy quit rates (Walsh et al., 2001). If our trend continues, this opens the possibility of much larger NIH grants, which would allow for collaboration on a multi-site basis.

Forty-two percent of patients in both interventions had hypertensive disorders of pregnancy (HDP) defined as gestational hypertension, preeclampsia, preeclampsia with severe features, eclampsia, or postpartum preeclampsia. A meta-analysis in 2013 suggested **only a 4.6% incidence of preeclampsia in all deliveries** (Abalos et al., 2013). Putting this into perspective, in multiple meta-analyses, pooled data from cohort and case-control studies as well as other prospective trials showed a **lower risk of preeclampsia associated with cigarette smoking during pregnancy**, including a dose response (i.e. the more someone smoked, the lower the OR of having a diagnosis of HDP) (Conde-Agudelo et al., 1999; Wei et al., 2015). This suggests that, at least with the limited number of participants, the higher rate of HDP represents the effect of **decreased smoking** regardless of intervention.

Smoking in pregnancy has been associated with PTD, SGA and a higher rate of NICU admissions. Preterm birth rate in Nebraska for most recent 2020 data on the CDC website last updated February 22, 2022, is 10.5%, the low birth weight is 7.4 %. The preterm birth rate was 10.5% for both interventions and the low birth weight was 5% for both interventions. Studies have shown the NICU admission rate to be between 10-15% and with our current study population the NICU admission rate was 5%. Studies have suggested that the rate of low birth rate in smokers can be double the normal rate, preterm birth rates in smokers can be as high as 25% and NICU relative risk in smokers increases 20% over baseline. While none of these outcomes have been powered for within this study, the pooled data suggests **lower rates of all three of these outcomes, which suggests lower smoking in both interventions.**

As of this writing, we are awaiting our cotinine lab results for these 19 patients and will be continuing to recruit participants if we can acquire funding. Of important note, we want to emphasize that there is not a lack of funding **but** a lack of time to complete our study due to multiple unfortunate circumstances. If not for these unforeseen and difficult circumstances, we should have been able to recruit enough participants to meet our power calculations. Clinical trials are of paramount importance for the advancement and development of novel treatment interventions. The goal of such trials and studies is to ensure the best quality patient care with the highest and most favorable outcome, whilst decreasing the cost and suffering of (a burden to) the recipient. Such endeavors are vital to the medical community. For the functioning of a robust medical system wherein discoveries, drugs and technology can be applied to the system from the top tier and eventually diffuse out to primary and satellite care centers to ensure the best care for all.

Historically, clinical trials have usually been a laborious, expensive, and difficult task to undertake under normal circumstances; however, trials under the unprecedented COVID-19 conditions have indeed suffered its consequences.

The often-complicated design and intricate nature of clinical trials mean the likelihood of derailment is high. A cohort of multiple external factors must be controlled and maintained to ensure the sanctity of the data as well as the integrity of the trial. All the criteria mentioned above are nullified by a pandemic such as COVID-19.

For the commencement of a new clinical trial or even the maintenance of an existing trial, **funding is a priority** to drive it to completion. COVID-19 severely impeded funding of non-COVID-19 related trials as the acquisition of a cure for the virus took center stage for international funding bodies. Over and above the funding issue, the need to tightly monitor trial participants and subsequently ensure their safety through such a pandemic proved detrimental to many ongoing studies, or those recently started. The statistics show a sharp decline in the enrolment of new members for trials.

Clinical trials did not only compete for funding under COVID-19 conditions but simultaneously had to cope with lockdown regulations that made accessibility of the study participants virtually impossible. The fact that data from the ongoing studies will be incomplete or will have gaps could lead to either shutting down the trial, a severe delay (and hence additional cost), or the need to re-start the trial. The ramifications of COVID-19 causing such a massive international dearth in the commencement of new trials and the continuation of non-COVID-19 trials will not be evident in the immediate future. However, the impact thereof will become evident as the time lost will add up to years lost in research in the broader spectrum." (Sathian et al., 2020).
We are thankful for your support and generosity in allowing us to further scientific knowledge and move towards improving the lives of these pregnant women and their babies. We humbly request an extension for the use of the funds associated with the LB 595 grant. We do not want

or need more money, just more time to recruit and complete our work. Because funds not used return to the State all we request is the use of these funds for an extended period. Showing what advancements we have made and what we could do with the added time will be integral to elucidating the complex intricacies to smoking in pregnancy.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

None

III. List of extramural grants submitted from 7/1/2021–6/30/2022

1. Great Plains IDeA-CTR Team Research Pilot Grant
   PI: John Cote
   Title: Human Placental Lactogen (Human Chorionic Somatomammotropin) and Oxytocin during Pregnancy: Individual Patterns and Correlations with Maternal-Fetal Attachment, Anxiety, and Depression

2. Department of Defense Congressionally Directed Medical Research Program, Peer Reviewed Medical Research Program: Discovery Award
   PI: Gelineau-van Waes
   Title: Evaluation of the G Protein-Coupled Estrogen Receptor (Gper) as a Therapeutic Target in a Preclinical Mouse Model of Endometriosis

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

None

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Development Program
Program Director: Juliane Strauss-Soukup, PhD

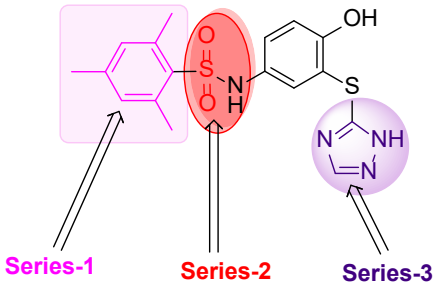
Chemical Optimization of Small Molecules to Probe Triggering Receptors Expressed on Myeloid Cell 1(TREM1): Novel Treatment for Hepato Cellular Carcinoma
Principal Investigator: Gopal P Jadhav, PhD

I. Progress Report Summary

A. Specific Aims

Aim 1: Structure Activity Relationships (SARs) of GPJCTS079 for improved potency and selectivity.

Aim 1.1 is a structure-guided approach to improve the potency of and selectivity toward TREM1 inhibition



Based on the above modifications, we have synthesized a total of 58 analogs of GPJCTS079.

Aim 1.2. SPR throughput screening (see studies and results).

Aim 2: To evaluate potency of GPJCTS analogs to inhibit the TREM1 protein and their functional studies in hepatic cancer cells.

Aim 2.1 Development of *in vitro* assay of Trem-1 inhibition.

We have designed and outsourced preparation of gene vector clone TREM1-DAP12_pcDNA3.1 (+)P2A that can simultaneously express TREM1 and DAP12 gene after *in vitro* transfection into the BWZ.36 cell line or HEPG2 cells.

Assay development is on hold as our research associate left the job due to health issues. Thus, we modified this sub-aim as follows:

Aim 2.2 To perform molecular docking and simulation studies of LR12-TREM1 to understand interactions between them in the development of more stable semi-peptide TREM1 inhibitors.

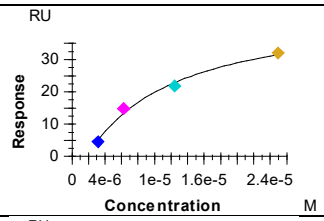
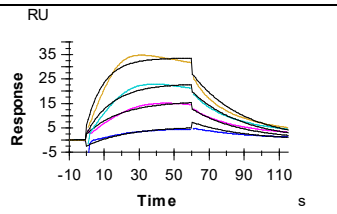
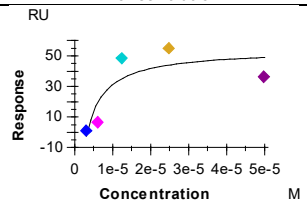
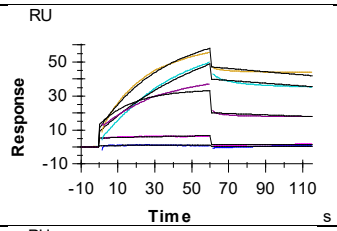
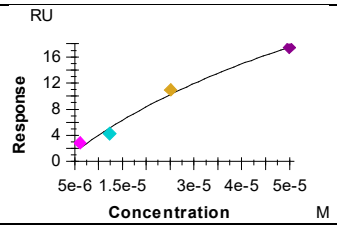
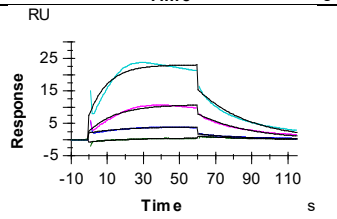
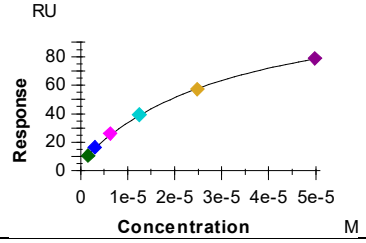
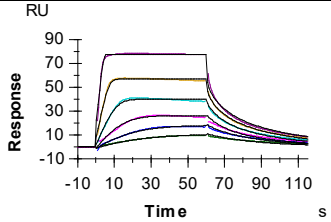
B. Studies and Results

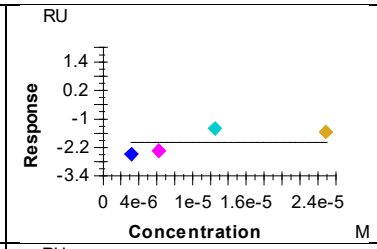
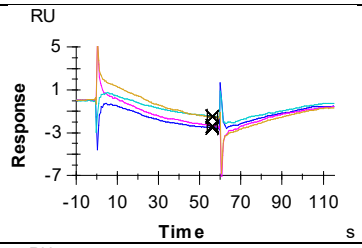
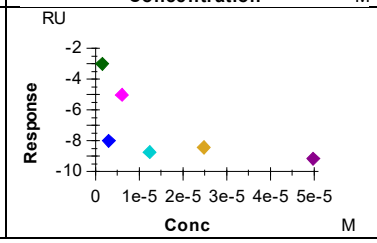
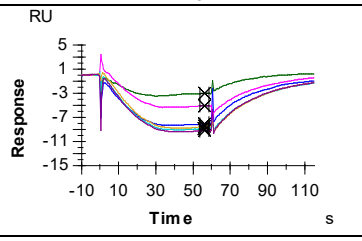
Aim 1.2. SPR throughput screening:

The main objective of performing SPR screening was to determine Kd (binding coefficient) of the newly developed molecules and compare them with GPJCTS079. Based on XP-docking (extra precision docking) results of these analogs in the TREM1 structure we selected:

a) 3 analogs (GPJCTS103, GPJCTS106, and GPJCTS159) that exhibited better or equivalent docking scores (more negative values are considered better scores) as compared to GPJCTS079.
b) 2 analogs (GPJCTS155 and GPJCTS161) that exhibited bad scores (positive values) as negative controls.

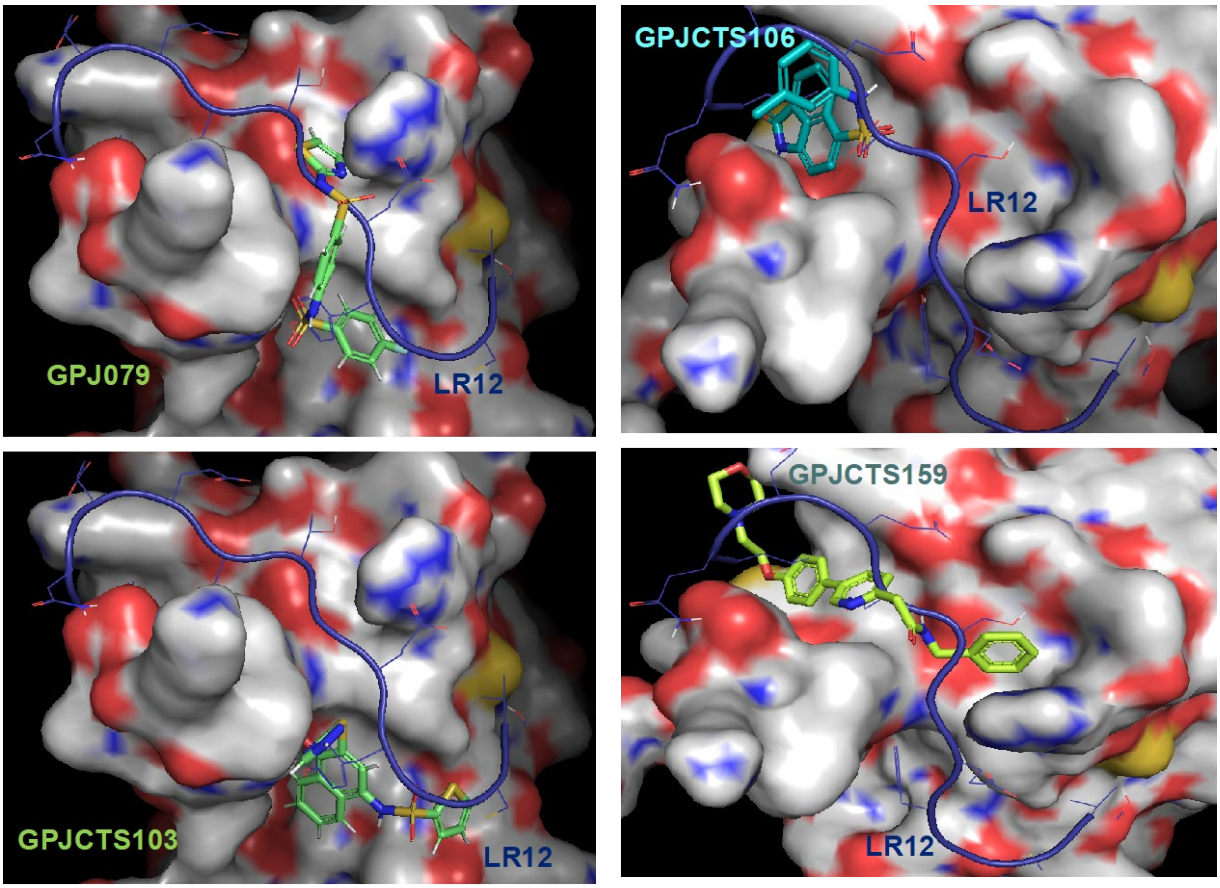
Here, TREM1 protein was immobilized on a CM5 sensor chip using the standard protocol. PBS pH 7.4 was used as the running buffer to make a series of dilutions from DMSO stock (5 mM) of inhibitors/positive and negative controls. Each compound was injected and passed on to TREM1 fixed on a sensor chip for 30 s association and 30 s dissociation time. The dose response curve was generated from SPR sensograms as follows.

Compound/Kd	Steady-state affinity fitting curves	Sensograms
GPJCTS079 Kd = 14.3 uM		
GPJCTS103 Kd = 1.3 uM		
GPJCTS106 Kd = 23.2 uM		
GPJCTS159 Kd = 5.1 uM		

GPJCTS155 Kd = No Binding		
GPJCTS161 Kd = No Binding		

Two molecules showed better binding than GPJCTS079.

Aim 2.2 To perform molecular docking and simulation studies of LR12-TREM1 to understand interactions between them in the development of more stable semi-peptide TREM1 inhibitors.



The above figure shows docking poses with an overlay of GPJCTS molecules and LR12 peptide in the TREM1 binding pocket.

Herein GPJCTS079 and GPJCTS103 bury within complementary determining regions (CRD1, CDR2 and CDR3) covering the C-terminal part of the (6 amino acids) of LR12 peptide.

Whereas, GPJCTS106 and GPJCTS159 bury within middle region pocket that is involved in TREM1 dimerization (or multimerization) of TREM1 and covering N-terminal part of the (6 amino acids) of LR12 peptide.

These data indicate that the LR-12 peptide, whose half-life is just 2 min, can be modified into a hybrid peptide containing the GPJCTS small chemical molecule either on the N- or C-terminal, with better half-life pharmacokinetics and higher potency.

C. Significance

Aim 1.2: SPR is expected to eliminate practically all nonspecific binders. We anticipate that kinetics determination will provide novel information of GPJCTS-TREM1 specific binding affinity and analysis would support our hypothesis of developing small molecule TREM1-interacting antagonists.

Aim 2.2: LR-12 peptides that have a very small half-life cannot be attractive drugs for TREM1 inhibition. Moreover, being peptides, they may attract other biological targets, leading to more serious side effects.

Thus, information obtained from the molecular simulation of the LR-12-TREM1 complex would lead us to understand that LR-12 interactions inhibit deleterious TREM1 functions. Moreover, binding poses of GPJCTS molecules covering CDRs regions and middle regions of TREM1 binding pockets would enable us to design and develop hybrid, synthetic, or semi-peptide analogs with half-lives greater than that of LR-12 and also with potent TREM1 inhibitors.

Overall, if successful, the proposed research is relevant to public health because hepatocellular carcinoma (HCC) is the most common form of liver cancer. It has high mortality, is increasing in prevalence, and represents a major national burden in terms of cost in both patient suffering and economic cost. This warrants an investigation into unconventional targets, such as TREM1, a receptor responsible for hepatic disorders. This project aims to develop novel TREM1 antagonists with effective pharmacokinetics necessary for oral route availability. We will develop new lead(s), devoid of side effects, to tackle complications of HCC. This discovery will stimulate the opening of a new avenue in therapeutics directed at dysfunctional liver functions.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

Pharmacological modulation of TREM-1 represents a novel therapeutic approach to treat inflammatory disorders. Neerja Trivedi, Neerja Tiwari, Amit Pant, Sonali Suryavanshi, Gopal P. Jadhav. Submitted to the journal Molecules, MDPI.

III. List of extramural grants submitted from 7/1/2021–6/30/2022

COBRE NIGMS Pilot Project under P20GM139762 -02
COBRE PI: Peter Steyger; Pilot project PI: Gopal Jadhav
Inhibition of TREM1 as novel treatment against NIHL and associated neurodegeneration

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

COBRE NIGMS Pilot Project under P20GM139762 -02
COBRE PI: Peter Steyger; Pilot project PI: Gopal Jadhav
Inhibition of TREM1 as novel treatment against NIHL and associated neurodegeneration

Creighton University Cancer & Smoking Disease Research Program FY20/21 Progress Report
(July 1, 2021 – June 30, 2022)

Development Program Awards
Juliane K. Strauss-Soukup, PhD, Principal Investigator

The Development Program assists faculty in developing pilot research projects related to cancer and smoking diseases. The goal of this program is to provide two years of support to investigators so they can develop fully realized projects meriting inclusion in one of the three Cancer and Smoking Disease Research Program Projects; in other cases, the project may develop into its own Research Program Project for a future inclusion in Creighton's Cancer and Smoking Disease Research Program. Investigators for the Development Pilot Projects are chosen through a competitive process that selects for funding the most promising and innovative research. Each year, a call for Pilot Projects is distributed for proposals. We received two applications for Developmental Pilot Projects; they were reviewed by the Chair of the External Advisory Committee and one was deemed appropriate for funding. The newly funded project follows:

PI: Gajanan Shelkar, PhD, Department of Pharmacology and Neuroscience
Title: Glutamate Delta-1 Receptor in Cisplatin-Induced Neuropathic Pain and Anorexia

Creighton University
LB595 Development Program
Application Guidelines
Application Deadline: 4:30 p.m., Friday, April 22, 2022

INTRODUCTION: LB595 Cancer and Smoking Disease Research Program Development Grants are to assist faculty to develop new research projects in cancer and smoking-related diseases that would ultimately be competitive for extramural funding. These grants are for $60,000/year for a total of $120,000 and a maximum of two years.

ELIGIBILITY: The following eligibility requirements apply to the LB595 Development Program:

- Omaha Campus School of Medicine tenured or tenure-track faculty or resident assistant professors/research assistant professors are eligible for funding. Faculty who hold contributed service, special rank, or visiting designations are not eligible for this program.
- Preference will be given to those faculty who have not previously been funded by this mechanism.
- Recipients of a LB595 Cancer and Smoking Disease Research Program Development Grant in the past two years are not eligible to apply this cycle.
- Principal Investigators (PIs) or co-investigators cannot be funded concurrently by other LB692 or LB595 support mechanisms.
- Investigators should not submit more than one grant proposal to be considered.
- The two-year award is not allowed an extension or renewal.

DEADLINE AND APPLICATION FORMAT: Proposals must be uploaded and routing started in the InfoEd submission system no later than 4:30 PM, Friday, April 22, 2022. Please see the non-system to system instructions for using InfoEd, located on the Sponsored Programs Administration website at https://www.creighton.edu/researchservices/grants/infoed/.

Please create one PDF with all documents in the following order:

- Research Plan
- Literature Cited
- Budget Justification
- Biographical Sketches
- Statement of Project's Relevance to Cancer or Smoking Diseases

You may include up to two 1-page letters of support. Upload the single PDF to the Attachments tab in InfoEd.

APPROVALS: Internal grant applicants must follow established University approval procedures. The Principal Investigator must submit the application to routing via the InfoEd system before 4:30 p.m. on the deadline day.

PREPARATION OF APPLICATIONS: The full application must include the budget, budget justification, a biographical sketch for each investigator, no more than 6 pages for the research plan section, literature cited, and a statement about the project's relevance to cancer or smoking diseases. Use Arial font, size 11 points or larger, and no less than one-half inch margins (top, bottom, left, and right).

BUDGET: Use the InfoEd budget form for all budget information. All full-time Creighton personnel added to the budget will receive a salary release email. As faculty salary is not an allowable expense, they should disregard the email. Their name will be listed on the budget; do not indicate any person-months or salary in the budget for any participating faculty.

The following are not allowable expenses:

- Faculty salaries
- Space
- Travel

- Repairs
- Renovations
- Computer equipment
- Direct patient treatment costs
- Clinical trials (any human subject investigation that involves a drug or device and is conducted at multiple institutions)
- Indirect costs

BUDGET JUSTIFICATION: Describe the specific functions of all personnel. *(Do not indicate person-months for faculty on the budget justification).* Provide a complete justification for all non-personnel items requested. No specific form page is required for the budget justification.

PHS 398 BIOGRAPHICAL SKETCH: Provide a biographical sketch for all investigators involved in the proposed project. Use the current PHS 398 Biographical Sketch form. The Biographical Sketch form and a sample are available at: https://grants.nih.gov/grants/forms/biosketch.htm.

RESEARCH PLAN: *(No more than 6 pages for the following sections of the Research Plan)*
Please follow the outline below for the proposal narrative. This section should include sufficient information needed for evaluation of the project, independent of any other document. Be specific and informative and avoid redundancies. Discussion of the inclusion of human subjects or animals must be included within the 6 pages of the Research Plan. No abstract is required. There are no specific form pages for the research plan, but use the following format:

1. **Specific Aims:** Concisely state the goals of the proposed research and summarize the expected outcomes(s), including the impact that the results of the proposed research will have on the research field(s) involved. List succinctly the specific objectives of the research proposed, e.g., to test a stated hypothesis, create a novel design, solve a specific problem, challenge an existing paradigm or clinical practice, address a critical barrier to progress in the field, or develop new technology.
2. **Research Strategy:** Organize the Research Strategy in the specified order and using the instructions provided below. Start each section with the appropriate section heading—Significance, Innovation, Approach.
   a. **Significance:**
      - Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.
      - Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
      - Describe how the concepts, methods, technologies, treatments, services, or preventive interventions that drive this field will be changed if the proposed aims are achieved.

   b. **Innovation:**
      - Explain how the application challenges and seeks to shift current research or clinical practice paradigms.
      - Describe any novel theoretical concepts, approaches, or methodologies; instrumentation or intervention(s) to be developed or used; and any advantage over existing methodologies, instrumentation, or intervention(s).
      - Explain any refinements, improvements, or new applications of theoretical concepts, approaches or methodologies, instrumentation, or interventions.

   c. **Approach:**
      - Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. Include how the data will be collected, analyzed, and interpreted, as well as any resource sharing plans, as appropriate.
      - Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.
      - If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high-risk aspects of the proposed

work.

- Discuss your plans for potential sources of future support for continuing the research program initiated by this application. Specify extramural funding agencies to be approached. In addition, if this research is included in any currently pending external proposal, identify that proposal.

LITERATURE CITED: *(Not included in 6-page limitation)*
List all references. Each reference must include the title, names of all authors, book or journal, volume number, page numbers, and year of publication. Be concise and select only those literature references pertinent to the proposed research.

CANCER OR SMOKING DISEASES RELEVANCE STATEMENT: *(Not included in 6-page limitation)*
Include a clear statement of the project's relevancy to cancer or smoking disease as defined by Neb Rev Statute 81-637: "Cancer means all malignant neoplasm regardless of the tissue of origin, including malignant lymphoma and leukemia. Smoking disease means diseases whose causes are linked to smoking including, but not limited to, cardiovascular, pulmonary, and gastrointestinal diseases."

PROJECT START DATE: Grants will be awarded with a start date of July 1, 2022.

CERTIFICATIONS: University procedures for projects involving human subjects, vertebrate animals, or biohazardous materials must be observed. Approval must be received prior to the release of funds.

QUESTIONS: If you have any questions, please contact Sponsored Programs Administration: Beth Herr at 402-280-5769 or bherr@creighton.edu or Barb Bittner at 402-280-3209 or barbarabittner@creighton.edu.

Cancer & Smoking Disease Research Program Development Applications
April 2022

Principal Investigator	School	Department	Project Title	Total Requested
Kalyana C Nandipati	School of Medicine	Surgery	The Role of Protein Kinase C Delta (PKC-) and Downstream Signaling Pathways in Esophageal Adenocarcinoma	119,456
Gajanan Shelkar	School of Medicine	Pharmacology & Neuroscience	Glutamate delta-1 receptor in cisplatin-induced neuropathic pain and anorexia	120000

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

A grid of previous submissions and awards for the State LB506 program is included below.

Analysis of Submissions and Awards for the State of Nebraska LB 506 Funding		
Fiscal Year	**Submissions**	**Awards**
FY 03/04	4	4
FY 04/05	0	0
FY 05/06	6	1
FY 06/07	11	2
FY 07/08	7	1
FY 08/09	9	3
FY 09/10	14	4
FY 10/11	7	4
FY 11/12	11	1
FY 12/13	5	0
FY 13/14	4	2
FY 14/15	1	1
FY 15/16	7	0
FY 16/17	7	1
FY 17/18	3	1
FY18/19	6	2
FY 19/20	10	0
FY 20/21	3	2
FY 21/22	4	2
FY 22/23	4	3

Complete

Investigator and Proposal Information

Principal Investigator/Project Director/Fellowship Sponsor:
Shelkar, Gajanan

Email GajananShelkar@creighton.edu
Phone 402-280-5004
Department Pharmacology & Neuroscience - Omaha

Personnel:

PI	Name	Department	Role	Net Effort
✔	Shelkar, Gajanan	Pharmacology & Neuroscience - Omaha	PD/PI	0.000

Originating Sponsor: State of Nebraska - LB595

Sponsor: State of Nebraska - LB595

Budget:

	Period 1	Period 2	Total
Direct Costs	$60,000	$60,000	**$120,000**
Indirect Costs	$0	$0	**$0**
F&A Rate	0%	0%	-
Total	**$60,000**	**$60,000**	**$120,000**

Project Total Cost Sharing Direct Costs: 0

Project Total Cost Sharing F&A Costs: 0

Start Date: 01-Jul-2022 End Date: 30-Jun-2024

Identification

Proposal Title
Glutamate delta-1 receptor in cisplatin-induced neuropathic pain and anorexia

Brief description of project in plain language (1000 character limit).
Cisplatin is a widely used chemotherapeutic agent prescribed in nearly 50% of all tumor chemotherapies. Cisplatin is extensively used to treat breast, ovarian, lung, kidney, liver, thyroid, lymphoma and other cancers. Unfortunately, cisplatin produces painful neuropathy and anorexia through mechanisms that remain poorly understood. This proposal aimed to delineate the mechanism underlying cisplatin-induced neuropathic pain and anorexia. Identifying neural mechanisms regulating cisplatin-induced pain and anorexia could help to generate mechanism-based therapy to alleviate these side effects, allowing more effective doses of cisplatin that would likely increase the therapeutic efficacy and patient's quality of life.

Sponsor Guidelines: Please provide a link or upload the guidelines here.
Upload Guidelines

Please upload the Sponsor Guidelines:

Protocols

Will your project involve...

☐ Yes ☑ No Human Subjects?

☑ Yes ☐ No Laboratory Animals?

Protocol Status:
Not yet submitted

☑ Yes ☐ No Recombinant DNA or other biological agents?

Protocol Status:
Not yet submitted

☐ Yes ☑ No Radioactive materials/radiation-generating machines?

Special Situations

Will your project require...

☐ Yes ☑ No A reduction in current course load for yourself or any other investigator? Chair/Dean pre-approval required.

☐ Yes ☑ No A commitment of facilities/space in addition to what is currently available to you?

☐ Yes ☑ No Any capital equipment purchases?

☐ Yes ☑ No A computer hardware or software purchase requiring network connectivity and/or Division of Information Technology support?

☐ Yes ☑ No Has this grant application been through a scientific review and edit by a faculty peer?

☑ Yes ☐ No Will this project utilize any core facilities?

If yes, select all that apply:

☐ CU Biostatistical Core Facility

- [ ] CU Flow Cytometry Core Facility
- [ ] CU Histology Core Facility
- [x] CU Integrated Biomedical Imaging Facility
- [ ] CU Molecular Biology Research Core Facility
- [ ] Other Non-CU Core Facility

Export Control

- [ ] Yes [x] No Will any project participant travel to embargoed foreign countries?
- [ ] Yes [x] No Will this proposal involve participation of foreign nationals/entities (includes individuals who are not US citizens and those who do not have permanent US residency)?
- [ ] Yes [x] No Do you anticipate transporting or shipping any research materials or equipment related to this project outside of the United States?

Keywords

Select up to three.

- [ ] Business
- [ ] Diversity
- [ ] Global Issues
- [ ] Law/Policy
- [x] Science (Biomedical)
- [ ] Translational
- [x] Cancer
- [ ] Education
- [ ] Humanities
- [x] Neuroscience
- [ ] Science (Non-Health)
- [ ] Undergraduate Research
- [ ] Community Health
- [ ] Faith-Based
- [ ] Interdisciplinary
- [ ] Other
- [ ] Sustainability

A statement of the project's relevancy to cancer or smoking disease as defined by Neb Rev Statute 81-637: **"Cancer means all malignant neoplasm regardless of the tissue of origin, including malignant lymphoma and leukemia. Smoking disease means diseases whose causes are linked to smoking including, but not limited to, cardiovascular, pulmonary, and gastrointestinal diseases."**

Project's Relevance to Cancer or Smoking Diseases:

The clinical challenges of managing cancer- and chemotherapy-associated pain has increased over the last decades. Cancer-induced pain can be divided into pain related to advanced cancer, active cancer, and cancer treatments. Cancer itself can cause peripheral neuropathy by direct tumor compression of peripheral nerves or producing substances that damage peripheral nerves (paraneoplastic syndrome). Although there have been significant advances in the types of tumoricidal treatments available, most chemotherapeutic drugs used in chemotherapy develop devastating adverse effects, including neuropathic pain and anorexia, resulting in premature discontinuation of therapy, suboptimal effects on cancer cell destruction, and reduced chemotherapeutic efficacy. Cisplatin is a widely used chemotherapeutic agent prescribed in nearly 50% of all tumor chemotherapies. Cisplatin is extensively used to treat breast, ovarian, lung, kidney, liver, thyroid, lymphoma, and other cancers. Unfortunately, cisplatin produces painful neuropathy and anorexia through mechanisms that remain poorly understood. Identifying neural mechanisms regulating cisplatin-induced pain and anorexia could help generate mechanism-based therapy to alleviate these cancer- and cisplatin-induced side effects, allowing more effective doses of cisplatin that would likely increase the therapeutic efficacy and patient's quality of life. Therefore, this proposal aims to delineate the mechanism underlying cisplatin-induced neuropathic pain and anorexia and to find a novel therapy to alleviate chemotherapy-induced anorexia and unbearable neuropathic pain. As we found beneficial effects of Cbln1 treatment in cisplatin-induced mechanical hypersensitivity and weight loss, these findings of the proposal can be utilized as a potential treatment for cancer- and chemotherapy-induced neuropathic pain and anorexia. Further, the recent drug discovery efforts focusing on the GluD1 receptor selective agents may identify a therapeutically targetable strategy to mitigate cancer- and chemotherapy-induced neuropathic pain and anorexia.

Glutamate delta-1 receptor in cisplatin-induced neuropathic pain and anorexia

A. Specific Aims: Cisplatin is a commonly used anti-neoplastic agent to treat a variety of cancers; however, it's devastating side effects, including neuropathic pain and anorexia, result in discontinuation of treatment, thereby limiting its therapeutic efficacy. Despite valuable clinical importance, the neural mechanisms arbitrating these side effects have remained elusive.

Evidence shows that glutamate signaling in the central amygdala (CeA) is important for pain and cisplatin-induced anorexia. Glutamate delta-1 receptor (GluD1) and GluD2 are the delta family of ionotropic glutamate receptors (iGluRs). Unlike other iGluRs, GluDs regulate synapse formation and maintenance by forming a GluD-Cerebellin 1 (Cbln1)-Neurexin trans-synaptic complex. GluD1 is highly expressed in several forebrain nuclei, including the CeA. We have recently discovered that GluD1-Cbln1 signaling in parabrachial nucleus (PB)-central laterocapsular amygdala (CeLC) synapses (Fig. 1) play an important role in both complete Freund's adjuvant (CFA)-induced inflammatory pain and spinal nerve ligation (SNL)-induced neuropathic pain conditions. Importantly, we found downregulation of GluD1 and Cbln1 in CFA and SNL pain conditions. Intra-CeA recombinant Cbln1 injection led to the mitigation CFA- and SNL-induced pain parameters and normalized hyperexcitability of CeA neurons. In addition, studies have shown that reducing the activity of PB neurons that project to CeLC or inhibition of glutamate receptors in CeA reduces cisplatin-induced anorexia. Despite these promising results, a mechanistic understanding of the regulation of PB-CeLC glutamatergic synapses in cisplatin-induced neuropathic pain and anorexia is lacking.

Recently we have shown that PB-CeLC synapses express GluD1 receptor at postsynaptic terminals (Fig. 2). Moreover, in agreement with the potential role of GluD1 and Cbln1, which is enriched in PB, in synapse formation and maintenance, GluD1 knockout (KO) mice display reduced PB-CeLC neurotransmission (Fig. 3) and a significantly lower frequency and amplitude of mEPSC in the CeLC neurons (Fig. 4). Together these data suggest that the GluD1-Cbln1 complex is obligatory for normal functioning of PB-CeLC synapses. Importantly, in our preliminary studies, intra-lateral ventricle (ICV) recombinant Cbln1 protein injection in cisplatin-treated mice showed reduced cisplatin-induced mechanical hypersensitivity (Fig. 5) and increased body weight (Fig. 6). However, the anatomical substrate and molecular mechanisms underlying the effect of Cbln1 in cisplatin-induced neuropathic pain and anorexia is remained unexplored. *We hypothesize that cisplatin will modulate GluD1-Cbln1 signaling at PB-CeLC synapses and thereby affect synaptic neurotransmission and behaviors.* Using a multidisciplinary approach including genetic mice, electrophysiology, immunohistochemistry, and confocal imaging, we will address the following aims:

Specific Aim 1: Determine cisplatin-induced changes in GluD1-Cbln1 signaling and neuroplasticity in the PB-CeLC circuitry and to test a rescue approach using recombinant Cbln1.

We will first evaluate the specific role of GluD1 and Cbln1 in the formation and maintenance of PB-CeLC synapses. We will address whether systemic cisplatin treatment leads to changes in expression and localization of GluD1 and Cbln1 at PB-CeLC synapses using immunohistochemistry and confocal imaging. Secondly, we will determine the effect of systemic cisplatin on excitatory neurotransmission at PB-CeLC synapses using electrophysiology in brain slices. We will determine whether there are changes in the excitability of CeLC and PB neurons and whether ablation of GluD1 affects cisplatin-induced neuroplasticity. Finally, we will also test whether overexpression of the GluD1 receptor by injecting AAV-hSyn-DIO-mGRID1 in PKCδ cre mice or injection of recombinant Cbln1 protein in CeA will rescue cisplatin-induced neuroplasticity. Thus, using a pharmacological approach together with electrophysiology, immunohistochemistry and confocal imaging, we will determine the mechanism for cisplatin-induced neuroplasticity in CeA synapses.

Specific Aim 2: Determine the effect of restoration of GluD1-Cbln1 signaling in the CeA on cisplatin-induced neuropathic pain and anorexia behaviors.

Using conditional region-specific deletion of GluD1 from CeA, we will address whether GluD1-Cbln1 function is critical for cisplatin-induced neuropathic pain and anorexia. Then we will test whether restoring GluD1-Cbln1 signaling by overexpression of GluD1 in CeLC or injection of recombinant Cbln1 protein will rescue cisplatin-induced neuropathic pain and anorexia.

Overall, the expected outcomes of the proposal will identify novel mechanisms regulating cisplatin-induced neuropathic pain and anorexia. The rescue strategy using recombinant Cbln1 and recent drug discovery efforts focusing on the GluD1 receptor selective agents may identify a therapeutically targetable strategy to mitigate chemotherapy-induced neuropathic pain and anorexia.

B. Significance and background:

Cisplatin is a widely used chemotherapeutic agent prescribed in nearly 50% of all tumor chemotherapies[1]. However, deleterious side effects, including neuropathic pain and anorexia associated with cisplatin treatment, remain major clinical problems and result in premature discontinuation of cisplatin, thereby limiting its chemotherapeutic efficacy. Identifying neural mechanisms regulating cisplatin-induced pain and anorexia could help to generate mechanism-based therapy to alleviate these side effects, allowing more effective doses of cisplatin that would likely increase the therapeutic efficacy and patients' quality of life. We have identified a therapeutically targetable GluD1-Cbln1 signaling as a pain-related CeA plasticity mechanism that can be targeted to restore synaptic function in chronic pain[2]. Owing to the role of CeA in chronic pain and anorexia[2–11], it is plausible that GluD1-Cbln1 signaling in the CeA may serve as an important therapeutic target to treat cisplatin-induced neuropathic pain and anorexia.

CeA, a common target for chronic pain and cisplatin-induced anorexia: Accumulating evidence showed the critical role of CeA in pain modulation and anorexia[2–11]. CeLC neurons receive monosynaptic excitatory inputs from lateral PB, a pontine nucleus that contains a rich expression of calcitonin gene-related peptide (CGRP)[12,13]. CeLC mainly consists of two non-overlapping cell populations; namely, protein kinase C delta (PKCδ) and somatostatin (SOM)[14]. Evidence suggests that PKCδ(+) neurons are the main targets of synapses from PB-CGRP terminals[15–18], while SOM(+) neurons receive inputs primarily from CGRP-negative PB cells[19–21]. Interestingly, an increased PB-CeLC neurotransmission and CeLC neuron hyperexcitability have shown in inflammatory[7,22,31,23–30] and SNL pain[25,32,33] models. Notably, consistent with the cell-type specific innervations of PB-CeLC, hyperexcitability in neuropathic pain and increased neurotransmission in the inflammatory pain model were specifically observed in the CeLC PKCδ(+) neuron[19,27]; however; no change in excitability[19], but a decrease in synaptic efficacy[21], were found in CeLC SOM(+) neurons in neuropathic pain models. These results suggest that the CeA can function as a pain rheostat, amplifying or suppressing pain-related behaviors in a cell type-specific manner[19]. Additionally, it was recently discovered that a neural circuit consisting of vagal afferents-nucleus tractus solitarius (NTS)-PB mediates anorexia. Notably, cisplatin treatment has been shown to activate neurons in the NTS, PB, and CeA[4,34–36]. Further, reducing activity of the PB CGRP neurons that project to CeLC or inhibition of glutamate receptors in CeA was found to reduce cancer- and cisplatin-induced anorexia[3,4,10]. Altogether, it seems that the PB is a core en-route center transmitting nociceptive and feeding signals to CeLC that integrate and interpret the sensory information. However, despite these promising results, a mechanistic understanding of the regulation of PB CGRP-CeLC glutamatergic synapses is lacking. Recently, we have discovered a novel role of GluD1-Cbln1 signaling in PB-CeLC synapses in CFA and SNL pain conditions[2].

