NEBRASKA

Good Life. Great Mission.

DEPT. OF HEALTH AND HUMAN SERVICES

Jim Pillen, Governor

December 31, 2023

Mr. Brandon Metzler
Clerk of the Legislature
State Capitol Room 2028
Lincoln, NE 68509

Subject: Cancer and Smoking Disease Research Report

Dear Mr. Metzler:

In accordance with Nebraska Revised Statute § 81-638(3)(b), please find attached copies of two 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 Buffett 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 Buffett Cancer Center.

Sincerely,

Charity Menefee

Charity Menefee
Director, Division of Public Health

Attachment

Creighton UNIVERSITY
Research and Scholarship

September 21, 2023

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 FY23. 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*
T 402.280.3265 | jksoukup@creighton.edu
Rigge Science Building
780540 California Plaza Omaha, NE 68178-0540
creighton.edu

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

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 2022-2023 has been productive for the investigators at Creighton University. Manuscripts were published in such journals as *The Journal of Allergy and Clinical Immunology*, *Journal of Biological Chemistry, Journal of Obstetric Gynecologic and Neonatal Nursing, 3D Printing in Medicine, Frontiers in Genetics, Frontiers in Cellular Neuroscience, Journal of Cell Biology,* and *Science Advances*.

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 28 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 $39,310,340 to Creighton University through LB595. This, coupled with Creighton's contribution of $17,493,101 through unrecovered indirect costs and $42,953,296 in internal seed grant funding, has led to $169,538,911 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 $4.3 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, 2023)

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			439,239	195,461	1,298,760
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			50,000			210,000	93,450	353,450
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	7,162,397	221,206	442,346	30,000	50,000	120,000	53,400	8,079,349
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	81,653					120,000	53,400	255,053
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	5,441,805	79,897	1,378,714		15,000	2,281,753	1,015,380	10,212,548
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	1,511,695			761,420	338,832	3,083,047
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			588,636	261,943	3,129,259
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	1,244,654		300,000		75,000	344,113	153,130	2,116,897
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
Shelkar, Gajanan					24,911	60,000	26,700	111,611
Smith, Derek	525,589			5,000	10,000	775,201	344,964	1,660,754
Strauss-Soukup, Juliane	800,051		361,353		5,000	526,011	234,075	1,926,490
Stessman, Holly	1,011,315		543,492			1,431,848	637,172	3,623,827
Swanson, Patrick	5,973,485	237,481	1,575,171	15,000	50,000	1,370,000	609,650	9,830,787
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	5,861,208	20,000	256,732		50,000	2,110,000	938,950	9,236,890
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
Xia, Jun	249,000		220,000			60,000	26,700	555,700
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	**$169,538,911**	**$23,593,067**	**$18,938,540**	**$80,000**	**$341,689**	**$39,310,340**	**$17,493,101**	**$269,295,649**

Creighton University Cancer & Smoking Disease Research Program FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

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

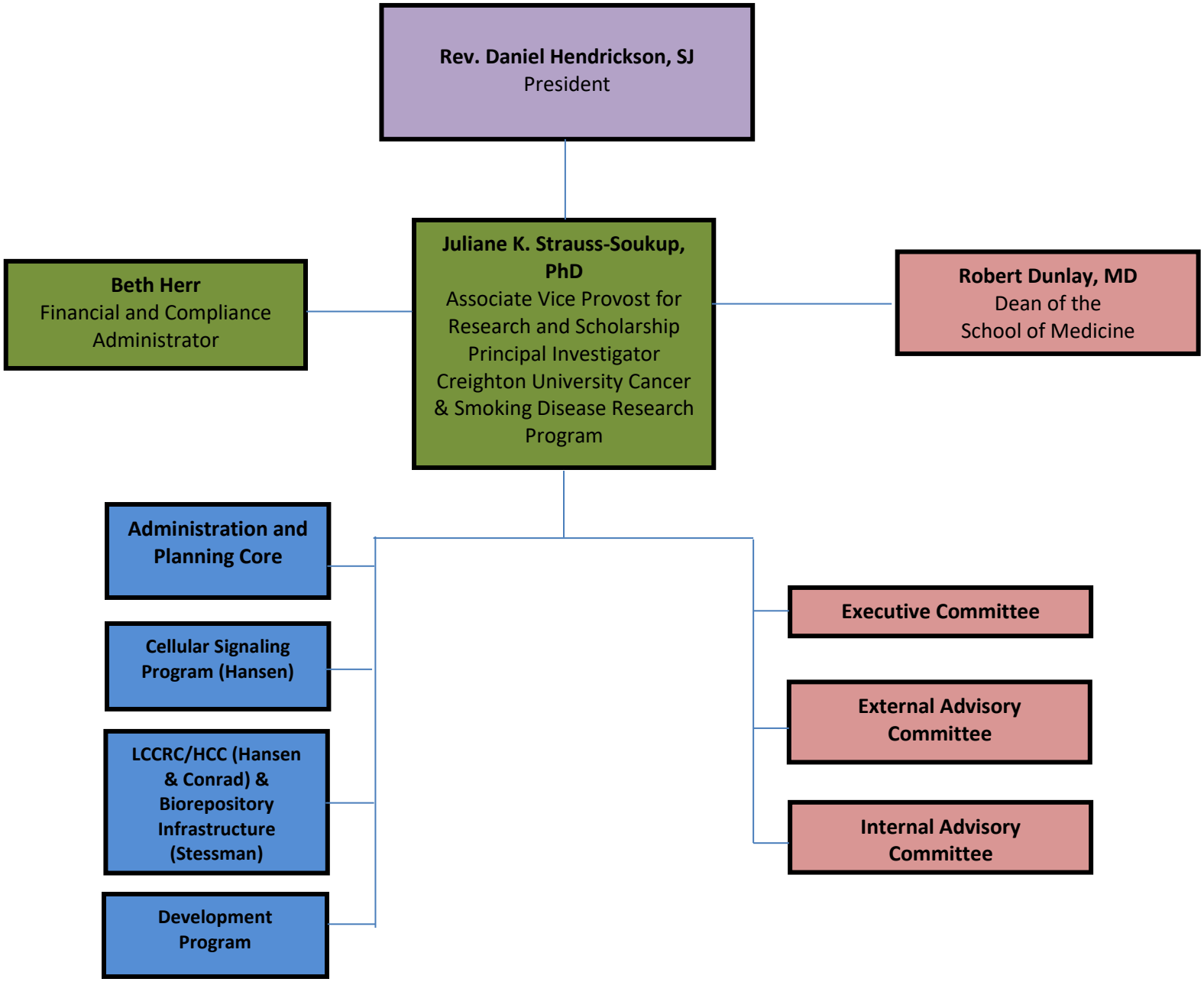
Juliane K. Strauss-Soukup, PhD
Associate Vice Provost for Research and Scholarship
Principal Investigator, Cancer and Smoking Disease Research Program

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, 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
- 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. Reynold Panettieri and Dr. Christine M. Eischen are co-chairs of the External Advisory Committee and participate in the State of Nebraska site visit. Additionally, Dr. Panettieri and Dr. Eischen provide 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 2022-2023 took place on

the Creighton University campus on July 31, 2023. See the agenda and External Advisory Committee report at the end of this Administration and Planning report.

James P. Calvet, PhD, University of Kansas Medical Center, notified us that he was retiring from the committee after the 2022 meeting. and two new members joined during the 2022-2023 year, as detail below.

Members of the External Advisory Committee are as follows:

- Reynold Panettieri, Jr., MD: Rutgers, The State University of New Jersey
- Christine Eischen, PhD, Thomas Jefferson University
- Ralf Krahe, PhD: University of Texas MD Anderson Cancer Center
- Stephen Hecht, PhD, University of Minnesota
- Tomoo Iwakuma, MD, PhD, Children's Mercy Research Institute
- Christy Hagan, PhD, University of Kansas Medical Center

4. Seminars

During the 2022-2023 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 is a list of speakers and seminar topics for the 2022-2023 year:

- Sushil Kumar, PhD, Associate Professor, University of Nebraska Medical Center
  Seminar Topic: A Sticky Wicket: Mucins in Pancreatic Cancer Inception and Stromal Hetreogeneity
- Ashwarya Prakash, PhD, Associate Professor, University of South Alabama
  Seminar Topic: A structural approach to understanding the function of DNA repair complexes
- Christopher Amos, PhD, Professor, Baylor College of Medicine
  Seminar Topic: Genomics Approaches for Cancer Precision Prevention

5. Cancer Journal Access at Library

During the 2022-2023 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, July 31, 2023

External Advisory Committee: Reynold Panettieri, MD (Co-Chair), Rutgers, The State University of New Jersey; Christine M. Eischen, PhD (Co-Chair, added 2022), Thomas Jefferson University; Ralf Krahe, PhD, University of Texas MD Anderson Cancer Center; Stephen Hecht, PhD, University of Minnesota (added 2022); Tomoo Iwakuma, MD, PhD, Children's Mercy Research Institute (added 2023); and Christy Hagan, PhD, University of Kansas Medical Center (added 2023). All attended the site visit face-to-face except Dr. Krahe (will resign 2023), who attended by Zoom video conferencing.

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

ADMINISTRATION & PROGRAM PLANNING – Juliane Strauss-Soukup, PhD

Juliane Strauss-Soukup, PhD (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 throughout Creighton University adds an important new dimension to the LB595 program. She appears to be committed to the high 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 additional cancer-related investigators and faculty. 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 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 FY22/23, seven of the LB595 investigators were awarded a total of 6 new NIH grants for a total of $1,817,835. Over the last year, Creighton received $10 million from NIH; 42% of LB595 basic scientists (13/33) were NIH-funded. In the last year, there were 11 peer-reviewed publications by 11 LB595-supported faculty. Although four LB506 grants were submitted, none were funded.

Despite some marketing of the program, grant submissions have decreased. To build on recent successes and accelerate growth, it is suggested that the administration consider a webinar about the LB595 Development Program to further 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 to recruit new faculty into the program, 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. It would be beneficial to further encourage multi-PI proposals between School of Medicine faculty and traditionally non-medical school departments. The recent incentive to return a portion of F&A to PIs may also enhance NIH grant submissions.

A seminar series, directed by Dr. Laura Hansen, was supported by LB595 during 2022-2023. Seminars were focused on cancer and smoking-related diseases and included basic science and translational research presented by external speakers.

It should be noted that the publication productivity of the LB595 investigators seemed somewhat modest this year, for unclear reasons. Although the investigators are still making progress, publishing appears to have slowed a bit. Only 11 publications originating from the LB595 program were reported. 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 count submitted manuscripts to get a better idea of ongoing publication activity. Also, external grant funding success was modest, so additional mentoring, greater use of the School of Medicine's mock review services, and more grant submissions of competitive applications should be encouraged. For example, applications to the Department of Defense and cancer- and smoking-related disease foundation grants could be encouraged in addition to NIH grants.

Another challenge is the Lynch "Comprehensive" Cancer Research Center (LCCRC). The recent departure of Dr. Robin Farias-Eisner as Director of the LCCRC after only two years represents a major challenge. The term "Comprehensive" should be deleted. The appointment of Dr. Laura Hansen, a PhD basic cancer scientist, as Director will foster stability. The vison is to create a co-directorship with Lesley Conrad, MD, a physician-scientist. Dr. Conrad, a recently recruited gynecologic oncologist, along with Dr. Laura Hansen, [together] co-chair the oversight committee for the LCCRC. The Center's plans will need clarification over the next year. Careful attention and focus by the co-Directors will be needed to define how the LCCRC and LB595 can mutually benefit each other and 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. LB595 funds will also continue to support the LCCRC Biorepository, which is considered an important and unique asset for cancer research.

BIOREPOSITORY INFRASTRUCTURE – Holly Stessman, PhD

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 cataloging of viable samples for future research, as well as the transition from paper records to a computerized database. The goals have largely remained unchanged. The previously noted IRB issues have been resolved, resulting in retainment of ~80% of high value samples from the "legacy collection." Patient consents have been manually audited and confirmed for accuracy and searchability. There are plans to re-consent existing patients with IRB protocol approval to allow future use of the samples.

The Biorepository Core's goal was to audit stored participant samples, implement a LIMS (Lab

Vantage), upgrade the databases to an electronic format, and establish RNA and DNA sequencing platforms. These goals were accomplished. Overall, substantial progress has been made, showing that 85% of ~9,000 donor specimens are accessible in a patient de-identified manner. A cost sharing model is in place. Moving forward, the PI should establish metrics in consultations and develop a sustainability plan. Some extramural funding that offsets a portion of the costs to run this Core has already occurred. A plan to market this unique biorepository both within and outside of Creighton is recommended. The EAC has no concerns regarding this Core. Dr. Stessman needs to be assured of protected effort for the management of such a complex and valuable asset.

CELLULAR SIGNALING & MOLECULAR TRAFFICKING IN CANCER – Laura Hansen, PhD

The overall program under the leadership of Dr. Hansen, Associate Dean for Research for the School of Medicine and co-Director of the LCCRC, consists of five projects. The program is well-established and continues to be solid. All projects made significant progress, and there appear to be some inter-programmatic interactions among the investigators. Dr. Hansen provides strong leadership for the program. Despite these strengths, several challenges exist. The overall numbers of extramural grant submissions and publications are modest. There is a mock review service in place, but it may be underutilized, especially for amended grant applications.