GluD1-Cbln1 signaling in the PB-CeLC neurotransmission in pain: Neuroanatomical studies have shown enriched expression of GluD1 in CeLC[37] but the expression of Cbln1, Cbln2 or Cbln4 mRNA is absent in this region[38,39]. Interestingly, Cbln1 is highly expressed in PB neurons, which is a main source of glutamatergic inputs to CeLC[38,39], suggesting that the GluD1-Cbln1 complex may contribute to the formation/maintenance of PB-CeLC glutamatergic synapses. We have recently shown that GluD1 is preferentially associated with PB-CGRP synapses on PKCδ(+) neurons in CeLC (Fig. 2). Furthermore, we also showed that GluD1 is critical for normal glutamatergic neurotransmission in CeLC neurons, such that deletion of GluD1 leads to a reduction in the frequency and amplitude of mEPSC (Fig. 4) and a decrease in the amplitude of evoked EPSC at PB-CeLC synapses (Fig. 3). Interestingly, we observed a downregulation of somatic and punctate GluD1 expression in CeLC PKCδ(+) neurons in both CFA and SNL pain models. Notably, intra-CeA injection of recombinant Cbln1 restored GluD1 downregulation and mitigated pain-like behavior in CFA and SNL pain models[2]. Additionally, in our preliminary studies, ICV injection of Cbln1 reduced cisplatin-induced mechanical hypersensitivity (Fig. 5) and increased body weight (Fig. 6), suggesting a possible role of GluD1-Cbln1 signaling in cisplatin-induced neuropathic pain and anorexia. *Based on our previous results and preliminary data, we hypothesize that GluD1-Cbln1 is downregulated at PB-CeLC synapses following cisplatin treatment, which leads to neuroplasticity responsible for persistent pain and anorexia. Therefore, we predict that restoration of GluD1-Cbln1 signaling may serve as a potential novel therapeutic strategy to mitigate cisplatin-induced neuropathic pain and anorexia.*

C. Innovation: This proposal is innovative because it seeks to understand novel mechanisms regulating the plasticity at PB-CeLC synapses following cisplatin treatment. It is also innovative because it will address the novel concept that a GluD1-Cbln1 trans-synaptic complex is a critical regulator of the PB-CeLC synapses and impairment of this structural signaling contributes to cisplatin-induced neuropathic pain and anorexia. This

knowledge may lead to the identification of therapeutic targets to overcome cisplatin-induced anorexia and weight loss. In addition, the study seeks to identify therapeutic potentials of Cbln1 in cisplatin-induced neuropathic pain and anorexia. Finally, the data generated in this project will be utilized in developing broader projects that can eventually serve as a general theme for future research on drug development.

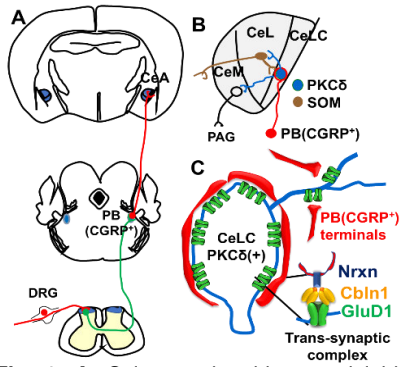


Fig. 1: A. Spino-parabrachio-amygdaloid pathway. **B.** CeLC division of the CeA receives glutamatergic inputs from PB-CGRP+ terminals. **C.** Preferential localization of GluD1, postsynaptic to CGRP terminals on soma and dendrites of PKCδ(+) cells. This, together with known expression of Cbln1 in PB neurons, forms the premise of the trans-synaptic signaling complex as the basis for structural integrity at the PB-CeLC synapse[2].

D. Approach:

Specific Aim 1: Determine cisplatin-induced changes in GluD1-Cbln1 signaling and neuroplasticity in the PB-CeLC circuitry and to test a rescue approach using recombinant Cbln1.

Rationale: An elegant set of data showed a specific expression of GluD1 in CeLC and its binding partner Cbln1 in PB[37–40]. Importantly, ablation of the GluD1 receptor reduced excitatory transmission in CeA and a decrease in the amplitude of evoked EPSC at PB-CeLC synapses[2], suggesting a critical role of GluD1-Cbln1 signaling in the regulation of PB-CeLC synapses. Interestingly, inflammatory or neuropathic pain conditions resulted in downregulation of somatic and punctate GluD1 expression in CeLC PKCδ(+) neurons and upregulation of GluA1 AMPA receptor[2]. Exogenous injection of recombinant Cbln1 into CeA rescues mechanical hypersensitivity in a CFA pain model and normalizes upregulation of the GluA1 subunit. Notably, cisplatin treatment has shown to activate neurons in the PB and CeA and reducing the activity of PB CGRP neurons that project to CeLC or inhibition of glutamate receptors in CeA was found to reduce cancer- and cisplatin-induced anorexia[3,4,10]. On a similar line, ICV injection of recombinant Cbln1 protein reduced mechanical hypersensitivity and rescued reduction in body weight in cisplatin-treated mice, suggesting a potential role of GluD1-Cbln1 signaling in cisplatin-induced pain and anorexia. However, the anatomical substrate and functional role of these interactions remain to be established in cisplatin's effects. To address this knowledge gap, we will use confocal imaging, and electrophysiology. The effect of conditional deletion of GluD1 in CeA neurons on cisplatin-induced pain and anorexia will also be tested. Finally, we will test the rescue strategy using recombinant Cbln1 injection in CeA.

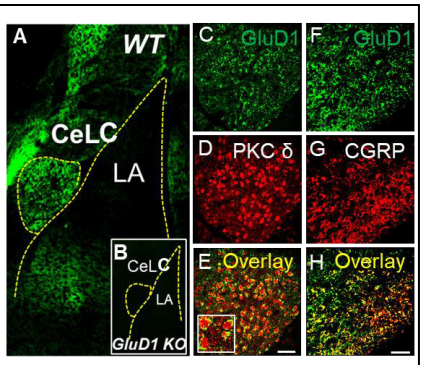


Fig 2: A. Predominant expression of GluD1 in the CeL and CeLC. **B.** No labeling seen in GluD1 KO. **C-E.** GluD1 is preferentially localized to PKCδ in the CeLC and CeL. GluD1 appears perisomatic (inset) and punctate in PKCδ-(+) neurons. **F-H.** Soma and punctate apposition of GluD1 with CGRP terminals in CeLC[2]. Scale bar 10 µM.

Experiment 1.1: Evaluation of cell-type and input-specific changes in GluD1 and Cbln1 following cisplatin.

Animal models/Interventions: We will utilize wildtype (WT), GluD1 KO, and Cbln1-Cre mice. WT mice will be treated with saline or cisplatin and assessed for neuropathic pain (mechanical hypersensitivity) using the von Frey filament test, and anorexia (food intake and body weight). Cisplatin will be injected (3-5 mg/kg, intraperitoneal (ip)) once a week for 4-6 weeks (cumulative dose: 20-30 mg/kg, ip) to induce neuropathy[41–45]. Cisplatin will be diluted in sterile 0.9% saline and injected at a volume of 10 mL/kg of body weight. Prior to each cisplatin treatment, each mouse will be treated subcutaneously with 1 mL of 4% sodium bicarbonate to prevent nephrotoxicity-induced lethality[41]. Pain induction and stability will be monitored by testing behaviors every 4 days after the initial injection using von Frey filament (IITC Life Sciences, Woodland Hills, CA). The control group will receive an equivalent volume of saline (ip) in lieu of cisplatin. After establishing significant behavioral effects (increased mechanical hypersensitivity and reduction in body weight), these mice will be perfused, and brains will be used for immunohistochemistry. GluD1 KO will be used to confirm the specificity of GluD1 immunolabeling and Cbln1-Cre line in combination with AAV-hSyn-DIO-eGFP to fluorescently label Cbln1-expressing neurons in PB.

Experimental Design: Confocal imaging will be performed as described in previous publications[2,46]. Cell-type-specific changes in expression of GluD1 in the CeLC will be determined using a highly specific GluD1 antibody[37] together with antibodies for PKCδ, SOM, or CRF, a marker of a subset of neurons involved in pain modulation[21,47–49]. We will conduct similar co-localization studies using a commercially available highly specific

Cbln1 antibody[39]. Input-specific expression of GluD1 will be examined by performing co-labeling of GluD1 with vGluT1 (for BLA projections) and vGluT2 or CGRP (for PB projections). To specifically label terminals of Cbln1-expressing PB neurons, we will use Cbln1-Cre mice (MMRRC, Stock No. 043695-JAX) and Cre-dependent AAV-hSyn-DIO-eGFP injection into the PB. We have standardized AAV injections in PB (Fig. 7). The CeLC tissue will be processed for dual localization of GFP and GluD1. Approximately 25 random z-stack images will be captured per animal using Leica TCS SP8 MP or Nikon spinning disc confocal microscope and the cell-type and input specific changes in expression will be analyzed using Volocity and Imaris software.

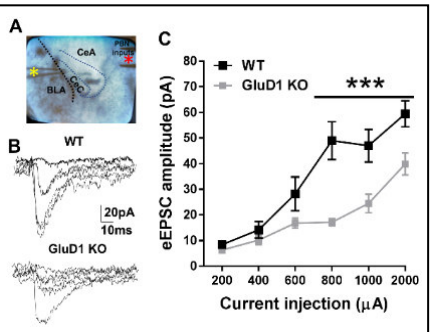


Fig 3: Lower neurotransmission at PB-CeLC synapses in GluD1 KO. **A.** Experimental strategy. **B.** Example traces at various magnitude of current injection. **C.** Significantly lower evoked EPSC amplitude in GluD1 KO mice[2]. N=16-25 from 4 mice. Repeated measure ANOVA, ***p<0.001

Expected outcomes: We predict that the cisplatin will progressively reduce the GluD1 and Cbln1 expression in CeLC neurons and PB terminals, respectively. In confocal microscopy studies, we predict a preferential changes in localization of GluD1 on the PKCδ neurons in cisplatin injected mice. Based on our preliminary data, we do not expect to find GluD1-Cbln1 expression in SOM(+) neurons; we will determine whether CRF(+) neurons that also receive PB input express GluD1.

Experiment 1.2: Determine the effect of cisplatin-induced changes in neuroplasticity in the PB-CeLC circuitry and the effect of a rescue strategy using recombinant Cbln1.

Animal models/Interventions: We will utilize WT (PKCδ-Cre) and GluD1$^{flox/flox}$PKCδ-Cre mice with or without Cbln1 treatment. The animals will be treated similarly as described in experiment 1.1. The recombinant Cbln1 (250-500 ng/side, intra-CeA) will be injected after each cisplatin injection. For Cbln1 injections, mice will be cannulated at CeA as described previously[2].

Experimental Design: Electrophysiology studies will be conducted to determine functional consequences of molecular and structural changes, particularly at PB-CeLC synapses and in PKCδ(+) neurons in pain models and rescue by Cbln1. Since our previous data suggest preferential expression of GluD1 on PKCδ(+) neurons, our electrophysiology studies will focus on this cell type. Experiments will be conducted in tissue from cisplatin- and saline-treated mice using WT (PKCδ-Cre) and GluD1$^{flox/flox}$PKCδ-Cre mice. Four groups for each pain model and genotypes will be used: saline-vehicle, cisplatin-vehicle, saline-Cbln1, and cisplatin-Cbln1. Patch-clamp recordings of synaptic transmission (mEPSC, mIPSC and evoked PB synaptic responses) and neuronal properties (excitability) will be obtained from fluorescently labeled PKCδ neurons in the PKCδ-cre mouse line to determine changes in the cisplatin-induced pain models and rescue with Cbln1. We will utilize electrical stimulation of the PB track to evoke responses at the PB-CeLC synapses. sIPSCs will be recorded from non-PKCδ neurons to assess potential changes in feedforward inhibition from PKCδ(+) neurons in cisplatin-induced pain models and rescue by Cbln1.

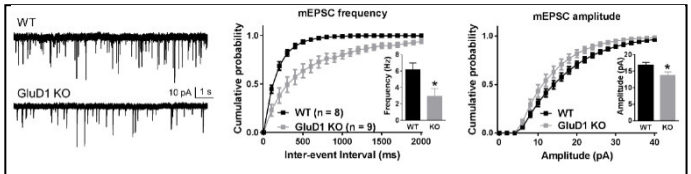


Fig 4: Lower excitatory neurotransmission in CeLC of GluD1 KO. Whole-cell recordings from CeLC neurons revealed significantly lower mEPSC frequency and amplitude in GluD1 KO. N=8-9 from 2 mice/genotype. Unpaired t-test, *p<0.05.

Expected outcomes: We predict that the cisplatin-treated mice will show a dysfunction of excitatory neurotransmission in PKCδ(+) neurons, which will be reversed by the Cbln1 injection into CeA. Furthermore, we predict that the beneficial effect of Cbln1 treatment will not be observed in GluD1 conditional KO animals, suggesting a requirement of GluD1 for the synaptic restoration effect of Cbln1. Irrespective of the direction of these synaptic changes, the validation of recombinant Cbln1 in normalizing synaptic function and behavior will identify novel pain mechanisms and therapeutic targets.

Limitations and alternative approaches: (1) The GluDs mediate neuroplasticity may involve both pre and post-synaptic structural and functional mechanisms such as the subunit composition or localization of postsynaptic AMPA or mGluR1/5 receptor, two receptor subtypes known to interact with GluDs[50]. Thus, additional confocal studies could be conducted to further address these issues to analyze changes in the subcellular and subsynaptic localization of these glutamate receptor subtypes in the CeLC of CFA and SNL animals. (2) Our proposal primarily focuses on evaluating the role of GluD1-Cbln1 signaling in PB-CeLC synapses. However, it is possible that cisplatin-induced neuropathic pain and anorexia may be regulated by separate neural circuits. In that case, we will search for an alternative pathway that may control one or other functions. Indeed, enriched

expression of GluD1 is shown in the oval region of bed nucleus of stria terminalis (ovBNST), which receives dense innervations from Cbln1 containing PB[51]. Notably, a role of ovBNST has been shown in inflammation-associated feeding behavior[51]. Therefore, alternatively, we will evaluate the role of the PB-ovBNST circuit in cisplatin-induced anorexia.

Specific Aim 2: Determine the effect of restoration of GluD1-Cbln1 signaling in the CeA on cisplatin-induced neuropathic pain and anorexia behaviors.

Rationale: The role of CeA in pain and cisplatin-induced anorexia has been well established[9,42,52,53]. Recently, we have demonstrated a critical role of GluD1-Cbln1 signaling in CeLC in CFA and SNL-induced pain behaviors[2]; however, the role of GluD1-Cbln1 in PB-CeLC circuit in cisplatin-induced neuropathic pain and anorexia remains to be tested. Our preliminary data demonstrated that, ICV injection of Cbln1, reduced mechanical hypersensitivity and increased body weight in cisplatin injected mice, however, the mechanisms underlying Cbln1 effects remained unidentified. Experiments in this aim will determine whether restoring the GluD1-Cbln1 signaling complex with intra-CeA injection of recombinant Cbln1 or overexpression of GluD1 in CeA PKCδ(+) neurons inhibits cisplatin-induced neuropathic pain and prevent anorexia and weight loss.

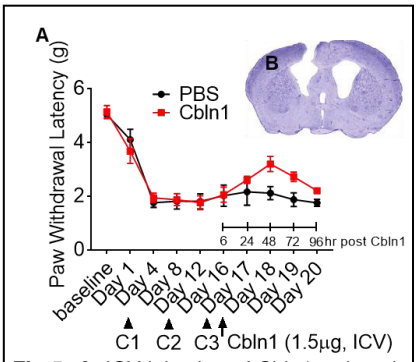


Fig 5: A. ICV injection of Cbln1 reduced mechanical hypersensitivity in cisplatin injected (5 mg/kg, ip) WT mice. **B.** Placement of guide cannula into lateral ventricle. C1, C2, and C3 shows cisplatin injections on day 1, 7, and 14 respectively. N=5 mice/group

Experiment 2.1: Determine the effect of intra-CeA injection of recombinant Cbln1 (rescue strategy) on cisplatin-induced neuropathic pain and anorexia behaviors.

Experimental Design: WT and global GluD1 KO mice will be cannulated into the CeA as described[2]. Behavioral testing will start 1 week after surgical implantation of guide cannula bilaterally into the CeA. Animals will be divided into 4 treatment groups: saline-vehicle, cisplatin-vehicle, saline-Cbln1, and cisplatin-Cbln1. Animals will be habituated to the testing location, handling, and the testing apparatus. After that, we will perform baseline measurement of nocifensive responses (day -3) to assess mechanical sensitivity (von Frey filament test) and cold allodynia (acetone test) of the paw. *Mechanical sensitivity* will be assessed as follows: *von Frey filament test:* We will analyze mechanical withdrawal threshold using an electronic von Frey apparatus (IITC Life Science Inc.) applied to plantar surface hind paws to identify the force needed to obtain a paw withdrawal response. Two replicates will be obtained for each paw. *Cold allodynia* will be assessed using the acetone test by applying a drop (approximately 20 µL) of acetone to the plantar surface of the right and left hind paws. Time spent attending to the acetone-stimulated paw will be measured over a 60-s observation period after acetone application is recorded. *Bodyweight and food consumption* as a measure of anorexia will be assessed daily by weighing the animal and the food. Bodyweight changes will be expressed as percentage changes from baseline, and food consumption will be expressed cumulatively as grams consumed[45]. Cisplatin will be administered as described in experiment 1.1. Recombinant Cbln1 (or vehicle) will be injected into the CeA (250-500 ng/side) 2 days after cisplatin injection, based on preliminary analysis (see Fig. 6). Additional time points will be included, if necessary, based on further analysis.

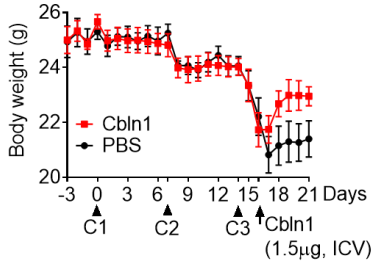


Fig 6: ICV injection of Cbln1 restored body weight in cisplatin injected (5 mg/kg, ip) WT mice. C1, C2, and C3 shows cisplatin injections on day 1, 7, and 14 respectively. N=5 mice/group.

Expected outcomes: We predict that intra-CeA injection of recombinant Cbln1 will mitigate cisplatin-induced neuropathic pain and anorexia behaviors. Such findings would be consistent with our preliminary data (Fig. 5-6) and support our hypothesis that downregulation of GluD1 and, consequently, synaptic dysfunction contribute to the persistence of neuropathic pain conditions but can be rescued by the synapse maintenance effects of Cbln1. We predict that Cbln1 will not be able to mitigate pain-like behaviors in GluD1 KO, demonstrating the requirement of GluD1 for the beneficial effects of Cbln1.

Experiment 2.1: Determine the effect of specific manipulations of the PB-PKCδ(+) circuitry on cisplatin-induced neuropathic pain and anorexia behaviors via overexpression of GluD1 receptor in CeLC.

Experimental Design: We will conduct cell-type-specific overexpression studies through bilateral stereotaxic injections of either AAV2/9-hSyn-DIO-mGluD1 vector (generated commercially, Lot#2494) or AAV2/9-hSyn-

mGluD1 (Lot#2495, Canadian neurophotonic platform) into the CeA of PKCδ-Cre mice or WT mice. Three weeks after delivery of the GluD1 AAVs or AAV-mCherry (control vectors), the cisplatin will be administered, and neuropathic pain and anorexia behaviors will be tested as described in Exp 2.1.

Expected outcomes: We predict that the overexpression of GluD1 in CeA PKCδ(+) neurons will reduce mechanical hypersensitivity and anorexia behaviors in the cisplatin-treated mice. These effects will be absent in control virus-injected mice.

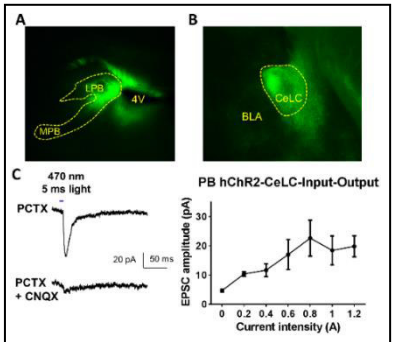


Fig 7: Optogenetic stimulation of PB-CeLC synapses. A. AAV-ChR2 was injected into the PB. **B.** Labeled fibers from PB in the CeLC. **C.** Light (470 nm) stimulated AMPA EPSCs from CeLC neurons in WT mice (N=3).

Limitations and alternative approaches: We have proposed to investigate the PB-CeLC circuit based on our preliminary data obtained using ICV administration of Cbln1. Therefore, to test the selectivity of GluD1-Cbln1 signaling for the PB-CeLC amygdala pain pathway, we will assess the effect of recombinant Cbln1 or GluD1 injection in the ventrobasal thalamus, the main target of the spinothalamic pain pathway, or in other PB projection sites, such as the ovBNST.

Feasibility, sample size, and statistical analysis:

Feasibility: The experimental procedures proposed in this study, including immunohistochemistry, confocal imaging, electrophysiology, AAV injections, and behavioral analysis, are well characterized and standardized in the laboratory and have been published previously[2,46,54] (preliminary data); therefore, we do not foresee any obstacles in carrying out the proposed experiments. Dr. Dravid (Mentor) has extensive experience in GluD1, and NMDA physiology and pharmacology. Dr. Dravid has extend his support for conducting proposed experiments, any guidance for experimental design, troubleshooting, interpretation of results and new ideas.

Sample size: For electrophysiology experiments, 12 or more recordings will be obtained from 6 or more mice in each group. For *in vivo* analyses, 10 or more animals per group will be utilized. Approximately equal numbers of males and females will be used for the experiments. If a significant difference is observed, sex will be included as a variable for the remainder of the experiments.

Statistical Analysis: Data will be analyzed by unpaired t-test and one-way or two-way ANOVA followed by *post hoc* multiple comparison tests, such as the Bonferroni's or Student-Neuman Keuls test. For all statistical analyses, $P<0.05$ will be considered statistically significant. Individuals conducting *in vivo* studies or conducting brain slice electrophysiology will be blinded to the treatment and genotype.

Overall, accomplishing the proposed study will identify crucial role of GluD1-Cbln1 signaling in chemotherapy-induced neuropathic pain and anorexia. I am confident that valuable information about GluD1-Cbln1 function would be assimilated during the award period of two years. Finally, the data generated in this project will be utilized in developing broader projects for submission to extramural funding (NIH R21/R01). Besides, this grant will provide a steppingstone for establishing independent laboratory to work on interdisciplinary research in the field of pharmacology and neuroscience and establishing collaborations amongst the scientist community.

Literature cited:

1. Galanski, M., Jakupec, M. A. & Keppler, B. K. Update of the preclinical situation of anticancer platinum complexes: novel design strategies and innovative analytical approaches. *Curr. Med. Chem.* **12**, 2075–2094 (2005).
2. Gandhi, P. J. *et al.* Dysfunction of Glutamate Delta-1 Receptor-Cerebellin 1 Trans-Synaptic Signaling in the Central Amygdala in Chronic Pain. *Cells* **10**, (2021).
3. Campos, C. A. *et al.* Cancer-induced anorexia and malaise are mediated by CGRP neurons in the parabrachial nucleus. *Nat. Neurosci.* **20**, 934–942 (2017).
4. Alhadeff, A. L., Holland, R. A., Nelson, A., Grill, H. J. & De Jonghe, B. C. Glutamate Receptors in the Central Nucleus of the Amygdala Mediate Cisplatin-Induced Malaise and Energy Balance Dysregulation through Direct Hindbrain Projections. *J. Neurosci. Off. J. Soc. Neurosci.* **35**, 11094–11104 (2015).
5. Gauriau, C. & Bernard, J.-F. Pain pathways and parabrachial circuits in the rat. *Exp. Physiol.* **87**, 251–258 (2002).
6. Neugebauer, V., Li, W., Bird, G. C. & Han, J. S. The amygdala and persistent pain. *Neurosci. a Rev. J. bringing Neurobiol. Neurol. psychiatry* **10**, 221–234 (2004).
7. Han, J. S., Li, W. & Neugebauer, V. Critical role of calcitonin gene-related peptide 1 receptors in the amygdala in synaptic plasticity and pain behavior. *J. Neurosci. Off. J. Soc. Neurosci.* **25**, 10717–10728 (2005).
8. Han, J. S., Adwanikar, H., Li, Z., Ji, G. & Neugebauer, V. Facilitation of synaptic transmission and pain responses by CGRP in the amygdala of normal rats. *Mol. Pain* **6**, 10 (2010).
9. Neugebauer, V. Amygdala pain mechanisms. *Handb. Exp. Pharmacol.* **227**, 261–284 (2015).
10. Alhadeff, A. L. *et al.* Excitatory Hindbrain-Forebrain Communication Is Required for Cisplatin-Induced Anorexia and Weight Loss. *J. Neurosci. Off. J. Soc. Neurosci.* **37**, 362–370 (2017).
11. Thompson, J. M. & Neugebauer, V. Amygdala Plasticity and Pain. *Pain Res. Manag.* **2017**, 8296501 (2017).
12. Han, S., Soleiman, M. T., Soden, M. E., Zweifel, L. S. & Palmiter, R. D. Elucidating an Affective Pain Circuit that Creates a Threat Memory. *Cell* **162**, 363–374 (2015).
13. Palmiter, R. D. The Parabrachial Nucleus: CGRP Neurons Function as a General Alarm. *Trends Neurosci.* **41**, 280–293 (2018).
14. Haubensak, W. *et al.* Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* **468**, 270–276 (2010).
15. Schwaber, J. S., Sternini, C., Brecha, N. C., Rogers, W. T. & Card, J. P. Neurons containing calcitonin gene-related peptide in the parabrachial nucleus project to the central nucleus of the amygdala. *J. Comp. Neurol.* **270**, 398-399,416-426 (1988).
16. Kruger, L., Mantyh, P. W., Sternini, C., Brecha, N. C. & Mantyh, C. R. Calcitonin gene-related peptide (CGRP) in the rat central nervous system: patterns of immunoreactivity and receptor binding sites. *Brain Res.* **463**, 223–244 (1988).
17. de Lacalle, S. & Saper, C. B. Calcitonin gene-related peptide-like immunoreactivity marks putative visceral sensory pathways in human brain. *Neuroscience* **100**, 115–130 (2000).
18. Dobolyi, A., Irwin, S., Makara, G., Usdin, T. B. & Palkovits, M. Calcitonin gene-related peptide-containing pathways in the rat forebrain. *J. Comp. Neurol.* **489**, 92–119 (2005).
19. Wilson, T. D. *et al.* Dual and Opposing Functions of the Central Amygdala in the Modulation of Pain. *Cell Rep.* **29**, 332-346.e5 (2019).
20. Ye, J. & Veinante, P. Cell-type specific parallel circuits in the bed nucleus of the stria terminalis and the central nucleus of the amygdala of the mouse. *Brain Struct. Funct.* **224**, 1067–1095 (2019).

21. Li, J.-N. & Sheets, P. L. Spared nerve injury differentially alters parabrachial monosynaptic excitatory inputs to molecularly specific neurons in distinct subregions of the central amygdala. *Pain* **161**, 166–176 (2020).

22. Neugebauer, V., Li, W., Bird, G. C., Bhave, G. & Gereau, R. W. 4th. Synaptic plasticity in the amygdala in a model of arthritic pain: differential roles of metabotropic glutamate receptors 1 and 5. *J. Neurosci. Off. J. Soc. Neurosci.* **23**, 52–63 (2003).

23. Han, J. S. & Neugebauer, V. Synaptic plasticity in the amygdala in a visceral pain model in rats. *Neurosci. Lett.* **361**, 254–257 (2004).

24. Bird, G. C. *et al.* Protein kinase A-dependent enhanced NMDA receptor function in pain-related synaptic plasticity in rat amygdala neurones. *J. Physiol.* **564**, 907–921 (2005).

25. Ikeda, R., Takahashi, Y., Inoue, K. & Kato, F. NMDA receptor-independent synaptic plasticity in the central amygdala in the rat model of neuropathic pain. *Pain* **127**, 161–172 (2007).

26. Cheng, S.-J. *et al.* Role of extracellular signal-regulated kinase in synaptic transmission and plasticity of a nociceptive input on capsular central amygdaloid neurons in normal and acid-induced muscle pain mice. *J. Neurosci. Off. J. Soc. Neurosci.* **31**, 2258–2270 (2011).

27. Sugimura, Y. K., Takahashi, Y., Watabe, A. M. & Kato, F. Synaptic and network consequences of monosynaptic nociceptive inputs of parabrachial nucleus origin in the central amygdala. *J. Neurophysiol.* **115**, 2721–2739 (2016).

28. Sato, M. *et al.* The lateral parabrachial nucleus is actively involved in the acquisition of fear memory in mice. *Mol. Brain* **8**, 22 (2015).

29. Watabe, A. M. *et al.* Synaptic potentiation in the nociceptive amygdala following fear learning in mice. *Mol. Brain* **6**, 11 (2013).

30. Crock, L. W. *et al.* Central amygdala metabotropic glutamate receptor 5 in the modulation of visceral pain. *J. Neurosci. Off. J. Soc. Neurosci.* **32**, 14217–14226 (2012).

31. Carrasquillo, Y. & Gereau, R. W. 4th. Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. *J. Neurosci. Off. J. Soc. Neurosci.* **27**, 1543–1551 (2007).

32. Nakao, A., Takahashi, Y., Nagase, M., Ikeda, R. & Kato, F. Role of capsaicin-sensitive C-fiber afferents in neuropathic pain-induced synaptic potentiation in the nociceptive amygdala. *Mol. Pain* **8**, 51 (2012).

33. Ji, G. *et al.* 5-HT(2C) Receptor Knockdown in the Amygdala Inhibits Neuropathic-Pain-Related Plasticity and Behaviors. *J. Neurosci. Off. J. Soc. Neurosci.* **37**, 1378–1393 (2017).

34. Horn, C. C., Ciucci, M. & Chaudhury, A. Brain Fos expression during 48 h after cisplatin treatment: neural pathways for acute and delayed visceral sickness. *Auton. Neurosci.* **132**, 44–51 (2007).

35. De Jonghe, B. C. & Horn, C. C. Chemotherapy-induced pica and anorexia are reduced by common hepatic branch vagotomy in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, R756-65 (2008).

36. Horn, C. C. Brain Fos expression induced by the chemotherapy agent cisplatin in the rat is partially dependent on an intact abdominal vagus. *Auton. Neurosci.* **148**, 76–82 (2009).

37. Konno, K. *et al.* Enriched expression of GluD1 in higher brain regions and its involvement in parallel fiber-interneuron synapse formation in the cerebellum. *J. Neurosci. Off. J. Soc. Neurosci.* **34**, 7412–7424 (2014).

38. Miura, E., Iijima, T., Yuzaki, M. & Watanabe, M. Distinct expression of Cbln family mRNAs in developing and adult mouse brains. *Eur. J. Neurosci.* **24**, 750–760 (2006).

39. Otsuka, S. *et al.* Roles of Cbln1 in Non-Motor Functions of Mice. *J. Neurosci. Off. J. Soc. Neurosci.* **36**, 11801–11816 (2016).

40. Hepp, R. *et al.* Glutamate receptors of the delta family are widely expressed in the adult brain. *Brain Struct. Funct.* **220**, 2797–2815 (2015).

41. Deng, L. *et al.* The maintenance of cisplatin- and paclitaxel-induced mechanical and cold allodynia is suppressed by cannabinoid CB_2 receptor activation and independent of CXCR4 signaling in models of

chemotherapy-induced peripheral neuropathy. *Mol. Pain* **8**, 71 (2012).

42. Thompson, J. M. *et al.* Front and hind paw differential analgesic effects of amitriptyline, gabapentin, ibuprofen, and URB937 on mechanical and cold sensitivity in cisplatin-induced neuropathy. *Mol. Pain* **15**, 1744806919874192 (2019).

43. Guindon, J., Deng, L., Fan, B., Wager-Miller, J. & Hohmann, A. G. Optimization of a cisplatin model of chemotherapy-induced peripheral neuropathy in mice: use of vitamin C and sodium bicarbonate pretreatments to reduce nephrotoxicity and improve animal health status. *Mol. Pain* **10**, 56 (2014).

44. Guindon, J., Lai, Y., Takacs, S. M., Bradshaw, H. B. & Hohmann, A. G. Alterations in endocannabinoid tone following chemotherapy-induced peripheral neuropathy: effects of endocannabinoid deactivation inhibitors targeting fatty-acid amide hydrolase and monoacylglycerol lipase in comparison to reference analgesics following . *Pharmacol. Res.* **67**, 94–109 (2013).

45. Garcia, J. M. *et al.* Inhibition of cisplatin-induced lipid catabolism and weight loss by ghrelin in male mice. *Endocrinology* **154**, 3118–3129 (2013).

46. Shelkar, G. P., Liu, J. & Dravid, S. M. Astrocytic NMDA Receptors in the Basolateral Amygdala Contribute to Facilitation of Fear Extinction. *Int. J. Neuropsychopharmacol.* **24**, 907–919 (2021).

47. McCullough, K. M., Morrison, F. G., Hartmann, J., Carlezon, W. A. J. & Ressler, K. J. Quantified Coexpression Analysis of Central Amygdala Subpopulations. *eNeuro* **5**, (2018).

48. Andreoli, M., Marketkar, T. & Dimitrov, E. Contribution of amygdala CRF neurons to chronic pain. *Exp. Neurol.* **298**, 1–12 (2017).

49. Harrigan, E. A., Magnuson, D. J., Thunstedt, G. M. & Gray, T. S. Corticotropin releasing factor neurons are innervated by calcitonin gene-related peptide terminals in the rat central amygdaloid nucleus. *Brain Res. Bull.* **33**, 529–534 (1994).

50. Yuzaki, M. & Aricescu, A. R. A GluD Coming-Of-Age Story. *Trends Neurosci.* **40**, 138–150 (2017).

51. Wang, Y. *et al.* A bed nucleus of stria terminalis microcircuit regulating inflammation-associated modulation of feeding. *Nat. Commun.* **10**, 2769 (2019).

52. Kato, F., Sugimura, Y. K. & Takahashi, Y. Pain-Associated Neural Plasticity in the Parabrachial to Central Amygdala Circuit : Pain Changes the Brain, and the Brain Changes the Pain. *Adv. Exp. Med. Biol.* **1099**, 157–166 (2018).

53. Veinante, P., Yalcin, I. & Barrot, M. The amygdala between sensation and affect: a role in pain. *J. Mol. psychiatry* **1**, 9 (2013).

54. Liu, J. *et al.* Facilitation of GluN2C-containing NMDA receptors in the external globus pallidus increases firing of fast spiking neurons and improves motor function in a hemiparkinsonian mouse model. *Neurobiol. Dis.* **150**, 105254 (2021).

Budget for: Glutamate delta-1 receptor in cisplatin-induced neuropathic pain and anorexia
Proposal: P2200319
Sponsor: State of Nebraska - LB595
Investigator: Gajanan Shelkar
Project Period: 7/1/2022-6/30/2024

Category	Item	Period 1	Period 2	Total
Salary	Gajanan Shelkar	0	0	0
	Research Associate	35,608	36,644	72,252
	Subtotal Personnel:	**35,608**	**36,644**	**72,252**
Other Costs	Animal Housing	13,490	13,250	26,740
Supplies	Supplies	10,902	10,106	21,008
	Subtotal Non-Personnel:	**24,392**	**23,356**	**47,748**
	Total Sponsor Direct Costs:	60,000	60,000	120,000
	Sponsor F&A:	0	0	0
	Total Sponsor Costs:	**60,000**	**60,000**	**120,000**

Budget

Requesting a 2-year award of $60,000 per year.

	Year 1	**Year 2**	**Total**
Personnel	35,608	36,644	72,252
Animal care cost	13,490	13,250	26,740
Supplies	10,902	10,106	21,008
	60,000	60,000	120,000

The Research Associate will conduct the proposed experiments in this proposal. The PI will discuss with the Research Associate about the experimental design, expected outcomes and future direction. Salary and fringe benefits for 9.6 calendar months is requested for the Research Associate for both Year 1 and Year 2.
Funds are also requested for supplies for electrophysiology, behavioral, immunohistochemistry, and confocal imaging studies and for upkeep of mice (Year 1: $24,392 and Year 2: $23,356).

The award of this grant will substantially increase the productivity of the PI and growth towards independence. It will be helpful in generating publications and preliminary results that would be necessary to achieve federal funding (NIH R21/RO1 scheme). Beside this grant will provide a steppingstone for establishing independent laboratory to work on interdisciplinary research in the field of pharmacology and neuroscience and establishing collaborations amongst the scientist community.

Year 1:
Personnel: Research Associate (9.6 calendar months): Salary $28,000 + Fringe $7,608 = $35,608

Animal: Per diem charge: 112 mice X 365 days X $0.33/day = $13,490

Supplies: Electrophysiology, behavioral, immunohistochemistry, and confocal imaging studies: $10,902

Total Year 1 = $60,000

Year 2:
Personnel: Research Associate (9.6 calendar months): Salary $28,630 + Fringe $8,014 = $36,644

Animal: Per diem charge: 110 mice X 365 days X $0.33/day = $13,250

Supplies: Electrophysiology, behavioral, immunohistochemistry, and confocal imaging studies: $10,106

Total Year 2 = $60,000

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Shelkar, Gajanan P.

eRA COMMONS USER NAME (credential, e.g., agency login): gshelkar

POSITION TITLE: Resident Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
MSBTE, India	Diploma of Pharmacy	06/2005	Pharmacy
Aurangabad University, India	Bachelor of Pharmacy	07/2008	Pharmacy
Nagpur University, India	Master of Pharmacy	11/2010	Pharmacology
Nagpur University, India	Ph.D.	10/2015	Pharmacology
Creighton University, USA	Research Associate	10/2017	Pharmacology and Neuroscience
Creighton University, USA	Postdoctoral Research Associate	01/2022	Pharmacology and Neuroscience
Creighton University, USA	Resident Assistant Professor	Present	Pharmacology and Neuroscience

A. Personal Statement

My main research interest is in understanding the neurobiology of central glutaminergic systems and associated clinical conditions, emphasizing GluN2C- and GluN2D-containing NMDA receptors and Glutamate delta-1 (GluD1) receptor. During my post-graduation in pharmacology, I got an opportunity to work on a neuroscience-related topic for my dissertation. While working on the project, behavioral assays attracted my attention, and I developed a strong interest in neuropharmacology. From then to my Ph.D., I have worked on finding mechanistic circuits of various neurological disorders and their correlates with pharmacotherapy.

Currently, I am working on several projects which aim to study the physiology and pharmacology of NMDA receptors GluN2C and GluN2D subunits and GluD1 receptor in some central affective disorders like inflammatory and neuropathic pain, addiction, autism, and schizophrenia by using multidisciplinary approaches such as genetic models, electrophysiology, immunohistochemistry and confocal imaging, optogenetics, viral vectors, and behavioral assays.

The present study aims to understand the novel role of GluD1 and its transsynaptic binding partner cerebellin-1 (Cbln1) in the regulation of cisplatin-induced neuropathic pain and anorexia. The present study is based on our recent findings on GluD1-Cbln1 signaling in the lateral parabrachial nucleus (LPB)-central lateral part of central amygdala (CeLC) synapses which regulate inflammatory and neuropathic pain conditions. In preliminary data, we found that intra-cranial administration of recombinant Cbln1 protein reduced pain-like conditions and restored cisplatin-induced weight loss. In the proposed study, using molecular biology and electrophysiology methods, I will identify a specific role of the GluD1-Cbln1 signaling in the regulation of cisplatin-induced neuropathic pain and anorexia. We will also test a rescue strategy using recombinant Cbln1 protein to mitigate these devastating chemotherapy-induced adverse effects. These findings will further help develop broader projects that can eventually serve as a general theme for my independent laboratory.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022-present Resident Assistant professor, Department of Pharmacology and Neuroscience, Creighton University, Omaha, Nebraska, USA

2017-2022 Postdoctoral Research Associate, Department of Pharmacology and Neuroscience, Creighton University, Omaha, Nebraska, USA

2016-2017 Research Associate, Department of Pharmacology and Neuroscience, Creighton University, Omaha, Nebraska, USA

2011-2016 Contributory Lecturer, Department of Pharmaceutical Sciences, Nagpur University, Nagpur, India

2013-2016 PhD Scholar, Department. of Pharmaceutical Sciences, Nagpur University, Nagpur, India

2012-2013 Senior Research Fellow, DBT funded research project, Nagpur University, Nagpur, India

2011-2012 Junior Research Fellow, DBT funded research project, Nagpur University, Nagpur, India

2010-2011 Lecturer, S. K. B. college of Pharmacy, Nagpur University, Nagpur, India

Honors

2021 Received the prestigious ***2021 Trainee Professional Development Award (TPDA)*** from Neuroscience 2021, Society for Neuroscience conference", Chicago, IL, USA.

2019 Selected as a ***Molecular Pharmacology*** **Highlighted Trainee Author for the August 2019** issue for the article published in the Molecular Pharmacology entitled "Modulation of Burst Firing of Neurons in Nucleus Reticularis of the Thalamus by GluN2C-containing NMDA Receptors" https://www.aspet.org/aspet/news/news/2019/07/17/molecular-pharmacology-highlighted-trainee-author-august-2019.

2016 Awarded with **Uvnas Prize** at 49th Annual Conference of Indian Pharmacological Society organized by PGIMER, Chandigarh, India for the research article published in Addiction Biology (2016; 21, 766–775) entitled "Neuropeptide Y system in accumbens shell mediates ethanol self-administration in posterior ventral tegmental area" by Borkar CD, Upadhya MA, Shelkar GP, Subhedar NK, Kokare DM.

2016 The research paper entitled "Nitric oxide-α2-adrenergic receptor interaction within locus coeruleus underlies facilitation of inhibitory avoidance memory by agmatine. **British Journal of Pharmacology**, [2016, 173(17):2589-2599] was considered as **editorial pick in hot content as best paper** in an editor's choice and made free for the readers.

2013 Worked as a **member local organizing committee** at the 45th annual conference of Indian Pharmacological Society and International Conference on 'Navigating Pharmacology towards safe and effective therapy' held at Nagpur.

2013 **Resource person in pre-conference workshop** entitled 'Techniques in immunocytochemistry' (**45th Annual Conference of Indian Pharmacological Society** and International Conference on 'Navigating Pharmacology towards safe and effective therapy') held at Nagpur.

2012 Conferred with **first prize in poster presentation** at one-day national conference on "Pharmaceutical science for shaping the future of India" held at Nagpur for the research work entitled "Influence of melanocortin system on ethanol self-administration in high-fat diet-fed rats" by **Shelkar GP**, Kale AD, Kokare DM, Singru PS, Subhedar NK.

2012 **Teaching Assistant in the sixth SERB School in Neurosciences**, organized by the National Institute of Science Education and Research, Bhubaneshwar.

2010 **Teaching Assistant in the workshop** entitled 'Surgical and behavioral techniques in neuropharmacology' organized by Smt. Kishoritai Bhoyar College of Pharmacy, New Kamptee, Nagpur in association with Indian Pharmacological Society.

2006-07 **Best poster award** at National Pharmacy Week, December 2007 organized by Indian Pharmaceutical Association, Aurangabad branch.

Citations:

1. Gandhi PJ, Gawande DY, Shelkar GP, Gakare SG, Kiritoshi T, Ji G, Misra B, Pavuluri R, Liu J, Neugebauer V, Dravid SM (2021). Dysfunction of glutamate delta-1 receptor-cerebellin 1 trans-

synaptic signaling in the central amygdala in chronic pain. Cells, 10(10):2644. PMCID: PMC8534524.
2. Shelkar GP, Liu J and Dravid SM (2021). Astrocytic NMDA receptors in the basolateral amygdala contribute to facilitation of fear extinction. Int J Neuropsychopharmcol. 24(11): 907-919. PMCID: PMC8598288.
3. Liu J, Shelkar GP, Gandhi PJ, Gawande DY, Hoover A, Villalba RM, Pavuluri R, Smith Y, Dravid SM (2020). Striatal glutamate delta-1 receptor regulates behavioral flexibility and thalamostriatal connectivity. Neurobiology of Disease, 137:104747. PMCID: PMC7204410.
4. Gawande DY*, Shelkar GP*, Liu J*, Ayala A, Pavuluri R, Choi D, Smith Y, Dravid SM (2021). Glutamate Delta-1 receptor regulates inhibitory neurotransmission in the nucleus accumbens core and anxiety-like behaviors. Molecular Neurobiology, 58(10):4787-4801. PMCID: PMC8500932.

C. Contributions to Science

Complete List of Published Work in MyBibliography:

https://pubmed.ncbi.nlm.nih.gov/?term=Shelkar+G

1. **Astrocytic NMDA receptors in physiology and function:** During my postdoctoral training, we have identified unique expression of GluN2C-containing NMDA receptor in mouse brain using a novel reporter mouse line and then explored their role in fear extinction in basolateral amygdala.
   a) Ravikrishnan A, Gandhi PJ, **Shelkar GP**, Liu J, Pavuluri R, Dravid SM (**2018**). Region-specific expression of NMDA receptor GluN2C subunit in parvalbumin-positive neurons and astrocytes: analysis of GluN2C expression using a novel reporter model. Neuroscience, 380:49-62
   b) **Shelkar GP***, Liu J, Dravid SM **(2021)**. Astrocytic NMDA Receptors in the Basolateral Amygdala Contribute to Facilitation of Fear Extinction. Int J Neuropsychopharmacol, 24(11):907-919 (*corresponding author)
2. **NMDA receptor in relation to thalamic circuit and Schizophrenia:** In these projects during my postdoctoral training, we have investigated the role of GluN2C- and GluN2D-containing NMDA receptors in regulation of cortico-limbic-thalamic circuitry. The following articles highlight our findings:
   a) Liu J*, Shelkar GP*, Zhao F, Clausen RP, Dravid SM (2019). Modulation of burst firing of neurons in nucleus reticularis of the thalamus by GluN2C-containing NMDA receptors. Molecular Pharmacology, pii: mol.119.116780. doi: 10.1124/mol.119.116780 (*equal contribution)
   b) **Shelkar GP**, Pavuluri R, Gandhi PJ, Ravikrishnan A, Gawande DY, Liu J, Stairs DJ, Ugale RR, Dravid SM (**2019**). Differential effect of NMDA receptor GluN2C and GluN2D subunit ablation on behavior and channel blocker-induced schizophrenia phenotypes. Scientific Reports, 9(1):7572.
3. **Neurocircuitry of addiction:** During PhD and postdoc, we have investigated the neural circuits involved in reward and reinforcement**.** The following research articles summarizes our work:
   a) Liu J, Gandhi PJ, Pavuluri R, **Shelkar GP**, Dravid SM **(2018)**. Glutamate delta-1 receptor regulates cocaine-induced plasticity in the nucleus accumbens. Translational Psychiatry, 8(1):219.
   b) **Shelkar GP,** Kale AD, Singh U, Singru PS, Subhedar NK, Kokare DM (**2015**). Alpha-melanocyte stimulating hormone modulates ethanol self-administration in posterior ventral tegmental area through melanocortin-4 receptors. Addiction Biology 20; 302-315.
   c) **Shelkar GP**, Kumar S, Singru PS, Subhedar NK, Kokare DM (**2017**). Noradrenergic inputs from locus coeruleus to posterior ventral tegmental area are essential to support ethanol reinforcement. Addiction Biology, 22(2):291-302.
   d) Somalwar AR*, **Shelkar GP*,** Subhedar NK, Kokare DM (**2017**). Cocaine- and amphetamine-regulated transcript (CART) neurons in the lateral hypothalamic nucleus drive the reward behavior. Behavioural Brain Research, 317:340-349. (*equal contribution)
4. **Neurodegeneration, Neuroinflammation and pain:** We investigated the neuroprotective and anti-inflammatory role of endogenous some neuroactive pharmacological agent in neuropathic pain, spinal cord injury and Parkinson's disease.

a) Bharne AP, Upadhya MA, **Shelkar GP**, Singru PS, Subhedar NK, Kokare DM (**2013**). Neuroprotective effect of cocaine- and amphetamine-regulated transcript peptide in spinal cord injury in mice. Neuropharmacology 67; 126-135.
b) Upadhya MA*, **Shelkar GP***, Subhedar NK, Kokare DM (**2016**). CART modulates the effects of levodopa in rat model of Parkinson's disease. Behavioural Brain Research, 301:262-272. (*equal contribution)
c) Liu J*, **Shelkar GP***, Sarode LP, Zhao F, Clausen RP, Ugale RR, Dravid SM (2021). Facilitation of GluN2C-containing NMDA receptors in the external globus pallidus increases firing of fast spiking neurons and improves motor function in a hemiparkinsonian mouse model. Neurobiology of Disease, 150:105254 (*equal contribution)

Learning and Memory: My initial studies aimed to investigate the role of agmatine in learning and memory**.** The following research article summarizes our work:

a) Shelkar GP, Gakare SG, Chakraborty S, Dravid SM, Ugale RR (**2016**). Interactions of nitric oxide with α2 -adrenoceptors within the locus coeruleus underlie the facilitation of inhibitory avoidance memory by agmatine. Br J Pharmacol, 173(17):2589-2599.

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Cellular Signaling and Molecular Trafficking in Cancer
Laura A. Hansen, PhD

Checkpoint Signaling and Cell Survival in Normal and Tumorigenic Skin Keratinocytes

Principal Investigator: Laura A. Hansen, PhD

I. Progress Report Summary

A. Specific Aims

The original aims:

Aim 1. To delineate the UV-activated checkpoint signaling pathways downstream of CDC25A and P53 involving FWE isoforms and Survivin in normal keratinocytes.

Aim 2. To evaluate the mechanisms and consequences of dysregulated checkpoint signaling in initiated keratinocytes, leading to upregulation of cytoplasmic Survivin and an increased FWE^{WIN}/FWE^{LOSE} ratio, that allow for survival of initiated cells following UV- and chemotherapy-induced DNA damage.

To understand how FWE isoforms interact and impact cell cycle checkpoints and carcinogenesis, we have expanded the scope of the work to assess FWE localization and membrane topology.

B. Studies and Results

Flower (FWE) proteins are small, highly conserved, putative membrane proteins produced from a single alternatively spliced *FWE* gene. The human *FWE* locus encodes four isoforms: hFWE1, hFWE2, hFWE3, and hFWE4. It has been proposed that, as in *Drosophila* models, differential expression of hFWE isoforms in neighboring cells is necessary and sufficient to elicit cell competition, a process whereby less-fit cells are non-autonomously eliminated from heterogenous tissues (Madan et al 2019, Rhiner et al 2011). In human cancers, epithelial cells or fibroblasts expressing high levels of hFWE1 or hFWE3 undergo apoptotic cell death only when neighbored by epithelial cells expressing high levels of hFWE2 or hFWE4. However, the molecular mechanisms mediating this hFWE-dependent competition in human cells are entirely unknown. Intriguingly, FWE isoforms appear to have cell competition-independent function, as well. In both fly and mouse, the canonical FWE isoform has been suggested to either act directly as a calcium channel (Yao et al 2009) or to indirectly facilitate calcium-dependent synaptic vesicle endocytosis in terminal boutons at neuromuscular junctions (Yao) and cytotoxic granule endocytosis in cytotoxic T-lymphocytes (Chang et al, 2018). Our RNA sequencing of wild-type (WT) squamous cell carcinoma (SCC) cells during competitive elimination revealed dysregulation of many genes encoding Ca^{2+}-responsive proteins, including matrix metalloproteinases, *S100* family members, as well as *Calreticulin*, *Calmodulin 2* and *Calbindin 2* (data not shown). In experiments completed last year and previously reported, we identified S100A7 as a FWE3-interacting protein and S100A6 as a FWE4-interacting protein, and in other

work discovered that xenografted SCC cells overexpressing tagged FWE4 are underrepresented compared to wild-type SCC cells in the highly proliferative, less differentiated basal cell layer within tumors (data not shown). Analysis of publicly available transcriptomic data for human cutaneous SCC revealed significantly increased expression of S100A7 in the epithelial cell population of skin cancer compared to skin (Fig. 1A-B), while our analysis showed increased transcripts of S100A8/9 in a panel of cutaneous SCC cell lines compared to nontumorigenic HaCaT keratinocytes (Fig. 1C). We also showed that SCC cells overexpressing FWE isoforms found in skin cancers (hFWE2/3/4) have reduced S100A7/A8 transcripts, and S100A9 was reduced in FWE2 and FWE4 overexpressing cell lines (Fig. 1D). S100A7 protein levels were reduced in FWE-overexpressing cells, as well (Fig. 1E).

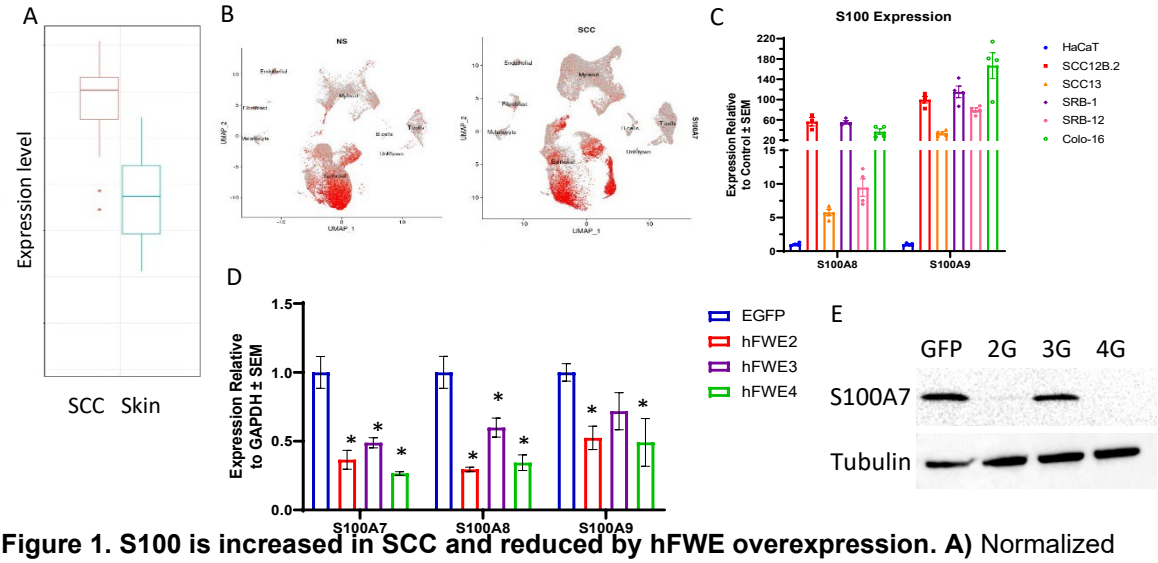


Figure 1. S100 is increased in SCC and reduced by hFWE overexpression. A) Normalized expression values of S100A7 transcripts in SCC and normal skin (NS) samples from four publicly available GEO RNAseq datasets (GSE84293, GSE125285, GSE139505, and ERP106532). **B)** Single cell RNA-seq GEO dataset GSE144236 shows that S100A7 expression is primarily in the epithelial portion of the NS and SCC samples. **C)** RT-qPCR was performed on a non-tumorigenic epithelial cell line (HaCaT) and five human SCC cell lines. S100A8 and S100A9 were all upregulated in the cancer cell lines vs HaCaT. N ≥ 5. **D)** RT-qPCR was performed on stable SCC13 cells overexpressing EGFP or hFWE2/3/4 and EGFP. Values were normalized to GAPDH and then compared to the EGFP control cell line. N = 4, *p ≤ 0.001 by one-way ANOVA with Bonferroni correction. **E)** Immunoblotting on SCC cells overexpressing EGFP or hFWE2/3/4 for S100A7 in SCC13. G=EGFP, 2G=hFWE2 EGFP, 3G=hFWE3 EGFP, 4G=hFWE4 EGFP.

Exposure of cutaneous SCC cells (SCC13 cell line) to ultraviolet irradiation (UV), the main etiologic agent for skin cancer development, increased hFWE2/4 transcripts, while hFWE1/3 trended upward but was not significantly altered under these conditions (Fig. 2). We hypothesize that this UV-induced increase in FWE expression may enhance cell cycle arrest in response to UV. This hypothesis is based on our data showing that increased FWE expression causes a G_0/G_1 cell cycle arrest in SCC cells. As shown in Fig. 3, the G_0/G_1 phase was significantly increased in FWE2, FWE3, and FWE4 overexpressing cells compared to control, with the largest magnitude of effect in the FWE4 cells. Current experiments are focused on testing this hypothesis.

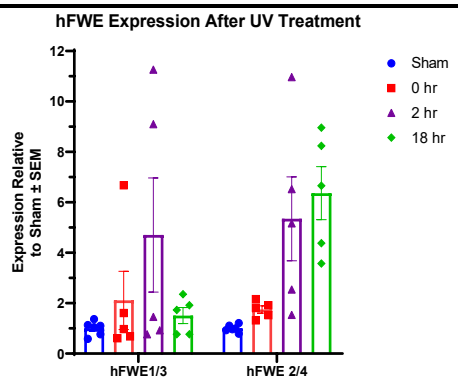


Figure 2. UV irradiation increases FWE expression. SCC13 cells exposed to 600 J/m^2 UVA/B and RNA collected 0, 2, or 18 h later. RT-qPCR for hFWE with GAPDH as a control. N ≥ 5.

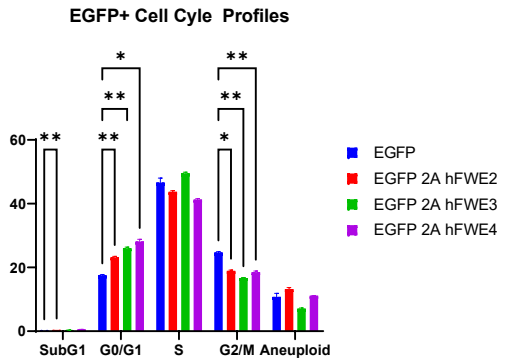


Figure 3. Overexpression of FWE in SCC13 leads to G_0/G_1 arrest. SCC13 were infected with lentivirus encoding EGFP alone or EGFP 2A hFWE2/3/4. Cells were subject to 1.5 h EdU pulse and assayed for cell cycle stage using dual EdU/DNA content labeling.

importance to elucidating FWE function in human cells. To determine FWE3 and FWE4 subcellular localization, lentivirus encoding C-terminal FWE3- or FWE4-EGFP fusion constructs were transduced into HEK293 cells, followed by transfection with constructs expressing fluorescently tagged markers of various subcellular compartments, including the cytosol, ER, endosomes, and mitochondria. Our analyses of these populations are consistent with membrane localization of FWE3/4 in internal organelles, with some signal appearing colocalized with the plasma membrane for FWE4. FWE3-EGFP signal was colocalized with the ER-mCherry marker, while FWE4-EGFP signal colocalized with early and recycling endosomal markers Rab4, Rab5, and Rab11 (Fig. 4).

In addition to precise subcellular distribution, correct determination of FWE isoform membrane topologies is critical to understanding FWE function. FWE isoforms share a common N-terminal domain encoded by exon 1 and exhibit variable inclusion of domains encoded by exons 3 and 5. Hydropathy-based topological prediction (Madan et al 2019) has suggested that this produces multiple transmembrane

The separate functions of FWE isoforms in the published literature appear to rely on different subcellular localizations of FWE, as cell competition reportedly requires plasma membrane localization to facilitate extracellular exposure of isoform specific C-terminal epitopes, while models for calcium-related FWE activity suggest that integration into yet-undefined vesicular and organellar membranes is essential. The subcellular localizations of FWE isoforms have not been experimentally determined and are of critical

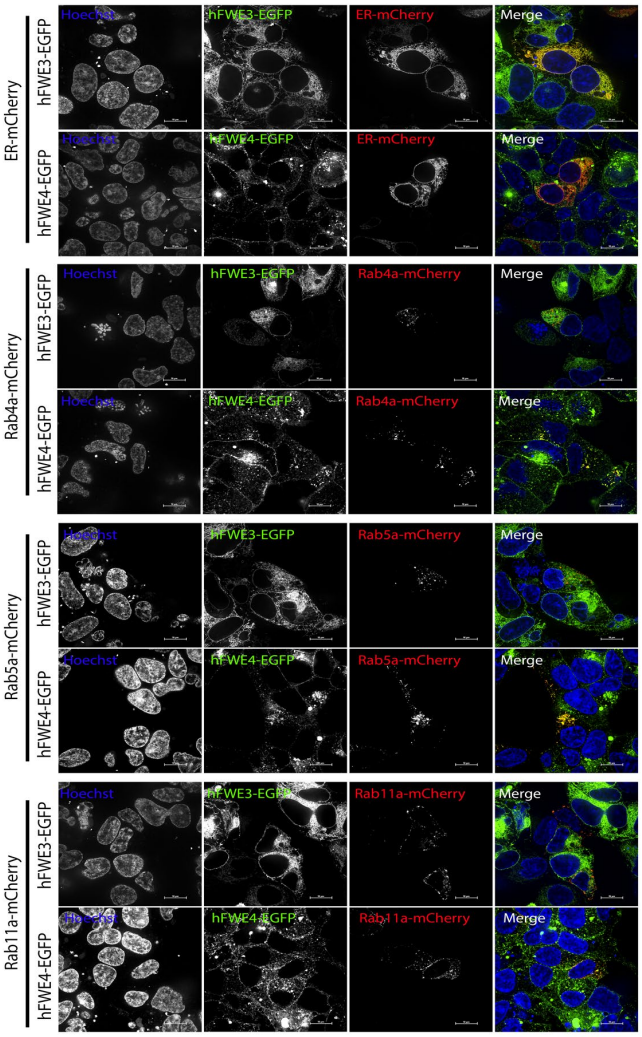


Figure 4. Subcellular localization of FWE3 and FWE4. HEK293 were transduced with lentivirus encoding C-terminal hFWE3 or hFWE4-EGFP fusion constructs. hFWE3- and hFWE4-EGFP-expressing cells were transfected with plasmid-encoding Rab4a-mCherry, Rab5a-mCherry, Rab11a-mCherry or KDEL-mCherry (ER) fusion constructs. 24h post transfection, cells were live imaged at 100x magnification in phenol red free medium on a spinning disc confocal microscope.

domains. Since X-ray structures for the various hFWE isoforms are not available, in collaboration with Dr. Sandor Lovas, we used MD 250 ns long simulations to determine the membrane-embedded structure of FWE3 and FWE4. For the simulations, the initial structures of both FWE3 and hFWE4 were predicted by Alphafold and RoseTTAfold methods. The predicted structure for FWE3 includes three transmembrane (TM) helices. The FWE3 N- and C-terminal tails, residues 1-18 and 103-132, respectively, are predicted to be on opposite sides of the membrane (Fig. 5). For FWE4, models predict four TM helices, with both the N- and C-terminal tails on the same side of the membrane (Fig. 5A-B). The sequences of the N- and C-terminal tails of FWE3 and FWE4 are the same; therefore, their secondary structures are the same. Furthermore, FWE4 residues 51-57 form a membrane surface helical loop on the same side of the of the membrane as the two terminal domains. The N-terminal tail is unordered, while the C-terminal tail is α-helical. CD spectropolarimetry data of the synthetic FWE(1-18)-NH_2 indicates that it has mainly unordered structure in PBS, which is good agreement with the MD simulation results. Moreover, we showed that in 30% 2,2,2-trifluoro-ethanol (TFE) in PBS, the peptide has 46% helicity (Fig. 5C).

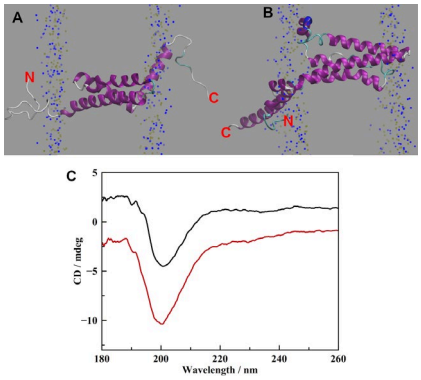


Fig. 5. MD-predicted structure of hFWE3 (A) and hFWE4 (**B**) Red-colored N and C indicate the N- and C-terminal ends of the proteins, respectively. For clarity, only N (blue) and P (gray) atoms are shown in the membrane bi-layer. CD Spectra (**C**) of hFWE(1-18)-NH_2 N-terminal fragment in PBS (black) and in 30% TFE (red) in PBS.

To experimentally determine whether and which domains of FWE3/4 isoforms are present on the surface of SCC cells, FWE3 and FWE4 constructs were epitope-tagged at the N or C-terminus, as well as at various putative loop motifs between predicted transmembrane domains (L1-2, L2-3, etc.). LGR5-834del, a mutant LGR5 that is not internalized from the plasma membrane and presents an extracellular HA or FLAG tagged N-terminus, served as a positive control for surface labeling (top panels Fig. 6A-B). Immunofluorescence analysis using antibodies for the FLAG or HA tags was performed on intact live cells or permeabilized cells. As shown in Fig. 6A-B, neither terminus of FWE3 or FWE4 is detectable on the surface of the cells, although the LGR5 positive control is clearly detected in the intact cells. The loop motif L1-2 and L3-4 did, however, reveal cell surface localization of FWE4 (Fig. 6B). These experiments reveal for the first time the membrane topology of FWE3 and FWE4 experimentally and provide useful information for the design of FWE-FWE binding and functional studies.

Last year, we reported construction of a Crainbow vector suitable for generation of a Crainbow transgenic mouse. The system is designed to allow for lineage tracing of each of the FWE isoforms expressed in skin (FWE2/3/4). The Crainbow transgene contains four distinct cassettes encoding a fluorogen activating protein (FAP) MARS1 in position 1, and unique fluorescent reporters co-expressed with FWE2, 3, and 4 isoforms in positions 2, 3, and 4. Each cassette is flanked by a pair of orthogonal lox sites, enabling stochastic expression of a single FWE isoform and the corresponding fluorescent barcode in CRE+ cells, or MARS1-FAP in CRE- cells. These mice were generated in the past year and crossed with an MMTV-Cre line driving expression in the mammary gland to confirm expression of the fluorescent markers *in vivo* (Fig. 7). These animals will be used for lineage tracing during development of the skin and eventually for skin carcinogenesis experiments in the future.

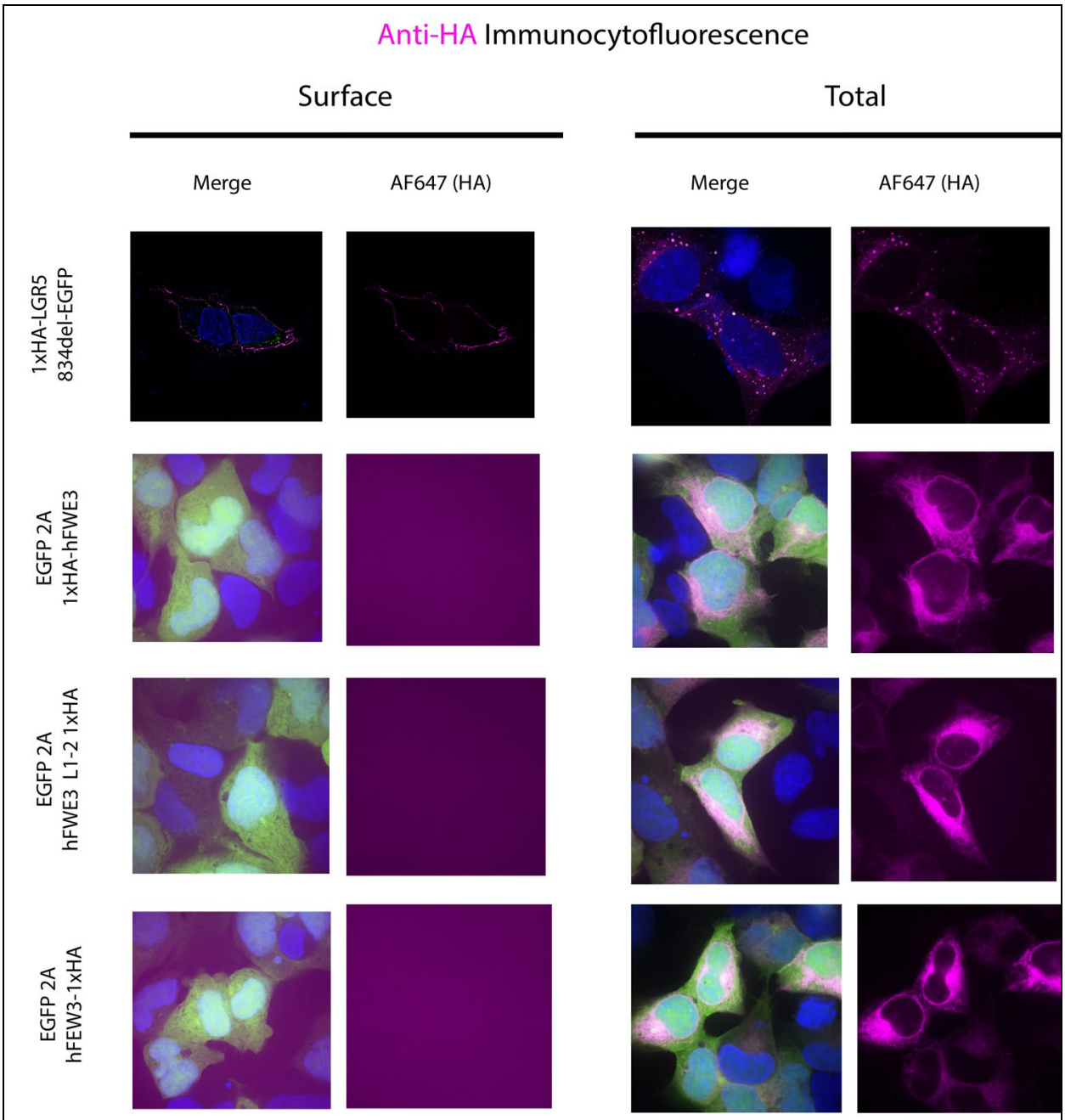


Figure 6A – legend on page 7.

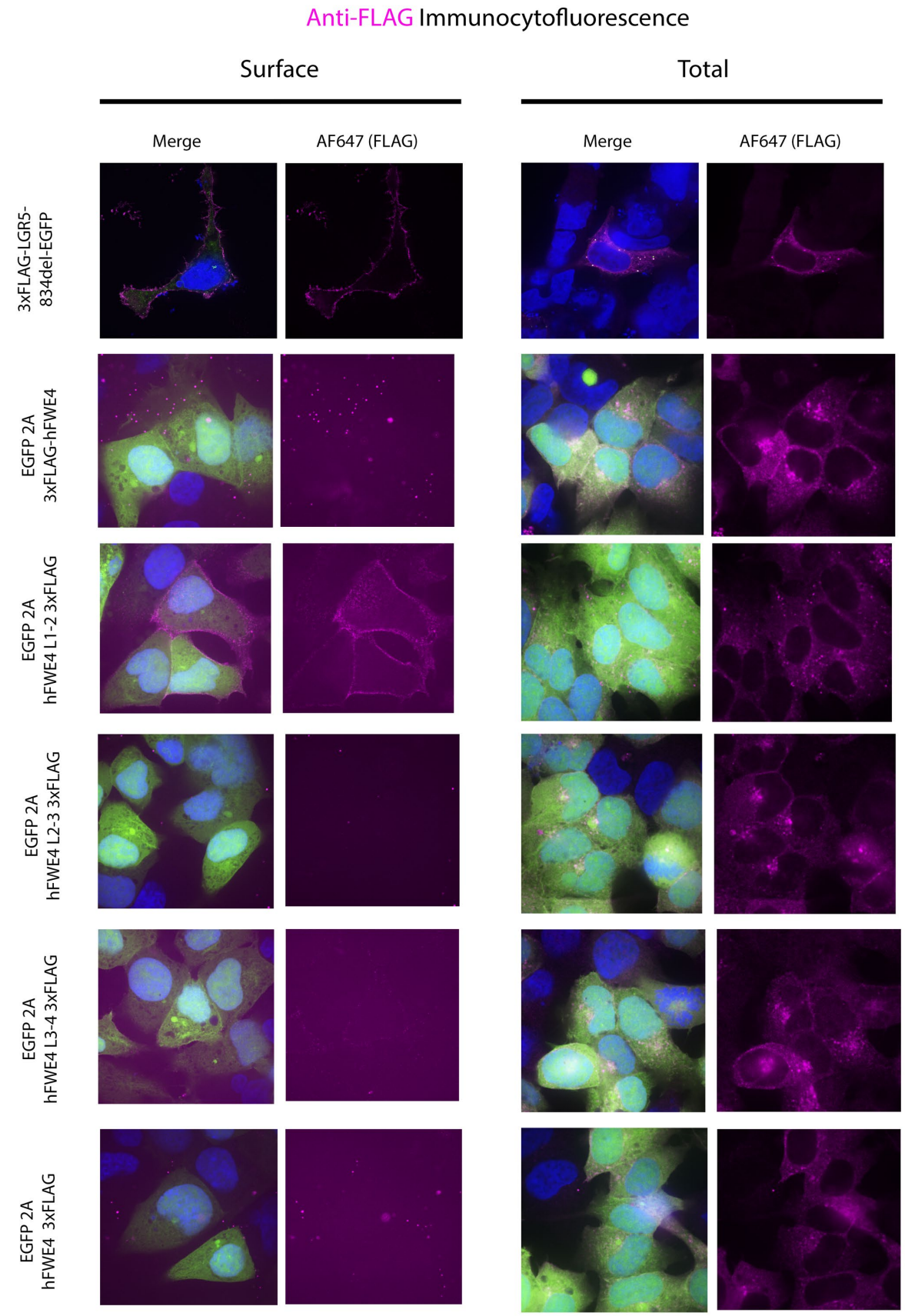


Figure 6B – legend on next page.

Figure 6. Surface detection of hFWE3 and hFWE4.
HEK293 were seeded into CC2-coated chamber slides and transfected with plasmids encoding 1xHA-LGR5-834del-EGFP, 3xFLAG-LGR5-834del-EGFP, EGFP-2A-1xHA-hFWE3, EGFP-2A-hFWE3-L1-2-1xHA, EGFP-2A-hFWE3-1xHA, EGFP-2A-3xFLAG-hFWE4, EGFP-2A-hFWE4-L1-2-3xFLAG, EGFP-2A-hFWE4-L2-3-3xFLAG, EGFP-2A-hFWE4-L3-4-3xFLAG, or EGFP-2A-hFWE4-3xFLAG. For surface labeling, cells were washed in cold staining medium, and pulsed on ice with anti-HA or anti-FLAG primary antibodies, then washed, fixed, incubated in anti-rabbit AF647 secondary antibody, and counterstained with Hoechst. For total protein labeling, cells were fixed and permeabilized in 0.3% Tx-100 prior to immunofluorescence.

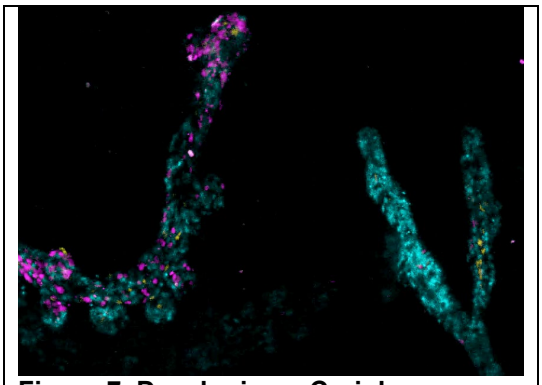


Figure 7. Developing a Crainbow lineage tracing model to evaluate hFWE driven cell competition *in vivo*.
Our CRAINBOW Flower mouse was crossed to a mammary specific MMTV-Cre, revealing recombination and expression of all three colors/isoforms in the mammary gland. FWE2 = teal, FWE3 = yellow, FWE4 = fluorescent orange but false-colored magenta.

C. Significance

Our experiments reveal UV regulation of FWE expression and suggest mechanisms of FWE function. We showed that FWE isoform overexpression causes a G_1-phase cell cycle arrest. We have also shown that FWE isoforms bind to and suppress S100A7/8/9 expression. Our analysis reveals upregulation of S100A7 during skin carcinogenesis, suggesting that FWE suppression of S100A7 may have a tumor suppressive effect. Further, our analyses of FWE localization and membrane topology reveal a complex pattern of FWE3 and FWE4 localization in ER and endosomes, respectively, including some FWE4 cell surface localization. These data were used as preliminary data in our recently funded LB506 award. Generation of a mouse model enabling lineage tracing of FWE isoform overexpressing cells will allow for future studies documenting FWE phenotypes *in vivo*.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

None.

III. List of extramural grants submitted from 7/1/2021–6/30/2022

1. State of Nebraska LB506 Cancer and Smoking Disease Research PI: Hansen
   Title: Molecular determinants of Flower protein-mediated cell competition
   Dates: 7/1/2022-6/30/2023
   Award: $50,000 direct

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

1. State of Nebraska LB506 Cancer and Smoking Disease Research PI: Hansen
   Title: Molecular determinants of Flower protein-mediated cell competition
   Dates: 7/1/2022-6/30/2023
   Award: $50,000 direct

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Cellular Signaling and Molecular Trafficking in Cancer
Program Director: Laura Hansen, PhD

Cellular Pathways Targeting BubR1 to the Proteasome for Degradation: Implications for Skin Cancer
Principal Investigator: Brian North, PhD

I. Progress Report Summary

A. Specific Aims

The specific aims will stay the same for the upcoming budget period.

B. Studies and Results

During this budget year, we have continued to focus our attention on Aims 1 and 2. Aim 1 was set out to "*Define the regulation of the NAD^+/SIRT2/β-TRCP/BubR1 pathway in skin cells following carcinogen exposure*." We have made progress to define downregulation of BubR1 following UV exposure. In particular, we have found that the loss of BubR1 following UV exposure is greater in HaCAT cells (an immortalized but not transformed skin cell line), compared to a cutaneous squamous cell carcinoma line SSC13, which suggests that the loss of BubR1 may have more pronounced effects early in the UV-induced carcinogenesis process. We are currently working on defining the pathway controlling BubR1 loss following UV exposure. We have confirmed that this loss is reversed when blocking the proteosome pathway using MG132, suggesting a role for the ubiquitin-proteosome pathway, as we initially hypothesized. In addition, treating with the compound MLN4924, an NAE inhibitor that blocks neddylation of the Cullin component of these multiprotein ligase complexes, which is a required modification to activate Cullin-RING E3 ubiquitin ligases. Again, this is consistent with our initial hypothesis that β-TRCP may serve as the F-box functioning as the substate recognition subunit of the Skp1-Cul1-F-box (SCF) E3 ubiquitin ligase. We are now in the process of depleting and overexpressing *β-TRCP* to confirm its direct role in this process.

Aim 2 was set out to "*Determine the biological significance of the NAD^+/SIRT2/β-TRCP/BubR1 pathway in carcinogen-induced skin cancer with age and the protective effect of CR* in vivo." All our mouse strains (*SIRT2*, *β-TRCP,* and *BubR1*) are now at least backcrossed to N7; we would anticipate our mice will be ~99% that of the SKH-1 or FVB/N background. During this reporting period, we have focused on determining the effects of nicotinamide mononucleotide (NMN) on UV-induced skin tumorigenesis. This particular set of experiments have been postponed until subsequent years of this grant. In this upcoming budget period, we will be performing crosses with *K14-cre* lines to establish skin-specific deletion of *SIRT2*, *β-TRCP,* and *BubR1* lines to carry out our proposed studies looking at the effect of deletion of components of this pathway on carcinogen (UV and DMBA/TPA)-induced tumorigenesis.