Checkpoint Signaling and Cell Survival in Normal and Tumorigenic Skin Keratinocytes – Laura Hansen, PhD

Dr. Hansen's project on function and expression of Flower (FWE) isoforms in skin keratinocytes and squamous cell carcinoma (SSC) cells is multifaceted, quite novel, and potentially high impact. The effects of the expression of different isoforms of FWE and where they reside inside skin cells were studied. The results showed that human FWE4 expression modulates SSC differentiation. Additionally, data show that human FWE4 is involved with endocytic processes. Additionally, to study the FWE isoforms *in vivo*, a Crainbow mouse model for lineage tracing has been developed. 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. The project is proceeding well, but the results are quite complex. While overall progress has been substantial, the exact goals need clarification. Overall, productivity of the PI continues to be substantial, with an additional NE DHHS LB506 pilot grant awarded, originating from the LB595 project. Her publication of a JBC manuscript is impressive, as well is her previous receipt of an LB506 for the Crainbow mouse studies.

Cellular Pathways Targeting BubR1 to the Proteosome for Degradation: Implications for Skin Cancer – Brian North, PhD (Niki Kumari, Ph.D., presenter)

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 several 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 (SKH-1 mice) treated with NMN showed an increase in tumor burden, but overall tumor incidence was not affected.

Bulk RNA-sequencing studies were performed, which revealed upregulated EMT-associated gene signatures when UV-treated mice were supplemented with NMN; NMN also down-regulated interferon alpha and gamma expression. Further studies to elucidate the underlying mechanisms relative to the pathway are in progress. No publications or grants were generated; the PI did submit 2 R01s on unrelated topics.

Localization of RAG1 Degradation and Implication of RAG1 Stabilization on Genome Instability and Cancer – Patrick Swanson, PhD

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 whether impairing RAG1 turnover increases the frequency of aberrant 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. Investigations into the mechanism of RAG1 degradation are continuing. This year, other targets of RACK1, including Bim and HIF1alpha, were evaluated in B cells. Interestingly, IkappaBa was modulated with RACK1 loss, which was unexpected and will be evaluated. It is a challenging project that, once completed, should be impactful for B cell biology and lymphoid malignancies. He has two funded R21s based on these studies, which were originally supported by LB595 funding. Over the last 12 months, there were no publications, likely due to challenges with staffing his laboratory; however, he did submit an R01. He is a tremendous asset to the LB595 program.

Dysregulated Mitochondrial Dynamics and Cancer Metastasis – Yaping Tu, PhD

Dr. Tu's project explores miR-133a upregulation, which increases Drp1-dependent mitochondrial fusion and respiration 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 3 R01s submitted and one with a fundable score awaiting a notice of grant award. A mouse experiment is also in the planning stages. Of note, however, while the investigation into the mechanism of miR-133a regulation of Parkin was scientifically rigorous, it could have included a study of other genes in the same pathway, since miRNAs do not typically regulate a single mRNA. Two investigators (Drs. Tu and Abel) are studying miRNAs and their target genes, which could lead to a future collaborative study. It should be noted that miRNA studies are less scientifically impactful unless RNA modifications and how they are regulated are also the focus.

Inhibition of GBM Invasion with Highly Selective and Proteolytically Stable Peptide Analogs – Sandor Lovas, PhD

Due to last year's awarded R01 and to avoid overlap, the focus of the current grant was changed 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 previously showed 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. Over the last year, Dr. Lovas has further characterized analogs of the MMP-2 inhibitory peptides. However, significant concerns remain and were raised. Although Dr. Lovas uses state-of-the-art peptide engineering, the clinical, pharmacological, and physiological relevance remains unclear. Further, the development of an invasion assay rather than a migration assay would be advantageous.

Additionally, 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 considerable data on the project. He is a co-investigator on several recent grant submissions and is a co-author on one published paper with Dr. Hansen.

DEVELOPMENT PROGRAM – Juliane Strauss-Soukup, PhD

LB595 grant proposals require a statement of the project's relevance 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: John Cote, MD, 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 project 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. Dr. Cote recognizes that recruiting the

requisite number of participants is challenging, considering the current low rate of smoking during pregnancy in Omaha. In the past year, Dr. Cote continued his study, suggesting MAAS scores correlate with smoking cessation via fetal imaging; 34 participants were recruited with impressive URM participant recruitment. Intriguing data was observed, although no difference was seen between interventions. Power calculations suggest >90 participants are needed to show differences in group. Plans to increase satellite recruitment sites would increase patient numbers and enhance power to show clinically relevant findings. If successful, the impact on maternal-fetal health of smoking in expecting mothers could be significant. Overall, the committee was impressed by the progress and direction, but no publication was generated.

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

This project 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. 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 miRNA caused increased proliferation and differentiation in mouse lung fibroblasts and exacerbated lung fibrosis. The project is progressing well but is somewhat early, so its impact at this stage is uncertain. Thus, it is a positive aspect of this research 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. No publications were generated this year; however, given the development of a mouse with a global KO of miR-146b, the PI is poised to answer whether miR-146b serves as a susceptibility gene for lung fibrosis by bleomycin. This represents a critical experiment for proof of concept. The PI should also link his IPF work with smoking exposure to show compliance with the award requirements. Smoking is a risk factor for development of IPF.

PI: Yusi Fu, PhD, 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 project focused on the identification of molecular signatures of pre-cancerous lesions in endometrial cancer (EC). Dr. Fu has 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 the current approach. Endometrial aspirates from patients in the CHI hospital system were obtained with IRB approval from 4 patients with true-positive endometrial cancer diagnoses and 4 patients with false-positive diagnoses. Cell samples were 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. 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 whether recurring mutations are associated with endometrial cancer.

Dr. Fu's project is innovative, high-impact, and potentially 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? Substantial work showed that 16,064 cells were studied, with interesting translational work identifying EC in biopsies. There were alterations in oncogene expression and DNA copy number identified in subgroups. Monocyte subpopulations may also predict carcinogenesis. Next steps should focus on understanding the molecular pathways regulating this immunophenotype and the relevance of immune dysregulation in the pathogenesis of EC. Dr. Fu coauthored two published manuscripts, submitted one NIH grant and a cancer foundation grant, and contributed to two other NIH grants as a co-investigator.

PI: Gajanan Shelkar, PhD, Resident Assistant Professor, Department of Pharmacy and Neuroscience
Title: Glutamate Delta-I receptor in Cisplatin-Induced Neuropathic Pain and Anorexia

Dr. Shelkar's project utilizes a multidisciplinary approach that includes genetically engineered 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. Dr. Shelkar 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 whether cisplatin affects glutamatergic mechanisms and whether these effects can be reversed and involve GluD1. The project also includes the examination of 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. Cisplatin is frequently used in cancer management but is associated with significant side effects. GluD1-Cbln 1 signaling through spino-parabrachio-amygdala pathways modulates inflammatory and neuropathic pain. Data indicate that GluD1-Cbln-1 activation is downregulated and is associated with pain and anorexia after cisplatin treatment. Significant progress has been made showing that cisplatin decreases GluD1-Cbln-1 activation. Injection of Cbln-1 reversed cisplatin effects. No manuscript was published, but one R21 was submitted with a plan to submit an R01 this year.

PI: Jun Xia, PhD, Department of Biomedical Sciences
Title: Mechanism of Lung Cancer Risk Gene FUBP1-Induced DNA Damage

This pilot grant's goal is to understand the pathogenesis of lung cancer via DNA repair processes. Dr. Xia has studied endogenous DNA damage and the mechanisms that regulate repair. FUBP1 is one of the highest expressing proteins related to DNA injury. Dr. Xia focuses on transcriptional independent DNA repair-injury pathways, such as FUBP1 variant expression. Mutant, dysfunctional FUBP1 engenders a susceptibility to DNA injury. Overexpression of FUBP1 inhibits DNA replication. The overall approach is interesting, and significant progress has been realized regarding methods development. The PI published two collaborative manuscripts and submitted one NIH R01 grant and is a co-investigator on two grants submitted by Dr. Fu.

LYNCH COMPREHENSIVE CANCER RESEARCH CENTER (LCCRC)- Laura Hansen

The Institute has evolved and was renamed and reimagined. The term “Comprehensive” should be avoided. The Directorship will be a partnership between clinicians and scientists. Several issues should be addressed. With the reimagined Institute, what is the mission and vision? What is the sustainability model? A formal SWOT analysis and retreat with a goal to formulate a 5-year strategic plan seems to be a reasonable next step. New recruitment efforts could include cancer imaging, precision therapy, informatics, and community engagement foci.

Creighton University Cancer & Smoking Disease Research Program FY22/23 Progress Report

(July 1, 2022 – June 30, 2023)

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 2022-2023:

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

PI: Jun Xia, PhD, Department of Biomedical Sciences
Title: Mechanism of Lung Cancer Risk Gene RUBP1-Induced DNA Damage

These are the year two reports for the 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

This is the final report for a Development project that was 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

The full reports follow this page.

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

DEVELOPMENT GRANTS
Juliane K. Strauss-Soukup, PhD

Project Title: Glutamate delta-1 receptor in cisplatin-induced neuropathic pain and anorexia
Principal Investigator: Gajanan Shelkar

I. Progress Report Summary

Cisplatin, a commonly used anti-neoplastic agent for various cancers, is associated with severe side effects, such as neuropathic pain and anorexia, which often lead to treatment discontinuation and reduce its therapeutic effectiveness. This proposal aims to investigate novel mechanisms that regulate plasticity at the parabrachial nucleus (PB)-central laterocapsular amygdala (CeLC) synapses following cisplatin treatment. Our goal is to elucidate the role of the therapeutically targetable GluD1-Cerebellin 1 (Cbln1) signaling pathway as a mechanism for pain-related plasticity in the central amygdala (CeA), which can be potentially targeted to restore synaptic function in cisplatin-induced neuropathic pain and anorexia.

A. Specific Aims: (No modifications from the original specific 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.

- Experiment 1: To address whether systemic cisplatin treatment leads to changes in expression and localization of GluD1 and Cbln1 at PB-CeLC synapses.
- Experiment 2: To determine the effect of systemic cisplatin on excitatory neurotransmission at PB-CeLC synapses using electrophysiology in brain slices.
- Experiment 3: To determine whether there are changes in the excitability of CeLC and PB neurons and whether ablation of GluD1 affects cisplatin-induced neuroplasticity.
- Experiment 4: To 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.

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

- Experiment 1: To address whether GluD1-Cbln1 function is critical for cisplatin-induced neuropathic pain and anorexia using conditional region-specific deletion of GluD1 from CeA.
- Experiment 2: To 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.

B. Studies and Results

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. Experiment 1: To address whether systemic cisplatin treatment leads to changes in expression*

and localization of GluD1 and Cbln1 at PB-CeLC synapses.

Accomplishment: To address this question, we conducted immunohistochemical (IHC) studies. Brain tissues from three groups of mice, namely saline-PBS, cisplatin-PBS, and cisplatin-Cbln1, were obtained and processed for GluD1 immunolabeling in the CeLC region. Our results revealed a significant reduction in GluD1 expression in CeLC neurons following systemic administration of cisplatin. Importantly, intra-cerebroventricular administration of recombinant Cbln1, which acts as a transsynaptic binding partner to GluD1, significantly restored the expression of GluD1. These findings suggest that cisplatin treatment induces abnormal changes in GluD1 signaling, while the administration of Cbln1 mitigates these changes and restores GluD1 expression.

Experiment 2: To determine the effect of systemic cisplatin on excitatory neurotransmission at PB-CeLC synapses using electrophysiology in brain slices.

Accomplishment: To achieve this objective, we conducted electrophysiological studies in the brain slices obtained from saline-PBS, cisplatin-PBS, and cisplatin-Cbln1 treated mice. The mice were cannulated into the CeA, and after sufficient recovery period, they were tested for baseline mechanical sensitivity using the Von Frey filament test. After obtaining a stable baseline, these mice were divided into saline-PBS, cisplatin-PBS, and cisplatin-Cbln1 groups. Saline or cisplatin (5 mg/kg) were injected via the intraperitoneal route and tested for mechanical hypersensitivity via the Von Frey filament test. After significant behavioral effects were observed (increased mechanical hypersensitivity in cisplatin-treated mice), these mice received PBS or Cbln1 treatment in the CeA and were assessed for reversal of behavioral effects. Subsequently, the brains from these mice were utilized for electrophysiological studies.

Our findings demonstrated that cisplatin treatment significantly increased excitatory neurotransmission in the CeLC neurons, as evidenced by an increase in miniature excitatory post-synaptic currents (mEPSC) frequency. Importantly, intra-CeA administration of recombinant Cbln1 in mice that received cisplatin (ip) injection significantly restored the cisplatin-induced changes in excitatory neurotransmission in CeLC neurons. This suggests that the restoration of GluD1-Cbln1 signaling reversed the increased excitatory neurotransmission observed in cisplatin-induced neuropathic pain conditions within CeA neurons.

We will conduct knock-out studies to establish GluD1 specific effects in cisplatin-induced neuropathic pain conditions.

Experiment 4: To 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.

Accomplishments: In different sets of studies, we have validated the overexpression of GluD1 by injecting AAV-hSyn-DIO-mGRID1 in the CeA.

We will conduct electrophysiological studies to accomplish the objectives.

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

Experiment 2: To 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.

Accomplishments: We have conducted preliminary studies to test the therapeutic effect of Cbln1 injection in mitigating cisplatin-induced neuropathic pain. The animals were prepared following the procedure described in Experiment 2. In our initial findings, we observed an increased mechanical hypersensitivity in the cisplatin injected mice. Importantly, intra-CeA administration of Cbln1 significantly reduced mechanical hypersensitivity in cisplatin-treated wild-type mice. To substantiate our preliminary results, we plan to increase the group size to 7-8 mice per group in future experiments.

Subsequent experiments will be conducted to assess whether the overexpression of GluD1 in CeLC can alleviate cisplatin-induced mechanical hypersensitivity.

C. Significance

Collectively, our findings support the hypothesis and demonstrate that cisplatin treatment decreases GluD1 expression, resulting in increased excitatory neurotransmission in CeLC neurons, leading to mechanical hypersensitivity and neuropathic pain-like symptoms. However, the administration of exogenous recombinant Cbln1 effectively restores GluD1 expression, normalizes excitatory neurotransmission, and alleviates the pain-like condition, as indicated by reduced mechanical hypersensitivity. These results underscore the crucial role of GluD1-Cbln1 signaling in the CeA for regulating the effects of cisplatin.

These findings have significant implications for the development of potential therapeutic interventions for cisplatin-induced neuropathic pain. By elucidating the underlying mechanisms and identifying a therapeutic target in the GluD1-Cbln1 signaling pathway, our research offers insights into strategies that can potentially mitigate the devastating side effects associated with cisplatin treatment. By restoring GluD1 expression and normalizing excitatory neurotransmission, these interventions could enhance the efficacy of cisplatin therapy while minimizing the occurrence of neuropathic pain and improving patient outcomes.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

None

III. List of extramural grants submitted from 7/1/2022–6/30/2023

None

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

None

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

DEVELOPMENT PROGRAM
Program Director: Juliane Strauss-Soukup, PhD

Mechanism of lung cancer risk gene FUBP1-induced DNA damage
Principal Investigator: Jun Xia, PhD

I. Progress Report Summary

A. Specific Aims

Aim 1: DNA breakage and DNA binding maps of FUBP1 overproduction.

Aim 2: Mutagenesis maps of FUBP1 overproduction: insights into FUBP1 overproduction-induced genome instability and evolution.

B. Studies and Results

This project was funded Nov 2022, in the past eight months, we have made significant progress to advance the proposed research:

a. **FUBP1 constructs have been made.** We have created different N-terminal fusion constructs of FUBP1. In total, we have generated GFP fusions of the following variants of FUBP1: variant 1, variant 2, FUBP1var2 aa1-510, aa100-447, aa100-644, aa511-644, and aa1-99_448-644. These constructs are crucial for identifying the domains/activities responsible for FUBP1-induced DNA damage.

b. **FUBP1 overproduction induces DNA damage.** In the MRC5-SV40 cell line, a lung fibroblast cell line, overproduction of the central binding domain mutant still leads to similar

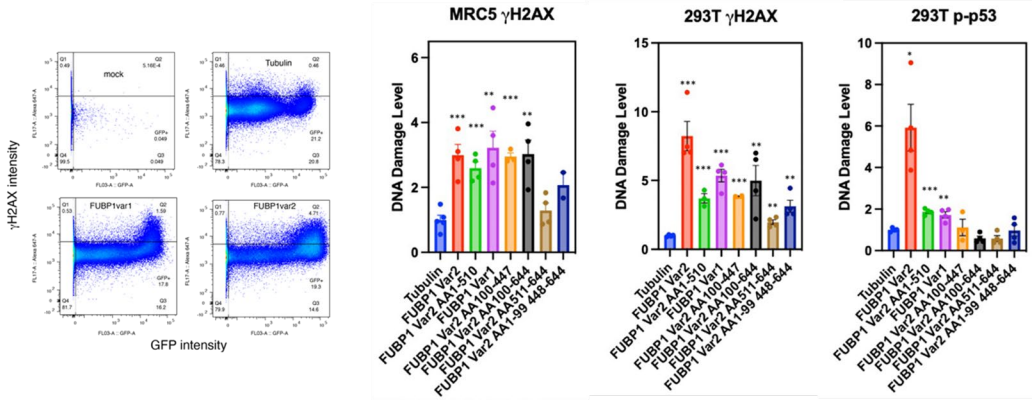


Figure 1. Both FUBP1 variants 1 and 2 promote DNA damage when overproduced. Left: representative flow cytometric assay. Right: quantification of DNA damage: gH2AX and phospho-p53 (Ser15) in various FUBP1 mutants. Central binding domains activities are sufficient for increased DNA damage.

levels of DNA damage compared to overproduction of wild-type FUBP1. However,

activation of the transactivating domain alone does not induce DNA damage. FUBP1deltaDBD induces a limited amount of DNA damage compared to the Tubulin-only control, but significantly less compared to wild-type FUBP1 variant 1 and variant 2. We have confirmed these DNA damage-promoting phenotypes in the 293T cell line. Most of the observed phenotypes align with the MRC5 model.

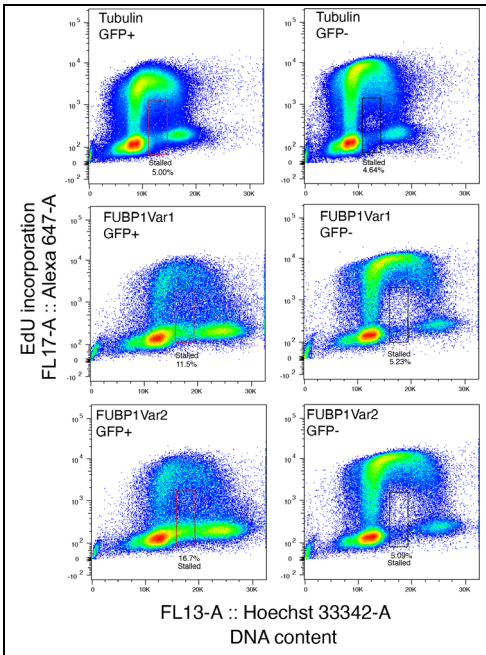


Figure 2: FUBP1 variant 1 and 2 overproductions inhibit DNA replication and G2/M cell cycle arrest.

c. **FUBP1var2 appears to induce higher levels of DNA damage compared to var1 overproduction alone**, indicating a potentially distinct mechanism of action for FUBP1var2. This will be further investigated through downstream experiments, including CUT&Tag, DSB mapping, and mutagenesis studies. We are currently working on additional validation of these findings in the lung adenocarcinoma A549 cell line.

d. **FUBP1 overproduction inhibits DNA replication.** We conducted DNA staining and EdU click chemistry assays to estimate the number of cells experiencing inhibited DNA replication (stalled replication forks) in 293T cells. Additional replicates are currently being performed. Specifically, the overexpression of FUBP1 var2 results in the highest level of stalled forks, surpassing the effect of FUBP1 var1 overexpression. Var1 exhibits a higher level of stalled forks compared to the Tubulin control overproduction. Interestingly, GFP-negative cells display similar profiles with elevated stalled forks, while only the GFP-positive (transfected) cells exhibit the expected phenotypes.

e. **We have performed CUT&Tag (cleavage under target and tagmentation) experiments in MRC5 cells to map FUBP1 var1, var2, binding sites.** As a control, we detected high levels of H3K4me3 peaks in the NUP214 promoter regions. This region consistently exhibits detection across different cell lines using various DNA-protein interaction approaches, including ChIP-Seq and CUT&RUN. The CUT&Tag method requires a minimal number of cells and sequencing. Currently, we have completed one replicate of CUT&Tag for FUBP1 var1, var2, Tubulin, and AQP3 (a transporter that is not expected to directly interact with DNA). Detailed peak calling is in progress, and we have identified variant-specific binding

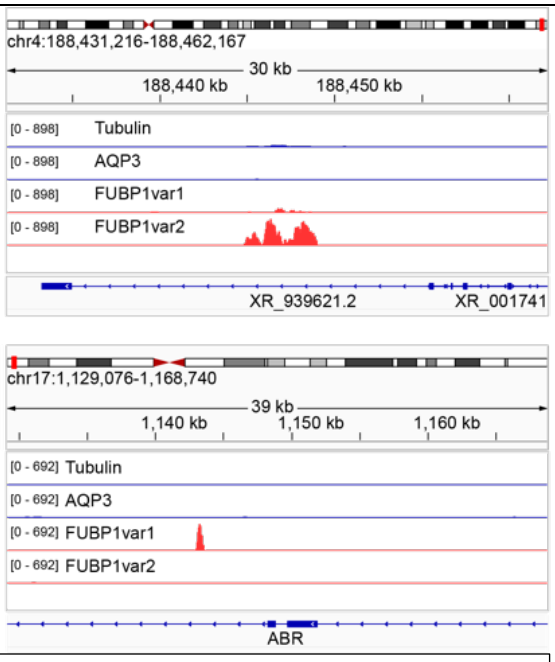


Figure 3: FUBP1 variant 1 and 2 overproduction binds to different genomic locations. Measured by CUT&Tag.

regions such as *XR_939621.2* for variant 2 and *ABR* intron for variant 1.

f. **Duplex-seq has been performed on neutral regions of FUBP1 overproduction.** A total of 48kb was sequenced, spanning 20 regions across different chromosomes, with each region being 2.4kb in size. These regions have undergone extensive validation to ensure they are neither positively nor negatively selected, and they do not include any known cancer driver genes. We observed a slight increase of 2.66 x 10^{-7}/informed base (FUBP1 overproduced) compared to 2.08 x 10^{-7}/informed base (mock), although a more significant difference is expected when we design capture panels targeting the FUBP1 binding regions. Additionally, a larger number of somatic mutations is required to accurately compute the mutational signatures.

g. **Another TF MYBL1 overproduction has unique signatures in MYBL1 binding regions using duplex-sequencing approach.** In collaboration with Susan Rosenberg's lab from Baylor College of Medicine, we have observed unique mutational signatures induced by MYBL1 overproduction. Capture panels were designed from 550kb MYBL1 binding sites (3,212 regions) using MYBL1 CUT&Tag data. MYBL1 overproduction results in a 2-fold increase in overall mutation frequencies within 72 hours post-transfection. More interestingly, MYBL1 overproduction promotes a single base substitution signature 39 (SBS39), dominated by C>G mutations. SBS39 has no known etiology, although it is reported to be enriched in BCL6 super-enhancer regions in B cells. Our findings suggest a potentially new etiology of TF-induced DNA damage and mutagenesis. We expect FUBP1 overproduction will have similar effects.

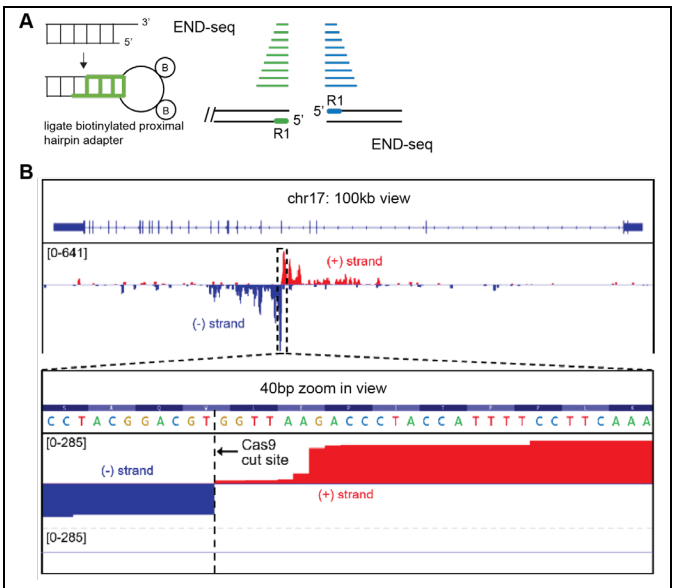


Figure 4. (A) END-Seq scheme. END-Seq is a sensitive approach to map double strand breaks. (B) END-Seq can detect Cas9-induced DSBs in single-base resolution.

h. **We have mastered the END-Seq approach (see iCas9 induced DSB induction).** We can detect Cas9-induced DSB in a single-base resolution. We have purchased and installed the Biorad S3e sorter to increase our sorting capacity by a factor of 10. We aim to perform END-Seq DSB analysis in the next fiscal year.

i. We have started to used SHERRY (a sensitive single-cell RNA-seq) to detect whether certain genes were supposed be dysregulated (for example, c-Myc) and identify potential alternative splicing events induced by FUBP1 var 1 and var 2 because the central binding domain can bind to DNA and also to RNA.

C. Significance

The significance of this project is as follows:

1. The findings in this project will expand the DNA damageome proteins, overproduction of which can cause high DNA damage. This project will assign functions of lung cancer

associated GWAS/TWAS loci and potentially offer a pipeline for other GWAS/TWAS nominated genes/loci.

2. This project will provide new mechanistic insights into how TFs promote genome instability independent of transcription dysregulation.

3. This study will generate single-base resolution maps for the TF mapping, DNA damage, and mutations induced by FUBP1 overproduction. This is critically to dissect the mechanisms on how TF binding can cause DNA damage and its distance/frequency of associated mutations.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

Ashour ME, Byrum AK, Meroni A, **Xia J**, Singh S, Galletto R, Rosenberg SM, Vindigni A, Mosammaparast N. Rapid profiling of DNA replication dynamics using mass spectrometry–based analysis of nascent DNA. ***Journal of Cell Biology***. 2023 Feb 16;222(4):e202207121.