During this reporting period, our animal studies been focused on our experiment to assess the ability of modulators of this pathway, namely nicotinamide mononucleotide (NMN), to suppress UV-induced tumorigenesis. To this end, we have treated young and aged mice with NMN

(administered via drinking water). They were then sham irradiated or irradiated 5 times per week with UV and monitored for tumor development in water- and UV-treated groups in both males and females. Contrary to our hypothesis, we observed that tumors began earlier and progressed more quickly in the NMN-treated mice compared to the control animals. These cohorts were recently euthanized, and we are now processing tissue samples and analyzing for NAD^+ levels, proliferation, DNA damage and cell death markers, as well as expression levels of BubR1, SIRT2, and β-TRCP1. In addition, sections will be stained for hematoxylin and eosin to assess the pathology of tumorigenesis, as well as dermal and epidermal thickness.

Aim 3 was set out to "*Identify novel therapeutic methods to stabilize BubR1 by disrupting its interaction with β-TRCP.*" As discussed in our prior reporting periods, the interaction between these proteins appears to be more complex than previously planned. This interaction is being studied under various conditions, such as UV exposure based on these studies and glucose deprivation based on another area of investigation in the lab, which may promote the interaction between BubR1 and its E3 ubiquitin ligase.

C. Significance

NAD^+ boosters have gained notoriety lately due to their potential to delay aging at the molecular and cellular level. Due to this, compounds such as NMN and nicotinamide riboside (NR), which are cell permeable molecules that are components of the NAD^+ salvage pathway, and are available over-the-counter, have been heavily marketed to the public. Our studies suggest that long term utilization of these compounds to boost NAD^+ may in fact have adverse effects in certain circumstances, such as UV-induced skin tumorigenesis. Thus, our focus is to define how NMN promotes skin tumorigenesis following UV irradiation and to publish this work in the coming reporting period.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

None

III. List of extramural grants submitted from 7/1/2021–6/30/2022

1. **Department of Defense (DoD)** 04/01/2022 - 03/31/2026
   Project Number: CA210355 – Impact Award
   PI: Brian J. North
   Title: Targeting Myc in Lymphoma with a Novel Combination Therapeutic Regimen
   Major Goals: The goal of this research proposal is to determine the combination efficacy and mechanism of action of HDAC6-selective inhibitors and Aurora A kinase inhibitors for the treatment of T- and B-cell lymphomas.
   Effort: 3.0 Calendar Months
   Direct Costs: $1,250,000
   Indirect Costs: $568,750

2. **Department of Defense (DoD)** 04/01/2022 - 03/31/2025
   Project Number: CA210704 – Idea Award
   PI: Brian J. North
   Title: Identification of Novel E3 Ubiquitin Ligases Regulating Hepatocellular Carcinoma Tumorigenesis and Metastasis
   Major Goals: The goal of this research proposal is to utilize an *in vivo* CRISPR/Cas9 screen to identify E3 Ubiquitin Ligases that promote liver cancer (hepatocellular carcinoma) growth

and metastasis.
Effort: 2.0 Calendar Months
Direct Costs: $500,000
Indirect Costs: $227,500

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

National Cancer Institute/NIH 6/01/2022 - 3/31/2027
Project Number: R01 GM145828
PI: Brian J. North
Title: Regulatory Mechanisms Governing BubR1 Protein Stability During Stress and Aging
Major Goals: The goals of these studies are to elucidate the molecular basis of BubR1 decline with age and under conditions of cellular stress.
Effort: 2.0 Calendar Months
Direct Costs: $1,025,000
Indirect Costs: $466,375

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Cellular Signaling and Molecular Trafficking in Cancer
Director: Laura A. Hanson, PhD

Localization of RAG1 Degradation and Implications of RAG1 Stabilization on Genome Instability and Cancer
Principal Investigator: Patrick C. Swanson, PhD

I. Progress Report Summary

A. Specific Aims
The original specific aims are as follows:

1. Establish the cellular localization of RAG1 degradation and identify factors required for this process.
2. Determine if impairing RAG1 turnover increases the frequency of aberrant V(D)J rearrangement and lymphoid cell neoplasia.

B. Studies and Results
For specific aim 1, we focused on several sub-aims.

The first involved determining the mechanism(s) by which treatment of cells expressing full-length RAG1 by the NEDD8 activating enzyme inhibitor, MLN4924, extends RAG1 half-life. Recall that cullin E3 ubiquitin ligases are thought to require neddylation for activation. In the previous cycle, we had worked to address reviewer comments received from a manuscript submitted to PLoS One entitled “The CRL4VPRBP(DCAF1) E3 Ubiquitin Ligase Directs Constitutive RAG1 Degradation in Non-Lymphoid cells.” That manuscript was published during this cycle (Schabla *et al.* 2021).

The second involved establishing a role for the Receptor of activated protein C kinase 1 (RACK1), a protein we identified in a proteomics screen as preferentially binding full-length RAG1, in B cell development and RAG1 turnover. RACK1 was of interest because it functions as a signaling node and an adaptor to an E3 ubiquitin ligase in other systems. Recall that the LB595 grant enabled us to acquire mice harboring a floxed *Rack1* allele and begin breeding them to ultimately generate conditional B cell lineage RACK1 knockout (RACK1-BKO) mice, which enabled us to submit a competitive NIH R21 grant entitled “Role of RACK1 in RAG1 degradation and B cell development” that was funded in June 2021. As a result, the analysis of RACK1-BKO mice transitioned to the NIH grant.

The departure of Dr. Schabla from my laboratory to an industry position late last cycle (spring 2021) left a void in this project. I hired a new technician and she has worked to learn the RAG1 degradation assays. Two new MS students also joined my laboratory in 2021/22 academic year, and have been working to learn these assays as well. One student is working to identify determinants of RAG1 required for mediating RAG1 degradation, focusing on establishing whether a newly identified nucleolar localization signal in the amino-terminal region of RAG1 regulates RAG1 turnover. The second student, working toward a combined PharmD/MS, is working to develop a screening assay to identify potential drug classes from a protein-protein interaction inhibitor library that modulate RAG1 levels selectively for full-length RAG1 (which supports RAG1 degradation), and not an amino-terminal truncated form of RAG1

(which does not associate with $CRL4^{VPRBP(DCAF1)}$ E3 ubiquitin ligase complex and is stabilized against degradation). The approach relies on using GFP-tagged forms of the two RAG1 proteins, and monitoring drug-dependent changes in GFP expression and/or localization using confocal microscopy.

We are also pursuing two new sub-aims relating to Aim 1, which extend from preliminary studies of RACK1-BKO mice. We found that loss of RACK1 expression in Bcl2-transgenic B cells did not reproduce the B cell phenotype observed in comparable mice lacking VprBP(DCAF1) in the B lineage (specifically inversion of the ratio of B cells expressing Igκ or Igλ light chain a Bcl2-transgenic background). These results raised the possibility that RACK1 does not facilitate RAG1 degradation, as might have been expected if RACK1 functions as a co-factor with the $CRL4^{VprBP(DCAF1)}$ E3 ubiquitin ligase. Therefore, the first new sub-aim was to determine whether loss of RACK1 affected levels of other putative targets of RACK1-dependent degradation. Included among those reported were HIF1α and Bim. To test this possibility, we evaluated intracellular levels of both proteins using flow cytometry in RACK1-proficient and RACK1-deficient B cells. Preliminary data suggest that loss of RACK1 in B cells leads to increased levels of Bim, but not HIF1α. Because Bim is a pro-apoptotic factor and RAGs introduce DNA double-strand breaks during V(D)J recombination, this finding raised the intriguing possibility that full-length RAG1 associates with RACK1 to recruit Bim to the $CRL4^{VprBP(DCAF1)}$ E3 ubiquitin ligase for degradation to reduce pro-apoptotic signaling during V(D)J recombination. We will further investigate this possibility during the coming year.

A second new sub-aim, partly related to the new sub-aim above, is to acquire and begin breeding RAG1 knock-in mice that harbor deletions or mutations in the N-terminal region of RAG1 that regulate RAG1 protein levels and putative E3 ligase activity. The long-term goal of these studies will be to determine whether loss or mutation of the N-terminus of RAG1 leads to Bim accumulation and/or an inversion of the ratio of $Ig\kappa^+$:$Ig\lambda^+$ B cells in a Bcl2-transgenic background. We anticipate that gaining preliminary data from the two new sub-aims will provide a strong foundation for submitting a competitive NIH R01 proposal.

For specific aim 2, as noted in the previous progress report, we changed course and developed and applied a next-generation sequencing pipeline to analyze expressed light chain gene usage in immunoglobulin VH12 transgenic mice. Based on preliminary data funded by the LB595 grant, we submitted a new NIH R21 grant, entitled “A novel form of light chain gene replacement” in June 2020, which was awarded in January 2021. Hence, further activity on this project has shifted to being supported by the NIH grant for the current cycle. Notably, a major manuscript reporting the results from this research was published in *Cell Reports* during the current cycle (Worth *et al.* 2022).

C. Significance

The findings in specific aim 1 are significant because they suggest that the N-terminus of RAG1 plays a key role in regulating RAG activity and potentially the response to DNA double-strand breaks. Furthermore, the data establish that the RAG1 degradation mechanism is constitutive and not restricted to lymphoid cells, and also shows it is evolutionarily conserved between mice and humans. Data and reagents obtained with LB595 support were used to support publication of one manuscript and the submission of a new R21 proposal, which was funded in 2021.

The findings in specific aim 2 are significant as they support a model in which many of the phosphatidylcholine-reactive B cells developing in VH12 mice are removed by receptor editing, and that expressing catalytically inactive RAG1 (dnRAG1) in this background blocks this process and enforces receptor specificity against phosphatidylcholine.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

Schabla NM and **Swanson PC**. The CRL4VPRBP(DCAF1) E3 ubiquitin ligase directs constitutive RAG1 degradation in a non-lymphoid cell line. PLoS One. 2021 Oct 14;16(10):e0258683. doi: 10.1371/journal.pone.0258683. eCollection 2021. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0258683

Worth AN, Palmer VL, Schabla NM, Perry GA, Fraser-Philbin AN, **Swanson PC**. Receptor editing constrains development of phosphatidyl choline-specific B cells in VH12-transgenic mice. Cell Rep. 2022 Jun 14;39(11):110899. doi: 10.1016/j.celrep.2022.110899. https://www.sciencedirect.com/science/article/pii/S221112472200674X?via%3Dihub

III. List of extramural grants submitted from 7/1/2021–6/30/2022

Agency: NIH/NIGMS, T32 training grant, submitted January 2022
Role: Faculty/Graduate Program Director. PI: J. Zuo
Title: G-RISE at Creighton University
Dates: 02/01/2023 to 01/31/2028
Amount: $2,101,770 total (5% FTE).

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

Agency: NIH/NIAID R21 AI153688-01A1
Role: PI
Title: Role of RACK1 in RAG1 degradation and B cell development
Dates: 07/01/2021 to 06/30/2023
Amount: $400,125 total

Agency: Great Plains IDeA-CTR Network Team Research Pilot Grant.
Role: PI
Title: Light chain contributions to specificity and pathogenicity of VH4-34+ B cells in lupus
Dates: 07/01/2021 to 06/30/2022
Amount: $50,000 total

Agency: NIH/NIGMS/NIAID 1R01AI153090-01A1.
Role: Consultant (Buckley, PI; University of Nebraska Medical Center)
Title: Role of E3 ligase UBR5 in alternative splicing during B cell development and activation
Dates: 08/25/2021 to 07/31/2025
Amount: $32,019 total

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Cellular Signaling and Molecular Trafficking in Cancer
Director: Laura Hansen, PhD

Dysregulated Mitochondrial Dynamics and Cancer Metastasis
Principal Investigator: Yaping Tu, PhD

I. Progress Report Summary

A. Specific Aims

- Aim 1: To assess the pathological importance of Drp1 upregulation in CRC metastasis.
- Aim 2: To determine the molecular mechanism of Drp1 upregulation in metastatic CRC.

B. Studies and Results

Metastasis is the major cause of cancer death. One of the **major challenges** in the management of cancer is to identify cancer cells with high metastatic potential, and to confine the cancer cells to their current location for destruction once detected. Understanding the molecular mechanism that allows cancer cells to acquire migratory and invasive abilities can lead to development of novel therapies. Mitochondria are organelles that supply energy required for cellular functions. They exist as dynamic networks that often change size and distribution, and these dynamics are maintained by two opposing processes: fission and fusion, regulated by Drp1 and mitofusin (Mfn) proteins, respectively. Significant efforts in recent years have implicated dysregulated mitochondrial dynamics (unbalanced fission or fusion) as critical for cancer progression. We previously reported that increased fission activity of mitochondria promotes cancer metastasis (1). More recently, we identified upregulated dynamin-related protein 1 (Drp1) to be responsible for dysregulation of mitochondrial fission in colorectal cancer (CRC), the second leading cause of cancer deaths in the USA. More importantly, we found that aberrantly upregulated miR-133a increases Drp1 expression and promotes mitochondrial fission of CRC cells. Interestingly, miR-133a expression correlates with metastasis and poor prognosis of CRC patients (2). We also reported that miR-133a orchestrates epithelial-mesenchymal transition (EMT) (3) that endows epithelial cancer cells with enhanced motility and invasiveness. Therefore, **we hypothesize** that miR-133a-dependent upregulation of Drp1 promotes mitochondrial fission, which in turn promotes CRC metastasis. During the past year, we focused our efforts on the mechanism for Drp1 upregulation in metastatic CRC (Aim 2). We hypothesize that upregulated miR-133a increases Drp1 expression via down-regulation of Parkin protein. We first examined miR-133a regulation of Drp1 expression and metastatic abilities of SW480 cells. We then determined if anti-miR-133a inhibitor reduces metastatic ability of HCT-116 via inducing Parkin-dependent Drp1 degradation.

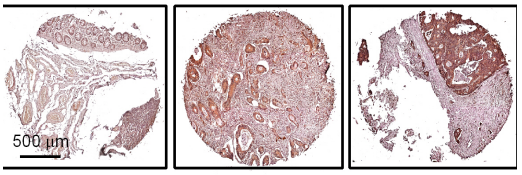


Fig.1. Upregulation of Drp1 protein expression in human CRC and metastases to lymph node. Representative immunostaining of Drp1 protein in human CRC tissue microarray using a mouse anti-Drp1 antibody.

1) Increased Drp1 protein levels in human invasive CRC and metastases to lymph nodes: To examine the clinical relevance of our data, we performed immunohistochemical (IHC) analysis of Drp1 protein expression in commercial microarrays of 266 human CRC specimens and adjacent normal tissues (US Biomax Inc., BC05115, BC05112, CO702b, CO1002b). We used a mouse anti-Drp1 antibody (BD Biosciences) with non-immune mouse IgG as the negative control. **Fig.1** shows that Drp1 immunostaining was very weak in normal tissue, increased in carcinoma, and much more intense in lymph node metastases. To semi-quantify these differences, expression levels of Drp1 protein in all microarray cases were graded from 1–4 based on overall staining intensity. As shown in **Table 1**, average Drp1 staining intensities in carcinoma were increased compared with normal or adjacent normal tissues

Table 1. Drp1 protein expression by immunohistochemistry staining in human CRC and metastases to lymph node

Colon specimens	n	Staining intensity				Average ± s.e.
		1	2	3	4	
Normal/Adjacent Normal	16	14	2	0	0	1.12 ± 0.08
Adenocarcinoma	202	32	66	78	4	2.34 ± 0.05 **
Lymph Node Metastases	50	2	17	12	19	2.96 ± 0.13 ** #

Statistical significance was determined using a Kruskal-Wallis test and Dunn post-test. **P < 0.001 vs Normal/Adjacent Normal. # P < 0.01 vs Adenocarcinoma.

(2.34±0.05 vs 1.12±0.08, *P*<0.001). Drp1 protein expression was further increased in lymph node metastases compared to carcinoma (2.96±0.13 vs 2.34±0.05, $^{\#}P$<0.01). These data suggest that upregulation of Drp1 mitochondrial fission protein is proportional to the degree of metastasis of these CRCs.

2) Upregulated miR-133a promotes EMT and migratory abilities of CRC cells. miR-133a has been reported to be reduced in cancers, and overexpression of miR-133a inhibits cancer cell proliferation. Interestingly, the higher expression of miR-133a correlates with metastases and poor prognosis in CRC patients (2). Indeed, expression levels of miR-133a were much higher in HCT-116 and LoVo cells compared to SW480 cells (**Fig.2a**). Transfection of miR-133a mimic caused changes in EMT markers (**2b**) and enhanced migratory potential of SW480 cells (**2c**).

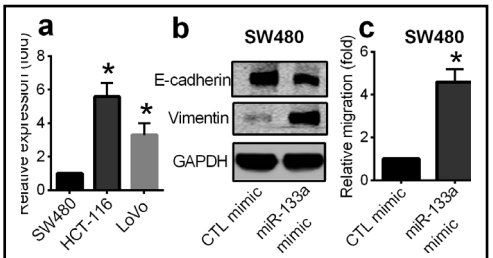


Fig.2. MiR-133a promotes EMT and migratory abilities of CRC cells.

3) Anti-miR-133a (a miR-133a hairpin inhibitor) reduces Drp1 expression and modulates CRC cell functions. Anti-miR-133a (50 nM, Thermo Scientific) or its negative control was introduced into HCT-116 cells for 72 h. As shown in **Fig.3**, inhibition of endogenous miR-133a by the anti-miR-133a reduced Drp1 but not Mfn1 expression (**3a**), promoted mitochondrial elongation (**3b**), decreased cell migratory ability (**3c**), and reduced the mitochondrial respiration of HCT-116 cells (**3d**).

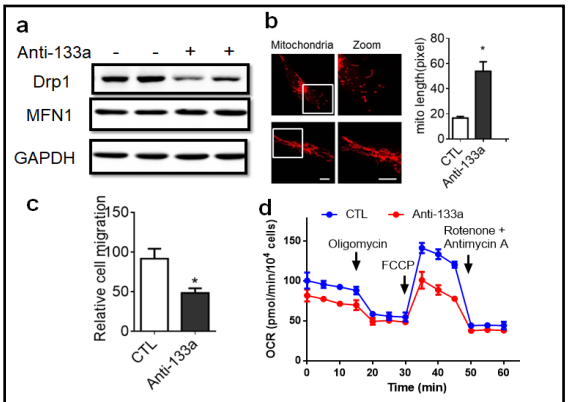


Fig.3. Effects of anti-miR-133a on HCT-116 cells.

4) Parkin is a target of miR-133a in CRC cells. We searched for the miR-133a direct targets that negatively regulate Drp1 expression. Among the putative targets of miR-133a predicted by online algorithms (Targetscan, mirSVR), Parkin fits our hypothesis because the 3-UTR of *Parkin* contains a binding site for miR-133a (**Fig.4**). It also induces Drp1 degradation (4) and loss of Parkin leads to mitochondria fragmentation (5). The mutation of the *Parkin* gene is a cause of familial Parkinson's disease. Parkin also functions as a tumor suppressor and its mutations were found in many types of cancers, but its roles in cancer metastasis are unknown.

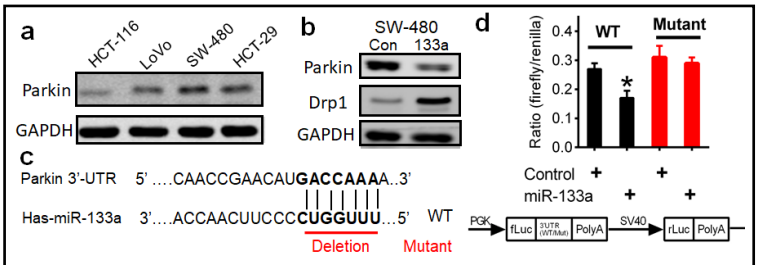


Fig.4. Parkin is a target of miR-133a. (a) Decreased Parkin expression in metastatic CRC cells**.** (b) miR-133a reduced Parkin protein expression with increased Drp1 expression. (**(b)** The putative miR-133a binding site of the 3′-UTR of the *Parkin* gene and its deletion mutant at the seeding region. **(d)** Firefly luciferase (fLuc) activity assays of the pmirGLO plasmids containing the Parkin-3′-UTR WT or its mutant with Renilla luciferase (rLuc) activity as the internal control. N=3, **p*<0.05.

We found that HCT-29 and SW-480 cells express higher levels of Parkin compared to HCT-116 and LoVo cells (**Fig.4a**). Expression of miR-133a downregulates Parkin expression with increased Drp1 protein in SW480 cells (**Fig.4b**). To test whether *Parkin* is directly targeted by miR-133a, we cloned and inserted the predicted *Parkin* 3′UTR-binding site and its mutant form at the seeding region into the pmirGLO dual-luciferase reporter plasmid, as we reported (3) (**Fig.4c**). SW480 cells were co-transfected with miR-133a and luciferase reporter plasmids. As shown in **Fig. 4d**, miR-133a represses wild-type Parkin-3′UTR reporter activity without inhibition of the mutant Parkin-3′UTR reporter activity, suggesting a direct regulation of miR-133a in the 3′UTR of *Parkin* mRNA.

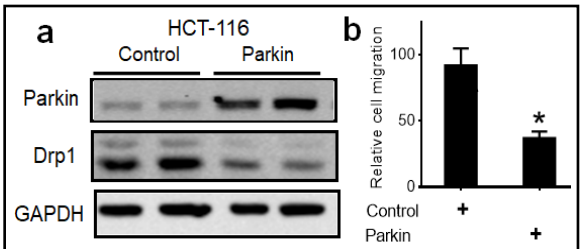


Fig.5. Parkin regulates Drp1 expression and HCT-116 cell migration**.** N=4, **p*<0.05

5) Parkin regulates Drp1 expression and HCT-116 cell migration. HCT-116 cells express lower levels of endogenous Parkin. Expression of recombinant Park1 decreases Drp1 expression (**Fig.5a**) and reduces HCT-116 cell migration (**Fig.5b**). We will further determine whether the inhibitory effect of Parkin is dependent on reduction of Drp1 by re-expressing recombinant Drp1 in CRC cells.

C. Significance

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related deaths in the USA. We recently found that Drp1 expression levels were markedly elevated in human

metastatic CRC specimens and CRC cell lines express different levels of Drp1 that correlated with their metastatic abilities. More importantly, we found that aberrantly upregulated miR-133a upregulates Drp1 expression and promotes mitochondrial fission of CRC cells. Dysregulation of miRNAs has been implicated in CRC, and has considerable potential for biomarkers and therapeutic targets. For example, miR-133a expression correlates with metastasis and poor prognosis of CRC patients. Since our recent data suggest that miR-133a orchestrates EMT, which endows epithelial cancer cells with enhanced motility and invasiveness. Therefore, **we hypothesize** that miR-133a-dependent upregulation of Drp1 promotes mitochondrial fission, which in turn promotes CRC metastasis. Our studies will address the following two questions: Does upregulated Drp1 induce mitochondrial fission and promote CRC metastasis (**Aim 1**)? What is the mechanism for Drp1 upregulation (**Aim 2**)? Our studies will provide new insights into the importance of Drp1-regulated mitochondrial dynamics in CRC metastasis. Completion of this project will allow us to identify biomarkers such as Drp1 for predicting CRC metastasis. It will also help identify exploitable vulnerabilities in metastatic CRC as new therapeutic targets. This may have significant therapeutic impact and change treatment paradigms to eliminate death and suffering from this all too often fatal metastatic CRC.

Reference:

1. Zhao J, Zhang J, Yu M, Xie Y, Huang Y, Wolff DW, Abel PW, Tu Y. (2013) Mitochondrial dynamics regulates migration and invasion of breast cancer cells. *Oncogene.* 32:4814-24.
2. Wan TM, Lam CS, Ng L, Chow AK, Wong SK, Li HS, Man JH, Lo OS, Foo D, Cheung A, Yau T, Poon JT, Poon RT, Law WL, Pang RW. (2014) The clinicopathological significance of miR-133a in colorectal cancer. Dis Markers. 919283.
3. Chen L, He X, Xie Y, Huang Y, Wolff DW, Abel PW**,** Tu Y. (2018) Up-regulated miR-133a orchestrates epithelial-mesenchymal transition of airway epithelial cells. *Sci Rep.* 8:15543.Wan TM, Lam CS, Ng L, Chow AK, Wong SK, Li HS, Man JH, Lo OS, Foo D, Cheung A, Yau T, Poon JT, Poon RT, Law WL, Pang RW. (2014) The clinicopathological significance of miR-133a in colorectal cancer. Dis Markers. 919283.
4. Wang H1, Song P, Du L, Tian W, Yue W, Liu M, Li D, Wang B, Zhu Y, Cao C, Zhou J, Chen Q. (2011) Parkin ubiquitinates Drp1 for proteasome-dependent degradation: implication of dysregulated mitochondrial dynamics in Parkinson disease. J Biol Chem. 286:11649-58.
5. Lutz AK, Exner N, Fett ME, Schlehe JS, Kloos K, Lämmermann K, Brunner B, Kurz-Drexler A, Vogel F, Reichert AS, Bouman L, Vogt-Weisenhorn D, Wurst W, Tatzelt J, Haass C, Winklhofer KF. (2009) Loss of parkin or PINK1 function increases Drp1-dependent mitochondrial fragmentation. J Biol Chem. 284:22938-51.

II. List of publications (7/1/2021– 6/30/2022)

1. Xie Y, Abel PW, Casale TB, Tu Y. (2022) T_H17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149:467-479. PMCID: PMC8821175. (Impact Factor: 14.29)
2. Zhang T, Wang R, Li Z, Wang L, Gao Z, Tu Y, Cao X. (2021) Anti-EGFR single-chain Fv antibody fragment displayed on the surface of ferritin H-chain protein nanoparticle for asthma therapy. ACS Appl Bio Mater. 4:6690-6702. PMID: 35006972. (Impact Factor: 3.25)
3. Zhang J, Yan L, Wei P, Zhou R, Hua C, Xiao M, Tu Y, Gu Z, Wei T. PEG-GO@XN nanocomposite suppresses breast cancer metastasis via inhibition of mitochondrial oxidative phosphorylation and blockade of epithelial-to-mesenchymal transition. Eur J Pharmacol. 895:173866. PMID: 33454376. (Impact Factor: 4.432)

III. List of extramural grants submitted from 7/1/2021 – 6/30/2022

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
R01 HL164593-01
Title: A novel approach to target neutrophilic airway inflammation and airway hyperresponsiveness in therapy-resistant (refractory) asthma
Dates: 7/2022 - 6/2027
Tu (PI): Role: Co-Investigator
Total funds requested: $2,049,764
Impact Score: 30; Percentile: 20

Nebraska Department of Health and Human Services Cancer and Smoking Disease Research
Title: New therapeutic agents for cigarette smoke-related pulmonary fibrosis
Dates: 7/2022 - 6/2023
PI: Yaping Tu
Total fund requested: $50,000
NIH Score: 1.8 (**funded**)

IV. List of extramural grants awarded from 7/1/2021 – 6/30/2022

Nebraska Department of Health and Human Services Cancer and Smoking Disease Research
Title: Aberrantly upregulated P-Rex1 confers chemoresistance in colorectal cancer
Dates: 7/2021 - 6/2022
PI: Yaping Tu
Award: $50,000

American Lung Association
American Lung Association ALA-AAAAI Allergic Respiratory Diseases Award
Title: Effects of RGS pathway polymorphisms on airway smooth muscle phenotype and asthma severity
Dates: 7/2021 - 6/2023
PI: Juan Carlos Cardet (USF)
Co-PI: Yaping Tu
Award: $150,000

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Cellular Signaling and Molecular Trafficking in Cancer Program
Director: Laura A. Hansen

Inhibition of Cancer Growth with Highly Selective and Proteolytically Stabile Peptide Analogs

Principal Investigator: Sándor Lovas

I. Progress Report Summary

A. Specific Aims

Glioblastoma multiforme (GBM) is the most common type of primary brain tumor. GBM is highly invasive, with a median overall survival of 1–2 years and a 5-year survival rate of less than 10%. The key step in glioma cell invasion appears to be breakdown of the extracellular matrix, which allows cancerous cells to migrate throughout the brain. The major molecular player in the GBM invasion is matrix metalloproteinase-2 (MMP-2). Chlorotoxin (CTX), a 36-amino acid peptide, has high-binding selectivity for glioma and other cancer cells, including squamous cell carcinoma (SCC) but not to healthy mammalian cells by specifically binding to MMP-2. Molecular docking studies of CTX to MMP-2 have shown favorable binding of the toxin to the collagen binding domain (CBD). Our molecular dynamics (MD) simulations shown that conformational stability of the C-terminal Gly^{24}-Arg^{36} residues of CTX is independent of the presence of disulfide bonds and inherently form stable low energy β-sheet-loop-β-sheet conformations. Furthermore, the C-terminal fragment of CTX (residues Gly^{24}-Arg^{36}) can be internally cross-linked by forming a disulfide bridge between Cys residues, thereby reinforcing conformational stability, which might enhance MMP-2 binding, and preventing proteolysis in serum. Our *central hypothesis* is that our lead C-terminal fragment of CTX is a good starting point for the development of a selective MMP-2 inhibitor using a quantitative structure-activity relationships approach. Therefore, we propose to develop a constrained short peptide derivative of CTX with demonstrated binding to MMP-2 and selective inhibition of Type IV collagen proteolysis, which would be a transformative therapy in patients with gliomas.

> **Aim 1. Optimize the current lead peptide for high affinity binding activity to MMP-2.** *Our working hypothesis is that the C-terminal fragment of CTX (residues Gly^{24}-Arg^{36}) can be converted to a proteolytically stable specific inhibitor of MMP-2.*
>
> *Sub-Aim 1A.* In the CTX(24-36) fragment, the optimal disulfide bridge configuration will be determined. Residues that are crucial for binding to MMP-2 will be determined by sequentially replacing each residue with Ala. Ala-scanned analogs will be docked to MMP-2 and ranked on the basis of their binding energy. Similar to Ala-scan, residues will be replaced with their D-enantiomer and docked to MMP-2. *Sub-Aim 1B.* Analogs with retained binding affinity will be synthesized and their MMP-2 inhibition activity will be determined. *Sub-Aim 1C.* The lead CTX(24-36) fragment will be sequentially truncated *N*-terminally. To protect against cleavage, the disulfide bridge will be replaced by various lactam bridges. MMP-2 inhibitory activity of the synthetic peptides will be determined. *Sub-Aim 1D.* To gain structural insight for further analog design, the structure of the

analogs with high affinity will be determined using CD spectropolarimetry and MD simulations.

Aim 2. Determine the effect of synthetic analogs on glioblastoma cells. *Sub-Aim 2A.* Analogs with nanomolar binding affinities from Aim 1 will be tested *in vivo* for their inhibition of glioblastoma cell invasion. *Sub-Aim 2B.* Analogs with submicromolar migration inhibition activity will be tested for their toxicity to glioblastoma cells. *Sub-Aim 2C.* The active analogs in cell culture assay will be further tested for their efficacy using animal models.

Aim 3. Determine the effect of synthetic analogs on SCC cells. Our *working hypothesis* is that CTX receptors, including MMP-2, in SCC can be targeted by CTX fragments. Analogs with nanomolar binding affinities from Aim 1 will be tested *in vivo* for their inhibition of SCC cell survival.

B. Studies and Results

Aim 1

1. In previous years we developed a 13 residue fragment of chlorotoxin (CTX) ,which is a 36-amino acid peptide, and has high-binding selectivity for glioma: Ac-[Ser^{35}]CTX(24-36)-NH_2 (P75). The sequence of P75: Ac-Gly^{24}-Arg-Gly-Lys-(Cys^{28}-Tyr-Gly-Pro-Gln-Cys^{33})-Leu-Ser^{35}-Arg-NH_2: the peptide has a Cys^{28}-Cys^{33} disulfide bridge. P75 at 1 μM concentration and the full length CTX had similar MMP-2 inhibitory efficacy.
2. Eight Ala-scanned analogs of P75 were synthesized to determine the residue contribution to inhibition of UG-87 glioblastoma cell invasion and migration.
3. Four P75 analogs were designed and synthesized, in which the turn-central -Gly-Pro- sequence was replaced with turn stabilizing dipeptides.
4. Structural properties of the turn-stabilized P75 analogs were analyzed by extensive molecular dynamics (MD) simulations and subsequent state-of-the-art Markov state modeling, as well as by circular dichroism (CD) spectropolarimetry. The peptides, similar to the parent P75, lost their native antiparallel hairpin structure (β-sheet-loop-β-sheet) but a β-bend structure at residues Tyr^{29} – Gln^{32} was stabilized.

Aim 2

1. P75 inhibited cell migration activity of U-87 glioblastoma cells. Also, P75 and its some of it synthetic analogs inhibited cell invasion activity of U-87 glioblastoma cells.
2. Previously in six replicates, we have shown that P75 inhibited U-87 glioblastoma cell survival at IC_{50} of 25.6 μM. However, we were not successful in reproducing the results; the cells no longer responded to peptide treatments. The peptide did not degrade as we have shown it using HPLC and subsequent MALDI-TOF mass spectrometry. To determine whether our U-87 cells are still functional and respond to drugs, we treated the U-87 cells with temozolomide (TMZ), which is clinically used to treat glioblastoma patients. At 100 μm concentration, TZM induced 50% cell death. To further confirm the functionality of our UG-87 cells, we treated them with 5 μM Selinexor, a novel anticancer agent that is under clinical trial against glioblastoma. After six days of treatments, Selinexor induced 100% cell death. Presently, we are optimizing the peptide assay conditions. In a few experiments, we have achieved 50% cell death at 100 μM P75 concentration.
3. In the previous LB595 cycle and with our NIH-supported project we are developing peptides that interfere with 14-3-3 interactions. Literature indicates that 14-3-3 proteins are present in glioblastoma. With our tetrapetide (ES1P2) that inhibit cutaneous

squamous cell carcinoma by interfering with 14-3-3ε heterodimerization, in preliminary experiments, we were able to show that the peptide inhibit SCC12 growth.

C. Significance

We have established that the C-terminal fragment analog of CTX (P75) has the same MMP-2 inhibitory activity as the full, native CTX. The peptide also inhibits the cell invasion activity of U-87 glioblastoma cells. These results established that Ac-[Ser^{35}]CTX(24-36)-NH_2 is a good lead compound to develop it with further modifications to a drug candidate.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

None

III. List of extramural grants submitted from 7/1/2021–6/30/2022

Nebraska LB506 Cancer and Smoking Disease Research Program PI: Laura Hansen
Dates: 07/01/22 - 06/30/23
Project title: Molecular determinants of Flower protein-mediated cell competition
Role: Co-I
Award: $50,000

US Army, USAMRDC, entitled "Novel antibiotics that selectively inhibit the essential protein-protein interfaces of the SSB interactome in ESKAPE-E bacteria."
Role: Co-I, PI: Piero Bianco, UNMC

US Army, Defense Threat Reduction Agency (DTRA), entitled "Novel antibiotics that inhibit the essential protein-protein interfaces of the SSB interactome, thereby nullifying infections of Tier 1 WMD bacteria"
Role: Co-I, PI: Piero Bianco, UNMC

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

NIH/NCI 1R01 CA253573-01 PI: L. Hansen
Dates: 08/01/20-04/30/25
Project title: Targeting aberrant anti-apoptotic signaling for prevention of skin cancer
Role: co-PI
Award: $1,250,000 direct cost
The goal is to delineate aberrant prosurvival signaling pathways in skin cancer cells in order to develop candidate agents to disrupt this signaling.

NIH/NIGMS CoBRE, P20GM139762 PI: P. Steyger
Dates: 03/05/21-01/31/26
Project title: Drug Discovery and Delivery Core of the Translational Hearing Center.
Role: Co-director
Award: $638,700

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Lynch Comprehensive Cancer Research Center of Excellence
Program Director: Robin Farias-Eisner, MD, PhD, MBA, FACOG

Project Title: Dual Domain HDL-Mimetic Peptides: A Novel Targeted Anti-Tumorigenic Therapy
Principal Investigator: Robin Farias-Eisner, MD, PhD, MBA, FACOG

I. Progress Report Summary

As the former Director of Creighton University's Lynch Comprehensive Cancer Research Center (LCCRC), formerly the Hereditary Cancer Center (HCC), Robin Farias-Eisner, MD, PhD, utilized LB595 funding to develop a robust and diversified research infrastructure within the LCCRC. Key clinical, translational, and basic science faculty and personnel were hired, including next-generation sequencing expert Yusi Fu, PhD, cancer cell biologist James Grunkemeyer, PhD, and obstetrician-gynecologist Lesley Conrad, MD, who joined geneticist Holly Stessman, PhD, in the LCCRC. The LCCRC team then focused on securing extramural funding through grant applications, including an NIH Small Business Innovative Research (SBIR) grant. The SBIR initial submission was reviewed positively, and we are optimistic our resubmission will result in securing the necessary funding to support our cancer research. The LCCRC team also worked diligently to prepare the lab for the operations required for planned *in vitro* and *in vivo* experiments supported by extramural funding. The School of Medicine has also separately established the Innovative Genomics Core Facility to support the group's planned cancer genetics research. The LCCRC LB595 funds originally awarded to Dr. Farias-Eisner will support the collaborative gynecologic oncology research projects of Drs. Fu, Conrad, and Stessman. The ongoing work of these researchers will provide a foundation for future applications for extramural funding in support of research projects with a focus on improving women's health.

A. Specific Aims

The aims have been modified to focus on the collaborative projects with faculty members currently within LCCRC:

- Aim 1: Distinguish high- versus low-risk missense variants in PMS2 that affect its function in DNA mismatch repair.
- Aim 2: Characterize the population abundance and transcriptome change of cancer stem cells during endometrial cancer progression.

B. Studies and Results

Aim 1: Distinguish high- versus low-risk missense variants in PMS2 that affect its function in DNA mismatch repair.

Based on shared interest in genetic risk factors for developing cancer, Dr. Robin Farias-Eisner committed LB595 funds to supporting a PhD student stipend to pursue such work. Jocelyn Plowman was recruited through an interdepartmental collaboration with Dr. Holly Stessman (SOM – Pharmacology & Neuroscience) in 2021. Led by Dr. Stessman, Ms. Plowman has been

developing a project to resolve Lynch Syndrome variants of undetermined significance (VUSs) using a high-throughput *in vitro* approach. Dr. Stessman has been recently funded for this work by the State of Nebraska LB506 mechanism, including full salary support for this student for the next year (2022-2023).

Lynch syndrome (LS) is one of the most common hereditary cancer syndromes affecting an estimated 1 in 279 people. LS cases are known to be caused by germline mutations in the genes MLH1, MSH2, MSH6, and PMS2, which encode proteins that function in DNA mismatch repair (MMR). When DNA damage is introduced during replication, the MMR pathway halts the cell cycle until the damage can be repaired or signals the damaged cell to die. Individuals with loss-of-function mutations in the LS genes fail to cull damaged cells, resulting in the accumulation of excess somatic mutations over time, which increases cancer risk.

While genetic testing has become more common among individuals with a family history of cancer, the interpretation of identified variants remains difficult. Variants in PMS2 are most common among LS cases; however, they are the least understood. Disruptive PMS2 variants (nonsense, frameshifting, and canonical splice-site) are often confidently classified as pathogenic, resulting in an LS diagnosis. Individuals who carry known pathogenic variants can significantly reduce their cancer risk through early and frequent surveillance and prophylactic measures. However, most PMS2 missense variants are classified as variants of undetermined significance (VUSs). Certainly, a portion of these variants carry an increased cancer risk, but which ones? Not only are VUSs clinically unactionable, but they are unclear to patients and clinicians. Thus, there is a critical need to better classify the genetic risk associated with PMS2 missense variants.