Zhai Y, Pribis JP, Dooling SW, Garcia-Villada L, Minnick PJ, **Xia J**, Liu JJ, Mei Q, Fitzgerald DM, Herman C, Hastings, PJ, Costa-Mattoli M, Rosenberg SM. Drugging evolution of antibiotic resistance at a regulatory network hub. ***Science Advances***. 9, eadg0188 (2023)

III. List of extramural grants submitted from 7/1/2022–6/30/2023

NIH/NIEHS R01ES035884
The Arsenic-Aquaglyceroporin Interactome in Genome and Transcriptomic Evolution at Single-Cell Level
Role: PI (Xia)
Total Award Amount (including Indirect Costs): $2,134,500

NIH/NCI R21CA286359
Evaluation of uterine blood as a liquid biopsy for endometrial carcinoma early diagnosis with single-cell RNA-seq
Role: Co-I (Xia), PI (Fu)
Total Award Amount (including Indirect Costs): $404,250

State of Nebraska LB692
Molecular classification of endometrial serous carcinoma with error-corrected sequencing
Role: Co-I (Xia), PI (Fu)
Total Award Amount (including Indirect Costs): $75,000

Mary Kay Ash Foundation
Genomic characterization of endometrial serous carcinoma with ultra-sensitive and accurate duplex DNA sequencing
Role: Co-I (Xia), PI (Fu)
Total Award Amount: $99,999

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

NIH/NIEHS R00ES033259
The Role of Aquaporin 3 in Arsenic-induced DNA Damage and Mutagenesis

Role: PI (Xia)
Total Award Amount (including Indirect Costs): $731,466

Creighton University Cancer & Smoking Disease Research Program FY22/23 Progress Report (July 1, 2022 – June 30, 2023)

DEVELOPMENT PROGRAM
Juliane K. Strauss-Soukup, PhD

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

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. The 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 in comparison 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 remains 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 myofibroblast that produces 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. Our proposal thus focused 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 proposed to further utilize clinically relevant *ex vivo* and human lung fibroblasts (HLF) from IPF

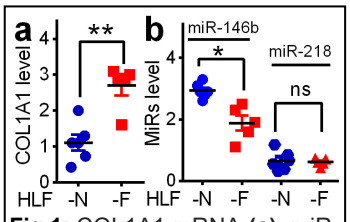


Fig.1: COL1A1 mRNA **(a)**, miR-146b and -218 **(b)** levels in HLF-N & HLF-F (*p<0.05; **p<0.01).

patients to define functions, mechanisms, and importance of miR-146b in IPF progression. The data we obtained will form the basis for the submission of a new R01 application.

1. Repression of miR-146b and more robust 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 age-matched 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 (**2b**).

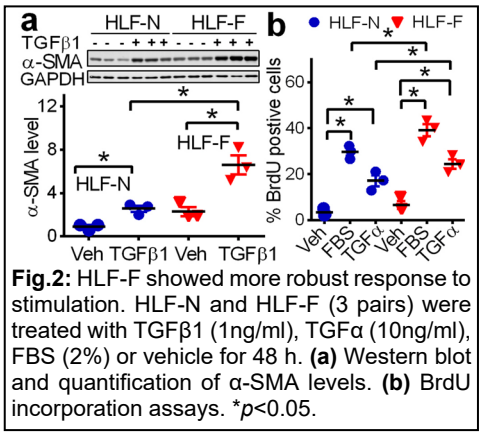


Fig.2: HLF-F showed more robust response to stimulation. HLF-N and HLF-F (3 pairs) were treated with TGFβ1 (1ng/ml), TGFα (10ng/ml), FBS (2%) or vehicle for 48 h. **(a)** Western blot and quantification of α-SMA levels. **(b)** BrdU incorporation assays. *p<0.05.

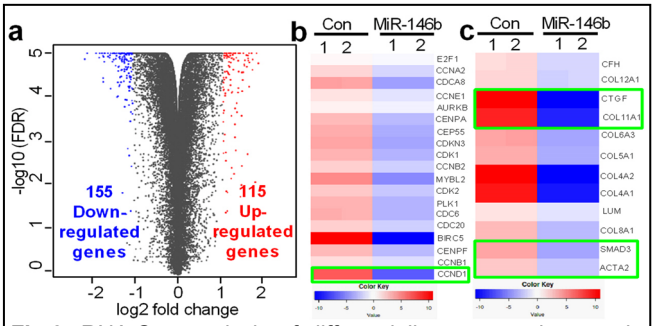


Fig.3: RNA-Seq analysis of differentially expressed genes in HLF-F transfected with miR-146b mimic or its control. **(a)** Volcano plot. The top downregulated genes involved in the cell cycle **(b)** and TGFβ pathways **(c)**.

2. miR-146b may inhibit pulmonary fibrosis via targeting proliferative and fibrogenic signaling in lung fibroblasts. RNA-Seq detected and quantified a total of 11127 mRNAs in HLF-F transfected with miR-146b mimic or its control. Using a *P*-value < 0.05 and fold change >2 as cutoffs, a total of 270 differentially expressed genes were identified in miR-146b-transfected cells (**Fig.3a**). KEGG pathway enrichment analysis revealed that miR-146b downregulates the cell cycle pathway (**3b**) and TGFβ signaling pathway (**3c**), suggesting that miR-146b inhibits pulmonary fibrosis via targeting proliferative and fibrogenic signaling in lung fibroblasts.

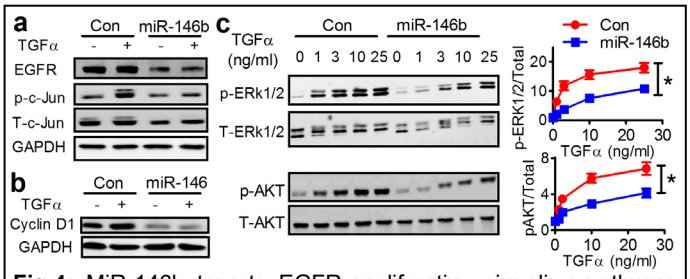


Fig.4: MiR-146b targets EGFR proliferative signaling pathway. HLF were transfected with miR-146b mimic or control. Serum-starved cells were stimulated without or with TGFα and subjected to western blot analysis. **(a)** 10 ng/ml TGFα for 10 min. **(b)** 10 ng/ml TGFα for 24h. **(c)** ERK1/2 and AKT phosphorylation.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

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. Interestingly, EGFR and c-Jun proteins were also reduced (**4a**), suggesting that miR-146b can inhibit HLF proliferation by targeting EGFR/c-Jun signaling.

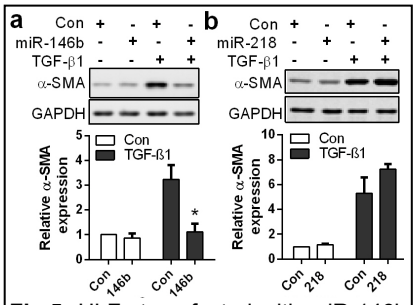


Fig.5: HLFs transfected with miR-146b **(a),** miR-218 **(b)** or control mimics (50nM) for 24h, and then stimulated with TGFβ1 (1ng/ml) for 48h. Western blot (top) and quantification (bottom) of α-SMA normalized by GAPDH. Data are means ± SEM (*p< 0.01, n=4).

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**).

4. miR-146b inhibited TGFβ1-induced HLF differentiation via targeting TGFβR1 and Smad3 protein expression. Western blot showed highly increased expression of α-SMA in TGFβ1-treated HLFs. Transfection with miR-146b, but not miR-218 mimic, abolished TGFβ1-induced α-SMA expression, indicating an attenuation of differentiation (**Fig.5**).

Phosphorylation of the transcription factor Smad3 by the TGFβR1/TGFβR2 complex is an essential step in TGFβ1 fibrogenic signaling. miR146b blocked TGFβ1-induced Smad3 but not Smad2 phosphorylation in HLF (**Fig.6**). Total Smad3 and TGFβR1 protein were also reduced, suggesting that miR-146b inhibits HLF differentiation via targeting TGFβR1/Smad3-dependent fibrogenic signaling.

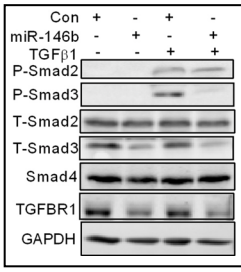


Fig.6: MiR-146b reduced TGFβR1 and Smad3 protein expression and suppressed TGFβ1 -induced Smad3 phosphorylation in HLF-N. WB images are representatives of 2 experiments.

5. EGFR, Jun, TGFBR1, and SMAD3 are putative miR-146b targets. Using two miRNA Target Prediction Tools (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 (**Fig. 7a**) and are conserved in humans and mice (**not shown**). Thus, the 3'-UTR containing the putative miR-146b site of *EGFR, Jun, TGFBR1*, and *Smad3* genes and their mutants with the seeding region deleted were each cloned into the pmirGLO dual-luciferase reporter vector (**Fig.7b**). HEK293 cells were co-transfected with control or miR-146b mimic (50nM) AND the reporter plasmids containing the miR-146b targeting site or its mutants. The effects of miR-146b on the luciferase activity were determined using a Dual-Glo® Luciferase Assay kit

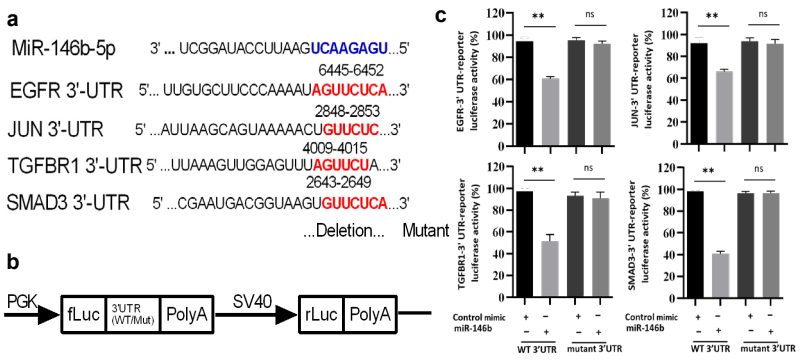


Fig.7: (a) The putative miR-146b binding site of the 3'-UTR of the EGFR, Jun, TGFBR1, and SMAD3 genes and their deletion mutants at the seeding region. **(b)** The pmirGLO plasmids containing the 3'-UTR WT or their mutants of the EGFR, Jun, TGFBR1, or SMAD3 genes. **(c)** WT and their mutant plasmids were co-transfected with control or miR-146b mimics into HEK293 cells for 24 h. The cells were harvested for Firefly luciferase (fLuc) activity assays with Renilla luciferase (rLuc) activity as the internal control. Data shown are means ± SEM from three separate experiments, *P < 0.05, **P < 0.01.

(Promega). As shown in **Fig. 7c**, miR-146b mimic inhibited luciferase activity by 42 ± 3%, which was abolished by deletion of the seed region.

6. Loss of miR-146b exacerbated fibrosis in mouse 3D-precision cut lung slices (3D-PCLS) *ex vivo* model of fibrosis. PCLS has emerged as a useful *ex vivo* translational model to study lung biology and disease pathogenesis. Alsafadi *et al*. recently established an *ex vivo* human 3D-PCLS model of early fibrosis induced by a combination of profibrotic mediators. Using a similar mouse 3D-PCLS model of fibrosis (**Fig.8a**), we found fibrosis-like characters, including increased *ACTA2* (**8b**) and *COLA1* mRNA expression (**8c**), increased collagen (**8d**), deposition, and alveolar septal thickening (**8e**) in mouse lung slices. Importantly, loss of miR-146b exacerbated these fibrotic pathohistological changes (**8b-e**), suggesting the importance of miR-146b repression in IPF patients.

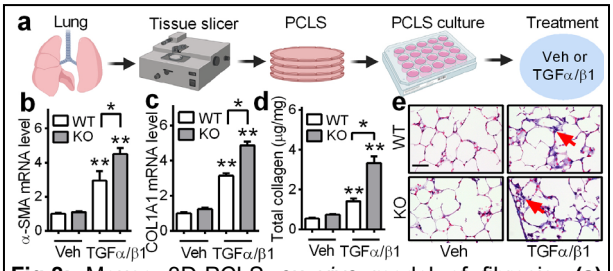


Fig.8: Mouse 3D-PCLS *ex vivo* model of fibrosis. **(a)** Experimental procedure. WT or miR-146b KO mouse PCLS (150 μm) were cultured in 0.1% FBS plus 10 ng/ml of TGFα and TGFβ1 or vehicle for 120h. Tissue lysate was subjected to analysis of *ACTA2* **(b)** and *COLA1* mRNA expression **(c)** and total collagen **(d)** measured by qRT-PCR and a Sirius red total collagen detection kit (Chondrex). **(e)** Sections (5μm) of fixed PCLS were stained with Masson's trichrome to visualize collagen deposition (blue). Scale bar: 100μm. Arrows: Alveolar areas showing septal thickening.

7. Krüppel-like factor 4 (KLF4) regulates miR-146b expression. A 338-bp DNA fragment encompassing the essential *miR-146b* gene promoter was cloned into the luciferase reporter pGL3 vector, designated as P(−275/+63). It has two consensus transcription factor Sp1 binding motifs and retained full promoter activity (**not shown**). However, the *Sp1 inhibitor* mithramycin A *(MMA)* had little effect on miR-146b levels in HLF-N (**Fig.9a**). KLF4, an Sp1-like transcription factor, also binds to the Sp1 sites to regulate gene transcription. Knockdown of KLF4 in HLF-N by its siRNAs (*Dharmacon*) reduced miR-146b levels by 60% (**9b**). KLF4 repression was found in lung tissues of human IPF and KLF4 overexpression inhibited bleomycin-induced PF in mice. We found less KLF4 protein in HLF-F than in HLF-N (**9c**), positively correlating with miR-146b expression, and treatment with TGFβ1 decreased KLF4 expression in HLF-N (**9d**).

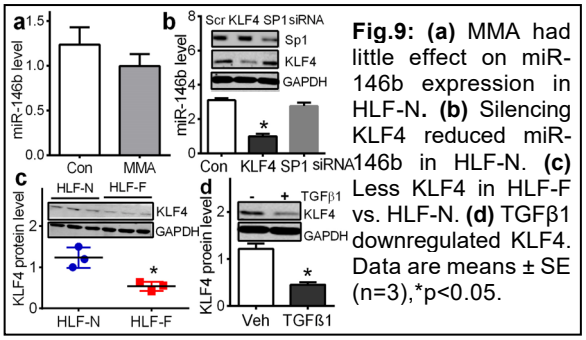


Fig.9: (a) MMA had little effect on miR-146b expression in HLF-N. **(b)** Silencing KLF4 reduced miR-146b in HLF-N. **(c)** Less KLF4 in HLF-F vs. HLF-N. **(d)** TGFβ1 downregulated KLF4. Data are means ± SE (n=3),*p<0.05.

8. Identification of small molecules N20 as a new anti-fibrotic drug. Small molecules are broadly pursued as potential drugs targeting fibrotic signaling pathways. Using a miR-146b promoter-driven luciferase reporter assay, we recently screened our Small Molecule Compound Library (**Fig.10a**) and identified the compound N20, but not its analog N19, which induces the miR-146b promoter activity (EC_{50} of 3 μM, Vmax =15 fold) (**10b**). Expression of miR-146b in mouse lung fibroblasts is at least 10-fold higher than in other mouse airway cells. Treatment of mouse ASM and epithelial cells with N20 caused less than 2-fold stimulation of miR-146b expression as compared to 8-fold in mouse lung fibroblasts (**10c**). Treatment with 10 μM N20 increased miR-146b expression in four HLF cells by 3-8-fold (**20d**). Importantly, treatment of HLF-F with 10 μM of N20, also inhibited TGFβ1-induced fibroblast differentiation (**10e**), which was largely prevented by anti-miR-146b. Thus, N20 could be an effective prototype drug for increasing fibroblast miR-146b to suppress pulmonary fibrosis progression.

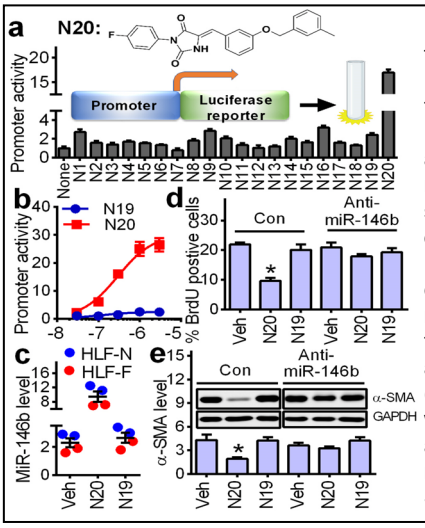


Fig.10: Identification of N20 as a new anti-fibrotic drug. HLF-N transfected with the P(-275/+63) luciferase plasmid were treated with drugs (10 μM) of our Small Molecule Compound Library **(a)**, N20 or its analog N19 (0-25 μM) **(b)** for 48h. A representative diagram of screening data sets was shown. **Inset:** Chemical structure of N20. **(c)** N20 (10 μM) increased miR-146b expression in HLF-N and HLF-F (n=2 each). HLF-F pre-transfected with anti-miR-146b (50 nM) or its control were treated with N20 or N19 (10μM) for 24h and then stimulated with TGFα (10 ng/ml) **(d)** or TGFβ1 (1ng/ml) **(e)** for 48h. Cells were subjected to BrdU cell proliferation assay or western blot analysis of α-SMA, normalized by GAPDH. Data are means ± SE (n=3, **p*<0.05).

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 will be a major advance by providing target-directed therapy against both excessive fibroblast proliferation and differentiation, which will have significant long-term clinical impact by changing treatment paradigms for lethal IPF. A critical strength of this project is that the original data 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.

II. List of publications and patent (7/1/2022 – 6/30/2023)

Hulen J, Kenny D, Black R, Hallgren J, Hammond KG, Bredahl EC, Wickramasekara RN, Abel PW, Stessman HAF. KMT5B is required for early motor development. Front Genet. 2022;13:901228. doi: 10.3389/fgene.2022.901228. eCollection 2022. PubMed PMID: 36035149; PubMed Central PMCID: PMC9411648.

Xie Y, Abel PW, Casale TB, Tu Y. (2022) TH17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149(2):467-479. PMCID: PMC8821175.

III. List of extramural grants submitted from 7/1/2022 – 6/30/2023

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

R01 HL164593-01A1
Title: A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-Resistant (Refractory) Asthma
Dates: 4/2023 - 3/2028
Tu (PI): Role: Co-Investigator
Total funds requested: $ 2,092,677
Impact Score: 29; Percentile: 15

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
R01 HL164593-01
Title: Targeting Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Refractory Asthma
Dates: 12/2023 - 11/2028
Tu (PI): Role: Co-Investigator
Total funds requested: $2,236,132
Impact Score: 29; Percentile: 13 (Current payline: 14)

IV. List of extramural grants awarded from 7/1/2022 – 6/30/2023

None

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

DEVELOPMENT PROGRAM
Juliane K. 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

The **central hypothesis** of this proposal is that, using sensitive and accurate high-throughput single-cell methods, we will identify endometrial cancer (EC)-related molecular signatures for early differential diagnosis of EC from pre-cancer and benign conditions.

We tested our central hypothesis by pursuing the following two 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

Endometrial cancer is the uncontrollable growth of the endometrium, which is the lining of the uterus. The incidence rate of EC has increased during the past two decades, becoming the third most common cancer in U.S. women. EC is frequently preceded by a pre-invasive precursor lesion. When EC is confined within the uterus upon diagnosis, patients experience a 5-year relative survival rate of 96%. In contrast, the survival rate drops to a mere 20% when cancer has metastasized to distant parts of the body, underscoring the critical significance of early detection and diagnosis. Current diagnostic procedures for EC include endometrial biopsy by Pipelle and directed dilation and curettage (D&C). Both involve limited tissue sampling of the uterus cavity, resulting in a high false-negative EC diagnostic rate and leading to overtreatment with total hysterectomy for patients with premalignant lesions. According to retrospective studies with hysterectomy specimens, 40% of patients diagnosed with EIN have concurrent carcinomas. This is due to sampling limitations: approximately 60% of D&C specimens sampled less than one-half of the uterine cavity, and the small volume of tissue obtained limits the ability to accurately assess the cancer risk. On the other hand, overtreatment by way of a hysterectomy results in early physiological and psychological postmenopausal changes and is not suitable for patients who wish to preserve fertility. In addition, failing to exclude a coexisting EC at an early stage will delay proper treatment. Thus, this proposal aims to identify EC-related molecular signatures with sensitive high-throughput single-cell methods for early differential diagnosis of endometrial cancer from pre-cancer and benign conditions, which will potentially provide diagnostic biomarker(s) for coexisting carcinomas and reveal EC etiology, enabling personalized treatment for better patient care.

Sample collection and process. During the past two years, in collaboration with Dr. Lesley B.

Conrad, a gynecologic oncologist at CHI Health Creighton University Medical Center - Bergan Mercy, we collected the uterine blood fresh (uterine blood is the blood in the uterus and is currently collected through the aspiration of blood in the vaginal canal or within the uterus using an endometrial Pipelle at the time of hysterectomy) from three groups of individuals who (1) are cancer-free (N=2), (2) have precursor lesions (pre-cancer, N=3), or (3) have EC at International Federation of Gynecology and Obstetrics (FIGO) stage 1A (N=2). Stage 1A EC has grown less than halfway through the underlying muscle layer of the uterus, and is an early stage of EC.

We performed **high-throughput single-cell RNAseq** to profile the cell types and transcriptomes of the single cells within the uterine blood. To keep the original cell population distribution, we only removed the red blood cells and directly performed high-throughput single-cell RNA-seq with Chromium Single cell 3' reagent kits within one hour after the uterine blood was extracted to ensure high-quality RNA. The mRNAs from each cell were separated into water-in-oil droplets and tagged with unique cell barcodes that were identifiable through downstream sequencing data analysis. After filtering out the low-quality cells with less than 300 transcripts, we obtained the transcriptome of at least 4,000 cells from each group, with a total of 16,064 single cells from seven individuals (Table 1).

Table 1. Number of single cells profiled for each individual and group.

Progression to EC	(1) Cancer-free		(2) Pre-cancer			(3) EC	
Individual ID	Ind 1	Ind 2	Ind 3	Ind 4	Ind 5	Ind 6	Ind 7
Profiled cell number	863	5,126	1,245	983	2,220	2,804	2,823
Total cell number	5,989		4,448			5,627	

Reconstruct cell populations in the endometrium with uterine blood. We integrated our data with the published single-cell dataset (data from Wang et al. Nature Medicine, 2020) from

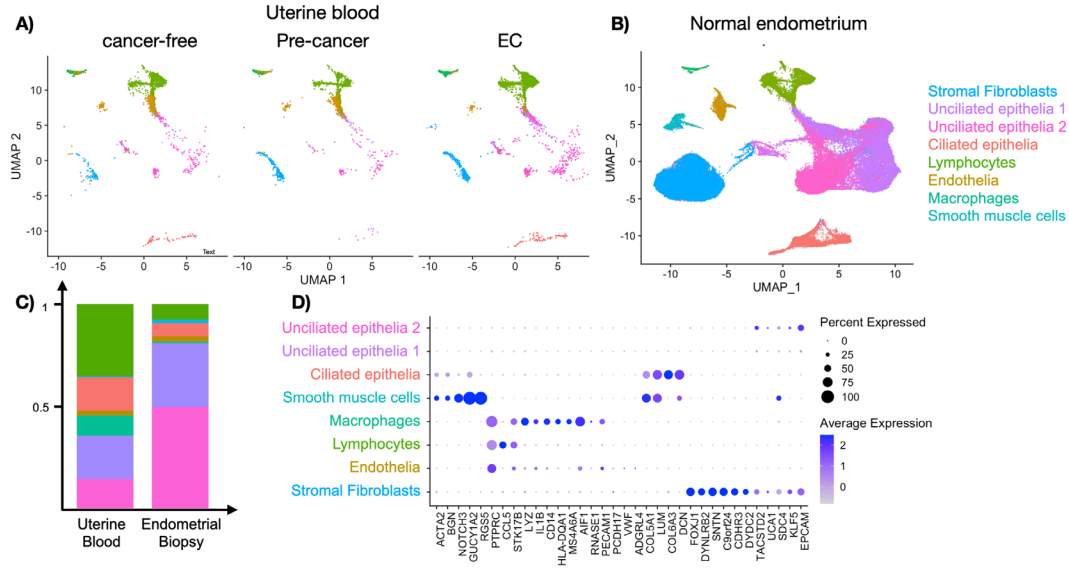


Figure 1. A) Major cell types identified from uterine blood of different groups of patients and B) from normal endometrium. C) Cellular composition comparison between uterine blood and endometrial biopsy. D) The expression pattern of known cell-type-specific markers; a darker dot represents a higher expression level; a larger dot represents higher ratio of cells with expression. Cells are color-coded the same way in all panels.

endometrium biopsies as a reference. We mapped the cells from uterine blood to cells in the endometrium based on whole transcriptome similarities on 2-dimensional Uniform Manifold

Approximation and Projection (UMAP) to reveal cell type information. We verified the cell types independently with the reference transcriptomic datasets of the Human Cell Atlas. The results show we can recapitulate the main cell types in normal endometrium from the uterine blood for all three groups (Figure 1A, B), including epithelial cells, endothelial cells, stromal fibroblasts, and immune cells, such as macrophages and lymphocytes. We found a higher ratio of immune cells from uterine blood than in endometrial biopsy (Figure 1C). These results indicate that uterine blood is a good representation of the endometrium microenvironment and can profile local cell types residing within the endometrium. The expression patterns of known markers for different cell types in our dataset match with existing knowledge (Figure 1D), validating our cell type identification process and the feasibility of using single-cell RNAseq with uterine blood as a sensitive method to identify EC-related molecular signatures. The signatures we focus on in this study are immune cell composition and expression changes, as well as genomic mutations accumulated along EC development.

The monocyte cellular compositional changes during EC development. Based on the expression of CD14 and CD16, monocytes can be classified into three subpopulations: classical monocytes ($CD14^{++}CD16^{-}$), nonclassical monocytes ($CD14^{+}CD16^{++}$), and intermediate monocytes ($CD14^{++}CD16^{+}$). We can detect all three subsets in cancer-free individuals' uterine blood (Figure 2A). The relative ratio of the three monocyte subsets changes with development of EC (Figure 2B, C); the ratio is consistent among the individuals of the same group. For the pre-cancer group, the abundance of nonclassical monocytes increased (Figure 2B), which has been reported in peripheral blood from breast cancer patients and has been proposed as an early diagnostic biomarker. At stage 1A, nonclassical monocytes are almost completely depleted (figure 2B), and monocytes are dominated by the intermediate subset (Figure 2C). The intermediate monocytes express high levels of receptors for immunosuppressive molecules expressed on

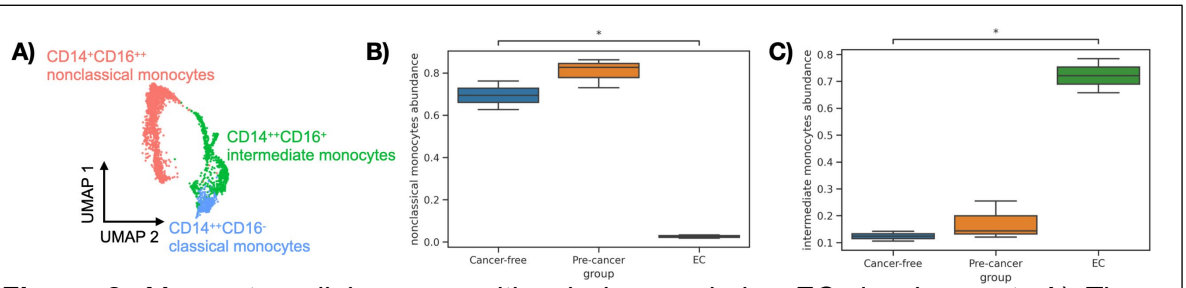


Figure 2. Monocyte cellular compositional change during EC development. A) Three subsets of monocytes in all patients. The changes in the relative abundance of B) nonclassical monocytes and C) intermediate monocytes for three groups of patients along progression to EC. *P < 0.05 (t-test).