The long-term goal of our work is to understand the contribution of genetic variation to LS risk. Our overall objective in this proposal is to establish an *in vitro* system by which PMS2 missense variants can be tested for MMR activity in a high-throughput way. Our hypothesis is that a portion of PMS2 missense VUSs cause decreased DNA MMR activity which is known to increase cancer risk (i.e., somatic mutations) over time. This hypothesis is supported by studies of several other hereditary cancer syndrome genes, including MSH2 and BRCA1. The rationale for this proposal is that once the underlying genetic architecture of LS is fully understood, better diagnostic, management, and family planning approaches can be developed that will significantly decrease cancer risk over time. We plan to test our central hypothesis through distinguishing high- versus low-risk missense variants in PMS2. We will establish a robust *in vitro* assay in human cell lines, in which PMS2 missense VUSs can be tested for MMR function. Variants with reduced MMR function have been shown to increase one’s risk for cancer.

Aim 2: Characterize the population abundance and transcriptomic changes of cancer stem cells during endometrial cancer progression

We set up a local Linux server for data analysis and report generation for high-throughput single-cell RNA sequencing with a computing power of 5TB storage and 64GB RAM, single node. Ongoing: we are in the process of moving the analysis pipeline to cloud-based on-demand computing resources, which will streamline data transfer to results automation, and offer easier adaptation to similar projects.

We set up the newest Illuminia Nextseq 2000 sequencer. In the past few months, five labs have successfully run their samples on the sequencer with applications ranging from single-cell RNA sequencing to whole-genome DNA sequencing. We are now able to generate 1.3 billion sequencing reads within 24 hours. The LCCRC has sequenced the transcriptome of 7,394 single-cells on this machine.

We purchased a Qubit Flex fluorometer and the TapeStation 4200 system and established quality control steps for RNA extraction, library quantification, etc. Qubit detects fluorescent dyes that will specifically bind to the target of interest, thus enabling it to detect DNA, RNA and protein at low concentrations. The TapeStation is a DNA and RNA electrophoresis system that detects the size distribution of the input materials. It works with 1 to 96 samples, thus offering more flexibility compared to the bioanalyzer system.

We streamlined sample processing procedures for single-cell suspension and high-throughput single-cell RNA amplification from uterine blood. We also developed a standard operating procedure to snap-freeze and methanol-fix tissue and blood samples for long-term storage and future access.

We applied single-cell high-throughput RNA-seq to uterine blood samples from endometrial cancer patients and sequenced 7,394 single cells from four individuals, recovered immune cells and endometrial cells from uterine blood. Ongoing: we plan to retrieve FFPE blocks from the Biorepository core and use single nuclei sequencing to identify the driver mutations in uterine cancer.

In our dataset, we found an enrichment of a type of epithelial cells that has an expression pattern between luminal epithelial and glandular epithelial cells based on unsupervised clustering. The ratio of this special cell type compared to epithelial cells or to all cells is correlated with the cancer stage. Gene ontology analysis of this population suggested an enrichment of stem cell markers, implying that this population is cancer stem cell. Among all the significant upregulated genes that expressed in this cluster, *ALDH1A1* ranked first and there is a clear, higher expression of *ALDH1A1* compared to other epithelial clusters

C. Significance

- Our results will help to understand the contribution of genetic variation to LS risk, and the outcomes of this study will establish a scalable *in vitro* assay for the functional testing of PMS2 variants.
- Our results can verify clinically classified pathogenic and benign PMS2 missense variants (i.e., high-quality positive and negative controls), and functionally re-classify a VUS identified in a family from our local Lynch hereditary cancer biorepository that may explain their cancer risk. The clinical reclassification of VUSs supported by functional data is expected to have a positive impact on cancer prevention and management for LS families carrying a pathogenic PMS2 variant.
- The results will fill the gap and provide a non-invasive, sensitive way to identify CSCs based on whole transcriptome assessment, predict cancer risk with single-cell technologies and liquid biopsy, as well as help to overcome the inadequate sampling and analysis issues plaguing other methods.
- The findings can affect treatment decisions for patients and provide opportunities for transcriptome-guided drug development. Aside from endometrial cancer, the methods and analysis pipeline used here can be applied to other cancer types and might be useful as a general early detection and diagnostic procedure.

II. List of refereed publications germane to this project from 7/1/2021–6/30/2022

Submmited to *International Journal of Molecular Sciences*
Pharmacokinetic/Pharmacodynamic Characterization of the Novel Anti-Cancer HM-10/10 HDL-

Mimetic Peptide. Michael P. Dempsey, Katelyn E. Andersen, Clay L. Cashman, Lesley B. Conrad, Claire A. Kearney, Brittney M. Wells, Mitchell A. Taylor, Mary B. Conklin, Emily R. Via, Emily M. Doe, Srinivasa T. Reddy, Robin Farias-Eisner

III. List of extramural grants submitted from 7/1/2021–6/30/2022

1. National Institutes of Health, Small Business Innovation Research
   Title: HM-10/10: A Novel Treatment for Chemotherapy-Resistant High--Grade Epithelial Ovarian Cancer
   PI: Robin Farias-Eisner
2. Department of Defense, Peer Reviewed Cancer Research Program
   Title: np-HM-10/10: A Novel Apolipoprotein Mimetic Peptide-Based Treatment for Endometrial Cancer
   PI: Robin Farias-Eisner

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

None

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

Biorepository Infrastructure
PD/PI: Holly A. F. Stessman

I. Progress Report Summary

A. Specific Aims

- **Specific Aim 1. Audit of stored participant biospecimens.** We will perform a systematic inventory of all stored specimens to better estimate availability for research.
- **Specific Aim 2. Data migration from paper to digital records.** Conversion of historic records will be required for more efficient data mining efforts. This will occur in three phases: (1) digital conversion of existing paper records, (2) data extraction of relevant information, and (3) modernization of participant contact.
- **Specific Aim 3. Modernize laboratory techniques.** Based on new trends in the cancer genetics field, we will establish cutting-edge techniques locally as a resource to Creighton investigators.

The original aims have not been modified.

B. Studies and Results

Specific Aim 1. Audit of stored participant biospecimens. A full-time research coordinator (Bridget Sefranek) began employment July 1, 2021, with the Biorepository. A decision was made in September 2021 with the new head of the IRB (Kindra Cooper) to de-identify all samples in the former Lynch collection (based on LB595 external committee recommendation) to preserve as much data and as many samples as possible. Approximately 7,134 individuals have a sample in the Biobank; many have consented more than once and provided many samples over time. Bridget and staff have manually audited ~2,700 individuals and identified that ~1,620 (60%) of these are good for future use under a de-identified structure. Only 820 (30%) are not approved for future use and 267 (~10%) require additional IRB guidance/ruling, which is forthcoming. For all possible reapproved participants, a high-quality DNA sample has been identified, quantified, and stored for request. All blocks and slides in storage have now been inventoried. A manual audit of all associated files is underway, as are requests to keep these resources from their originating hospitals.

Specific Aim 2. Data migration from paper to digital records. (1) All paper records were converted to digital copy in 2020-2021. These records have been used exclusively for the consent checking associated with SA1. During this process, discrepancies have been noted. Spot-checking of original patient files is currently underway, with the goal of disposing of all paper records by December 2022. SA2-1 is considered complete. (2) The LabVantage LIMS environment has been populated only with allowable data from the legacy database for those participants/samples with IRB approval. This work continues with the ongoing audit of historic specimens. Mark Stacey (Programmer) is developing and implementing training materials for use of the new LIMS environment for all legacy data and new samples. (3) The Creighton research participant contact portal is finalizing customizations (from beta testing) prior to a

contracted security audit that will be conducted on the app. IRB consenting will be performed going forward using this interface. An updated biorepository consent form is currently under review with the IRB. There were significant delays with SA2-3 this year due to turnover of administrative staff and all Creighton IRB staff.

Specific Aim 3. Modernize laboratory techniques. To support other LB595-funded work on campus, we added extraction of viral RNA from clinical discarded nasopharyngeal swabs to the laboratory service line in 2020-2021. Because of this, the laboratory was able to support (with 50% personnel cost share) contract work with the Centers for Disease Control and Prevention (CDC) from August 2021-July 2022. There was a turnover of laboratory staff in April-May 2022. However, new staff (Cynthia Watson, MS) have already worked to optimize a 10X genomics single-cell preparation and sequencing protocol to add to the core service line. External contracts have significantly increased over the last fiscal year, supporting the sustainability of this laboratory going forward. Importantly, cost sharing of personnel was built into our original budget to support the laboratory and former Lynch collection as a core resource. We are currently meeting that original goal. Finally, an ultra-low freezer was purchased this year to consolidate the LN2 Lynch specimen collection. This is an equipment upgrade that has a lower risk of failure than LN2 dewers. Also, this freezer has an option for LN2 backup, which will be purchased in the next fiscal year, to further reduce risk of sample loss.

C. Significance

Both Dr. Henry Lynch and Creighton University have contributed substantially to hereditary cancer research advances, owing in large part to the biobanking of participant specimens and data over the past 40+ years. While substantial progress has been made in the field, there is likely still genetic fruit to be found. The success of these approaches hinges entirely on the quality of the biological specimens from which the DNA or RNA molecules for genetic testing are obtained. Biorepositories that store and maintain these specimens and their derivatives play a critical role in ensuring the integrity of the samples used by cancer researchers. Advancements in our understanding of the genetic predisposition for cancer can only be achieved through the utilization of well-preserved and well-characterized biospecimens.

II. List of refereed publications germane to this project from 7/1/2021–6/302022

None.

III. List of extramural grants submitted from 7/1/2021–6/30/2022

NSF REU (Co-PI: Stessman)
“Developmental Neuroscience: From Molecules to Brain Networks”

LB 506 State of Nebraska (DHHS) (PI: Stessman)
“Resolving variants of undetermined significance (VUSs) in Lynch Syndrome.”

R21 NIH (PD/PI: Stessman)
“Defining Disease-Relevant Gene Targets of KMT5B”

R01 NIH (PD/PI: Stessman)
“KMT5B Regulation of Motor Development in Autism”

R21 NIH/NICHD (PD/PI: Stessman)
“H4K20 Regulation of H19 Expression in Skeletal Muscle”

P20 NIH (Co-I: Stessman)
“Neuroinflammation in Neurodegeneration and Cognitive Impairment Associated with Global Ischemia”

R01 NIH (Co-I: Stessman)
“Neuroinflammation in Global Ischemia”

DoD (Co-I: Stessman)
“Evaluation of the G Protein-Coupled Estrogen Receptor (Gper) as a Therapeutic Target in a Preclinical Mouse Model of Endometriosis”

CURAS Summer Research Scholar (PI: Stessman; Student PI: Scavuzzo)
“Characterization of PMS2 Missense Variants in Lynch Syndrome”

R15 NIH (Co-I: Stessman)
“Regulation of the Microglial Neuroimmune Response by Long Non-Coding RNAs”

R21 NIH (Co-I: Stessman)
“Alternate Porin Production of OpdP vs OprD Influences Carbapenem Susceptibility in Pseudomonas aeruginosa Through Regulatory Pathways Affected by Nutrient Availability”

GP IDeA-CTR Network NIH (Co-I: Stessman)
“Human Placental Lactogen (Human Chorionic Somatomammotropin) and Oxytocin during Pregnancy: Individual Patterns and Correlations with Maternal-Fetal Attachment, Anxiety, and Depression”

IV. List of extramural grants awarded from 7/1/2021–6/30/2022

LB 506 State of Nebraska (DHHS) (PI: Stessman)
“Resolving variants of undetermined significance (VUSs) in Lynch Syndrome.”

P20 NIH (Co-I: Stessman)
“Neuroinflammation in Neurodegeneration and Cognitive Impairment Associated with Global Ischemia”

GP IDeA-CTR Network NIH (Co-I: Stessman)
“Human Placental Lactogen (Human Chorionic Somatomammotropin) and Oxytocin during Pregnancy: Individual Patterns and Correlations with Maternal-Fetal Attachment, Anxiety, and Depression”

Creighton University
Cancer & Smoking Disease Research Program
Total External Submissions & Awards

Investigator	Submitted FY 21/22	# Submitted
Juliane Strauss-Soukup	$75,000	1
Holly Stessman	$2,038,675	6
Laura Hansen	$50,000	1
Brian North	$1,800,000	3
Patrick Swanson	$0	0
Yaping Tu	$1,515,109	2
Sandor Lovas	$1,107,287	2
John Cote	$63,013	1
Yusi Fu	$110,000	1
Peter Abel	$0	0
Gopal Jadhav	$0	0
TOTAL SUBMISSIONS	$6,759,084	

Investigator	Awarded FY 21/22	# Awarded
Juliane Strauss-Soukup	$480,833	2
Holly Stessman	$180,000	3
Laura Hansen	$623,537	4
Brian North	$45,750	5
Patrick Swanson	$304,145	4
Yaping Tu	$52,146	2
Sandor Lovas	$0	0
John Cote	$0	0
Yusi Fu	$0	0
Peter Abel	$0	0
Gopal Jadhav	$131,424	1
TOTAL AWARDS	$1,817,835	

LB595 Investigator Awards FY21/22

Principal Investigator	Originating Sponsor Name	Project Title	Awarded Project Period Start Date	Awarded Project Period End Date	Directs	Indirects	Total
Holly Feser Stessman	State of NE - LB506	Resolving Variants of Undetermined Significance (VUSs) in Lynch Syndrome	01-Jul-2022	30-Jun-2023	$50,000.00	$0.00	$50,000.00
	Simons Foundation	In Vitro Modeling of Genetic Subtypes of Autism	01-Dec-2017	30-Nov-2023	$55,000.00	$11,000.00	$66,000.00
	State of NE - LB692	KMT5B as a Novel Analgesic Target	01-Jul-2021	30-Jun-2022	$75,000.00	$0.00	$75,000.00
					Total: $180,000.00	Total: $11,000.00	Total: $191,000.00
Laura Hansen	State of NE - LB506	Molecular Determinants of Flower Protein-Mediated Cell Competition	01-Jul-2022	30-Jun-2023	$50,000.00	$0.00	$50,000.00
	National Institutes of Health	Targeting Aberrant Anti-Apoptotic Signaling for Prevention of Skin Cancer	01-Aug-2020	30-Apr-2025	$224,175.00	$102,000.00	$326,175.00
	National Institutes of Health	A Novel Antioxidant Delivery System, Pro-NPTM, for Protection Against UV-Related Skin Cancer	01-Jun-2021	31-May-2023	$41,062.00	$18,683.00	$59,745.00
	State of NE - LB692	Core Facility Equipment	01-Jun-2022	30-Jun-2022	$308,300.00	$0.00	$308,300.00
					Total: $623,537.00	Total: $120,683.00	Total: $744,220.00
Gopan Jadhav	National Institutes of	Development of Small Chemical-Molecule Inh	11-Sep-2020	31-Aug-2023	$131,424.00	$55,975.00	$187,399.00
					Total: $131,424.00	Total: $55,975.00	Total: $187,399.00
Brian North	National Institutes of Health	Characterizing Upstream Regulators of Glucosylceramide Metabolism for Parkinson's Disease and Lewy Body Dementia	15-Jan-2021	31-Dec-2022	$90,000.00	$40,950.00	$130,950.00
	National Institutes of Health	Regulatory Mechanisms Governing BubR1 Protein Stability During Stress and Aging	01-Jun-2022	31-Mar-2027	$205,000.00	$93,275.00	$298,275.00
	National Institutes of Health	Characterizing Upstream Regulators of Glucosylceramide Metabolism for Parkinson's Disease and Lewy Body Dementia	15-Jan-2021	31-Dec-2022	$10,000.00	$4,550.00	$14,550.00
	State of NE - LB606	Targeting SIRT2 in Glioblastoma Cancer Stem Cells	01-Jul-2021	30-Jun-2022	$95,750.00	$0.00	$95,750.00
	State of NE - LB692	Role of Protein Homeostasis Factors in Regulating the Interrelationship Between Aging and Cancer	15-Jan-2019	31-Dec-2022	$50,000.00	$0.00	$50,000.00
					Total: $450,750.00	Total: $138,775.00	Total: $589,525.00
Juliane Strauss-Soukup	National Institutes of Health/University of NE Medical Center	Detection and Characterization of Compounds that Target the glmS Riboswitch and Act as Antibiotics	01-May-2020	30-Apr-2025	$82,059.00	$16,381.00	$98,440.00

	National Institutes of Health/University of NE Medical Center	Nebraska Research Network in Functional Genomics	01-May-2020	30-Apr-2025	$398,774.00	$76,821.00	$475,595.00
					Total: $480,833.00	Total: $93,202.00	Total: $574,035.00
Patrick Swanson	State of NE - LB692	GP IDeA-CTR Network: Light Chain Contributions to Specificity and Pathogenicity of VH4-34+ B Cells in Lupus	01-Jul-2021	30-Jun-2022	$50,000.00	$0.00	$50,000.00
	National Institutes of Health/University of NE Medical Center	Role of E3 Ligase UBR5 in Alternative Splicing During B Cell Development and Activation	25-Aug-2021	31-Jul-2025	$4,145.00	$1,886.00	$6,031.00
	National Institutes of Health	A Novel Form of Light Chain Gene Replacement	19-Jan-2021	31-Dec-2022	$125,000.00	$56,875.00	$181,875.00
	National Institutes of Health	Role of RACK1 in RAG1 Degradation and B Cell Development	16-Jun-2021	31-May-2023	$125,000.00	$56,875.00	$181,875.00
					Total: $304,145.00	Total: $115,636.00	Total: $419,781.00
Yaping Tu	State of NE - LB506	New Therapeutic Agents for Cigarette Smoke-Related Pulmonary Fibrosis	01-Jul-2022	30-Jun-2023	$50,000.00	$0.00	$50,000.00
	American Lung Assoc/University of South Florida	Effects of RGS Pathway Polymorphisms on Airway Smooth Muscle Phenotype and Asthma Severity	01-Jul-2021	30-Jun-2022	$2,146.00	$0.00	$2,146.00
					Total: $52,146.00	Total: $0.00	Total: $52,146.00

LB595 Submitted Report FY21/22

Principal Investigator	Originating Sponsor Name	Project Title	Requested Project Period Start Date	Requested Project Period End Date	Directs	Indirects	Total
John Cote	National Institutes of Health/University of NE Medical Center Great Plains IDeA-CTR	GP IDeA-CTR Network: Human Placental Lactogen (Human Chorionic Somatomammotropin) and Oxytocin during Pregnancy: Individual Patterns and Correlations with Maternal-Fetal Attachment, Anxiety, and Depression	01-Jul-2022	30-Jun-2023	$43,307.59	$19,704.96	$63,012.55
					Total: $43,307.59	Total: $19,704.96	Total: $63,012.55
Holly Feser Stessman	State of NE - LB506	Resolving Variants of Undetermined Significance (VUSs) in Lynch Syndrome	01-Jul-2022	30-Jun-2023	$50,000.00	$0.00	$50,000.00
	National Science Foundation	Developmental Neuroscience: From Molecules to Brain Networks	01-Mar-2022	28-Feb-2025	$120,300.00	$0.00	$120,300.00
	Simons Foundation	In Vitro Modeling of Genetic Subtypes of	01-Dec-2021	30-Nov-2023	$55,000.00	$11,000.00	$66,000.00
	National Institutes of Health	KMT5B Regulation of Motor Development in Autism	01-Apr-2023	31-Mar-2028	$1,250,000.00	$568,750.00	$1,818,750.00
	National Institutes of Health	Defining Disease-Relevant Gene Targets of KMT5B	01-Dec-2022	30-Nov-2024	$288,375.00	$125,125.00	$413,500.00
	National Institutes of Health	H4K20 Regulation of H19 Expression in Skeletal Muscle	01-Apr-2023	31-Mar-2025	$275,000.00	$125,125.00	$400,125.00
					Total: $2,038,675.00	Total: $830,000.00	Total: $2,868,675.00
Yusi Fu	State of NE - LB606	ALDH1A1+ Cancer Stem Cells Abundance in Uterine Blood as a Potential Diagnostic	01-Jul-2022	30-Jun-2023	$110,000.00	$0.00	$110,000.00
					Total: $110,000.00	Total: $0.00	Total: $110,000.00
Laura Hansen	State of NE - LB506	Molecular Determinants of Flower Protein-Mediated Cell Competition	01-Jul-2022	30-Jun-2023	$50,000.00	$0.00	$50,000.00
					Total: $50,000.00	Total: $0.00	Total: $50,000.00
Sandor Lovas	Defense Threat Reduction Agency/University of NE Medical Center	Novel Antibiotics that Inhibit the Essential Protein-Protein Interfaces of the SSB Interactome, Thereby Nullifying Infections of Tier 1 WMD Bacteria	01-Jul-2022	30-Jun-2027	$519,530.30	$236,386.29	$755,916.59
	U.S. Department of Defense/University of NE Medical Center	Novel Antibiotics That Selectively Inhibit the Essential Protein-Protein Interfaces of the SSB Interactome in ESKAPE-E	01-Jul-2022	30-Jun-2027	$587,756.75	$267,429.32	$855,186.07
					Total: $1,107,287.05	Total: $503,815.61	Total: $1,611,102.66
Brian North	U.S. Department of the Army	Identification of Novel E3 Ubiquitin Ligases Regulating Hepatocellular Carcinoma	01-Jul-2022	30-Jun-2025	$500,000.00	$227,500.00	$727,500.00

	U.S. Department of the Army	Targeting Myc in Lymphoma with a Novel Combination Therapeutic Regimen	01-Jul-2022	30-Jun-2026	$1,250,000.00	$568,750.00	$1,818,750.00
	Health Sciences Strategic Investment Fund	Regulation of cardiac development and function through BubR1 control of the potassium channel adaptor Kcne1.	01-Jul-2022	30-Jun-2024	$50,000.00	$0.00	$50,000.00
					Total: $1,800,000.00	Total: $796,250.00	Total: $2,596,250.00
Juliane Strauss-Soukup	State of NE - LB692	Bridge Funding for NIH project: Examination of Ornithine Decarboxylase Antizyme RNA Structure and Function for	01-Jul-2022	30-Jun-2023	$75,000.00	$0.00	$75,000.00
					Total: $75,000.00	Total: $0.00	Total: $75,000.00
Yaping Tu	National Institutes of Health	A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-	01-Jul-2022	30-Jun-2027	$1,465,109.00	$584,655.00	$2,049,764.00
	State of NE - LB506	New Therapeutic Agents for Cigarette Smoke-Related Pulmonary Fibrosis	01-Jul-2022	30-Jun-2023	$50,000.00	$0.00	$50,000.00
					Total: $1,515,109.00	Total: $584,655.00	Total: $2,099,764.00

Creighton University Cancer & Smoking Disease Research Program
FY21/22 Progress Report
(July 1, 2021 – June 30, 2022)

PUBLICATIONS
Juliane K. Strauss-Soukup, PhD, Principal Investigator

Cellular Signaling and Molecular Trafficking in Cancer Program

Publications for Hansen:

None in this cycle.

Publications for North:

None in this cycle.

Publications for Swanson:

1. Schabla NM and **Swanson PC**. The CRL4VPRBP(DCAF1) E3 ubiquitin ligase directs constitutive RAG1 degradation in a non-lymphoid cell line. PLoS One. 2021 Oct 14;16(10):e0258683. doi: 10.1371/journal.pone.0258683. eCollection 2021. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0258683

2. Worth AN, Palmer VL, Schabla NM, Perry GA, Fraser-Philbin AN, **Swanson PC**. Receptor editing constrains development of phosphatidyl choline-specific B cells in VH12-transgenic mice. Cell Rep. 2022 Jun 14;39(11):110899. doi: 10.1016/j.celrep.2022.110899. https://www.sciencedirect.com/science/article/pii/S221112472200674X?via%3Dihub

Publications for Tu:

1. Xie Y, Abel PW, Casale TB, Tu Y. (2022) T_H17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149:467-479. PMCID: PMC8821175. (Impact Factor: 14.29). https://www.sciencedirect.com/science/article/pii/S0091674921026725?via%3Dihub
2. Zhang T, Wang R, Li Z, Wang L, Gao Z, Tu Y, Cao X. (2021) Anti-EGFR single-chain Fv antibody fragment displayed on the surface of ferritin H-chain protein nanoparticle for asthma therapy. ACS Appl Bio Mater. 4:6690-6702. PMID: 35006972. (Impact Factor: 3.25). https://pubmed.ncbi.nlm.nih.gov/35006972/
3. Zhang J, Yan L, Wei P, Zhou R, Hua C, Xiao M, Tu Y, Gu Z, Wei T. PEG-GO@XN nanocomposite suppresses breast cancer metastasis via inhibition of mitochondrial oxidative phosphorylation and blockade of epithelial-to-mesenchymal transition. Eur J

Pharmacol. 895:173866. PMID: 33454376. (Impact Factor: 4.432). https://linkinghub.elsevier.com/retrieve/pii/S0014-2999(21)00019-4

Publications for Lovas:

None in this cycle.

Biorepository Infrastructure

Publications for Stessman Project:

None in this cycle.

Development Program

Publications for Coté project:

None in this cycle.

Publications for Jadhav project:

Publications for Abel project:

1. Xie Y, Abel PW, Casale TB, Tu Y. (2022) T_H17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149(2):467-479. PMCID: PMC8821175. https://www.sciencedirect.com/science/article/pii/S0091674921026725?via%3Dihub
2. Hulen J, Kenny D, Black R, Hallgren J, Hammond KG, Wickramasekara RN, Abel PW, Stessman HF. (2022) KMT5B is required for early motor development. Frontiers in Genetics. doi: 10.3389/fgene.2022.901228. https://www.frontiersin.org/articles/10.3389/fgene.2022.901228/abstract

Creighton University
Cancer & Smoking Disease Research Program
Report of Expenditures
July 1, 2021 - June 30, 2022

	Approved Budget	Total Expenses	Remaining Budget
Personnel	$811,711.00	$662,689.38	$149,021.62
Consultant	26,000.00	13,050.00	12,950.00
Equipment	246,980.00	332,064.99	(85,084.99)
Supplies	23,000.00	26,722.00	(3,722.00)
Other Expenses	192,309.00	118,530.76	73,778.24
Total	$1,300,000.00	$1,153,057.13	$146,942.87

FRED & PAMELA
BUFFETT CANCER CENTER

NCI Cancer Center
A Cancer Center Designated by the National Cancer Institute

Fred & Pamela Buffett Cancer Center

LB 595 Annual Program Progress Report

Program Period: July 2021 – June 2022

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PROGRAM OVERVIEW

Mission: The Fred & Pamela Buffett Cancer Center (BCC), the only NCI-designated cancer center in Nebraska, is a matrix cancer center at the University of Nebraska Medical Center and our affiliated healthcare network, Nebraska Medicine. The Mission of the BCC is to promote innovative translational cancer research, excellence in cancer education and training, and outstanding patient-centered cancer care, and to reduce the burden of cancer and cancer health disparities across Nebraska and beyond.

The BCC continues to make substantial progress in pursuing our mission by advancing scientific and clinical research, expanding BCC facilities and research infrastructure, promoting transdisciplinary collaborations, inclusive of their close integration with clinical research and care, strengthening and expanding cancer training and educational programs for trainees and faculty development, and expanding our community outreach and engagement with community partners across the Nebraska.

The BCC has the following Specific Aims:

Aim 1: To provide a research environment, including innovative shared resources, that promotes interdisciplinary cancer research.

Aim 2: To promote outstanding scientific discovery in the mechanisms of cancer initiation and progression, and to identify associated biomarkers for risk, prognosis, and therapy.

Aim 3: To lead the development of new therapeutic strategies and innovative clinical trials regionally and nationally.

Aim 4: To drive research in our Catchment Area, the state of Nebraska, related to prevention, early detection, and control of cancer.

Aim 5: To provide leadership in cancer research and education, and to enhance cancer-related research and the clinical workforce regionally and beyond.

Aim 6: To promote community outreach and engagement activities to understand and address cancer burden lth disparities, particularly in the underserved and underrepresented communities in Nebraska and nationally.

OVERALL

Cancer Center Organization and Senior Leadership

To most effectively support the BCC's progress towards its prioritized strategic goals, several important changes have been made to the Cancer Center's senior leadership team. First, Quan Ly, MD, was appointed the Associate Director for Diversity, Equity and Inclusion. Dr. Ly is a surgical oncologist with a strong background in basic, translational, and clinical cancer research, particularly in the area of pancreas cancer. She also serves as Vice Chair of Diversity, Equity and Inclusion in the UNMC Department of Surgery. She is committed to initiating and fostering DEI initiatives at the Buffett Cancer Center and to working with University leadership to collaborate with similar activities at the campus-wide level. Dr. Ly will work closely with Dr. Joyce Solheim, BCC Associate Director for Cancer Research Training and Education and Dr. Shinobu Watanabe-Galloway, BCC Associate Director for Community Outreach and Engagement, to enhance diversity, equity and inclusion across the BCC and to reduce cancer health disparities across the state. Second, Heather Jensen-Smith, PhD, was appointed the Assistant Director for Shared Resources. Dr. Jensen-Smith will be working closely in this role with the Associate Director for Basic Research and component lead for Shared Resource Management, Dr. Tony Hollingsworth. Much of Dr. Jensen-Smith's research has focused on the development and use of various imaging methodologies for representation of biological phenomena during normal and

disease states. She has extensive experience in the advanced technology, equipment, and operations of the Cancer Center core facilities. The BCC looks forward to Dr. Jensen-Smith assisting Dr. Hollingsworth with overseeing the Cancer Center-support shared resources and helping investigators who are seeking to enhance their research with the extensive array of services available within these core facilities. Third, Pankaj Singh, PhD, departed UNMC for a new leadership opportunity at the University of Oklahoma. Led by the BCC Director, Deputy Director, and other senior leaders, the process is underway to identify a replacement for Dr. Singh as Co-Leader of the Cancer Biology Program, in coordination with Program Co-Leader Dr. Hamid Band.

DIRECTOR'S OVERVIEW AND SIX ESSENTIAL CHARACTERISTICS

Physical Space

The new BCC cancer research and cancer care facility celebrated its grand opening in June 2017, with President Biden as the keynote speaker. This 615,000 sq ft facility contains 98 research laboratories, a 108-bed cancer hospital, multidisciplinary clinics, a 24/7 infusion center, radiation treatment facilities, surgical suites, an imaging center, offices for clinical and research faculty, and the BCC Clinical Trials Office. This uniquely integrated building was specifically designed to create an environment that fosters advances in transdisciplinary research through enabling efficient scientific innovation, while facilitating the delivery of state-of-the-art multidisciplinary patient care in the context of an optimal patient experience. The new facility provides the BCC Director with authority over space for integration of the programs and recruitment planning, that the access of the BCC membership to one another as well as to shared resources is of great benefit to the science, and that the environment also provides a great benefit to the patients and the clinical interface. The BCC will continue to build on its strengths in space and facilities in the coming project period.

Organizational Capabilities

The Cancer Center has two principal advisory boards for planning and evaluation: the External Advisory Board and the Senior Leadership Council. The EAB is comprised of nationally recognized leaders in basic, translational, clinical, and population research and cancer center administration with leadership roles at NCI-designated Cancer Centers. They meet regularly with Center leadership to review research, outreach and engagement, as well as training and education. The BCC Senior Leadership Council includes the Deputy Director and Associate Directors and program leaders. It meets monthly and at an annual retreat to review Cancer Center activities and update/revise strategic initiatives.

Transdisciplinary Collaboration and Coordination

The unique BCC research space promotes collaboration and interaction among members of basic, clinical and population science programs, as evidenced by the success of publication and funding efforts. This component was evaluated as having strong leadership to promote these efforts and considerable transdisciplinary research activities as it relates to interactive publications and funding of multi-investigator grants. BCC members remain highly collaborative, with almost half of all program member publications since August 2020 being CCSG interactive. The BCC membership currently holds multiple multi-investigator project awards. Recent strategic recruitments in the translational/clinical research space as well as in cancer prevention and control and population science will be instrumental in growing the success of transdisciplinary collaboration at the BCC. Those recruits are briefly summarized as follows:

- **Joshua Mammen, MD, PhD,** was named professor and chief in the UNMC Division of Surgical Oncology, Department of Surgery. An established leader, clinician, and researcher with a focus on melanoma and soft tissue sarcomas, Dr. Mammen is looking forward to working with colleagues and collaborators throughout the region to also address issues beyond treatment, including cancer prevention, early detection, and addressing cancer care disparities.

- **Melodi Whitley, MD, PhD,** was recruited as assistant professor and director of transplant dermatology

in the UNMC Department of Dermatology. Dr. Whitley's research and clinical interests focus on studying cutaneous squamous cell carcinoma and caring for patients with high-risk skin cancers.

- **Joseph Khoury, MD,** was named professor and chair of the UNMC Department of Pathology and Microbiology. Dr. Khoury is an internationally recognized leader in the field of hematopathology, with expertise in the fields of molecular pathology, flow cytometry and immunohistochemistry, with particular emphasis on clinical applications of these tools for biomarker discovery and detection.

- **Edward Peters, DMD, ScD,** was recruited as professor and chair of the UNMC Department of Epidemiology. Dr. Peters' research interests use classic and molecular epidemiologic tools to examine molecular and biologic heterogeneity and susceptibility of cancer and other chronic diseases. This research examines how social determinants of health influence disparate disease outcomes through a transdisciplinary social-genomic perspective. His lab is currently studying the interaction between the built environment, stress, inflammation, and the development of ovarian, colorectal, and oral cancers.

- **Ronnie Horner, PhD,** was named professor and chair of the UNMC Department of Health Services Research and Administration. Dr. Horner's research interest focus on mHealth technology support in shared decision-making, provider resiliency, and precision clinical management of disease, particularly neurological disease. These interests are components of precision health care delivery that focuses on incorporating patient preferences and desires regarding received health care for the purpose of maximizing health outcomes.

- **Sunil Hingorani, MD, PhD,** was recruited as the inaugural Nancy Armitage Presidential Chair and as the inaugural director of a newly established Pancreatic Cancer Center of Excellence. The center was instituted via the passing of LB 766 by the Nebraska state legislature which allocated $15 million in funding to be matched by another $15 million in private philanthropy. The bill was sponsored by state Senator Kolterman, a member of the BCC Community Outreach and Engagement EAB. Dr. Hingorani has extensive experience in animal histopathology and pancreatic cancer clinical trials, and he established an influential murine clinical trials program. The BCC anticipates Dr. Hingorani's research program will collaborate with several Cancer Center members and established and developing shared resources, including Tony Hollingsworth and Paul Grandgenett and the Pancreatic Cancer Rapid Autopsy Program, Kurt Fisher and Adrian Black and their organoids models, Heather Jensen-Smith and the Preclinical Imaging Shared Resource, and DJ Murry and translational drug development efforts.

A primary mechanism to facilitate collaboration is the successful pilot grant program involving BCC leadership at all levels, with each area getting to prioritize funding. In 2021, the BCC funded 21 collaborative pilot projects (out of 59 applications received), totaling nearly $1,200,000, with Pis represented from all the major cancer research-focused departments at UNMC. Funding was provided by various sources including philanthropic support, specifically the Cattlemen's Ball of Nebraska, as well as partnerships with the American Cancer Society, the UNMC Great Plains IDeA-CTR, and the UNMC Pediatric Cancer Research Group. In the coming project period, the BCC will continue to refine the implementation of this program and the tracking of its success.

The Cancer Center will also work to continue improving the translation of basic science discoveries into clinical trial accruals. There was a 19% increase in patient accruals to interventional protocols in 2021 as compared to 2020. Importantly, although most NCI cancer centers' accrual decreased during the COVID-19 pandemic, the Buffett Cancer Center was able to increase its accrual more than the national average in 2020 and above 2019 numbers in 2021.

Cancer Focus

The BCC has established and maintained a long history of outstanding cancer research. Grants awarded by the NCI to BCC members continue to provide a significant source of funding. In the coming project period, the

BCC will continue to focus on increasing cancer-focused funding and facilitating the publishing of papers in high-impact cancer research journals.

Institutional Commitment

Recently, there has been greater integration of the cancer research efforts and clinical activities, via the BCC Director's reporting relationship to the UNMC Chancellor and the Nebraska Medicine CEO which allows for better integration of the matrix responsibilities of the Director and linking of efforts in research and in practice. These new levels of administrative linkage, oversight, and integration an important demonstration of commitment of UNMC to the mission and success of BCC. Of particular note is the following important authorities of the BCC Director: the Director is a member on the most important leadership cabinets for UNMC (Chancellor's Council) and NM (Senior Leadership Team); the BCC Director has the authority to appoint memberships in the BCC to faculty from all NU academic units; the BCC Director has authority over the state budget for the Eppley Institute and the BCC and over fundraising and all philanthropic funds for the BCC; the BCC Director has complete authority over all BCC controlled space for research and administration; the BCC Director has the authority to recruit faculty with primary academic appointments into the Eppley Institute and oversees promotion and tenure in the Institute; and the BCC Director controls 400,000 net square feet for research and administration, a 67% increase since 2015. The BCC Director is currently recruiting one or more junior investigators for faculty positions in the Eppley Institute, and he is also actively supporting the recruitment of research faculty in other cancer research departments, including Genetics, Cell Biology and Anatomy. Successfully completing this recruitment cycle will be a focus for the coming project period.

Center Director

The BCC Director, Dr. Cowan, has had an extraordinary cancer career in research, practice, and as a center director. He has achieved much progress in several areas, including significant philanthropy, growth in lab research space, integration of the BCC in UNMC/NM clinical practice, increases in cancer-focused, peer-reviewed funding and in NCI direct funding specifically, and improvements in clinical trial functionality and enrollment. To support his endeavors, Dr. Cowan expanded cancer center leadership, developing additional delegate roles like Deputy Director and Associate Directors for Basic Research, Translational Research, Clinical Research, Cancer Research Training and Education Coordination, and Community Outreach and Engagement. Recently, Dr. Cowan further expanded the BCC senior leadership team by appointing the inaugural Associate Director for Diversity, Equity and Inclusion.

Dr. Cowan has communicated his plan to step down as Director during the next funding period, and there is institutional commitment for a robust search and selection process. The BCC plans an upcoming transition period in which Dr. Cowan will remain at UNMC to facilitate the change successfully.

PROJECT UPDATES

PLANNING AND EVALUATION

Specific Aims: Planning and Evaluation

1) Develop a strong senior leadership team to establish the BCC vision and goals:

   A strong BCC senior leadership team will work collaboratively through an effective organization with key advisory committees to establish the BCC vision and goals. The leadership team will oversee cancer research, cancer care and cancer education at the University of Nebraska and Nebraska Medicine and cultivate research collaborations across the University and throughout our catchment area (Nebraska);

2) Advance effective strategies to achieve BCC objectives:

   The BCC senior leadership team will leverage existing scientific strengths and prioritize research in specific strategic areas key to the center's future goals. The senior leaders will steward and allocate BCC resources and cultivate collaborations across the university and the state to: 1) enhance transdisciplinary research through strategic recruitment of key research and clinical faculty; 2) expand shared resources and research infrastructure with advanced technologies and services; 3) promote training and education for students, trainees, and faculty; and 4) develop effective partnerships with diverse communities across Nebraska to address the cancer burden and disparities in the state; and

3) Implement processes to evaluate progress and refine strategies to achieve BCC objectives:

   The leadership will review outcomes throughout the year and at an annual BCC leadership retreat. Feedback will be provided by an External Advisory Board composed of experts from NCI-designated cancer centers, university leadership, Nebraska Medicine leadership, community and internal advisory committees, and state-wide partners. Periodic surveys of the users of Shared Resources will be used to drive improvements and growth.

The Fred & Pamela Buffett Cancer Center (BCC) has a strong leadership team that works collaboratively to develop the Center's strategic plan, in consultation with internal and external committees, and to implement the BCC mission and goals. The BCC works to continually reaffirm its mission to promote innovative translational cancer research, excellence in cancer education and training, and outstanding patient-centered cancer care, and to reducing the burden of cancer and cancer health disparities across Nebraska and beyond. The goals of the Planning and Evaluation activities are to have a team of strong leaders that: 1) envision, develop, and implement innovative and collaborative programs to advance the mission and goals of the BCC; 2) review and assess the progress through a reiterative process; and 3) refine and revise strategies as needed to achieve desired outcomes.