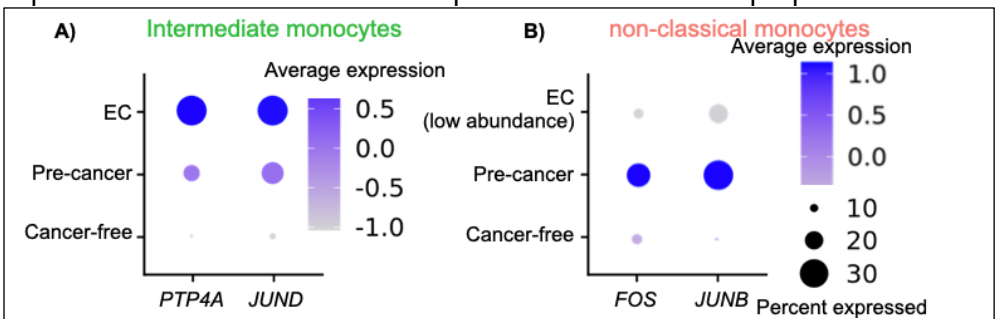


Figure 3. Gene expression levels in A) classical monocytes and B) non-classical monocytes in three groups of patients along progression to EC. The number of non-classical monocytes in the EC group is too low for calculation. A darker dot represents a higher expression level; a larger dot represents a higher ratio of cells with expression.

malignant cells, facilitating the malignant cells to evade immune surveillance. The enrichment of immunosuppressive intermediate monocytes in the EC group also exists in other types of cancer. Nonclassical monocytes are characterized by the capacity to produce proinflammatory cytokine tumor necrosis factor (TNF)-α and are the main effector of the inflammatory response during cancer development, matched with their reduced abundance in the EC group. Thus, the monocyte compositional changes indicate EC development.

Transcriptomic changes in monocyte subsets during progression to EC. We found the intermediate monocytes in the EC group were altered compared to cancer-free patients, with an increased expression of tumorigenesis-related *PTP4A2* and cancer proto-oncogene *JUND* (Figure 3A). When comparing the non-classical monocytes between the pre-cancer group and the cancer-free group, we identified higher levels of the proto-oncogenes *FOS*, *JUNB* (Figure 3B). Both genes are involved in the development of multiple types of cancer, indicating early onset of malignancy in the microenvironment immune cells.

Genomic copy number changes during EC progression. We obtained genomic DNA from a total of 10^6 cells derived from cancer tissue. To identify any copy number alterations throughout the genome, we prepared a DNA sequencing library from the genomic DNA and performed low-depth sequencing. The entire genome was divided into 50k-base pair regions, and we employed the circular binary segmentation (CBS) algorithm to analyze the sequencing depth data within these segments. This approach enabled us to identify genomic regions exhibiting abnormal copy numbers. However, our analysis did not reveal any significant copy number changes in the individual with EC. In Figure 4, we present a representative copy number distribution along chromosome 14. Notably, this visualization demonstrates no significant copy number alterations across chromosome 14. The copy number remains consistent at 2, indicating the presence of mostly normal diploid cells in the tissue.

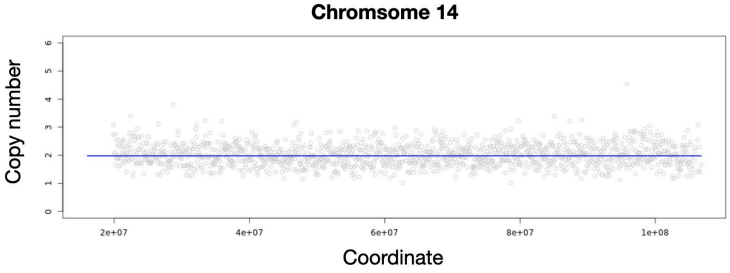


Figure 4. No copy number variation is detected on Chromosome 14 of one EC patient. Gray dots are sequencing depth in each genomic region on chr14; blue line is inferred copy number from sequencing depth.

Figure 4 is a representative copy number distribution along chromosome 14, which shows no significant copy number change along the whole chromosome, with a normal copy number of 2 for the normal diploid cells.

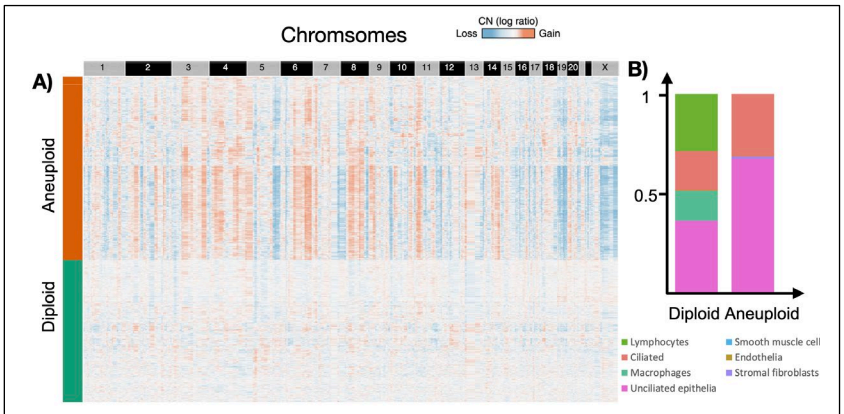


Figure 5. Copy number changes inferred from scRNA-seq datal A) The whole genome copy number alternations distribution of normal diploid cell and aneuploid cells. B) Cell type distribution for diploid cell and aneuploid cells.

We then applied an integrative Bayesian segmentation approach called copy number karyotyping of aneuploid tumors (CopyKAT) to estimate genomic copy number profiles at an average genomic resolution of 5 Mb from read depth from the high-throughput single-cell RNAseq data from the same individual. We successfully identified clonal subpopulations displaying normal copy numbers, as well as clusters of cells exhibiting distinct copy number changes throughout the genome (Figure 5A). By analyzing the expression profile, we were able to determine the cell type of the aneuploid cells. The majority of these cells were epithelial cells, including both ciliated and unciliated cells. On the other hand, most immune cells maintained normal diploid karyotypes. This method proved advantageous as it allowed us to uncover changes that may have been obscured in the bulk sequencing method.

C. Significance

Overall, our findings demonstrate changes in monocyte cellular composition along EC development, as well as an early onset of malignancy in the microenvironment immune cells with a changed transcriptome toward supporting the overgrowth of the cancer cells. We also identified the existence of clonal subpopulations with varying copy numbers, predominantly composed of epithelial cells, while immune cells largely maintain normal diploid karyotypes. The utilization of single-cell sequencing in uterine blood samples provided us with increased sensitivity in detecting changes and enabled the identification of alterations in cells for early differential diagnosis of endometrial cancer from pre-cancer and benign conditions.

Endometrial suction curette and D&C are the current diagnostic procedures for EC; both techniques are invasive and have high false-positive detection rates for EC. Because it is easy to access, uterine blood can act as a liquid biopsy of the endometrium. With uterine blood, we can detect endometrium cells and monitor EC development in a potentially non-invasive manner, making it feasible to be used in regular screening tests for EC and provide higher sensitivity and accuracy to identify true malignancy at an early stage. With high-throughput single-cell sequencing, we can simultaneously profile the compositional, transcriptomic, and genomic changes in the endometrium and microenvironment of the uterus. When profiling immune cells in uterine blood, we find both compositional and transcriptomic changes among monocyte subsets during progression to EC, indicating those cells are altered to reduce local immune response and facilitate cancer cell transformation. Single-cell methods are more accurate than bulk sequencing as cross-validations between single cells are possible. When cells are profiled at the single-cell level, cell heterogeneity is resolved. The large portion of normal cells in the samples will not affect the molecular signatures of rare cancerous cells. The progression-correlated changes can be markers for cancer risk screening and potential drug targets.

This study filled the gap and provided a potential non-invasive, sensitive, and molecular way to assess cancer risk with single-cell technologies and uterine blood, helping to overcome the inadequate sampling and analysis issues plaguing other methods. Compared to sequencing on bulk samples, single-cell analysis requires fewer sequencing reads, so mutations can be identified with lower false-positive and false-negative rates. The molecular signatures identified from this proposal can also be used as a reference for cancer classification, as it will have a direct effect on treatment decisions for patients and provide opportunities for genome-guided clinical trials and drug development. Aside from endometrial cancer, the methods and analysis pipeline used in this study 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 7/1/2022–

6/30/2023

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 Z. Z. He and Jian Zuo

Submitted to *Nature Aging.* Available on bioRxiv, Aging Atlas Reveals Cell-Type-Specific Effects of Pro-longevity Strategies, Authors: Shihong Max Gao, Yanyan Qi, Qinghao Zhang, Aaron S. Mohammed, Yi-Tang Lee, Youchen Guan, Hongjie Li*, Yusi Fu*, Meng C. Wang*

III. List of extramural grants submitted from 7/1/2022–6/30/2023

LB692 New Initiative
Title: Molecular classification of endometrial serous carcinoma with error-corrected sequencing
PI: Yusi Fu

National Institutes of Health: NCI R21 (1R21CA286359-01)
Title: Evaluation of uterine blood as a liquid biopsy for endometrial carcinoma early diagnosis with single-cell RNA-seq
PI: Yusi Fu

Mary Kay Ash Foundation
Title: Genomic characterization of endometrial serous carcinoma with ultra-sensitive and accurate duplex DNA sequencing
PI: Yusi Fu

COBRE NIGMS Pilot Project
Title: WGS to identify candidate genetic variants associated with susceptibility to AIHL
COBRE PI: Peter Steyger; Pilot project PI: Yusi Fu

Kicks for a Cure Cancer Research Program
Title: Genomic characterization of endometrial serous carcinoma with ultra-sensitive and accurate duplex DNA sequencing.
PI: Yusi Fu

Creighton University – Dr. George F. Haddix President's Faculty Research Fund
Title: Creighton Women’s Health Initiative
PI: Lesley Conrad
Co-I: Yusi Fu

National Institutes of Health: NIEHS R01 (1R01ES035884-01)
Title: The arsenic-aquaglyceroporin interactome in genome and transcriptomic evolution at single-cell level
PI: Jun Xia
Co-I: Yusi Fu

National Institutes of Health: NIAID R01 (1R01AI179878-01)
Title: RACK1 in B cell development and V(D)J recombination
PI: Patrick Swanson
Co-I: Yusi Fu

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

Nebraska Stem Cell Research Project – LB606
Title: ALDH1A1^{+} cancer stem cells abundance in uterine blood as a potential diagnostic marker for endometrial cancer
PI: Yusi Fu

Creighton University – Dr. Dr. George F. Haddix President's Faculty Research Fund
Title: Creighton Women’s Health Initiative
PI: Lesley Conrad
Co-I: Yusi Fu

Creighton University Cancer & Smoking Disease Research Program
FY20/21 Progress Report
(July 1, 2022 – June 30, 2023)

DEVELOPMENT PROGRAM
Juliane K. 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 pursuing 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 presentation 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 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.

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. As such, our current study does serve as a pilot study upon which a larger multicenter trial can be built.

Table 1 Baseline demographic characteristics

	3D Picture (*n*=16)	3D Model (*n*=17)	*p*
Age	26.8 ± 5.4	28.5 ± 5.8	.370
Race			
White	69	65	
Black	25	35	
Native	6	0	
Marital Status			
Single	94	81	
Married	6	19	
Gestational Age	28.6 ±1.6	28.9 ±1.2	.650
Primigravida	25	25	
Multigravida	75	75	
Nulliparous	38	44	
Multiparous	62	56	
Education			
Some Highschool	25	18	
Grad Highschool	56	59	
College	10	22	
Insurance			
Medicaid	100	94	
Commercial	0	6	

Note: Data presented as mean ± SD, or percent

Table 2. Attachment stratified by intervention

	3D Picture-Pre (*n* =14)	3D Model-Pre (*n* =17)	3D Picture-Post (*n* =14)	3D Model-Post (*n* =17)	*p*-value
MAAS					
Global					
Total	83± 8.6	81.5 ±8.0	86.4 ±7.3	83.2 ±5.5	
3D picture pre vs 3D model pre					0.627
3D picture post vs 3D model post					0.177
3D picture pre vs 3D picture post					0.134
3D model pre vs 3D model post					0.190
Combined pre vs post					**0.043**

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* =15)	3D Model (*n* =17)	3D Picture (*n* =15)	3D Model (*n* =17)
TLFB				
Cig/day				
Total	7.5 ± 4.2	7.9 ± 7.5	5.9 ± 4.2	6.2 ± 6.4

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

	3D Picture (*n* =15)	3D Model (*n* = 16)	*p*
Weight of Baby (g)	3147±240	3109±257	0.775
EGA at Delivery	39.2±0.8	38.4±1.4	0.065
Weight %	38±16	34±29	0.732
Hypertension	38	31	0.893

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

C. Significance

There are multiple significant observations.

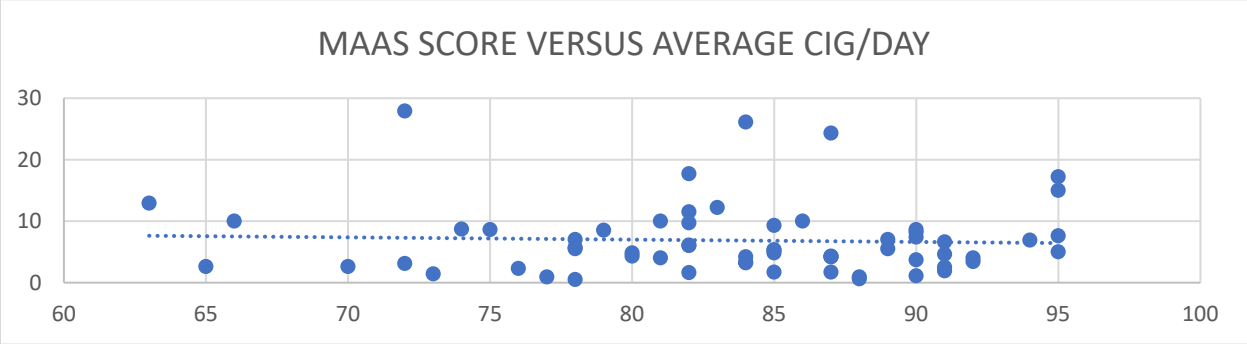
Attachment scores

First, the MAAS global scores pre intervention are not statistically different when comparing the two groups. Second, the MAAS global scores post intervention are not statistically different when comparing the two groups. Third, **the MAAS global score pre versus post intervention combining both groups is statistically significantly different**. 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. Second, the cigarettes smoked per day post-intervention are not statistically different when comparing the two groups. Third, the **cigarettes smoked per day pre- versus post-intervention in the 3D-print group was statistically significantly different**. Fourth, the **cigarettes smoked per day pre- versus post-intervention in the 3D model group was statistically significantly different**. **Fifth, the cigarettes smoked per day pre- versus post-intervention in the combined groups was statistically significantly different.**

Attachment versus cigarettes smoked per day



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

In both interventions, 35% of patients 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**. 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). 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 preterm delivery, small for gestational age, 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 6% for both interventions and the low birth weight was 6% for both interventions. Studies have shown the NICU admission rate to be between 10-15%; with our current study population, the NICU admission rate was 6%. Studies have suggested that the 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 suggest **lower rates of all three of these outcomes, which suggests lower smoking in both interventions.**

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

Coté, J.J., Côté-Arsenault, D., Handelzalts, J., Badura-Brack, A., Kalata, M., Walters, R.W., Kasinath, P., Herbig, K., Kump, D.A., Tampi, R. (2023). The effects of 3D printed models and 3D printed pictures on maternal and paternal-fetal attachment, anxiety, and depression. Journal of Obstetric, Gynecologic & Neonatal Nursing https://doi.org/10.1016/j.jogn.2023.02.002.

Coté, J. J., Coté, B. P., & Badura-Brack, A. S. (2022). 3D printed models in pregnancy and its utility in improving psychological constructs: a case series. 3D Printing in Medicine, *8*(1), 1-6. doi 10.1186/s41205-022-00144-w

III. List of extramural grants submitted from 7/1/2022–6/30/2023

March of Dimes
Cote (PI)
March 2023-February 2025
3D Printed Models and Psychological Constructs in Pregnancies With and Without Facial Clefts
Total Requested Costs: $94,033

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

Great Plains IDeA-CTR Team Research Pilot Grant/State of Nebraska – LB692
Cote (PI)
July 2022-June 2023
Human Placental Lactogen (Human Chorionic Somatomammotropin) and Oxytocin during Pregnancy: Individual Patterns and Correlations with Maternal-Fetal Attachment, Anxiety, and Depression
Total Award: $50,000

Creighton University Cancer & Smoking Disease Research Program FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

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.

No applications were received for the original call for proposals. Dr. Strauss-Soukup provided the applicants of the most recent State of Nebraska LB506 Cancer and Smoking Disease Research Program an opportunity to revise their applications based on reviewer comments and submit to the LB595 Development Program. Three applications were received. Three members of the External Advisory and one *ad hoc* member reviewer agreed that two of the three applicants adequately addressed the comments from the LB506 reviewers. These applications were funded:

PI: Janee Gelineau-van Waes, DVM, PhD, Department of Pharmacology and Neuroscience
Title: Bisphenol AF Exposure and Risk for Endometriosis-Associated Ovarian Cancer

PI: Brian North, PhD, Biomedical Sciences
Title: Identifying Regulators of Liver Cancer Metastasis

Creighton University
LB595 Development Program
Application Guidelines
Application Deadline: 4:30 p.m., Monday, May 8, 2023

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) 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, Monday, May 8, 2023. 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 single-spaced 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 person-months or salary for the participating faculty on the budget form.

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)
- U.S. visa fees
- Indirect costs

BUDGET JUSTIFICATION: ***Describe the specific functions and person-months for all participating personnel including faculty positions that are not allowed salary.*** 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, 2023.

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 spa@creighton.edu.

June 2023 Cancer & Smoking Disease Research Program Development Applications
June 2023

PI	PI School	PI Department	Project Title	Total Requested
Janee Gelineau-van Waes	School of Medicine	Pharmacology & Neuroscience	Bisphenol AF Exposure and Risk for Endometriosis-Associated Ovarian Cancer	65,000
Brian North	School of Medicine	Biomedical Sciences	Identifying Regulators of Liver Cancer Metastasis	65,000
Kalyana C Nandipati	School of Medicine	Surgery	Pathophysiology of Esophageal Adenocarcinoma	65,000

Complete

Investigator and Proposal Information

Principal Investigator/Project Director/Fellowship Sponsor:
Gelineau-van Waes, Janee

Email JaneeGelineau-vanWaes@creighton.edu
Phone 402-280-3457
Department Pharmacology & Neuroscience - Omaha

Personnel:

PI	Name	Department	Role	Net Effort
✔	Gelineau-van Waes, Janee	Pharmacology & Neuroscience - Omaha	PD/PI	0.000
	Howe, Christina	Research Compliance Office	Co-Investigator	0.000
	Abdulrahim, Ahmed	Resident & Fellow - Omaha	Co-Investigator	0.000
	Cote, John J	Obstetrics & Gynecology - Omaha	Consultant	0.000

Originating Sponsor: State of Nebraska - LB595

Sponsor: State of Nebraska - LB595

Budget:

	Period 1	Total
Direct Costs	$65,000	**$65,000**
Indirect Costs	$0	**$0**
F&A Rate	0%	-
Total	**$65,000**	**$65,000**

Project Total Cost Sharing Direct Costs: 0

Project Total Cost Sharing F&A Costs: 0

Start Date: 01-Jul-2023 End Date: 30-Jun-2024

Identification

Proposal Title
Bisphenol AF Exposure and Risk for Endometriosis-Associated Ovarian Cancer

Brief description of project in plain language (1000 character limit).
This project will use a novel translational mouse model of peritoneal and ovarian endometriosis to study the role of dietary exposure to the endocrine-disrupting chemical bisphenol AF on activation of the G protein-coupled estrogen receptor (GPER) signaling pathway and risk for progression to endometriosis-associated ovarian cancer.

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:
Approved

☑ Yes ☐ No Protocol is listed on Protocol Approvals page?

☐ Yes ☑ No Recombinant DNA or other biological agents?

☐ 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 Was this a review external to Creighton University?

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

If yes, select all that apply:

- ☐ CU Statistical Core Facility
- ☑ CU Histology Core Facility
- ☐ CU Integrated Biomedical Imaging Facility
- ☑ Other Non-CU Core Facility
- ☐ SOM COBRE Cell & Tissue Culture Core Facility
- ☐ SOM COBRE Auditory & Vestibular Electrophysiology Core Facility
- ☐ CU Flow Cytometry Core Facility
- ☐ CU Innovative Genomics Core Facility
- ☐ CU Molecular Biology Research Core Facility
- ☐ SOM COBRE Advanced Microscopy Core Facility
- ☐ SOM COBRE Mass Spectrometry Core Facility
- ☐ SOM COBRE Drug Discovery & Delivery Core Facility

If Other Non-CU Core Facility, please add the institution name(s) and the name(s) of the core facility:
Institution names(s):
UNMC

Core facility name(s):
Tissue Science Facility

Export Control

☐ Yes ☑ No Will any project participant travel to embargoed foreign countries?

☐ Yes ☑ 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 ☑ 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
- ☑ Science (Biomedical)
- ☑ Translational
- ☑ Cancer
- ☐ Education
- ☐ Humanities
- ☐ Neuroscience
- ☐ Science (Non-Health)
- ☐ Undergraduate Research
- ☐ Community Health
- ☐ Faith-Based
- ☐ Interdisciplinary
- ☐ Other
- ☐ Sustainability

Response to Reviewer Comments:

Comment: *Much of the data citing the risks of BPA exposure are somewhat old and there have been significant attempts to eliminate BPA exposure from the environment. Notwithstanding the 2021 report, BPA has been very widely studied and current BPA exposure may be decreasing and so a little overstated. However, this is offset by the greater focus here on BPAF, for which there appears to be much less information on its ability to affect the risk of some oral contraceptives.*

Response: Data citing the risks of BPA exposure on endometriosis and endometrial/ovarian cancers are presented for context because the adverse consequences of BPA exposure on female reproductive health have been widely studied. The use of BPA is decreasing, but it has been replaced by BPAF, a newer, fluorinated derivative (marketed as a 'BPA-free' substitute) that may be an even more potent endocrine disruptor. BPAF demonstrates a 9-fold stronger binding affinity and more potent activation of the G protein-coupled estrogen receptor (GPER) than the BPA parent compound. GPER activation has been implicated in both endometrial and ovarian cancers. In intact mice, the volume of *peritoneal* endometriotic (PE) lesions is significantly greater in response to BPAF exposure than to either BPA or vehicle (Jones, 2018). However, *nothing is known* about BPAF exposure and size or number of *ovarian* endometriotic (OE) lesions, or the role of BPAF activation of GPER in progression of OE to endometriosis-associated ovarian carcinoma (EAOC). Environmental risk factors and underlying mechanisms that contribute to EAOC are understudied, largely due to the paucity of appropriate animal models. The proposed work aims to fill these *critical knowledge gaps* by using a unique, translationally relevant mouse model of endometriosis (PE + OE) and *Gper* wildtype and knockout mice to study the role of BPAF activation of GPER in endometrial lesions and peritoneal macrophages and risk for EAOC. Oral contraceptives are not mentioned in the proposal, so it is unclear what the reviewers are referring to in the comments. Perhaps the acronym OC (ovarian carcinoma) was inadvertently transcribed as oral contraceptives?

Comment: *It is not clear if there will be five or six mice per group, nor what are the precise groups; the reviewers assumed four concentrations of BPAF in diet with control and control diet. However, a BPA positive control is lacking for comparison with the extensive body of literature.*

Response: In the Experimental Approach, it indicates that an n=5 mice/experimental diet will be examined at each time point. In the Expected Results for Aim 2, the sentence that reads: "Jones used an n=6 mice/group" (referencing their study on BPAF exposure and endometriosis) has been removed to avoid any confusion. In the section titled BPAF Exposure, it indicates that mice will be placed on "control diet or diet in which BPAF has been homogeneously incorporated into the feed at 30, 300 or 900 ppm." In other words, there are 4 experimental diets – one control diet (no BPAF) and 3 diets with variable concentrations of BPAF that span the range of human exposures for BPA because no corresponding data for BPAF are available. A BPA group was not included because a literature search of 'BPA and endometriosis-associated ovarian carcinoma" (EAOC) yields no results; despite the extensive body of literature on the relationship between BPA and endometriosis and endometrial/ovarian cancers, no studies have examined the role of BPA in progression of OE to EAOC. BPA is much less potent than BPAF at activating GPER, and therefore not expected to provide a robust *positive* control to test our hypothesis. Nevertheless, we recognize the importance of comparing BPAF to BPA in our paradigm. The budget and one-year timeframe for completion of the proposed work precludes doubling the size of the study to incorporate parallel dose-response studies for BPA; however, such studies will be included in a future proposal.

Comment: *The proposed work is largely descriptive in nature. The lack of clarity on how the results would be used to support additional funding, and a simple outline of what an application might seek to discover, is limiting.*

Response: Generating data that is descriptive in nature is a necessary first step, because nothing is known about the role of BPAF exposure on ovarian endometriosis (OE) or progression of OE to EAOC. The studies in Aim 1 will provide data on dose-response, time course for progression of OE to EAOC, and histopathological indices, as well as insight into underlying mechanisms (ie. oxidative stress, macrophage polarization). The studies in Aim 2 use reciprocal transplantation of *Gper* wildtype and knockout tissue in donor vs. recipient mice to investigate the underlying role of BPAF activation of GPER in (donor) endometrial vs. (recipient) peritoneal macrophages in disease progression. These results will provide further insight into potential mechanisms, and evidence-based information regarding GPER as a potential therapeutic target. We are currently conducting pharmacokinetic analyses with the available GPER agonist G1 and antagonist G36 in our mouse model to optimize dosing for promotion and inhibition of PE, OE and EAOC disease endpoints. We are also working with a medicinal chemist to develop additional GPER antagonists, including novel formulations that enable cell-type specific delivery. These endeavors, coupled with the data generated in this proposal will be used to apply for NIH funding to extend the BPAF studies, compare with BPA, and similarly investigate GPER antagonists as treatment options for OE and progression to EAOC. Verbiage to this effect has been added to this proposal.