The Buffett Cancer Center has two principal Advisory Boards for Planning and Evaluation: the External Advisory Board and the Senior Leadership Council. The EAB is comprised of nationally recognized leaders in basic, translational, clinical, and population research and cancer center administration with leadership roles at NCI-designated Cancer Centers. They meet regularly and at least annually with Center leadership to review research, outreach and engagement, as well as training and education. The most recent EAB meeting was held virtually on April 20, 2022, with all members except two attending. This meeting was successful, and the Board's useful advice will be used to clarify strategic priority setting for the BCC in the coming year. Plans are underway for the next EAB meeting, to be held sometime in early 2023. The Senior Leadership Council includes the BCC Deputy and Associate Directors and Program Leaders. It meets monthly and at an annual retreat to review Cancer Center activities and update, revise, and evaluate the progress of strategic initiatives. Meetings have been held in in April, May, June, July, August, October, and November of 2021, and in January, February, March, and May of 2022.

Buffett Cancer Center External Advisory Board, Current Membership:

- **Kerry Burnstein, PhD,** *University of Miami Miller School of Medicine and Sylvester Comprehensive Cancer Center*;
- **Victoria Champion, PhD, RN,** *Indiana University and IU Simon Comprehensive Cancer Center*;
- **Robert Diasio, MD,** *Mayo Clinic College of Medicine and Mayo Clinic Cancer Center*;
- **Eric Fearon, MD, PhD,** *University of Michigan Rogel Cancer Center*;
- **Robert Gerlach, MPA,** *Dartmouth-Hitchcock Norris Cotton Cancer Center*;
- **Stanton Gerson, MD,** *Case Western Reserve University and Case Comprehensive Cancer Center*;
- **David Goldman, MD,** (Chair), *Albert Einstein College of Medicine and Albert Einstein Cancer Center*;
- **Stanley Hamilton, MD,** *City of Hope Comprehensive Cancer Center*;
- **Ernest Hawk, MD, MPH,** *University of Texas MD Anderson Cancer Center*;
- **Patrick Loehrer, MD,** *Indiana University School of Medicine and IU Melvin and Bren Simon Cancer Center*;
- **Linda Malkas, PhD,** *City of Hope Comprehensive Cancer Center*;
- **James Mulé, PhD,** *Moffitt Cancer Center*; and
- **Douglas Yee, MD,** *University of Minnesota Medical School and UM Masonic Cancer Center*.

Buffett Cancer Center Senior Leadership Council:

- **Ken Cowan, MD, PhD—**Director and Physician-in-Chief
- **Ray Bergan, MD—**Deputy Director
- **Tony Hollingsworth, PhD—**Associate Director, Basic Research
- **Heather Jensen-Smith, PhD—**Assistant Director, Shared Resources
- **Surinder Batra, PhD—**Associate Director, Translational Research
- **Apar Ganti, MD—**Associate Director, Clinical Research
- **Shinobu Watanabe-Galloway, PhD—**Associate Director, Community Outreach and Engagement
- **Nicole Carritt, MPH—**Assistant Director, Community Outreach and Engagement
- **Joyce Solheim, PhD—**Associate Director, Training and Education
- **Quan Ly, MD—**Associate Director, Diversity, Equity and Inclusion
- **Matt Winfrey, MPP—**Associate Director, Administration and External Affairs
- **Hamid Band, MD, PhD—**Leader, Cancer Biology Program
- **Sarah Holstein, MD, PhD—**Co-Leader, Targets, Modulators and Delivery Program
- **Rob Lewis, PhD—**Co-Leader, Targets, Modulators and Delivery Program
- **Amar Natarajan, PhD—**Co-Leader, Targets, Modulators and Delivery Program
- **Jenny Black, PhD—**Co-Leader, Gastrointestinal Cancer Program
- **Chi Lin, MD, PhD—**Co-Leader, Gastrointestinal Cancer Program
- **Vimla Band, PhD—**Chair, UNMC Department of Genetics, Cell Biology and Anatomy

The Buffett Cancer Center has an extensive organizational structure is in place and clearly functions well, with the leadership team being effective at enhancing transdisciplinary research activities with impact. Moving forward, the BCC will focus on further clarifying the various leadership committees and their roles in the planning process and on illuminating the relationships between the BCC Director and campus leadership.

DEVELOPMENTAL FUNDS

Specific Aims: Developmental Funds

1) To link the use of developmental funds to the results of planning and evaluation activities as they relate to strategic faculty recruitment to the Buffett Cancer Center; and
2) To utilize discretionary funds to promote transdisciplinary cancer research and advance the BCC Research Programs.

The Buffett Cancer Center Senior Leadership has assessed and chosen to strategically support the recruitment and retention of various basic, translational, and clinical cancer research positions using developmental funds over the previous year. The recent recipients of developmental funds are summarized below, with descriptions of their individual research programs following.

Faculty Recruitment

Michael Baine, MD, PhD: Dr. Baine is an Assistant Professor in the Department of Radiation Oncology at UNMC and an Associate Member of the BCC Targets, Modulators and Delivery Program (TMDP). Dr. Baine's research focuses on the development and testing of novel and cutting-edge diagnostic and therapeutic strategies for GI and GU malignancies with specific focus on pancreas adenocarcinoma, prostate cancer, ad urothelial carcinoma of the bladder. Ongoing projects in Dr. Baine's laboratory include: clinical validation of a systematically developed combinatorial biomarker panel for pancreatic cancer (PC) diagnosis and prognosis; analysis of adjuvant versus salvage therapy following radical prostatectomy for prostate adenocarcinoma; and assessing utility of immune modulators with short course radiation therapy in unresectable urothelial carcinoma of the bladder. Recent collaborative publications to which Dr. Baine's research contributed can be found in: *American Journal of Surgery*, *Cancers (Basel)*, *Oncology*, *Medicine (Baltimore)*, *Neoplasia*, *Urology*, *World Journal of Urology*, and *Scientific Reports*. Dr. Baine currently has active funding from the Otis Glebe Medical Foundation through a partnership with the University of Nebraska Foundation.

Kishor Bhakat, PhD: Dr. Bhakat is an Associate Professor in the Department of Genetics, Cell Biology at UNMC and Anatomy. He is a Member of the BCC Cancer Biology Program. His major research foci are mitotic gene bookmarking—a novel dimension of epigenetic memory in post-mitotic transcriptional reactivation, as well as APE1 and its acetylation—a potential prognostic and predictive biomarker in cancer. Dr. Bhakat has recent papers in: *Nucleic Acids Research*, *DNA Repair*, *Diangostics*, and *Cancer Letters*. He has a pending NIGMS R01 application investigating "A Novel Mechanism of Preferential Repair of Endogenous Damage in the Transcribed Genes and Telomeres by APE1", and another pending NINDS R01 application looking at "Targeting G-quadruplex Structure and FACT Complex to Sensitize Medulloblastoma to Radiation and Chemotherapy", as well as pending applications with the U.S. Department of Defense Congressionally Directed Medical Research Programs and with Alex's Lemonade Stand Foundation.

Punita Dhawan, PhD: Dr. Dhawan is a Professor in the Department of Biochemistry and Molecular Biology, and a Member of the BCC Gastrointestinal Cancer Program. She was recruited from Vanderbilt University in October 2014. Her research focuses on identifying novel biomarkers and therapeutic targets for colorectal and pancreatic cancer progression and metastasis. The major focus is on understanding the role and associated mechanism/s of claudin cell-cell adhesion proteins and cell cycle molecules in cancer metastasis, cancer stem cells (CSC) and therapy resistance. Recent collaborative publications to which Dr. Dhawan's research contributed can be found in: *Biomarkers in Medicine*, *Cancer Communications (London)*, *Clinical & Experimental Metastasis*, *Tissue Barriers*, *Clinical and Translational Gastroenterology*, *ACS Nano*, *Biotechniques*, *Oncogene*, and *Cells*. Her currently active funding includes a Veterans Administration Merit Award and an NCI R01 grant.

Kristin Dickinson, PhD, RN: Dr. Dickinson was recruited to UNMC in 2018 as an Assistant Professor in the College of Nursing. She is a Member of the BCC Cancer Biology Program. Dr. Dickinson's research is focused on understanding and managing cancer-related fatigue (CRF). Dr. Dickinson came to UNMC with R00 funding focused on the investigation of the role of cellular adaptive mechanisms and mitochondrial function in CRF in

men with non-metastatic prostate cancer. The K99 phase of the grant investigated biomarkers in acute CRF that develops during radiation therapy for men with nonmetastatic prostate cancer. Findings from this study provide preliminary evidence that cell damage might be upregulated in the CRF phenotype. She then conducted the R00 phase of the project that focuses on validating the K99 findings, adding examination of the mitochondrial bioenergetic profile, and extending investigation to chronic CRF in survivorship. In addition to her clinical study, Dr. Dickinson has worked with multidisciplinary collaborators at UNMC to expand her program of research to include a preclinical model. This effort is aimed at providing the unique opportunity to take observations form her previous clinical studies to an animal model of CRF to provide access to mechanistic investigations of metabolic dysfunction, hypoxia, and oxidative stress in CRF. Enhanced understanding of the biology of CRF will help guide the future development of targeted mechanism-based interventions, resulting in improved quality of life for those with cancer. Dr. Dickinson has a recent paper in *Oncology Nursing Forum*, "Demographic, Symptom, and Lifestyle Factors Associated with Cancer-Related Fatigue in Men with Prostate Cancer".

Gargi Ghosal, PhD: Dr. Ghosal joined the Department of Genetics, Cell Biology and Anatomy as an Assistant Professor in 2016. She is a Member of the BCC Cancer Biology Program. The research focus of Dr. Ghosal's laboratory is on understanding the molecular basis of genome instability in cancer and premature aging syndromes. Using mouse genetics and cell and molecular biology techniques, the Ghosal lab has been investigating the molecular mechanism underlying the replication stress response upon DNA damage and oncogene activation, with a focus on: a) Oncogene-induced replication stress response in Ewing sarcoma pathogenesis; b) Elucidating the molecular and physiological functions of SPRTN and SPRTN mediated translesion synthesis (TLS) and DNA-protein crosslink (DPC) repair in DPC-induced cancer; and c) Identifying enzymes that regulate replication stress response signaling and DNA repair to identify new targets and biomarkers for cancer therapy and to overcome drug resistance. Recent collaborative publications to which Dr. Ghosal's research contributed can be found in: *Frontiers in Molecular Bioscience*, *Proceedings of the National Academy of Sciences of the United States of America*, and *FEBS Journal*. She was recently awarded two R01 grants, one from the National Cancer Institute and one from the National Institute of General Medical Sciences.

R. Kate Hyde, PhD: Dr. Hyde is an Associate Professor in the Department of Biochemistry and Molecular Biology. She was recruited to UNMC in 2013, and she is a Member of the BCC Targets, Modulators and Delivery Program. Her research focuses on the molecular mechanisms regulating gene expression and cell survival in leukemia, with an emphasis on the Core Binding Factors (CBF), CBFβ and RUNX1. Her work has identified novel targets for drug development and provided pre-clinical data on new treatment strategies for leukemia. Dr. Hyde's recent papers can be found in: *Transplantation and Cellular Therapy*, *Journal of Geriatric Oncology*, and *Leukemia*. Her current funding includes an NCI R01 grant.

Kyle Hewitt, PhD: Dr. Hewitt joined the Department of Genetics, Cell Biology and Anatomy as an Assistant Professor in 2018. He is a Member of the BCC Cancer Biology Program. His lab is focused on identifying gene regulatory networks and cell signaling mechanisms that control blood production in physiological contexts, and the deregulation of these networks during initiation and progression to myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Establishing fundamental principles that govern blood homeostasis (and genetic mutations that predispose illness) is an essential step towards developing personalized medicine approaches and advancing translational strategies to treat disease. While foundational work has revealed transcription factors (e.g. GATA2) that regulate AML progression, critical targets remain unknown. The Hewitt lab has developed several unique *in vivo* mouse models and *ex vivo* gene editing approaches to study blood regeneration in stress and leukemia progression. One GATA2-regulated target locus (SAMD14) stimulates cell signaling through the proto-oncogenic c-Kit signaling pathway, which is an important signaling pathway in blood regeneration, stem cell transplantation, hematopoietic/erythropoietic progenitor expansion, leukemia and anemia. Delineating the mechanism(s) whereby a GATA2-regulated network controls leukemia progression has a high potential to reveal new therapeutic strategies for treating hematologic diseases. Dr. Hewitt has a recent paper in *Elife*, "Functional requirements for an Samd14-capping protein complex in stress erythropoiesis". His is actively funded via an R01 grant from the National Heart, Lung, and Blood Institute titled "GATA Factor Mechanisms in Erythroid Regeneration".

So-Youn Kim, PhD: Dr. Kim was recruited to UNMC in 2018 as an Assistant Professor in the Department of Obstetrics and Gynecology. Dr. Kim is a Member of the BCC Cancer Biology Program, along with the UNMC Child Health Research Institute (CHRI), The Midlands Society of Physiological Sciences (MSPS), The Endocrine Society (ENDO), and the Society for the Study of Reproduction (SSR). Dr. Kim's laboratory focuses on understanding oocyte death mechanisms induced by chemotherapeutic agents using multiple oocyte-specific knockout mouse models. Dr. Kim's research discovered that oocytes have a unique mechanism for death against gonadotoxic agents, which led to an R01 award (HD096042, Development of Mechanism-Based Ovarian Reserve Protecting Adjuvant Therapies against Gonadotoxic Therapeutic Agents). Furthermore, Dr. Kim developed a new mouse model for studying granulosa cell tumors (GCT) and cancer cachexia and received funding twice from the Granulosa Cell Tumor Research Foundation (GCTRF). Recent collaborative publications to which Dr. Kim's research contributed can be found in: *microPublication Biology*, *Biofabrication*, *Journal of Cachexia, Sarcopenia and Muscle*, *Journal of Reproductive Immunology*, *Scientific Reports*, *Journal of Endocrinology*, and *International Journal of Molecular Sciences*. She has active R01 funding from the National Institute of Child Health and Human Development, along with ongoing funding from the Granulosa Cell Tumor Research Foundation.

Robin Lally, PhD, RN: Dr. Lally was recruited from the University of Buffalo as a Professor in the College of Nursing. Dr. Lally's background includes ICU nursing in the Mayo hospitals, Rochester, MN, clinical trials nursing, and two decades of oncology nursing, specializing in breast cancer and psycho-oncology concepts and development of an Internet-based clinical intervention to support the psychosocial wellbeing of women with breast cancer and their families. Dr. Lally also holds a minor in biomedical ethics and earned a certificate in applied cognitive behavioral therapy and related supportive oncology. Dr. Lally's research focuses on the psychological adjustment of people newly diagnosed and surviving cancer as well as their families/friends. She led a team in the development of CaringGuidance" After Breast Cancer Diagnosis (https://my.caringguidance.org), an Internet-based, self-guided psychoeducational program for women newly diagnosed with breast cancer to address distress and depressive-symptoms through the provision of information, coping strategies, and support accessed by women on their computers/mobile devices. Dr. Lally has contributed to recent papers in *Oncology Nursing Forum*: "Update to 2019-22 ONS Research Agenda: Rapid Review to Promote Equity in Oncology Healthcare Access and Workforce Development", and "Update to 2019-2022 ONS Research Agenda: Rapid Review to Address Structural Racism and Health Inequities". She is also participating on a pending NIH U01 application led by Principal Investigator Dr. Shinobu Watanabe-Galloway (BCC Associate Director for Community Outreach and Engagement) investigating "Reducing Pandemic-Related Health Disparities in Cancer Care: Use of Health Exchange Information Data to Conduct Social, Behavioral and Economic Research on COVID-19".

Aaron Mohs, PhD: Dr. Mohs is an Associate Professor and Associate Dean for Research and Graduate Studies in the Department of Pharmaceutical Sciences in the UNMC College of Pharmacy. He is a Member of the BCC Targets, Modulators and Delivery Program. He was recruited from Wake Forest University Health Sciences in 2015. Dr. Mohs' primary research area is the development of fluorescent imaging agents to guide the surgical removal of tumors. His goal is to design targeted fluorophores that bind to tumor or stromal biomarkers to highlight tumor margins during surgery. Improved margin detection and removal could significantly reduce recurrent disease. Dr. Mohs and his team have developed probes targeted to mucins and CD44, which are both overexpressed on multiple cancers. More recently, he has worked with industry to develop probes targeted to the TrkA receptor to highlight nerves in nerve-sparing surgery. Dr. Mohs has recently published papers in: *Molecular Pharmacology*, *Analytical Chemistry*, *Biofabrication*, *Biotechniques*, and *ACS Omega*. He has active R01 funding from the National Institute of Biomedical Imaging and Bioengineering as well as from the NCI.

Mohd Nasser, PhD: Dr. Nasser was recruited as an Assistant Professor in the Department of Biochemistry and Molecular Biology in 2018. He is a Member of the BCC Cancer Biology Program. Dr. Nasser has demonstrated the role of S100 family protein S100A7 and its receptor RAGE in enhancing breast cancer growth and metastasis (Nasser et al. 2012 Can Res and Nasser et al. 2015 Can Res). The current focus of his laboratory is to understand the role of microRNAs and mucin proteins, especially MUC5AC, in establishing brain metastasis of breast and lung cancers. He has also started to explore the tumor-suppressive role of

microRNA miR-1 in small cell lung cancer. In collaboration with BCC members Drs. David Oupicky and Surinder Batra (also BCC Associate Director for Translational Research), he has developed miR-1 conjugated CXCR4-antagonist based nanoparticles for the attenuation of SCLC growth and metastasis. Dr. Nasser has contributed to several recent publications in: *Biochimica et Biophysica Acta – Reviews on Cancer*, *Frontiers in Immunology*, *Seminars in Cancer Biology*, *Molecular Cancer*, *Cytokinase and Growth Factor Reviews*, *Seminars in Cell and Developmental Biology*, *Bone Research*, *Biomolecules*, *Acta Neuropathology Communications*, *Cellular Oncology (Dordrecht)*, *Molecular Cancer Therapeutics*, *Cancer Letters*, and *Molecular Oncology*. He has two active R01 grants from the National Cancer Institute and collaborates on two NCI P01 awards led by Principal Investigator Dr. Surinder Batra (BCC Associate Director for Translational Research).

Armen Petrosyan, MD, PhD: Dr. Petrosyan joined the Department of Biochemistry and Molecular Biology as an Assistant Professor in 2014. He is a Member of the BCC Cancer Biology Program. Research in Dr. Petrosyan laboratory is centered on three distinct but related areas involving: 1) fundamental studies of mistargeting and dysfunction of the Golgi residential proteins during carcinogenesis, 2) the application of novel microscopy approaches for characterization of Golgi disorganization in cancer tissue and its correlation with severity of prostate cancer, and 3) the impact of Golgi disruption on the metastatic potential of cancer cells. The long-term goals of his group are to define the persistent signaling profiles induced by Golgi fragmentation and associated with the progression of prostate cancer. In 2019, he received an R01 award (AA027242) to study the link between alcohol and the progression of prostate cancer. Dr. Petrosyan has recent papers in: *Biomolecules*, *Hepatology Communications*, *Journal of Experimental and Clinical Cancer Research*, and *American Journal of Physiology – Gastrointestinal and Liver Physiology*. He has a second active R01 award from the National Institute on Alcohol Abuse and Alcoholism looking at “The Role for Alcohol-Induced Golgi Disorganization in the Progression of Prostate Cancer”.

Moorthy Palanimuthu Ponnusamy, PhD: Dr. Ponnusamy is an Associate Professor in the Department of Biochemistry and Molecular Biology. He was recruited to UNMC in 2014 as an Assistant Professor. Dr. Ponnusamy is a Member of the BCC Gastrointestinal Cancer Program. His research focuses on identifying and characterizing cancer stem cell populations in pancreatic and ovarian cancers. His laboratory has recently identified a novel biomarker, Pancreatic Differentiation 2/Polymerase Associated Factors 1 (PD2/PAF1), that is involved in the maintenance of drug-resistance and self-renewal of cancer stem cells. His current research is focused on investigating the impact of PD2/PAF1 in the self-renewal and drugresistance of cancer stem cells. Dr. Ponnusamy has contributed to recent collaborative publications in: *Cell Death Discovery*, *Molecular Cancer Research*, *Clinical and Translational Discovery*, *Gastroenterology*, *Seminars in Cancer Biology*, *EbioMedicine*, *Biochimica et Biophysica Acta – Reviews on Cancer*, *Oncogene*, *Molecular and Cellular Biology*, *Journal of Experimental and Clinical Cancer Research*, *Clinical Cancer Research*, and *Molecular Oncology*. He has two active multi-PI R01 awards from the National Cancer Institute and collaborates on an NCI P01 award led by Principal Investigator Dr. Surinder Batra (BCC Associate Director for Translational Research).

Satyanarayana Rachagani, PhD: Dr. Rachagani is an Associate Professor in the UNMC Department of Biochemistry and Molecular Biology and a Member of the BCC Gastrointestinal Cancer Program (GICP). His major research foci are: identification of miRNA signature for diagnosis and prognosis of pancreatic cancer; genetically engineered mouse models with/without mucins to study pancreatic and colorectal cancer pathogenesis; and chemoprevention and novel combination therapies for pancreatic and colorectal cancer. Dr. Rachagani’s lab focuses on studying pathogenesis and targeting strategies for pancreatic and colorectal cancers through miRNAm natural agents, and other novel combination therapies using human- and mouse-derived cell lines and GEM models. An additional research focus of Dr. Rachagani’s lab examines the role of mucins in cancer pathogenesis and their targeting strategies using cell lines, organoids, and genetically engineered mouse models. Dr. Rachagani has contributed to recent collaborative papers in: *Cancer Letters*, *Nanomedicine*, *Cellular and Molecular Life Sciences*, *Aging (Albany, NY)*, *Metabolites*, *EbioMedicine*, *Oncogene*, *Clinical Cancer Research*, *Clinical Cancer Research*, *Biomedicine and Pharmacotherapy*, *Gastroenterology*, *Pharmaceutics*, *Cancers (Basel)*, *Journal of Experimental and Clinical Cancer Research*, *Clinical Cancer Research*, and *Molecular Oncology*. His active R01 grant from the NCI is titled “Targeting Tumor and Its Microenvironment Using Nanotherapeutics for Pancreatic Cancer”.

Micah Schott, PhD: Dr. Schott is an Assistant Professor in the Department of Biochemistry and Molecular Biology, as well as an Associate Member in the BCC Cancer Biology Program. He was recruited to UNMC from the Mayo Clinic in 2021. His major research focus is on cell biology of lipid metabolism in metabolic liver diseases, with interest areas in lipid droplets, autophagy, cAMP, vesicle trafficking, and metabolism. Dr. Schott was awarded a K99/R00 award from the National Institute on Alcohol Abuse and Alcoholism; the project looked at “Synergy of Lipolysis and Lipophagy in Alcoholic Liver Disease” (AA026877). Dr. Schott was also awarded a recent supplement to his R00 titled “. The purpose and scope of this supplement is to provide additional funds to mitigate disruptions caused by the COVID19 pandemic on research and training activities related to the parent grant, which seeks to define new mechanisms of lipid catabolism affecting alcoholic liver disease (ALD). The research activities during this period will address Specific Aim 2, which seeks to define a novel, endo-lysosome based mechanism of microlipophagy that is impacted by alcohol consumption. In addition, this supplement will allow me to complete my proposed training in the use of animal models of ALD. The results gained from the proposed research will provide a mechanistic understanding of lipid droplet catabolism in alcoholic fatty liver. Importantly, these studies will provide published research manuscripts and preliminary data in support of a future R01 proposal. The supplement project has significant relevance to public health, as fatty liver affects ~90% of heavy drinkers. This project uses microscopy, biochemistry, mass spectrometry, and rodent models of ALD to determine the interplay between lipolysis and lipophagy in the hepatocellular breakdown of lipid droplets. The goal of this work is to gain a comprehensive understanding of hepatic lipid catabolism to support the development of pharmacotherapies that mitigate fatty liver progression. Dr. Schott recently contributed to collaborative publications in the *Journal of Cell Science* and *Autophagy*. He has an active NIAAA R00 award that aims to investigate “Synergy of Lipolysis and Lipophagy in Alcoholic Liver Disease”, and he is participating as a Research Project Leader on a recently submitted NIGMS COBRE Phase 2 application (P20GM121316) led by Principal Investigator Dr. Robert Lewis (Co-Leader, BCC Targets, Modulators and Delivery Program).

Jawed Siddiqui, PhD: Dr. Siddiqui is an Assistant Professor in the UNMC Department of Biochemistry and Molecular Biology and a Member of the BCC Cancer Biology Program. His major research interests are in bone metastasis and therapeutics, chemokines and bone metabolism, and the tumor microenvironment, with focus areas in bone biology and chemokines. Dr. Jain has several recent papers in: *Gastroenterology*, *Seminars in Cancer Biology*, *Molecular Cancer*, *Phytochemistry*, *Cytokine and Growth Factor Reviews*, *Seminars in Cell and Developmental Biology*, *Aging (Albany NY)*, *Bone Research*, and *Cancer Letters*. He has active funding from the U.S. Department of Defense Congressionally Directed Medical Research Programs looking at “Targeting Novel CDF15/CFRAL/RET Axis in Prostate Cancer Bone Metastasis”, and from METAvivor Research & Support, Inc., examining “Therapeutic Targeting of GFRAL/RET Axis to Overcome Bone Metastasis of Breast Cancer”.

Amar Singh, PhD: Dr. Singh is a tenured Professor in the Department of Biochemistry and Molecular Biology and a Member of the BCC Gastrointestinal Cancer Program. He was recruited from the Vanderbilt Medical Center in 2014, where he ran an active research program focused on understanding the connection between inflammation and colon cancer progression. A major goal of his research is to understand the role of the claudin family of proteins in control of mucosal inflammation and neoplastic growth for therapeutic gains and improved clinical management. Dr. Singh has recently published in the journals: *Biomarkers in Medicine*, *Tissue Barriers*, *Clinical and Translational Gastroenterology*, *Biotechniques*, *Oncogene*, and *Cells*. He has been continuously funded since 2008 by the NIH, including his active R01 from the National Institute of Diabetes and Digestive and Kidney Diseases, and by the Veterans Administration.

Paul Trippier, PhD: Dr. Trippier joined UNMC in 2019 as an Associate Professor in Department of Pharmaceutical Sciences in the UNMC College of Pharmacy. He also serves as Director of the Pharmaceutical Sciences Graduate Program. Dr. Trippier is a Member of the BCC Targets, Modulators and Delivery Program. A synthetic chemist by training, his research focuses on small-molecule drug discovery for several malignancies. His program has synthesized the most selective aldo-ketoreductase 1C3 inhibitor known that counters drug resistance to clinical androgen receptor antagonists in prostate cancer and anthracycline therapeutics in leukemia. The Trippier lab developed potent succinate dehydrogenase inhibitors that show

selective cytotoxicity to prostate cancer cells. His lab is also developing potent VEGF inhibitors, and carbonic anhydrase IX and XII inhibitors. Dr. Trippier has recently published in: *Drug Discovery Today*, *Bioorganic and Medicinal Chemistry*, *ACS Chemical Neuroscience*, *Pharmaceutical Research*, *Bioorganic and Medicinal Chemistry Letters*, *Expert Opinion on Therapeutic Targets*, *The Journal of Organic Chemistry*, *Journal of Medicinal Chemistry*, *Journal of Pharmacology and Experimental Therapeutics*, *Molecules*, and *Pharmacological Reviews*. His active funding includes an R01 from the NCI, along with an R01 from the National Institute of Child Health and Human Development, as well as ongoing funding from the U.S. Department of Defense Congressionally Directed Medical Research Programs.

Pilot Projects

During the previous program period, the Buffett Cancer Center did not award pilot projects using LB 595 funds. Thanks to the generosity of community donors, pilot project awards were made using only philanthropic funds. All LB 595 developmental funds from this reporting period were used to recruit, retain, and support the development of the promising faculty investigators described above.

SHARED RESOURCES

Specific Aims: Shared Resources

1) Manage and provide support (space, funds, personnel) to BCC Shared Resources (e.g., Administrative Core, Biomedical Informatics, Clinical Research Support, Epigenomics, Laboratory Services, Molecular Biology, Pathology, Structural Biology, and Synthetic and Medical Chemistry) that are necessary and highly utilized by Cancer Center members;
2) Monitor quality and user satisfaction of BCC-supported Shared Resources and maintain state-of-the-art Shared Resource Facilities;
3) Determine emerging and future needs of BCC membership for new or enhanced resources and establish plans to fulfill these needs.

The overall goal of the Shared Resources continues to focus on providing access to specialized state-of-the-art technologies, services, and expertise that enhance scientific interaction and productivity in the Fred and Pamela Buffett Cancer Center. This is accomplished by providing support for centralized shared services for BCC investigators in a manner that ensures stability, reliability, cost-effectiveness, and quality control of these services. BCC Shared Resources supported are configured and managed to provide access to specialized state-of-the-art technologies, services, and expertise that enhance scientific interaction and productivity in the Buffett Cancer Center in a manner that ensures stability, reliability, cost-effectiveness, and quality control of these services. The Director of the BCC, Dr. Cowan, makes final decisions regarding the allocation of Cancer Center resources (space, funds, personnel) to Shared Resources. Dr. Cowan is assisted by the Associate Director for Basic Research, Dr. Michael A. (Tony) Hollingsworth, who manages policies and practices to ensure an effective and fair process for setting scientific and other priorities regarding Shared Resource support and usage, and assuring accessibility to members across campuses.

Fiscal management and day-to-day administrative support for the Shared Resources is provided by Mr. Matthew Winfrey, the Associate Director for Administration and External Affairs. Shared Resources supported by the BCC are a subset of many Shared Resources available at UNMC and all Shared Resources supported by the BCC are also supported in part by the Institution (UNMC), which allows us to leverage Cancer Center funds with institutional assets and support. Our management plan for all resources is cooperative and collaborative with the institutional oversight of UNMC-wide resources, which is housed in the Office of the Vice Chancellor for Research (VCR), Jennifer Larsen, MD. After Dr. Larsen decided to step down from her post as VCR, UNMC recently identified its new Vice Chancellor for Research, Kenneth Bayles, PhD. To ensure that supported shared resources are most effectively meeting the research services needs of its members, the Buffett Cancer Center employs regular user satisfaction surveys to evaluate quality, timeliness, upcoming needs, and comprehensiveness of shared resource service. Also, communication from users regarding Shared Resource functionality is encouraged on an ongoing as-needed basis for problems that arise in the daily operations of the resource. Dr. Hollingsworth and Mr. Winfrey conduct an annual review of Shared Resources with each Manager (and associated personnel that conduct cancer related activities). On an annual basis, subsequent to receiving results of the UNMC-wide and Cancer Center-specific surveys, reports of internal advisory boards, notes from presentations, and direct feedback from users, Dr. Hollingsworth and Mr. Winfrey meet with the leaders of each Shared Resource to review usage, ongoing and completed cancer related research projects, publications, grant support, quality and user satisfaction. We also solicit input from each user and resource leader regarding the need for improvement in equipment, personnel, or resources to maintain the state-of-the-art functional status for each facility. As part of the surveys of users and managers, Cancer Center members are asked to identify current and anticipated needs with respect to capabilities within the shared resources. Suggestions for new or enhanced resources are reviewed and prioritized by leadership in the Cancer Center, including Program Leaders, Associate Directors, and the Director. Funding plans (using Institutional or funding from sources other than Cancer Center-specific funds) for high-priority resources are developed and when possible enacted.

In addition, a new position was recently created, Assistant Director for Shared Resources in the Fred & Pamela Buffett Cancer Center, to assist Dr. Hollingsworth and Mr. Winfrey with administrative issues related to oversight of the shared resources. This position also provides assistance to investigators who are seeking to enhance their research with the array of services available within the Cancer Center core facilities. Following consideration of several candidates, Dr. Heather Jensen-Smith was selected for this position.

ADMINISTRATIVE CORE

The major aims of the Administrative Core focus on: (1) To provide administration support to the BCC director and BCC senior leadership team, including the associate directors and program leaders, to promote BCC initiatives as outlined in the strategic plan; and (2) To oversee and coordinate the management functions of the BCC.

Providing administration support to the BCC senior leaders consists of:

- Coordinating and supporting the governance, planning, and evaluation operations of the Cancer Center;
- Facilitating communication between BCC leadership, members, institutional partners, and the National Cancer Institute;
- Coordinating educational programs for the BCC to ensure clear and effective communication between the Cancer Center, UNMC, and Nebraska Medicine, the University's hospital network partner;
- Supporting recruitment, retention, and promotion activities of BCC faculty to strengthen research; and
- Monitoring the operations of CPDM and PRMS, through regular communication with BCC leadership and staff, to review staffing, budgeting, and overall functionality of each BCC component.

Overseeing and coordinating the management functions of the BCC includes:

- Managing and monitoring the Cancer Center's finances, including grants (pre- and post-award), contracts, and institutional and philanthropic funds;
- Managing and overseeing the BCC's research administrative processes and systems, including preparation of grant applications;
- Overseeing and monitoring BCC-managed shared resources, including usage and billing rates;
- Communicating and providing oversight of BCC-supported shared resources to ensure their continued benefit and added value to Cancer Center members;
- Assuming responsibility for the financial oversight and management of Eppley Institute (EI) faculty members, including grants, contracts, start-up funds, and budget forecasting for individual and collaborative scientific programs (EI is a basic science unit at UNMC and the Director of the BCC also serves as EI Director);
- Providing Human Resource administration for the BCC and EI, including all EI faculty and staff and BCC core facilities staff;
- Managing the Cancer Center's space, facilities, and equipment to facilitate collaboration;
- Coordinating and supporting the Cancer Center's membership application and review process; Managing the BCC pilot project program, including solicitation, receipt, review, award notification, and monitoring;
- Providing administrative support and documentation for meetings, seminars, symposia, retreats, and the planning and evaluation activities of the Center; and
- Overseeing and coordinating the BCC's data management system, EVAL, including grant and publication portfolios for each member.

Recent administrative accomplishments have included:

- Continued integration of EVAL: Buffett Cancer Center Administration unit implemented Advarra's data collection and evaluation system EVAL in January 2020. EVAL is being used by the BCC to manage and track membership, publications, grants, and investments (e.g., pilot project, faculty recruitments, and retention support, etc.)

- Pilot Projects Program: BCC Administration coordinated its annual cancer research pilot project programs in 2021. The BCC funded 21 collaborative pilot projects, totaling almost $1,200,000 in direct costs, with project principal investigators represented from all the major cancer research-focused departments at UNMC. Funding derived from philanthropic support, specifically the Cattlemen's Ball of Nebraska, as well as partnerships with the American Cancer Society, the UNMC Great Plans IDeA-CTR, and the UNMC Pediatric Cancer Research Group. An innovative peer-review process was used in the evaluation of a total of 59 pilot project applications, wherein the applicants serve as reviewers for the pool of proposals. This approach allows for greater transparency in the review process and serves as a potential generator for future collaborative cancer research projects.
- BCC Clinical Trials Office: Over the past year, the BCC has partnered with the College of Medicine and Vice Chancellor for Research office to review and assess the BCC Clinical Trials Office. The goals is to increase efficiency and decrease the length of time is takes to open clinical trials. BCC Administration has played a significant role during this on-going process.

BIOMEDICAL INFORMATICS

The major aims of the Biomedical Informatics Shared Resource continue to focus on: (1) To develop biomedical databases; (2) To provide data integration, mining, and sharing; and (3) To conduct cancerogenesis and cancer survival modeling.

The Biomedical Informatics Shared Resource (BMISR) continues to be led by Whitney Goldner, MD, in coordination with Oleg Shats, MS, Senior Informatics Systems Manager, Buffett Cancer Center. Dr. Goldner is a Professor in the UNMC Department of Diabetes, Endocrinology and Metabolism, as well as an Associate Member in the Buffett Cancer Center Targets, Modulators and Delivery Program (TMDP). She is also director of the BCC bioinformatics and biospeciment registry, iCaRe2. Dr. Goldner's research interests include development of a thyroid nodule and thyroid cancer registry and biospecimen bank, biomarkers and well-differentiated thyroid cancer, environmental etiologies for thyroid diseases and thyroid cancer, vitamin D and thyroid cancer, and vitamin D replacement following bariatric surgery. Dr. Goldner's efforts have contributed to recent collaborative publications in: *Journal of the National Comprehensive Cancer Network*, *Thyroid*, *Biological Research for Nursing*, *Journal of the Endocrine Society*, and *Journal of Surgical Research*.

CLINICAL RESEARCH SUPPORT

Clinical Protocol and Data Management

The BCC Clinical Trials Office (CTO) provides centralized management and oversight functions for BCC Research Programs and investigators including the management, coordination, and reporting on all cancer-focused trials. The BCC CTO supports all phases of clinical research (Phase I- IV) including Investigator-initiated, cooperative group, and industry-sponsored studies. The specific aims of the BCC CTO are: (1) To provide support for protocol development, research support, data management, and overall management of all BCC clinical research studies; (2) To assure the highest quality and compliance standards for BCC clinical research; (3) Provides effective training and education to research staff members and develop standard operating procedures and guidelines to ensure the use of best practices and improved processes and timeliness for cancer clinical trial activation and completion; (4) To support all BCC clinical research (Phase I–IV) including multi-site Investigator-initiated trials, cooperative group, and industry-sponsored studies; (5) To monitor clinical trial safety, the conduct and progress of research protocols, the validity and integrity of clinical trial data, accrual rates, serious adverse events, and protocol-specific endpoints such as fulfillment of criteria to advance to a sequenced trial stage, including the quarterly convening of a BCC Data Safety Monitoring Committee (DSMC) and the BCC Audit Committee (AC); (6) To administer required protocol amendments, suspend study enrollment and study activities, and recommend study closure, when needed to assure subject safety or scientific integrity, to the BCC Scientific Review Committee (SRC); and (7) To ensure clinical trial implementation, education and awareness and recruitment efforts across the BCC Catchment area

(Nebraska) and beyond focusing on women, children, underserved minorities and rural populations and ensures their enrollment onto BCC cancer clinical trials at frequencies that meet or exceed their proportion of the population in the BCC catchment area (Nebraska and areas surrounding the Omaha metropolitan area).

In an effort to become more inclusive of all Cancer Center activities, the CTO has newly developed additional disease-focused teams (DFTs). We have added DFTs in Survivorship, Pediatrics, and non-therapeutic trials sections with supporting staff. Each of these groups will have regular meetings and aim to grow our research opportunities.

In a continued effort to streamline regulatory requirements and decrease timelines, we have adopted additional strategies. UNMC now has the capability to grant external monitors with access to electronic medical records in EPIC. This has allowed us to stay competitive with other academic centers and to continue research activities during challenging times, such as the COVID-19 pandemic. UNMC has also recently launched a new section of our clinical trials management system that now houses the regulatory process electronically. This allows for remote off-campus access to our regulatory records. This system facilitates electronic signature capabilities on items such as delegation of authority logs and study-required training. This system is fully compliant with Part 11.

Protocol Review and Monitoring System

The aim of the Buffett Cancer Center Protocol Review and Monitoring System is to oversee the scientific aspects of cancer-related research involving human subjects conducted by members of the University of Nebraska Medical Center (UNMC) faculty and students, and members of the Fred & Pamela Buffett Cancer Center.

The Buffett Cancer Center‘s multidisciplinary Scientific Review Committee (SRC) facilitates the development of innovative, collaborative, and scientifically sound studies that focus on the prevention, detection, diagnosis, and treatment of cancer and its long-term follow-up and care.

The FPBCC SRC aims: (1) To provide a process and criteria for internal peer review of the scientific merit of all proposed clinical trials, and amendments to existing protocols, to confirm the validity of the study as proposed; (2) Provide suggestions, when appropriate, to the Principal Investigators (PIs) which would enhance the scientific merit and/or logistics of the proposed study; (3) Ensure that the safety monitoring plan for the proposed study is appropriate, in accordance with regulations, and assures the safety of patients and subjects enrolled in the proposed study; (4) Ensure accurate prioritization of the clinical research portfolios by the Oncology Disease Focused Teams (DFTs), based on scientific merit and subject population, and possible competing studies; and (5) Terminate a study when there is low accrual, lack of scientific progress, or, upon recommendation of the Data Safety Monitoring Committee (DSMC), for confirmed concerns for safety or quality.

There were two PRMS personnel changes in 2021. Michelle Desler, M.S., is now the PRMS/CTMS Administrator. Her role is to supervise the day-to-day operations of the PRMS, to oversee all aspects of clinical trial management and the three committees housed by the PRMS, to coordinate the functioning of the DSMC, and to supervise the PRMS staff. Coordination of these committees include providing administrative support, processing submissions, and compiling official committee correspondence. She is the Administrator for the Oncology OnCore applications team, responsible for the analysis, planning, design, development, validation, testing, implementation, evaluation, maintenance and ongoing troubleshooting and support of complex system components to achieve organizational goals. Ms. Desler is also responsible for monitoring site activity on CTRP and verifying trial data is accurate, and reports to and meets quarterly with Dr. Ganti. Erin Kaspar, BS, BA, is our Regulatory Specialist, and her primary function is to serve as the Clinical Auditor for all cancer therapeutic trials sponsored by the Institution and as the primary point of contact for the PRMS Audit Committee. In addition, Ms. Kaspar serves as the coordinator for the DSMC, responsible for processing both scheduled reviews and adverse events, as well as preparation of agendas, minutes and committee

correspondence. Her position has primary responsibility for monitoring the registration of cancer intervention trials and their amendments, and for monitoring postings to UNMC's Clinical Trials website.

EPIGENOMICS

The major aim of the Epigenomics Shared Resource remains: (1) To assist researchers with epigenetic analysis, including DNA methylation, chromatin immunoprecipitation, and real-time quantitative PCR gene expression analysis.

The Epigenomics Shared Resource (ESR) was previously led by David Klinkebiel, PhD. Dr. Klinkebiel retired from UNMC. Buffett Cancer Center investigators are currently working with external collaborators to facilitate ongoing epigenomics projects.

LABORATORY SERVICES

The major aims of the Laboratory Services Shared Resource continue to be: (1) To provide FPBCC members with cost-effective alternatives by providing cancer research infrastructure support services; (2) To maintain and ensure quality of FPBCC common equipment and services to support the scientific needs of FPBCC researchers; and (3) To provide quality customer service to FPBCC members to help facilitate the success of individual and collaborative scientific research programs.

The Laboratory Services Shared Resource (LSSR) continues to be led by Adrian Black, PhD. Dr. Black serves as Assistant Professor in the UNMC Eppley Institute for Research in Cancer and is an Associate Member in the Buffett Cancer Center Gastrointestinal Cancer Program (GICP). Dr. Black's research expertise is in the areas of molecular biology, cell cycle, and transcription. Dr. Black has contributed to recent collaborative publications in: *Journal of Biological Chemistry* and *Oncogene*.

MOLECULAR BIOLOGY

The major aims of the Molecular Biology Shared Resource are: (1) To provide functional genomics services to FPBCC investigators; (2) To provide BCC investigators state-of-the-art molecular biology technologies, instrumentation, resources, and expertise for high-throughput siRNA and chemical screening, high-content cell imaging and analysis, and multi-analyte profiling using Luminex xMAP technologies; (3) To maximize the effectiveness of our resources and skills by training and mentoring FPBCC users in core technologies; and (4) To continue to develop/update new procedures and instrumentation in order to assist BCC investigators.

The Molecular Biology Shared Resource (MBSR) continues to be led by David Kelly, PhD. Dr. Kelly is an Assistant Professor in the UNMC Eppley Institute for Research in Cancer, an Associate Member in the Buffett Cancer Center Targets, Modulators and Delivery Program; he also serves as a Co-Core Director for the Nebraska Center for Molecular Target Discovery and Development, an NIH P20-support Center for Biomedical Research Excellence at UNMC led by Principal Investigator and Co-Leader of the BCC Targets, Modulators and Delivery Program, Robert Lewis, PhD.

In conjunction with the MBSR, Dr. Kelly's efforts have recently contributed to a collaborative publication in the journal *Fetal Pediatric Pathology*.

PATHOLOGY

The major aims of the Pathology Shared Resource are: (1) To provide comprehensive tissue resources, histology, immunohistochemistry, and digital pathology services; (2) To maximize the effectiveness of the

resource by training and mentoring users; and (3) To provide long-term sustainability of core services through modernization and innovation.

The Pathology Shared Resource (PSR) continues to be led by Benjamin Swanson, MD, PhD. Dr. Swanson is an Assistant Professor in the Department of Pathology and Microbiology at the University of Nebraska Medical Center, and Member in the Buffett Cancer Center Gastrointestinal Cancer Program (GICP). Dr. Swanson's expertise is particularly in the area of pancreatic cancer pathology; he also has extensive experience in gastrointestinal pathology and tissue banking administration. Since 2018, Dr. Swanson has served as director of the formalin and frozen tissue banks at UNMC, and he has served as director of the tissue core for UNMC's Pancreas SPORE collaborating with SPORE Principal Investigator, Tony Hollingsworth, PhD.

In conjunction with the PSR, Dr. Swanson's efforts have contributed to recent collaborative publications in: *International Journal of Surgical Pathology*, *Cancer Biomarkers*, and *Journal of the National Comprehensive Cancer Network*.

STRUCTURAL BIOLOGY

The major aims of the Structural Biology Shared Resource are: (1) To apply structural techniques to the analysis of important cancer-related biological macromolecules; (2) To provide basic knowledge of disease mechanisms; and (3) To drive research and direct the synthesis of novel therapeutics.

The Structural Biology Shared Resource (SBSR) continues to be led by Gloria Borgstahl, PhD. Dr. Borgstahl serves as Professor in the UNMC Eppley Institute for Research in Cancer, and as a Member in the Buffett Cancer Center Cancer Biology Program. Dr. Borgstahl's work focuses on developing novel X-ray crystallography methods and on studying the macromolecules necessary for the protection of biological macromolecules and DNA maintenance and replication.

In conjunction with the SBSR, Dr. Borgstahl's group has contributed to recent collaborative publications in: *Review of Scientific Instruments*, *Proceedings of the National Academy of Sciences of the United States of America*, *Protein Science*, *Molecular and Cellular Biology*, and *Acta Crystallographica Section F Structural Biology Communications*.

SYNTHETIC AND MEDICINAL CHEMISTRY

A major goal of the TMDP is to develop small molecules that perturb validated cancer targets. The SMCSR continues to support the TMDP, as well as other Buffett Cancer Center, UNMC, and NU researchers, by providing: (1) consultation on chemistry-related problems such as structure activity relationship (SAR) by catalog; (2) access to small molecules that are not commercially available (including compounds found in patents) for probing molecular targets; (3) chemical biology tools (e.g., fluorescently labeled or biotinylated compounds) for assay development or target identification; and (4) scale-up (mg to g) for in vivo validation of targets or proof-of-concept studies.

The Synthetic and Medicinal Chemistry Shared Resource continues to be led by Amar Natarajan, PhD. Dr. Natarajan is the Ruth Branham Professor of Cancer Research in the UNMC Eppley Institute for Research in Cancer, and Co-Leader of the Buffett Cancer Center Targets, Modulators and Delivery Program (TMDP). Dr. Natarajan's laboratory has research interests focused on the discovery and development of small-molecule inhibitors to perturb disease relevant biomolecules.

In conjunction with the SMCSR, Dr. Natarajan's group has contributed to recent collaborative publications in: *Cell Reports*, *Journal of Cell Biology*, *Frontiers in Pharmacology*, *Proceedings of the National Academy of Sciences of the United States of America*, *Journal of Biological Chemistry*, *Nucleic Acids Research*, *RSC*

Chemical Biology, *Future Medicinal Chemistry*, *Oncogene*, *Cancers (Basel)*, *European Journal of Medicinal Chemistry*, and *Bioorganic & Medicinal Chemistry Letters*.

PROGRAM PUBLICATIONS

Listed below in reverse chronological order are program-related peer-reviewed journal articles from the previous reporting period. These include papers that cite the Buffett Cancer Center as providing direct grant support (typically those articles discussing research that utilized BCC-supported shared resources), as well as recent papers by BCC investigators who received developmental funds during the previous reporting period. BCC members are shown in **bold**.

1. **Lockridge O**, Schopfer LM. Naturally Occurring Epsilon Gamma Glutamyl Lysine Isopeptide Crosslinks in Human Neuroblastoma SH-SY5Y Cells. ACS Omega. 2022 Jun 16;7(25):21978-21986. doi: 10.1021/acsomega.2c02502. PMID: 35785306; PMCID: PMC9245130.

2. Xia Z, **Vellichirammal NN**, Han L, Gao L, Boesen EI, Schiller AM, Pellegrino PR, Lisco SJ, **Guda C**, Zucker IH, Wang HJ. Cardiac Spinal Afferent Denervation Attenuates Renal Dysfunction in Rats With Cardiorenal Syndrome Type 2. JACC Basic Transl Sci. 2022 Jun 22;7(6):582-596. doi: 10.1016/j.jacbts.2022.02.008. PMID: 35818505; PMCID: PMC9270585.

3. Lisova AE, **Baranovskiy AG**, Morstadt LM, Babayeva ND, **Tahirov TH**. Efficient discrimination against RNA-containing primers by human DNA polymerase ε. Sci Rep. 2022 Jun 17;12(1):10163. doi: 10.1038/s41598-022-14602-2. PMID: 35715491; PMCID: PMC9205888.

4. Schulze TT, Neville AJ, Chapman RC, Davis PH. Mouse splenocyte enrichment strategies via negative selection for broadened single-cell transcriptomics. STAR Protoc. 2022 May 17;3(2):101402. doi: 10.1016/j.xpro.2022.101402. PMID: 35600930; PMCID: PMC9120244.

5. Tang W, Panja S, Jogdeo CM, Tang S, Ding L, Yu A, **Foster KW**, Dsouza DL, Chhonker YS, **Jensen-Smith H**, Jang HS, Boesen EI, **Murry DJ**, Padanilam B, **Oupický D**. Modified chitosan for effective renal delivery of siRNA to treat acute kidney injury. Biomaterials. 2022 Jun;285:121562. doi: 10.1016/j.biomaterials.2022.121562. Epub 2022 May 2. PMID: 35552115; PMCID: PMC9133205.

6. Rana S, Kour S, Kizhake S, King HM, Mallareddy JR, Case AJ, Huxford T, **Natarajan A**. Dimers of isatin derived α-methylene-γ-butyrolactone as potent anti-cancer agents. Bioorg Med Chem Lett. 2022 Jun 1;65:128713. doi: 10.1016/j.bmcl.2022.128713. Epub 2022 Apr 1. PMID: 35367592; PMCID: PMC9037195.

7. Knoche SM, Larson AC, Brumfield GL, Cate S, Hildebrand WH, **Solheim JC**. Major histocompatibility complex class I molecule expression by pancreatic cancer cells is regulated by activation and inhibition of the epidermal growth factor receptor. Immunol Res. 2022 Jun;70(3):371-391. doi: 10.1007/s12026-022-09262-3. Epub 2022 Mar 18. PMID: 35303241; PMCID: PMC9203924.

8. Anbalagan S, Sadlon J, Weaver K. Regulation of Mannitol Metabolism in Enterococcus faecalis and Association with par_{EF0409} Toxin-Antitoxin Locus Function. J Bacteriol. 2022 May 17;204(5):e0004722. doi: 10.1128/jb.00047-22. Epub 2022 Apr 11. PMID: 35404112; PMCID: PMC9112947.

9. Tang W, Panja S, Jogdeo CM, Tang S, Yu A, **Oupický D**. Study of Renal Accumulation of Targeted Polycations in Acute Kidney Injury. Biomacromolecules. 2022 May 9;23(5):2064-2074. doi: 10.1021/acs.biomac.2c00079. Epub 2022 Apr 8. PMID: 35394757; PMCID: PMC9150723.

10. Yu A, Tang S, Ding L, Foley J, Tang W, Jia H, Panja S, Holbert CE, Hang Y, Stewart TM, **Smith LM**, Sil D, Casero RA Jr, **Oupický D**. Hyaluronate-coated perfluoroalkyl polyamine prodrugs as bioactive siRNA delivery systems for the treatment of peritoneal cancers. Biomater Adv. 2022 May;136:212755. doi: 10.1016/j.bioadv.2022.212755. Epub 2022 Mar 17. PMID: 35813988; PMCID: PMC9268001.

11. Struble LR, Smith AL, Lutz WE, Grubbs G, Sagar S, **Bayles KW, Radhakrishnan P**, Khurana S, **El-Gamal D**, **Borgstahl GEO**. Insect cell expression and purification of recombinant SARS-COV-2 spike proteins that demonstrate ACE2 binding. Protein Sci. 2022 May;31(5):e4300. doi: 10.1002/pro.4300. PMID: 35481636; PMCID: PMC8996471.

12. Wu S, Li Y, Zhang C, Tao L, Kuss M, Lim JY, Butcher J, **Duan B**. Tri-Layered and Gel-Like Nanofibrous Scaffolds with Anisotropic Features for Engineering Heart Valve Leaflets. Adv Healthc Mater. 2022 May;11(10):e2200053. doi: 10.1002/adhm.202200053. Epub 2022 Mar 21. PMID: 35289986.

13. Cole KE, **Ly QP**, **Hollingsworth MA**, **Cox JL**, **Fisher KW**, **Padussis JC**, **Foster JM**, Vargas LM, **Talmadge JE**. Splenic and PB immune recovery in neoadjuvant treated gastrointestinal cancer patients. Int Immunopharmacol. 2022 May;106:108628. doi: 10.1016/j.intimp.2022.108628. Epub 2022 Feb 21. PMID: 35203041; PMCID: PMC9009221.

14. Onder S, Schopfer LM, Jiang W, Tacal O, **Lockridge O**. Butyrylcholinesterase in SH-SY5Y human neuroblastoma cells. Neurotoxicology. 2022 May;90:1-9. doi: 10.1016/j.neuro.2022.02.006. Epub 2022 Feb 18. PMID: 35189179; PMCID: PMC9124689.

15. Caplan M, Wittorf KJ, Weber KK, Swenson SA, Gilbreath TJ, Willow Hynes-Smith R, Amador C, **Hyde RK**, Buckley SM (previous BCC member). Multi-omics reveals mitochondrial metabolism proteins susceptible for drug discovery in AML. Leukemia. 2022 May;36(5):1296-1305. doi: 10.1038/s41375-022-01518-z. Epub 2022 Feb 17. PMID: 35177813; PMCID: PMC9061297.

16. **Baranovskiy AG**, Babayeva ND, Lisova AE, Morstadt LM, **Tahirov TH**. Structural and functional insight into mismatch extension by human DNA polymerase α. Proc Natl Acad Sci U S A. 2022 Apr 26;119(17):e2111744119. doi: 10.1073/pnas.2111744119. Epub 2022 Apr 25. PMID: 35467978; PMCID: PMC9169922.

17. Clement EJ, Law HC, Qiao F, Noe D, Trevino JG, **Woods NT**. Combined Alcohol Exposure and KRAS Mutation in Human Pancreatic Ductal Epithelial Cells Induces Proliferation and Alters Subtype Signatures Determined by Multi-Omics Analysis. Cancers (Basel). 2022 Apr 13;14(8):1968. doi: 10.3390/cancers14081968. PMID: 35454872; PMCID: PMC9027648.

18. Metz EP, Wilder PJ, Popay TM, Wang J, Liu Q, Kalluchi A, **Rowley MJ**, Tansey WP, **Rizzino A**. Elevating SOX2 Downregulates MYC through a SOX2:MYC Signaling Axis and Induces a Slowly Cycling Proliferative State in Human Tumor Cells. Cancers (Basel). 2022 Apr 12;14(8):1946. doi: 10.3390/cancers14081946. PMID: 35454854; PMCID: PMC9025961.

19. Pramanik S, Chen Y, Song H, Khutsishvili I, **Marky LA**, **Ray S**, **Natarajan A**, **Singh PK**, **Bhakat KK**. The human AP-endonuclease 1 (APE1) is a DNA G-quadruplex structure binding protein and regulates KRAS expression in pancreatic ductal adenocarcinoma cells. Nucleic Acids Res. 2022 Apr 8;50(6):3394-3412. doi: 10.1093/nar/gkac172. PMID: 35286386; PMCID: PMC8990529.

20. Acharya A, Pathania AS, Pandey K, Thurman M, Vann KR, Kutateladze TG, **Challagundla KB**, Durden DL, **Byrareddy SN**. PI3K-α/mTOR/BRD4 inhibitor alone or in combination with other anti-virals blocks replication of SARS-CoV-2 and its variants of concern including Delta and Omicron. Clin Transl Med. 2022 Apr;12(4):e806. doi: 10.1002/ctm2.806. PMID: 35390226; PMCID: PMC8989379.

21. Sukreet S, Pereira Braga C, An TT, **Adamec J**, **Cui J**, **Zempleni J**. Ultrasonication of Milk Decreases the Content of Exosomes and MicroRNAs in an Exosome-Defined Rodent Diet. J Nutr. 2022 Apr 1;152(4):961-970. doi: 10.1093/jn/nxab452. PMID: 34982830; PMCID: PMC8970979.

22. Zhang C, Hang Y, Tang W, Sil D, **Jensen-Smith HC**, Bennett RG, **McVicker BL**, **Oupický D**. Dually Active Polycation/miRNA Nanoparticles for the Treatment of Fibrosis in Alcohol-Associated Liver

Disease. Pharmaceutics. 2022 Mar 18;14(3):669. doi: 10.3390/pharmaceutics14030669. PMID: 35336043; PMCID: PMC8949580.

23. Lagundžin D, Krieger KL, Law HC, **Woods NT**. An optimized co-immunoprecipitation protocol for the analysis of endogenous protein-protein interactions in cell lines using mass spectrometry. STAR Protoc. 2022 Mar 10;3(1):101234. doi: 10.1016/j.xpro.2022.101234. PMID: 35300004; PMCID: PMC8920916.

24. Ding L, Tang S, Yu A, Wang A, Tang W, Jia H, **Oupický D**. Nanoemulsion-Assisted siRNA Delivery to Modulate the Nervous Tumor Microenvironment in the Treatment of Pancreatic Cancer. ACS Appl Mater Interfaces. 2022 Mar 2;14(8):10015-10029. doi: 10.1021/acsami.1c21997. Epub 2022 Feb 21. PMID: 35188730; PMCID: PMC9153289.

25. Phillips AJ, Lobl MB, Hafeji YA, Safranek HR, Mohr AM, **Mott JL**. Glycosylation of FGFR4 in cholangiocarcinoma regulates receptor processing and cancer signaling. J Cell Biochem. 2022 Mar;123(3):568-580. doi: 10.1002/jcb.30204. Epub 2022 Jan 4. PMID: 34981854; PMCID: PMC8940645.

26. Ma MCJ, Tadros S, Bouska A, Heavican T, Yang H, Deng Q, Moore D, Akhter A, Hartert K, Jain N, Showell J, Ghosh S, Street L, Davidson M, Carey C, Tobin J, Perumal D, **Vose JM**, **Lunning MA**, Sohani AR, Chen BJ, Buckley S (previous BCC member), Nastoupil LJ, Davis RE, Westin JR, Fowler NH, Parekh S, Gandhi M, Neelapu S, Stewart D, Bhalla K, **Iqbal J**, **Greiner T**, Rodig SJ, Mansoor A, Green MR. Subtype-specific and co-occurring genetic alterations in B-cell non-Hodgkin lymphoma. Haematologica. 2022 Mar 1;107(3):690-701. doi: 10.3324/haematol.2020.274258. PMID: 33792219; PMCID: PMC8883549.

27. Macke AJ, **Petrosyan A**. Alcohol and Prostate Cancer: Time to Draw Conclusions. Biomolecules. 2022 Feb 28;12(3):375. doi: 10.3390/biom12030375. PMID: 35327568; PMCID: PMC8945566.

28. Haney SL, Varney ML, Williams JT, **Smith LM**, **Talmon G**, **Holstein SA**. Geranylgeranyl diphosphate synthase inhibitor and proteasome inhibitor combination therapy in multiple myeloma. Exp Hematol Oncol. 2022 Feb 9;11(1):5. doi: 10.1186/s40164-022-00261-6. PMID: 35139925; PMCID: PMC8827146.

29. Laliwala A, Svechkarev D, Sadykov MR, Endres J, **Bayles KW**, **Mohs AM**. Simpler Procedure and Improved Performance for Pathogenic Bacteria Analysis with a Paper-Based Ratiometric Fluorescent Sensor Array. Anal Chem. 2022 Feb 8;94(5):2615-2624. doi: 10.1021/acs.analchem.1c05021. Epub 2022 Jan 24. PMID: 35073053.

30. Grabowska K, Macur K, Zieschang S, Zaman L, Haverland N, Schissel A, Morsey B, **Fox HS**, Ciborowski P. HIV-1 and methamphetamine alter galectins -1, -3, and -9 in human monocyte-derived macrophages. J Neurovirol. 2022 Feb;28(1):99-112. doi: 10.1007/s13365-021-01025-4. Epub 2022 Feb 17. PMID: 35175539; PMCID: PMC9076712.

31. King RJ, **Shukla SK**, He C, Vernucci E, Thakur R, Attri KS, Dasgupta A, Chaika NV, Mulder SE, Abrego J, Murthy D, **Gunda V**, Pacheco CG, **Grandgenett PM**, **Lazenby AJ**, **Hollingsworth MA**, **Yu** F, Mehla K (previous BCC member), Singh PK (previous BCC member). CD73 induces GM-CSF/MDSC-mediated suppression of T cells to accelerate pancreatic cancer pathogenesis. Oncogene. 2022 Feb;41(7):971-982. doi: 10.1038/s41388-021-02132-6. Epub 2022 Jan 10. PMID: 35001076; PMCID: PMC8840971.

32. Xiao Y, Chen Y, **Peng A**, **Dong J**. The phosphatase CTDSPL2 is phosphorylated in mitosis and a target for restraining tumor growth and motility in pancreatic cancer. Cancer Lett. 2022 Feb 1;526:53-65. doi: 10.1016/j.canlet.2021.11.018. Epub 2021 Nov 20. PMID: 34813892; PMCID: PMC8702485.

33. **Mirza S**, Kalluchi A, Raza M, Saleem I, **Mohapatra B**, Pal D, **Ouellette MM**, Qiu F, Yu L, Lobanov A, Zheng ZM, Zhang Y, Alsaleem MA, Rakha EA, **Band H**, **Rowley MJ**, **Band V**. Ecdysoneless Protein Regulates Viral and Cellular mRNA Splicing to Promote Cervical Oncogenesis. Mol Cancer Res. 2022

Feb;20(2):305-318. doi: 10.1158/1541-7786.MCR-21-0567. Epub 2021 Oct 20. PMID: 34670863; PMCID: PMC9138181.

34. Xu PC, You M, Yu SY, Luan Y, Eldani M, Caffrey TC, **Grandgenett PM**, O'Connell KA, **Shukla SK**, Kattamuri C, **Hollingsworth MA**, **Singh PK**, Thompson TB, Chung S, **Kim SY**. Visceral adipose tissue remodeling in pancreatic ductal adenocarcinoma cachexia: the role of activin A signaling. Sci Rep. 2022 Jan 31;12(1):1659. doi: 10.1038/s41598-022-05660-7. PMID: 35102236; PMCID: PMC8803848.

35. Kambis TN, Shahshahan HR, Mishra PK. Metabolites and Genes behind Cardiac Metabolic Remodeling in Mice with Type 1 Diabetes Mellitus. Int J Mol Sci. 2022 Jan 26;23(3):1392. doi: 10.3390/ijms23031392. PMID: 35163316; PMCID: PMC8835796.

36. Saxena S, Molczyk C, Purohit A, Ehrhorn E, Goel P, Prajapati DR, Atri P, Kaur S (previous BCC member), Grandgenett PM, **Hollingsworth MA**, **Batra SK**, **Singh RK**. Differential expression profile of CXC-receptor-2 ligands as potential biomarkers in pancreatic ductal adenocarcinoma. Am J Cancer Res. 2022 Jan 15;12(1):68-90. PMID: 35141005; PMCID: PMC8822283.

37. Ye Z, Abdelmoaty MM, Curran SM, Dyavar SR, Kumar D, **Alnouti Y**, **Coulter DW**, Podany AT, **Singh RK**, **Vetro JA**. Direct Comparison of Chol-siRNA Polyplexes and Chol-DsiRNA Polyplexes Targeting STAT3 in a Syngeneic Murine Model of TNBC. Noncoding RNA. 2022 Jan 13;8(1):8. doi: 10.3390/ncrna8010008. PMID: 35076584; PMCID: PMC8788547.

38. Vance K, Alitinok A, Winfree S, **Jensen-Smith H**, **Swanson BJ**, **Grandgenett PM**, **Klute KA**, Crichton DJ, **Hollingsworth MA**. Machine learning analyses of highly-multiplexed immunofluorescence identifies distinct tumor and stromal cell populations in primary pancreatic tumors. Cancer Biomark. 2022;33(2):219-235. doi: 10.3233/CBM-210308. Erratum in: Cancer Biomark. 2022;34(4):693. PMID: 35213363; PMCID: PMC9278645.

39. Liu L, Kshirsagar PG, **Gautam SK**, Gulati M, Wafa EI, Christiansen JC, White BM, Mallapragada SK, Wannemuehler MJ, **Kumar S**, **Solheim JC**, **Batra SK**, Salem AK, Narasimhan B, **Jain M**. Nanocarriers for pancreatic cancer imaging, treatments, and immunotherapies. Theranostics. 2022 Jan 1;12(3):1030-1060. doi: 10.7150/thno.64805. PMID: 35154473; PMCID: PMC8771545.

40. Azadmanesh J, Lutz WE, Coates L, Weiss KL, **Borgstahl GEO**. Cryotrapping peroxide in the active site of human mitochondrial manganese superoxide dismutase crystals for neutron diffraction. Acta Crystallogr F Struct Biol Commun. 2022 Jan 1;78(Pt 1):8-16. doi: 10.1107/S2053230X21012413. Epub 2022 Jan PMID: 34981770; PMCID: PMC8725007.

41. Mandolfo NR, **Berger AM**, Struwe L, Hanna KM, **Goldner W**, **Klute K**, **Langenfeld S**, Hammer M. Glycemic Variability Within 1 Year Following Surgery for Stage II-III Colon Cancer. Biol Res Nurs. 2022 Jan;24(1):64-74. doi: 10.1177/10998004211035184. Epub 2021 Oct 5. PMID: 34610762; PMCID: PMC9248290.

42. Ouellette SP, Hatch ND, Wood NA, Herrera AL, Chaussee MS. Codon-Dependent Transcriptional Changes in Response to Tryptophan Limitation in the Tryptophan Auxotrophic Pathogens Chlamydia trachomatis and Streptococcus pyogenes. mSystems. 2021 Dec 21;6(6):e0126921. doi: 10.1128/mSystems.01269-21. Epub 2021 Dec 14. PMID: 34904862; PMCID: PMC8670374.

43. Perumal N, **Kanchan RK**, Doss D, Bastola N, Atri P, Chirravuri-Venkata R, Thapa I, Vengoji R, Maurya SK, Klinkebiel D (previous BCC member), **Talmon GA**, **Nasser MW**, **Batra SK**, **Mahapatra S**. MiR-212-3p functions as a tumor suppressor gene in group 3 medulloblastoma via targeting nuclear factor I/B (NFIB). Acta Neuropathol Commun. 2021 Dec 18;9(1):195. doi: 10.1186/s40478-021-01299-z. PMID: 34922631; PMCID: PMC8684142.

44. Cannon A, Thompson CM, Bhatia R, Kandy RRK, **Solheim JC**, **Batra SK**, **Kumar S**. Contribution of CXCR3-mediated signaling in the metastatic cascade of solid malignancies. Biochim Biophys Acta Rev Cancer. 2021 Dec;1876(2):188628. doi: 10.1016/j.bbcan.2021.188628. Epub 2021 Sep 22. PMID: 34560199; PMCID: PMC8595872.

45. Herskovitz J, Hasan M, Patel M, Blomberg WR, Cohen JD, Machhi J, Shahjin F, **Mosley RL**, McMillan J, Kevadiya BD, **Gendelman HE**. CRISPR-Cas9 Mediated Exonic Disruption for HIV-1 Elimination. EBioMedicine. 2021 Nov;73:103678. doi: 10.1016/j.ebiom.2021.103678. Epub 2021 Nov 10. PMID: 34774454; PMCID: PMC8633974.

46. **Mikuls TR**, Gaurav R, **Thiele GM**, England BR, Wolfe MG, Shaw BP, Bailey KL, Wyatt TA, Nelson AJ, Duryee MJ, Hunter CD, **Wang D**, **Romberger DJ**, Ascherman DP, Poole JA. The impact of airborne endotoxin exposure on rheumatoid arthritis-related joint damage, autoantigen expression, autoimmunity, and lung disease. Int Immunopharmacol. 2021 Nov;100:108069. doi: 10.1016/j.intimp.2021.108069. Epub 2021 Aug 27. PMID: 34461491; PMCID: PMC8551041.

47. Lone W, Bouska A, Sharma S, Amador C, Saumyaranjan M, Herek TA, Heavican TB, Yu J, Lim ST, Ong CK, Slack GW, Savage KJ, Rosenwald A, Ott G, Cook JR, Feldman AL, Rimsza LM, McKeithan TW, **Greiner TC**, Weisenburger DD, Melle F, Motta G, Pileri S, **Vose JM**, Chan WC (previous BCC member), **Iqbal J**. Genome-Wide miRNA Expression Profiling of Molecular Subgroups of Peripheral T-cell Lymphoma. Clin Cancer Res. 2021 Nov 1;27(21):6039-6053. doi: 10.1158/1078-0432.CCR-21-0573. Epub 2021 Aug 23. PMID: 34426436; PMCID: PMC8563427.

48. Veerappa A, Pendyala G, **Guda C**. A systems omics-based approach to decode substance use disorders and neuroadaptations. Neurosci Biobehav Rev. 2021 Nov;130:61-80. doi: 10.1016/j.neubiorev.2021.08.016. Epub 2021 Aug 17. PMID: 34411560; PMCID: PMC8511293.

49. Hu T, **Shukla SK**, Vernucci E, He C, Wang D, King RJ, Jha K, Siddhanta K, Mullen NJ, Attri KS, Murthy D, Chaika NV, Thakur R, Mulder SE, Pacheco CG, Fu X, High RR, **Yu F**, **Lazenby A**, Steegborn C, Lan P, Mehla K (previous BCC member), Rotili D, Chaudhary S, Valente S, Tafani M, Mai A, Auwerx J, Verdin E, Tuveson D, Singh PK (previous BCC member). Metabolic Rewiring by Loss of Sirt5 Promotes Kras-Induced Pancreatic Cancer Progression. Gastroenterology. 2021 Nov;161(5):1584-1600. doi: 10.1053/j.gastro.2021.06.045. Epub 2021 Jul 8. PMID: 34245764; PMCID: PMC8546779.

50. Sagar S, Singh S, Mallareddy JR, Sonawane YA, Napoleon JV, Rana S, Contreras JI, Rajesh C, Ezell EL, Kizhake S, **Garrison JC**, **Radhakrishnan P**, **Natarajan A**. Structure activity relationship (SAR) study identifies a quinoxaline urea analog that modulates IKKβ phosphorylation for pancreatic cancer therapy. Eur J Med Chem. 2021 Oct 15;222:113579. doi: 10.1016/j.ejmech.2021.113579. Epub 2021 May 30. PMID: 34098465; PMCID: PMC8373685.

51. Rookwood AC, Bronner LP, Abney MA, Butler-Robbins HS, Pocwierz-Gaines MS, Larson AC, Huckleby AM, **Solheim JC**, **Godfrey M, Idoate RE**. Tailoring Our Approach in Response to the SARS-CoV-2 Pandemic and Transcending Science Outreach Modalities for Native American Students in a Cancer Research Education Program. J STEM Outreach. 2021 Oct;4(4):10.15695/jstem/v4i4.08. doi: 10.15695/jstem/v4i4.08. Epub 2021 Oct 4. PMID: 35814349; PMCID: PMC9262249.

52. Onder S, van Grol M, Fidder A, Xiao G, Noort D, Yerramalla U, Tacal O, Schopfer LM, **Lockridge O**. Rabbit Antidiethoxyphosphotyrosine Antibody, Made by Single B Cell Cloning, Detects Chlorpyrifos Oxon-Modified Proteins in Cultured Cells and Immunopurifies Modified Peptides for Mass Spectrometry. J Proteome Res. 2021 Oct 1;20(10):4728-4745. doi: 10.1021/acs.jproteome.1c00383. Epub 2021 Sep 1. PMID: 34469172; PMCID: PMC8491160.

53. **Idoate R**, Gilbert M, King KM, Spellman L, McWilliams B, Strong B, Bronner L, **Siahpush M**, Ramos AK, Clarke M, Michaud T, **Godfrey M**, **Solheim J**. Urban American Indian Community Health Beliefs

Associated with Addressing Cancer in the Northern Plains Region. J Cancer Educ. 2021 Oct;36(5):996-1004. doi: 10.1007/s13187-020-01727-z. PMID: 32162283; PMCID: PMC7523620.

54. Pachikov AN, Gough RR, Christy CE, Morris ME, **Casey CA**, **LaGrange CA**, Bhat G, Kubyshkin AV, Fomochkina II, Zyablitskaya EY, Makalish TP, Golubinskaya EP, Davydenko KA, Eremenko SN, Riethoven JM, Maroli AS, Payne TS, **Powers R**, Lushnikov AY, Macke AJ, **Petrosyan A**. The non-canonical mechanism of ER stress-mediated progression of prostate cancer. J Exp Clin Cancer Res. 2021 Sep 14;40(1):289. doi: 10.1186/s13046-021-02066-7. PMID: 34521429; PMCID: PMC8439065.

55. Fatima I, Barman S, Uppada J, Chauhan S, Rauth S, **Rachagani S**, **Ponnusamy MP**, **Smith L**, **Talmon G**, **Singh AB**, **Batra SK**, **Dhawan P**. MASTL regulates EGFR signaling to impact pancreatic cancer progression. Oncogene. 2021 Sep;40(38):5691-5704. doi: 10.1038/s41388-021-01951-x. Epub 2021 Jul 30. PMID: 34331012; PMCID: PMC8817225.

56. Prahlad J, Struble LR, Lutz WE, Wallin SA, Khurana S, Schnaubelt A, Broadhurst MJ, **Bayles KW**, **Borgstahl GEO**. CyDisCo production of functional recombinant SARS-CoV-2 spike receptor binding domain. Protein Sci. 2021 Sep;30(9):1983-1990. doi: 10.1002/pro.4152. Epub 2021 Jul 16. PMID: 34191362; PMCID: PMC8376421.

57. Gowrikumar S, Primeaux M, Pravoverov K, Wu C, Szeglin BC, Sauvé CG, Thapa I, **Bastola D**, Chen XS, Smith JJ, **Singh AB**, **Dhawan P**. A Claudin-Based Molecular Signature Identifies High-Risk, Chemoresistant Colorectal Cancer Patients. Cells. 2021 Aug 26;10(9):2211. doi: 10.3390/cells10092211. PMID: 34571860; PMCID: PMC8466455.

58. Fairweather AER, Goetz DB, Schroeder CM, Bhuiyan NH, Varney ML, Wiemer DF, **Holstein SA**. Impact of α-modifications on the activity of triazole bisphosphonates as geranylgeranyl diphosphate synthase inhibitors. Bioorg Med Chem. 2021 Aug 15;44:116307. doi: 10.1016/j.bmc.2021.116307. Epub 2021 Jul 11. PMID: 34298413; PMCID: PMC8370032.

59. Luan H, Bailey TA, Clubb RJ, **Mohapatra BC**, Bhat AM, Chakraborty S, Islam N, Mushtaq I, Storck MD, Raja SM, **Band V**, **Band H**. CHIP/STUB1 Ubiquitin Ligase Functions as a Negative Regulator of ErbB2 by Promoting Its Early Post-Biosynthesis Degradation. Cancers (Basel). 2021 Aug 4;13(16):3936. doi: 10.3390/cancers13163936. PMID: 34439093; PMCID: PMC8391510.

60. England BR, Campany M, Sayles H, Roul P, Yang Y, **Ganti AK**, Sokolove J, Robinson WH, Reimold AM, Kerr GS, Cannon GW, Sauer BC, Baker JF, Thiele GM, **Mikuls TR**. Associations of serum cytokines and chemokines with the risk of incident cancer in a prospective rheumatoid arthritis cohort. Int Immunopharmacol. 2021 Aug;97:107719. doi: 10.1016/j.intimp.2021.107719. Epub 2021 Apr 29. PMID: 33933845; PMCID: PMC8324526.

61. Bhattacharya DS, Bapat A, Svechkarev D, **Mohs AM**. Water-Soluble Blue Fluorescent Nonconjugated Polymer Dots from Hyaluronic Acid and Hydrophobic Amino Acids. ACS Omega. 2021 Jul 9;6(28):17890-17901. doi: 10.1021/acsomega.1c01343. Erratum in: ACS Omega. 2021 Oct 05;6(41):27598. PMID: 34308024; PMCID: PMC8296014.

62. Brockett MR, Lee J, Cox JV, Liechti GW, Ouellette SP. A Dynamic, Ring-Forming Bactofilin Critical for Maintaining Cell Size in the Obligate Intracellular Bacterium Chlamydia trachomatis. Infect Immun. 2021 Jul 15;89(8):e0020321. doi: 10.1128/IAI.00203-21. Epub 2021 Jul 15. PMID: 33941579; PMCID: PMC8281209.

63. Schopfer LM, Onder S, **Lockridge O**. Evaluation of mass spectrometry MS/MS spectra for the presence of isopeptide crosslinked peptides. PLoS One. 2021 Jul 9;16(7):e0254450. doi: 10.1371/journal.pone.0254450. PMID: 34242352; PMCID: PMC8270460.

64. McFee RM, Romereim SM, Snider AP, Summers AF, Pohlmeier WE, Kurz SG, Cushman RA, **Davis JS**, Wood JR, **Cupp AS**. A high-androgen microenvironment inhibits granulosa cell proliferation and alters cell identity. Mol Cell Endocrinol. 2021 Jul 1;531:111288. doi: 10.1016/j.mce.2021.111288. Epub 2021 Apr 24. PMID: 33905753.

65. King HM, Rana S, Kubica SP, Mallareddy JR, Kizhake S, Ezell EL, Zahid M, Naldrett MJ, **Alvarez S**, Law HC, **Woods NT**, **Natarajan A**. Aminopyrazole based CDK9 PROTAC sensitizes pancreatic cancer cells to venetoclax. Bioorg Med Chem Lett. 2021 Jul 1;43:128061. doi: 10.1016/j.bmcl.2021.128061. Epub 2021 Apr 23. PMID: 33895280; PMCID: PMC8187316.

66. **Lakshmanan I**, Chaudhary S, Vengoji R, **Seshacharyulu P**, **Rachagani S**, Carmicheal J, Jahan R, Atri P, Chirravuri-Venkata R, Gupta R, Marimuthu S, Perumal N, Rauth S, Kaur S (previous BCC member), Mallya K, **Smith LM**, **Lele SM**, **Ponnusamy MP**, **Nasser MW**, Salgia R, **Batra SK**, **Ganti AK**. ST6GalNAc-I promotes lung cancer metastasis by altering MUC5AC sialylation. Mol Oncol. 2021 Jul;15(7):1866-1881. doi: 10.1002/1878-0261.12956. Epub 2021 May 1. PMID: 33792183; PMCID: PMC8253099.

67. Pandey K, Acharya A, Mohan M, Ng CL, Reid SP, **Byrareddy SN**. Animal models for SARS-CoV-2 research: A comprehensive literature review. Transbound Emerg Dis. 2021 Jul;68(4):1868-1885. doi: 10.1111/tbed.13907. Epub 2020 Dec 20. PMID: 33128861; PMCID: PMC8085186.

68. Uppada SB, Madduri LSV, Singu S, Lawson B, Bauer L, **Freifeld A**, **Bhatt VR**, **Byrareddy SN**. Modified Papanicolaou staining for oral swab samples stored long term. Biotech Histochem. 2021 Jul;96(5):359-363. doi: 10.1080/10520295.2020.1804075. Epub 2020 Aug 21. PMID: 32820964; PMCID: PMC7897329.

69. Wong J, **Baine M**, Wisnoskie S, **Bennion N**, Zheng D, Yu L, Dalal V, **Hollingsworth MA**, **Lin C**, **Zheng D**. Effects of interobserver and interdisciplinary segmentation variabilities on CT-based radiomics for pancreatic cancer. Sci Rep. 2021 Aug 11;11(1):16328. doi: 10.1038/s41598-021-95152-x. PMID: 34381070; PMCID: PMC8357939.

70. Huynh LM, Keit E, Schuller AA, Carrillo RC, Huang E, Ahlering TE, Boyle S (previous BCC member), **Enke C**, **Baine MJ**. Impact of statin use on overall and time to biochemical failure following radical prostatectomy or radiation therapy. World J Urol. 2021 Sep;39(9):3287-3293. doi: 10.1007/s00345-021-03600-0. Epub 2021 Jan 27. PMID: 33502557.

71. Huynh LM, Keit E, Huang E, Carrillo RC, Ahlering TE, Boyle S (previous BCC member), **Enke CA**, **Baine M**. Protective Effects of Metformin Against Biochemical Failure Following Radical Prostatectomy or Radiation Therapy in Localized Prostate Cancer. Urology. 2021 Sep;155:172-178. doi: 10.1016/j.urology.2021.01.030. Epub 2021 Jan 28. PMID: 33515557.

72. Madduri LSV, Brandquist ND, Palanivel C, **Talmon GA**, **Baine MJ**, **Zhou S**, **Enke CA**, **Johnson KR**, **Ouellette MM**, **Yan Y**. p53/FBXL20 axis negatively regulates the protein stability of PR55α, a regulatory subunit of PP2A Ser/Thr phosphatase. Neoplasia. 2021 Dec;23(12):1192-1203. doi: 10.1016/j.neo.2021.10.002. Epub 2021 Oct 30. PMID: 34731788; PMCID: PMC8570931.

73. Huynh LM, Bonebrake BT, DiMaio DJ, **Baine MJ**, **Teply BA**. Development of bullous pemphigoid following radiation therapy combined with nivolumab for renal cell carcinoma: A case report of abscopal toxicities. Medicine (Baltimore). 2021 Dec 10;100(49):e28199. doi: 10.1097/MD.0000000000028199. PMID: 34889301; PMCID: PMC8663808.

74. Coutu BG, Johnson KC, Bhirud A, **Baine MJ**, **Zhen W**, **Zhang C**, Trujillo KP, **Bennion NR**. Systemic Immune-Inflammatory Index Association with Survival in Patients Undergoing Trimodality Therapy for

Lung Cancer. Oncology. 2022;100(5):247-256. doi: 10.1159/000520989. Epub 2021 Nov 18. PMID: 34794142.

75. Motzer RJ, Jonasch E, Agarwal N, Alva A, **Baine M**, Beckermann K, Carlo MI, Choueiri TK, Costello BA, Derweesh IH, Desai A, Ged Y, George S, Gore JL, Haas N, Hancock SL, Kapur P, Kyriakopoulos C, Lam ET, Lara PN, Lau C, Lewis B, Madoff DC, Manley B, Michaelson MD, Mortazavi A, Nandagopal L, Plimack ER, Ponsky L, Ramalingam S, Shuch B, Smith ZL, Sosman J, Dwyer MA, Gurski LA, Motter A. Kidney Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2022 Jan;20(1):71-90. doi: 10.6004/jnccn.2022.0001. PMID: 34991070.

76. Preuss K, Thach N, Liang X, **Baine M**, Chen J, Zhang C, Du H, Yu H, **Lin C**, **Hollingsworth MA**, **Zheng D**. Using Quantitative Imaging for Personalized Medicine in Pancreatic Cancer: A Review of Radiomics and Deep Learning Applications. Cancers (Basel). 2022 Mar 24;14(7):1654. doi: 10.3390/cancers14071654. PMID: 35406426; PMCID: PMC8997008.

77. Zhang C, Wu R, **Smith LM**, **Baine M**, **Lin C**, **Reames BN**. An evaluation of adjuvant chemotherapy following neoadjuvant chemotherapy and resection for borderline resectable and locally advanced pancreatic cancer. Am J Surg. 2022 Jul;224(1 Pt A):51-57. doi: 10.1016/j.amjsurg.2021.12.018. Epub 2021 Dec 28. PMID: 34973686.

78. Song H, Xi S, Chen Y, Pramanik S, Zeng J, Roychoudhury S, Harris H, Akbar A, Elhag SS, **Coulter DW**, **Ray S**, **Bhakat KK**. Histone chaperone FACT complex inhibitor CBL0137 interferes with DNA damage repair and enhances sensitivity of medulloblastoma to chemotherapy and radiation. Cancer Lett. 2021 Nov 1;520:201-212. doi: 10.1016/j.canlet.2021.07.020. Epub 2021 Jul 14. PMID: 34271103; PMCID: PMC8440470.

79. **Ray S**, **Chaturvedi NK**, **Bhakat KK**, **Rizzino A**, **Mahapatra S**. Subgroup-Specific Diagnostic, Prognostic, and Predictive Markers Influencing Pediatric Medulloblastoma Treatment. Diagnostics (Basel). 2021 Dec 28;12(1):61. doi: 10.3390/diagnostics12010061. PMID: 35054230; PMCID: PMC8774967.

80. **Bhakat KK**, **Ray S**. The FAcilitates Chromatin Transcription (FACT) complex: Its roles in DNA repair and implications for cancer therapy. DNA Repair (Amst). 2022 Jan;109:103246. doi: 10.1016/j.dnarep.2021.103246. Epub 2021 Nov 16. PMID: 34847380.

81. **Ahmad R**, **Kumar B**, Tamang RL, Xu W, **Talmon GA**, **Mohs AM**, **Dhawan P**, **Singh AB**. Colonoscopy-based intramucosal transplantation of cancer cells for mouse modeling of colon cancer and lung metastasis. Biotechniques. 2021 Sep;71(3):456-464. doi: 10.2144/btn-2020-0172. Epub 2021 Aug 16. PMID: 34392706; PMCID: PMC8867397.

82. Bony BA, Tarudji AW, Miller HA, Gowrikumar S, Roy S, Curtis ET, Gee CC, Vecchio A, **Dhawan P**, **Kievit FM**. Claudin-1-Targeted Nanoparticles for Delivery to Aging-Induced Alterations in the Blood-Brain Barrier. ACS Nano. 2021 Nov 8:10.1021/acsnano.1c08432. doi: 10.1021/acsnano.1c08432. Epub ahead of print. PMID: 34748307; PMCID: PMC9079187.

83. Duryee MJ, **Ahmad R**, Eichele DD, Hunter CD, Mitra A, **Talmon GA**, **Singh S**, **Smith LM**, Rosen MJ, **Dhawan P**, Thiele GM, **Singh AB**. Identification of Immunoglobulin G Autoantibody Against Malondialdehyde-Acetaldehyde Adducts as a Novel Serological Biomarker for Ulcerative Colitis. Clin Transl Gastroenterol. 2022 Apr 1;13(4):e00469. doi: 10.14309/ctg.0000000000000469. PMID: 35287144; PMCID: PMC9038499.

84. Lama Tamang R, Juritsch AF, **Ahmad R**, Salomon JD, **Dhawan P**, **Ramer-Tait AE**, **Singh AB**. The diet-microbiota axis: a key regulator of intestinal permeability in human health and disease. Tissue Barriers. 2022 May 21:2077069. doi: 10.1080/21688370.2022.2077069. Epub ahead of print. PMID: 35603609.

85. Primeaux M, Gowrikumar S, **Dhawan P**. Role of CD44 isoforms in epithelial-mesenchymal plasticity and metastasis. Clin Exp Metastasis. 2022 Jun;39(3):391-406. doi: 10.1007/s10585-022-10146-x. Epub 2022 Jan 12. PMID: 35023031.

86. Bhat AA, Nisar S, Singh M, Ashraf B, Masoodi T, Prasad CP, Sharma A, Maacha S, Karedath T, Hashem S, Yasin SB, Bagga P, Reddy R, Frennaux MP, Uddin S, **Dhawan P**, Haris M, Macha MA. Cytokine- and chemokine-induced inflammatory colorectal tumor microenvironment: Emerging avenue for targeted therapy. Cancer Commun (Lond). 2022 Aug;42(8):689-715. doi: 10.1002/cac2.12295. Epub 2022 Jul 5. PMID: 35791509; PMCID: PMC9395317.

87. Goettsch KA, Zhang L, **Singh AB**, **Dhawan P**, **Bastola DK**. Reliable epithelial-mesenchymal transition biomarkers for colorectal cancer detection. Biomark Med. 2022 Aug;16(12):889-901. doi: 10.2217/bmm-2022-0071. Epub 2022 Jul 27. PMID: 35892269; PMCID: PMC9442548.

88. **Dickinson K**, Lim A, **Kupzyk KA**. Demographic, Symptom, and Lifestyle Factors Associated With Cancer-Related Fatigue in Men With Prostate Cancer. Oncol Nurs Forum. 2021 Jul 1;48(4):423-430. doi: 10.1188/21.ONF.423-430. PMID: 34142995.

89. Kelliher J, **Ghosal G**, Leung JWC. New answers to the old RIDDLE: RNF168 and the DNA damage response pathway. FEBS J. 2022 May;289(9):2467-2480. doi: 10.1111/febs.15857. Epub 2021 Apr 16. PMID: 33797206; PMCID: PMC8486888.

90. Napoleon JV, Sagar S, Kubica SP, Boghean L, Kour S, King HM, Sonawane YA, Crawford AJ, Gautam N, Kizhake S, Bialk PA, Kmiec E, Mallareddy JR, Patil PP, Rana S, Singh S, Prahlad J, **Grandgenett PM**, **Borgstahl GEO**, **Ghosal G**, **Alnouti Y**, **Hollingsworth MA**, **Radhakrishnan P**, **Natarajan A**. Small-molecule IKKβ activation modulator (IKAM) targets MAP3K1 and inhibits pancreatic tumor growth. Proc Natl Acad Sci U S A. 2022 May 3;119(18):e2115071119. doi: 10.1073/pnas.2115071119. Epub 2022 Apr 27. PMID: 35476515; PMCID: PMC9170026.

91. Perry M, **Ghosal G**. Mechanisms and Regulation of DNA-Protein Crosslink Repair During DNA Replication by SPRTN Protease. Front Mol Biosci. 2022 Jun 15;9:916697. doi: 10.3389/fmolb.2022.916697. PMID: 35782873; PMCID: PMC9240642.

92. Ray S, Chee L, Zhou Y, Schaefer MA, Naldrett MJ, **Alvarez S**, **Woods NT**, **Hewitt KJ**. Functional requirements for a Samd14-capping protein complex in stress erythropoiesis. Elife. 2022 Jun 17;11:e76497. doi: 10.7554/eLife.76497. PMID: 35713400; PMCID: PMC9282853.

93. Lam CK, **Hyde RK**, Patel SA. Synthetic Immunotherapy: Programming Immune Cells with Novel and Sophisticated Logic Capabilities. Transplant Cell Ther. 2022 Sep;28(9):560-571. doi: 10.1016/j.jtct.2022.06.001. Epub 2022 Jun 10. PMID: 35691572.

94. Luan Y, Xu P, Yu SY, **Kim SY**. The Role of Mutant p63 in Female Fertility. Int J Mol Sci. 2021 Aug 20;22(16):8968. doi: 10.3390/ijms22168968. PMID: 34445673; PMCID: PMC8396438.

95. Xu PC, Luan Y, Yu SY, Xu J, **Coulter DW**, **Kim SY**. Effects of PD-1 blockade on ovarian follicles in a prepubertal female mouse. J Endocrinol. 2021 Nov 24;252(1):15-30. doi: 10.1530/JOE-21-0209. PMID: 34647523; PMCID: PMC8630981.

96. Du Y, Carranza Z, Luan Y, Busman-Sahay K, Wolf S, Campbell SP, **Kim SY**, Pejovic T, Estes JD, Zelinski M, Xu J. Evidence of cancer therapy-induced chronic inflammation in the ovary across multiple species: A potential cause of persistent tissue damage and follicle depletion. J Reprod Immunol. 2022 Mar;150:103491. doi: 10.1016/j.jri.2022.103491. Epub 2022 Jan 31. PMID: 35176661; PMCID: PMC9224575.

97. Luan Y, Zhang Y, Yu SY, You M, Xu PC, Chung S, Kurita T, Zhu J, **Kim SY**. Development of ovarian tumour causes significant loss of muscle and adipose tissue: a novel mouse model for cancer cachexia study. J Cachexia Sarcopenia Muscle. 2022 Apr;13(2):1289-1301. doi: 10.1002/jcsm.12864. Epub 2022 Jan 19. PMID: 35044098; PMCID: PMC8977964.

98. Xue W, Yu SY, Kuss M, Kong Y, Shi W, Chung S, **Kim SY**, **Duan B**. 3D bioprinted white adipose model forin vitrostudy of cancer-associated cachexia induced adipose tissue remodeling. Biofabrication. 2022 May 26;14(3):10.1088/1758-5090/ac6c4b. doi: 10.1088/1758-5090/ac6c4b. PMID: 35504266; PMCID: PMC9205601.

99. Yu SY, Luan Y, Abazarikia A, Dong R, **Lee J**, **Kim SY**. Oocyte CTR1 is not essential for cisplatin-induced oocyte death of primordial follicle. MicroPubl Biol. 2022 Sep 1;2022:10.17912/micropub.biology.000632. doi: 10.17912/micropub.biology.000632. PMID: 36120475; PMCID: PMC9478746.

100. Jones RA, Hirschey R, Campbell G, Cooley ME, Somayaji D, **Lally R**, Rueter EK, Gullatte MM. Update to 2019-2022 ONS Research Agenda: Rapid Review to Address Structural Racism and Health Inequities. Oncol Nurs Forum. 2021 Nov 1;48(6):589-600. doi: 10.1188/21.ONF.589-600. PMID: 34673760; PMCID: PMC8674842.

101. Jones RA, Hirschey R, Campbell G, Cooley ME, Somayaji D, **Lally R**, Rueter EK, Gullatte MM. Update to 2019-2022 ONS Research Agenda: Rapid Review to Promote Equity in Oncology Healthcare Access and Workforce Development. Oncol Nurs Forum. 2021 Nov 1;48(6):604-612. doi: 10.1188/21.ONF.604-612. PMID: 34673761; PMCID: PMC8694266.

102. Shi W, Fang F, Kong Y, Greer SE, Kuss M, Liu B, Xue W, Jiang X, Lovell P, **Mohs AM**, Dudley AT, Li T, **Duan B**. Dynamic hyaluronic acid hydrogel with covalent linked gelatin as an anti-oxidative bioink for cartilage tissue engineering. Biofabrication. 2021 Dec 31;14(1). doi: 10.1088/1758-5090/ac42de. PMID: 34905737.

103. Olson MT, Aguilar EN, Brooks CL, Isder CC, Muilenburg KM, **Talmon GA**, **Ly QP**, **Carlson MA**, **Hollingsworth MA**, **Mohs AM**. Preclinical Evaluation of a Humanized, Near-Infrared Fluorescent Antibody for Fluorescence-Guided Surgery of MUC16-Expressing Pancreatic Cancer. Mol Pharm. 2022 Oct 3;19(10):3586-3599. doi: 10.1021/acs.molpharmaceut.2c00203. Epub 2022 May 31. PMID: 35640060.

104. Chaudhary S, Pothuraju R, **Rachagani S**, **Siddiqui JA**, Atri P, Mallya K, **Nasser MW**, Sayed Z, Lyden ER, **Smith L**, Gupta SD, Ralhan R, **Lakshmanan I**, Jones DT, **Ganti AK**, Macha MA, **Batra SK**. Dual blockade of EGFR and CDK4/6 delays head and neck squamous cell carcinoma progression by inducing metabolic rewiring. Cancer Lett. 2021 Jul 10;510:79-92. doi: 10.1016/j.canlet.2021.04.004. Epub 2021 Apr 17. PMID: 33878394; PMCID: PMC8153085.

105. Mirzapoiazova T, Xiao G, Mambetsariev B, **Nasser MW**, Miaou E, Singhal SS, Srivastava S, Mambetsariev I, Nelson MS, Nam A, Behal A, Arvanitis LD, Atri P, Muschen M, Tissot FLH, Miser J, Kovach JS, Sattler M, **Batra SK**, Kulkarni P, Salgia R. Protein Phosphatase 2A as a Therapeutic Target in Small Cell Lung Cancer. Mol Cancer Ther. 2021 Oct;20(10):1820-1835. doi: 10.1158/1535-7163.MCT-21-0013. Epub 2021 Jul 12. Erratum in: Mol Cancer Ther. 2022 Apr 1;21(4):700. PMID: 34253596; PMCID: PMC8722383.

106. Mehraj U, Ganai RA, Macha MA, Hamid A, Zargar MA, Bhat AA, **Nasser MW**, Haris M, **Batra SK**, Alshehri B, Al-Baradie RS, Mir MA, Wani NA. The tumor microenvironment as driver of stemness and therapeutic resistance in breast cancer: New challenges and therapeutic opportunities. Cell Oncol (Dordr). 2021 Dec;44(6):1209-1229. doi: 10.1007/s13402-021-00634-9. Epub 2021 Sep 16. PMID: 34528143.

107. Nam A, Mohanty A, Bhattacharya S, Kotnala S, Achuthan S, Hari K, Srivastava S, Guo L, Nathan A, Chatterjee R, **Jain M**, **Nasser MW**, **Batra SK**, Rangarajan G, Massarelli E, Levine H, Jolly MK, Kulkarni P, Salgia R. Dynamic Phenotypic Switching and Group Behavior Help Non-Small Cell Lung Cancer Cells Evade Chemotherapy. Biomolecules. 2021 Dec 21;12(1):8. doi: 10.3390/biom12010008. PMID: 35053156; PMCID: PMC8773639.

108. Maurya SK, Khan P, Rehman AU, **Kanchan RK**, Perumal N, **Mahapatra S**, Chand HS, **Santamaria-Barria JA**, **Batra SK**, **Nasser MW**. Rethinking the chemokine cascade in brain metastasis: Preventive and therapeutic implications. Semin Cancer Biol. 2021 Dec 27:S1044-579X(21)00302-3. doi: 10.1016/j.semcancer.2021.12.009. Epub ahead of print. PMID: 34968667; PMCID: PMC9234104.

109. **Siddiqui JA**, **Seshacharyulu P**, **Muniyan S**, Pothuraju R, Khan P, Vengoji R, Chaudhary S, Maurya SK, **Lele SM**, **Jain M**, **Datta K**, **Nasser MW**, **Batra SK**. GDF15 promotes prostate cancer bone metastasis and colonization through osteoblastic CCL2 and RANKL activation. Bone Res. 2022 Jan 20;10(1):6. doi: 10.1038/s41413-021-00178-6. PMID: 35058441; PMCID: PMC8776828.

110. Khan P, Ebenezer NS, **Siddiqui JA**, Maurya SK, **Lakshmanan I**, Salgia R, **Batra SK**, **Nasser MW**. MicroRNA-1: Diverse role of a small player in multiple cancers. Semin Cell Dev Biol. 2022 Apr;124:114-126. doi: 10.1016/j.semcdb.2021.05.020. Epub 2021 May 24. PMID: 34034986; PMCID: PMC8606619.

111. **Siddiqui JA**, Pothuraju R, Khan P, Sharma G, **Muniyan S**, **Seshacharyulu P**, **Jain M**, **Nasser MW**, **Batra SK**. Pathophysiological role of growth differentiation factor 15 (GDF15) in obesity, cancer, and cachexia. Cytokine Growth Factor Rev. 2022 Apr;64:71-83. doi: 10.1016/j.cytogfr.2021.11.002. Epub 2021 Nov 17. PMID: 34836750; PMCID: PMC8957514.

112. Rehman AU, Khan P, Maurya SK, **Siddiqui JA**, **Santamaria-Barria JA**, **Batra SK**, **Nasser MW**. Liquid biopsies to occult brain metastasis. Mol Cancer. 2022 May 10;21(1):113. doi: 10.1186/s12943-022-01577-x. PMID: 35538484; PMCID: PMC9088117.

113. Manevski M, Devadoss D, Long C, Singh SP, **Nasser MW**, Borchert GM, Nair MN, Rahman I, Sopori M, Chand HS. Increased Expression of LASI lncRNA Regulates the Cigarette Smoke and COPD Associated Airway Inflammation and Mucous Cell Hyperplasia. Front Immunol. 2022 Jun 14;13:803362. doi: 10.3389/fimmu.2022.803362. Erratum in: Front Immunol. 2022 Aug 08;13:988069. PMID: 35774797; PMCID: PMC9237255.

114. Khan P, **Siddiqui JA**, Maurya SK, **Lakshmanan I**, **Jain M**, **Ganti AK**, Salgia R, **Batra SK**, **Nasser MW**. Epigenetic landscape of small cell lung cancer: small image of a giant recalcitrant disease. Semin Cancer Biol. 2022 Aug;83:57-76. doi: 10.1016/j.semcancer.2020.11.006. Epub 2020 Nov 18. PMID: 33220460; PMCID: PMC8218609.

115. **Kanchan RK**, Doss D, Khan P, **Nasser MW**, **Mahapatra S**. To kill a cancer: Targeting the immune inhibitory checkpoint molecule, B7-H3. Biochim Biophys Acta Rev Cancer. 2022 Aug 24;1877(5):188783. doi: 10.1016/j.bbcan.2022.188783. Epub ahead of print. PMID: 36028149.

116. **Casey CA**, Macke AJ, Gough RR, Pachikov AN, Morris ME, Thomes PG, Kubik JL, Holzapfel MS, **Petrosyan A**. Alcohol-Induced Liver Injury: Down-regulation and Redistribution of Rab3D Results in Atypical Protein Trafficking. Hepatol Commun. 2022 Feb;6(2):374-388. doi: 10.1002/hep4.1811. Epub 2021 Sep 7. PMID: 34494400; PMCID: PMC8793998.

117. **Petrosyan A**, Palanjyan A, Melnichenko I, Hakobyan M, Dabikian A, Sargsyan L, Vagharshakyan L, Danielyan S, Tamamyan G, Shamilyan A. ALL-436 Association Between ABO and Rh Blood Groups and Pediatric Hematological Malignances in Armenia. Clin Lymphoma Myeloma Leuk. 2022 Oct;22 Suppl 2:S204-S205. doi: 10.1016/S2152-2650(22)01204-6. PMID: 36163746.

118. Nimmakayala RK, Rauth S, Chirravuri Venkata R, Marimuthu S, Nallasamy P, Vengoji R, **Lele SM**, **Rachagani S**, Mallya K, Malafa MP, **Ponnusamy MP**, **Batra SK**. PGC1α-Mediated Metabolic Reprogramming Drives the Stemness of Pancreatic Precursor Lesions. Clin Cancer Res. 2021 Oct 1;27(19):5415-5429. doi: 10.1158/1078-0432.CCR-20-5020. PMID: 34172498; PMCID: PMC8709878.

119. Vengoji R, Atri P, Macha MA, **Seshacharyulu P**, Perumal N, Mallya K, **Liu Y**, **Smith LM**, **Rachagani S**, **Mahapatra S**, **Ponnusamy MP**, **Jain M**, **Batra SK**, **Shonka N**. Differential gene expression-based connectivity mapping identified novel drug candidate and improved Temozolomide efficacy for Glioblastoma. J Exp Clin Cancer Res. 2021 Oct 25;40(1):335. doi: 10.1186/s13046-021-02135-x. PMID: 34696786; PMCID: PMC8543939.

120. Rauth S, Karmakar S, Shah A, **Seshacharyulu P**, Nimmakayala RK, Ganguly K, Bhatia R, **Muniyan S**, Kumar S, **Dutta S**, **Lin C**, **Datta K**, **Batra SK**, **Ponnusamy MP**. SUMO Modification of PAF1/PD2 Enables PML Interaction and Promotes Radiation Resistance in Pancreatic Ductal Adenocarcinoma. Mol Cell Biol. 2021 Nov 22;41(12):e0013521. doi: 10.1128/MCB.00135-21. Epub 2021 Sep 27. PMID: 34570619; PMCID: PMC8608017.

121. Nallasamy P, Nimmakayala RK, Karmakar S, Leon F, **Seshacharyulu P**, **Lakshmanan I**, **Rachagani S**, Mallya K, Zhang C, **Ly QP**, Myers MS, Josh L, Grabow CE, **Gautam SK**, **Kumar S**, **Lele SM**, **Jain M**, **Batra SK**, **Ponnusamy MP**. Pancreatic Tumor Microenvironment Factor Promotes Cancer Stemness via SPP1-CD44 Axis. Gastroenterology. 2021 Dec;161(6):1998-2013.e7. doi: 10.1053/j.gastro.2021.08.023. Epub 2021 Aug 19. PMID: 34418441.

122. Leon F, **Seshacharyulu P**, Nimmakayala RK, Chugh S, Karmakar S, Nallasamy P, Vengoji R, **Rachagani S**, **Cox JL**, Mallya K, **Batra SK**, **Ponnusamy MP**. Reduction in O-glycome induces differentially glycosylated CD44 to promote stemness and metastasis in pancreatic cancer. Oncogene. 2022 Jan;41(1):57-71. doi: 10.1038/s41388-021-02047-2. Epub 2021 Oct 21. PMID: 34675409; PMCID: PMC8727507.

123. Parte S, Nimmakayala RK, **Batra SK**, **Ponnusamy MP**. Acinar to ductal cell trans-differentiation: A prelude to dysplasia and pancreatic ductal adenocarcinoma. Biochim Biophys Acta Rev Cancer. 2022 Jan;1877(1):188669. doi: 10.1016/j.bbcan.2021.188669. Epub 2021 Dec 13. PMID: 34915061.

124. **Seshacharyulu P**, Halder S, Nimmakayala R, **Rachagani S**, Chaudhary S, Atri P, Chirravuri-Venkata R, **Ouellette MM**, Carmicheal J, **Gautam SK**, Vengoji R, Wang S, **Li S**, **Smith L**, **Talmon GA**, **Klute K**, **Ly Q**, **Reames BN**, **Grem JL**, Berim L, **Padussis JC**, Kaur S (previous BCC member), **Kumar S**, **Ponnusamy MP**, **Jain M**, **Lin C**, **Batra SK**. Disruption of FDPS/Rac1 axis radiosensitizes pancreatic ductal adenocarcinoma by attenuating DNA damage response and immunosuppressive signalling. EBioMedicine. 2022 Jan;75:103772. doi: 10.1016/j.ebiom.2021.103772. Epub 2021 Dec 28. PMID: 34971971; PMCID: PMC8718746.

125. Ganguly K, Shah A, Atri P, Rauth S, **Ponnusamy MP**, **Kumar S**, **Batra SK**. Chemokine-mucinome interplay in shaping the heterogeneous tumor microenvironment of pancreatic cancer. Semin Cancer Biol. 2022 Mar 26:S1044-579X(22)00078-5. doi: 10.1016/j.semcancer.2022.03.022. Epub ahead of print. PMID: 35346803.

126. Nimmakayala RK, Parte S, **Batra SK**, **Ponnusamy MP**. A p130Cas-Mediated Mechanism Governs Acinar to Ductal Metaplasia and the Initiation of Pancreatic Ductal Adenocarcinoma. Gastroenterology. 2022 Apr;162(4):1037-1040. doi: 10.1053/j.gastro.2022.01.042. Epub 2022 Feb 1. PMID: 35114204.

127. Marimuthu S, **Batra SK**, **Ponnusamy MP**. Pan-cancer analysis of altered glycosyltransferases confers poor clinical outcomes. Clin Transl Discov. 2022 Jun;2(2):e100. doi: 10.1002/ctd2.100. Epub 2022 Jun 26. PMID: 35875597; PMCID: PMC9302706.

128. Marimuthu S, **Lakshmanan I**, **Muniyan S**, **Gautam SK**, Nimmakayala RK, Rauth S, Atri P, Shah A, Bhyravbhatla N, Mallya K, **Grandgenett PM**, **Hollingsworth MA**, **Datta K**, **Jain M**, **Ponnusamy MP**, **Batra SK**. MUC16 Promotes Liver Metastasis of Pancreatic Ductal Adenocarcinoma by Upregulating NRP2-Associated Cell Adhesion. Mol Cancer Res. 2022 Aug 5;20(8):1208-1221. doi: 10.1158/1541-7786.MCR-21-0888. PMID: 35533267.

129. Nimmakayala RK, Ogunleye AO, Parte S, Krishna Kumar N, Raut P, Varadharaj V, Perumal NK, Nallasamy P, Rauth S, **Cox JL**, **Lele SM**, **Batra SK**, **Ponnusamy MP**. PAF1 cooperates with YAP1 in metaplastic ducts to promote pancreatic cancer. Cell Death Dis. 2022 Oct 1;13(10):839. doi: 10.1038/s41419-022-05258-x. PMID: 36180487; PMCID: PMC9525575.

130. Natesh NS, White BM, Bennett MMC, Uz M, Kalari Kandy RR, **Batra SK**, Mallapragada SK, **Rachagani S**. Emerging Role of miR-345 and Its Effective Delivery as a Potential Therapeutic Candidate in Pancreatic Cancer and Other Cancers. Pharmaceutics. 2021 Nov 23;13(12):1987. doi: 10.3390/pharmaceutics13121987. PMID: 34959269; PMCID: PMC8707074.

131. **Gunda V**, Chhonker YS, Natesh NS, Raut P, **Muniyan S**, **Wyatt TA**, **Murry DJ**, **Batra SK**, **Rachagani S**. Nuclear factor kappa-B contributes to cigarette smoke tolerance in pancreatic ductal adenocarcinoma through cysteine metabolism. Biomed Pharmacother. 2021 Dec;144:112312. doi: 10.1016/j.biopha.2021.112312. Epub 2021 Oct 19. PMID: 34678726; PMCID: PMC8599650.

132. Pothuraju R, Pai P, Chaudhary S, **Siddiqui JA**, **Cox JL**, Kaur S (previous BCC member), **Rachagani S**, Roy HK, Bouvet M, **Batra SK**. Depletion of transmembrane mucin 4 (Muc4) alters intestinal homeostasis in a genetically engineered mouse model of colorectal cancer. Aging (Albany NY). 2022 Mar 7;14(5):2025-2046. doi: 10.18632/aging.203935. Epub 2022 Mar 7. PMID: 35255004; PMCID: PMC8954958.

133. **Gunda V**, Genaro-Mattos TC, Kaushal JB, Chirravuri-Venkata R, Natarajan G, Mallya K, **Grandgenett PM**, Mirnics K, **Batra SK**, Korade Z, **Rachagani S**. Ubiquitous Aberration in Cholesterol Metabolism across Pancreatic Ductal Adenocarcinoma. Metabolites. 2022 Jan 7;12(1):47. doi: 10.3390/metabo12010047. PMID: 35050168; PMCID: PMC8779872.

134. Kaushal JB, **Batra SK**, **Rachagani S**. Hedgehog signaling and its molecular perspective with cholesterol: a comprehensive review. Cell Mol Life Sci. 2022 Apr 29;79(5):266. doi: 10.1007/s00018-022-04233-1. PMID: 35486193.

135. **Gautam SK**, Dalal V, Sajja BR, Gupta S, Gulati M, Dwivedi NV, Aithal A, **Cox JL**, **Rachagani S**, **Liu Y**, Chung V, Salgia R, **Batra SK**, **Jain M**. Endothelin-axis antagonism enhances tumor perfusion in pancreatic cancer. Cancer Lett. 2022 Sep 28;544:215801. doi: 10.1016/j.canlet.2022.215801. Epub 2022 Jun 19. PMID: 35732216.

136. **Schott MB**, Rozeveld CN, Weller SG, McNiven MA. Lipophagy at a glance. J Cell Sci. 2022 Mar 1;135(5):jcs259402. doi: 10.1242/jcs.259402. Epub 2022 Mar 9. PMID: 35260889; PMCID: PMC9014375.

137. Sharma G, Pothuraju R, **Kanchan RK**, **Batra SK**, **Siddiqui JA**. Chemokines network in bone metastasis: Vital regulators of seeding and soiling. Semin Cancer Biol. 2022 Feb 3:S1044-579X(22)00024-4. doi: 10.1016/j.semcancer.2022.02.003. Epub ahead of print. PMID: 35124194.

138. Mughees M, Kaushal JB, Sharma G, Wajid S, **Batra SK**, **Siddiqui JA**. Chemokines and cytokines: Axis and allies in prostate cancer pathogenesis. Semin Cancer Biol. 2022 Feb 16:S1044-579X(22)00041-4. doi: 10.1016/j.semcancer.2022.02.017. Epub ahead of print. PMID: 35181473.

139. Bhatia R, Thompson CM, Clement EJ, Ganguly K, **Cox JL**, Rauth S, **Siddiqui JA**, Mashiana SS, **Jain M**, **Wyatt TA**, Mashiana HS, **Singh S**, **Woods NT**, **Kharbanda KK**, **Batra SK**, **Kumar S**. Malondialdehyde-Acetaldehyde Extracellular Matrix Protein Adducts Attenuate Unfolded Protein Response During Alcohol and Smoking-Induced Pancreatitis. Gastroenterology. 2022 Oct;163(4):1064-1078.e10. doi: 10.1053/j.gastro.2022.06.071. Epub 2022 Jul 3. PMID: 35788346.

140. Penning TM, Jonnalagadda S, **Trippier PC**, Rižner TL. Aldo-Keto Reductases and Cancer Drug Resistance. Pharmacol Rev. 2021 Jul;73(3):1150-1171. doi: 10.1124/pharmrev.120.000122. PMID: 34312303; PMCID: PMC8318518.

141. Rahman MS, Kumari S, Esfahani SH, Nozohouri S, Jayaraman S, Kinarivala N, Kocot J, Baez A, Farris D, Abbruscato TJ, Karamyan VT, **Trippier PC**. Discovery of First-in-Class Peptidomimetic Neurolysin Activators Possessing Enhanced Brain Penetration and Stability. J Med Chem. 2021 Sep 9;64(17):12705-12722. doi: 10.1021/acs.jmedchem.1c00759. Epub 2021 Aug 26. Erratum in: J Med Chem. 2022 Jan 13;65(1):890. PMID: 34436882; PMCID: PMC9295256.

142. Morsy A, Carmona AV, **Trippier PC**. Patient-Derived Induced Pluripotent Stem Cell Models for Phenotypic Screening in the Neuronal Ceroid Lipofuscinoses. Molecules. 2021 Oct 15;26(20):6235. doi: 10.3390/molecules26206235. PMID: 34684815; PMCID: PMC8538546.

143. Jayaraman S, Kocot J, Esfahani SH, Wangler NJ, Uyar A, Mechref Y, **Trippier PC**, Abbruscato TJ, Dickson A, Aihara H, Ostrov DA, Karamyan VT. Identification and Characterization of Two Structurally Related Dipeptides that Enhance Catalytic Efficiency of Neurolysin. J Pharmacol Exp Ther. 2021 Nov;379(2):191-202. doi: 10.1124/jpet.121.000840. Epub 2021 Aug 13. PMID: 34389655; PMCID: PMC8626779.

144. Giustiniano M, Gruber CW, Kent CN, **Trippier PC**. Back to the Medicinal Chemistry Future. J Med Chem. 2021 Nov 11;64(21):15515-15518. doi: 10.1021/acs.jmedchem.1c01788. Epub 2021 Nov 1. PMID: 34719927.

145. Ashraf-Uz-Zaman M, Ji G, Tidwell D, Yin L, Thakolwiboon S, Pan J, Junell R, Griffin Z, Shahi S, Barthels D, Sajib MS, **Trippier PC**, Mikelis CM, Das H, Avila M, Neugebauer V, German NA. Evaluation of Urea-Based Inhibitors of the Dopamine Transporter Using the Experimental Autoimmune Encephalomyelitis Model of Multiple Sclerosis. ACS Chem Neurosci. 2022 Jan 19;13(2):217-228. doi: 10.1021/acschemneuro.1c00647. Epub 2022 Jan 3. PMID: 34978174; PMCID: PMC9365315.

146. Jonnalagadda SK, Huwaimel BI, Jonnalagadda S, **Garrison JC**, **Trippier PC**. Access to Highly Strained Tricyclic Ketals Derived from Coumarins. J Org Chem. 2022 Mar 18;87(6):4476-4482. doi: 10.1021/acs.joc.2c00018. Epub 2022 Mar 8. PMID: 35258961; PMCID: PMC8996706.

147. Esfahani SH, Abbruscato TJ, **Trippier PC**, Karamyan VT. Small molecule neurolysin activators, potential multi-mechanism agents for ischemic stroke therapy. Expert Opin Ther Targets. 2022 May;26(5):401-404. doi: 10.1080/14728222.2022.2077190. Epub 2022 May 19. PMID: 35543670.

148. Rahman MS, Esfahani SH, Nozohouri S, Kumari S, Kocot J, Zhang Y, Abbruscato TJ, Karamyan VT, **Trippier PC**. Structure-activity relationship studies of functionalized aromatic peptidomimetics as neurolysin activators. Bioorg Med Chem Lett. 2022 May 15;64:128669. doi: 10.1016/j.bmcl.2022.128669. Epub 2022 Mar 12. PMID: 35292343; PMCID: PMC8985228.

149. Nozohouri S, Esfahani SH, Noorani B, Patel D, Villalba H, Ghanwatkar Y, Rahman MS, Zhang Y, Bickel U, **Trippier PC**, Karamyan VT, Abbruscato TJ. In-Vivo and Ex-Vivo Brain Uptake Studies of Peptidomimetic Neurolysin Activators in Healthy and Stroke Animals. Pharm Res. 2022 Jul;39(7):1587-1598. doi: 10.1007/s11095-022-03218-w. Epub 2022 Mar 3. PMID: 35239135; PMCID: PMC9295206.

150. Morsy A, Maddeboina K, Gao J, Wang H, Valdez J, Dow LF, Wang X, **Trippier PC**. Functionalized Allopurinols Targeting Amyloid-Binding Alcohol Dehydrogenase Rescue Aβ-Induced Mitochondrial Dysfunction. ACS Chem Neurosci. 2022 Jul 20;13(14):2176-2190. doi: 10.1021/acschemneuro.2c00246. Epub 2022 Jul 8. PMID: 35802826.

151. Kumari S, Maddeboina K, Bachu RD, Boddu SHS, **Trippier PC**, Tiwari AK. Pivotal role of nitrogen heterocycles in Alzheimer's disease drug discovery. Drug Discov Today. 2022 Oct;27(10):103322. doi: 10.1016/j.drudis.2022.07.007. Epub 2022 Jul 19. PMID: 35868626.

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SUMMARY OF EXPENDITURES

The following summary table includes the original Proposed Budget, approved Revised Budget, and Actual Expenditures from July 1, 2021, to June 30, 2022.

Budget Category	Revised LB 595 Budget	LB 595 Expenditures
Salaries and Fringe Benefits	$1,058,079	$1,023,721
Equipment	$0	$6,538
Supplies	$131,256	$172,481
Other Expenses	$110,665	$97,260
Total	**$1,300,000**	**$1,300,000**