*recent publication added to Gelineau-van Waes biosketch

Abstract: List the application's specific aims and ***clearly state the project's relevancy to cancer or smoking disease***. Describe the research design and methods for achieving the aims. The abstract serves as a description of the proposed work when separated from the application. As much as possible, use non-technical language to convey intent.
Do not exceed the space provided.

Endometriosis is an estrogen-dependent gynecological disease that affects 10-15% of reproductive-age women. The disease is thought to originate from retrograde menstruation and growth of ectopic endometrial tissue on ovaries and internal organs. Women with endometriosis have a higher risk of developing endometriosis-associated ovarian carcinoma (EAOC), one of the deadliest gynecological malignancies worldwide. Malignant transformation of ovarian endometriomas (OE) to different types of ovarian cancer is well documented, but the environmental risk factors that play a role are largely unknown. Humans are ubiquitously exposed to Bisphenol A (BPA), a chemical used in polycarbonate plastics commonly found in food/beverage packaging. BPA is an endocrine-disrupting chemical (EDC) that alters normal hormone function through its action on estrogen receptors, including the G protein-coupled estrogen receptor (GPER). BPA exposure has been linked to a wide range of adverse female reproductive health issues in humans and experimental animals, including endometriosis, and increased risk for endometrial and ovarian cancer. GPER signaling mediates the immune response and neoplastic transformation of both endometrial and ovarian cancers, suggesting that BPA activation of this pathway may play a role in EAOC. In response to public health concerns surrounding the use of BPA, structurally similar 'BPA-free' chemical analogs such as BPAF are now being used in food packaging. However, BPAF binds with greater affinity and is a more potent activator of GPER than BPA, little is known about its toxicity..

There is therefore a critical need for further research to examine the role of BPAF exposure as an environmental risk factor for female reproductive cancers. However, animal models of ovarian endometriosis that phenocopy human anatomy and physiology to accurately study the impact of this EDC on risk for OE progression to ovarian cancer (EAOC) are currently lacking. Unlike humans, rodents do not menstruate, and have a membranous bursa that encapsulates the ovaries, separating it from the peritoneal cavity. Our innovative translational mouse model of ovarian endometriosis overcomes these limitations; both donor and recipient mice are induced to menstruate, and, using minimally invasive surgery, menstrual endometrium is implanted to establish peritoneal and ovarian endometriotic lesions. In addition, the ovarian bursa is opened so that the ovaries and ovarian lesions are in contact with immune cells from the peritoneal fluid. Our immunocompetent, physiologically relevant mouse model of endometriosis will be used to test the **hypothesis**: *BPAF exposure and activation of GPER-mediated signaling in ectopic ovarian endometriotic (OE) lesions and peritoneal macrophages coordinate different aspects of pathogenesis that increase risk for progression to EAOC.*

AIM 1: Determine the role of dietary BPAF exposure (dose-response) on OE lesion pathology and risk for progression to EAOC

AIM 2: Determine the role of BPAF activation of GPER signaling in (donor) OE lesions vs. (recipient) peritoneal macrophages in the establishment and progression of OE to EAOC

In Aim 2, *Gper* wildtype donor and knockout recipient mice (or vice versa) will be used to examine the role of BPAF activation of GPER in donor endometrial tissue vs. recipient (infiltrating) peritoneal macrophages on establishment of lesions and disease progression.

Endpoints include evaluation of BPAF exposure (dose-response) on lesion number, size, histopathology, and progression to EAOC over time, indicators of oxidative stress (DNA damage, lipid peroxidation) involved in transformation, and evaluation of markers indicative of BPAF activation of GPER-mediated signaling involved in tumor cell survival and disease progression, including HIF1a, HO-1, proliferation, angiogenesis, and macrophage polarization.

Key Personnel

- The Principal Investigator is listed first.
- Behavioral sketches are required for ALL listed.
- See Application, page 8 for more details.

Name	**Organization**	**Role on Project**
Janee Gelineau-van Waes, DVM, PhD	Creighton University	Principal Investigator
Christina Howe, DVM	Creighton University	Co-Investigator
Ahmed Abdulrahim, MD	Creighton University	Co-Investigator
John Cote, MD	Creighton University	Consultant

Budget Worksheet *Direct Costs Only*					From 7/1/2023		Through 6/30/2024
Personnel (Applicant Organization only)		Type of Appointment	% of Effort on Project	Institutional Base Salary	Amount Requested		
					Salary	Fringe Benefits	Totals
Name	Role in Project						
J. Gelineau-van Waes	Principal Investigator	FT	10%				
C. Howe	Co-Investigator	*3/4	2%				
A. Abdulrahim	Co-Investigator	FT	2%				
J. Cote	Consultant	FT	2%				
J. Hallgren	Technician	FT	40%		18,706	5,239	23,945.00
				Subtotals →	18,706	5,239	23,945.00
Consultant Costs							0.00
Equipment							0.00
Supplies							10,397.00
Travel							$0.00
Patient Costs	Inpatient						0.00
	Outpatient						0.00
Contractual or Third-Party							0.00
Other							30,658.00
Total Direct Costs for Budget Period *Also indicate at #6 on the Face Page.*							**$65,000.00**

KEY PERSONNEL:

Janee Gelineau-van Waes, DVM, PhD (PI)

Dr. Gelineau-van Waes is a tenured Associate Professor in the Dept. of Pharmacology & Neuroscience. She will oversee the project and project budget and be responsible for coordinating the overall schedule to ensure that benchmarks are achieved as planned. The laparoscopic endometriosis surgeries are conducted in the van Waes laboratory. On surgery days, Dr. van Waes will harvest donor uterus, separate the decidualized endometrium from the myometrium, and prepare the endometrial biopsy punches for transplant. She will assist Dr. Howe with laparoscopic surgery (peritoneal endometriosis), induction of ovarian endometriosis, and with lesion harvest, documentation, photography, and fixation on the day of sacrifice. Dr. van Waes (IACUC Chair) and Dr. Howe (Creighton attending veterinarian) will be responsible for submitting and making modifications to the animal protocols, if necessary to further optimize experimental details. Dr. van Waes will coordinate the embedding, section, and H&E staining of ovaries and lesions with Ms. Toni Howard in the CU histology core facility and will arrange to give slides to Dr. Abdulrahim for histopathological examination. Janee will also work with Melissa Holzapfel in the UNMC Tissue Sciences Facility to conduct the immunohistochemistry (IHC) experiments and will photograph stained sections and perform the quantitative image analysis. Dr. van Waes will mentor graduate and medical students who volunteer to help with the project, analyze data, prepare manuscripts and progress reports, and write follow-up grant proposals to secure additional funding from DoD, NIH and/or other extramural sources.

Christina Howe, DVM (Co-Investigator)

Dr. Howe is the Attending Veterinarian at Creighton University. She has been key in optimizing many aspects of the laparoscopic transplant model, including intubation, anesthesia, ventilation, biopsy placement in the recipient mouse, and post-op analgesia. She performs the decidualization procedure with intrauterine sesame oil injection on d4.5 in the donor mice. Christy also does post-op checks and daily rounding on the mouse colonies, performs vaginal cytology exams to determine stage of estrus and conducts laparotomy/lesion harvest from recipients on the day of sacrifice. Dr. Howe trains new graduate students and medical students who help with the project on all aspects of the animal procedures. Drs. Howe and van Waes will be responsible for making modifications to the IACUC protocol as necessary. Christy and Dr. van Waes will work together to write manuscripts and progress reports. [*Dr. Howe's appointment as the Attending Veterinarian at Creighton University is 12 months/year at .75% FTE (30 hrs/week)].

Ahmed Abdulrahim, MD (Co-Investigator)

Dr. Abdulrahim is a pathology resident in the Dept. of Pathology & Laboratory Medicine at Creighton University Medical Center. He will perform the histopathology reading of the H&E slides to confirm that the ectopic peritoneal lesions harvested are of endometrial origin (presence of stroma and endometrial epithelial glands, and hemosiderin-laden macrophages) and provide a detailed description of the ovaries, ovarian endometriomas and endometriosis-associated ovarian carcinomas. He will record the findings, including a complete description of the measurements and phenotype (irregular borders, fluid-filled cysts, hemosiderin, macrophage infiltration, atypical endometriosis, clear cell carcinoma, or endometrioid carcinoma subtypes) and will photograph and label the sections/slides. He will assist in the preparation of progress reports and manuscripts.

John Cote, MD (Consultant)

Dr. Cote is an accomplished Ob/Gyn physician and surgeon. He routinely diagnoses and treats patients with endometriosis and performs robotic surgery to remove ectopic lesions and/or perform ovariohysterectomies. John was key in obtaining the equipment needed for the laparoscopic surgeries, and coordinates ethylene oxide gas sterilization of the endoscope every week at Lakeside Hospital. He trained personnel on how to operate the endoscope and camera and optimize the insufflation. John assists with mouse surgeries on his day off from clinic duties and is instrumental in recruiting medical student (M2) volunteers to help with the project. He will be in regular communication with Drs. van Waes and Howe and assist with the preparation of manuscripts.

OTHER PERSONNEL:

Jodi Hallgren, Technician

Salary and fringe are requested for Ms. Jodi Hallgren (technician) to assist on this project. Jodi has worked in the van Waes and Stessman lab for several years and assists with this project on endometriosis and other projects on birth defects. She will be responsible for placing orders, helping with mouse colony maintenance, weaning, and genotyping, setting up timed matings with vasectomized males, checking for vaginal plugs, weighing mice, and making up drug and vehicle. She will perform vaginal cytology, collect blood samples via cheek bleed (for plasma drug analysis), and autoclave surgical supplies. Jodi will also assist with decidualization, surgical prep and post-op recovery on transplant surgery days.

[40% FTE salary and fringe]. Salary $18,706 + Fringe $5,239 = $23,945
Requested fringe benefit rate is 28.01%

Master's Degree Graduate Student

Ms. Evanjalina Matoy is a Master's Degree graduate student who joined the Dept. of Pharmacology & Neuroscience beginning in the fall semester 2022. She is currently rotating in the laboratory of Dr. Holly Stessman. She has observed the laparoscopic surgeries and is currently being trained on all aspects of the project, including mouse handling, colony management, weaning, genotyping, checking plugs, vaginal cytology, administering drugs via subcutaneous or intraperitoneal injection, oral gavage, decidualization, anesthesia, intubation, autoclaving instruments, and prepping animals for surgery, running the endoscope, and assisting with laparoscopic surgery and post-op monitoring. She will assist with lesion harvest, photographing ectopic lesions and recording their location, size, and phenotype prior to formalin fixation. She will present research findings at our weekly Departmental Research Rounds (students present to the faculty and other graduate students once per semester). Vanjie will prepare posters and/or platform presentations for local and national meetings and be involved in data analysis and manuscript preparation. [No funds requested]

M2 Medical Students

Several second year (M2) medical students from the Creighton University School of Medicine volunteer their time to help with the laparoscopic surgeries and gain experience in laboratory research. The medical students assist with surgical prep, anesthesia, intubation, post-op recovery, and cleaning and autoclaving of instruments. They are also present for lesion harvest when their class schedule allows. [No funds requested]

NON-PERSONNEL BUDGET JUSTIFICATION:

SUPPLIES

(1) Laboratory [$9617]

Surgical supplies, drugs, chemicals, reagents, general lab supplies including: sterile gloves, syringes, needles, ketamine, xylazine, isoflurane, buprenorphine, sterile saline, suture material (7-0 prolene, 4-0 silk), plasma collection tubes, 14G IV catheters, lancets, petri dishes, formalin specimen storage vials, autoclave bags and tape, biohazard bags, CO_2 tanks, Dumont forceps, gauze pads, alcohol prep pads, cotton swabs, dissection waste bags, iodine, ethanol, syringes, needles, transfer pipettes, animal clippers, Bisphenol AF, kimwipes, spill pads, non-sterile gloves, PBS

Histology supplies, Ab for IHC: slides, coverslips, slide boxes, mounting media (including vectashield with DAPI), xylene, ethanol, primary antibodies [~$500 each] (vimentin, cytokeratin, GPER1, Ki67, CD31, CD68, CD163, CD11c, HIF1α, 4-HNE, d-OHdG), + secondary antibodies, Prussian blue stain.

(2) Mice [$780]

Funds are requested for the purchase of 6 vasectomized C57BL/6J male mice for induction of pseudopregnancy in donor and recipient experimental female mice. Vasectomized males are $130 each from Jackson Laboratories ($780).

OTHER

(1) Animals [$25,098]

Live animals are needed to model the disease process and test the hypothesis. We will use an established mouse model of endometriosis exposed to dietary bisphenol AF to accomplish the proposed specific aims. Mice in which *Gper1* has been genetically inactivated (knockout) will be used to accomplish the objectives in Aim 2. The B6.129S6-*Gper1*^{tm1Cwan}/J mice needed for this project were previously cryorecovered (Jackson Laboratories) and a breeding colony established at Creighton University. This breeding colony will be used to generate the mice needed for experiments. Funds are requested to support the breeding colony and experimental animals.

Animal Facility Charges:

Feed and Bedding: Funds are requested to pay for the purchase of Envigo global soy protein-free extruded autoclavable (control) rodent diet (2020SX) and TEK Fresh (paper) bedding to ensure that exposure to exogenous estrogens (ie. the mycotoxin zearalenone in corncob bedding (standard bedding used in ARF), and/or phytoestrogens in soy based rodent diets) will not interfere with and/or mask determination of the role of Bisphenol AF and activation of the G protein-coupled estrogen receptor GPER1 on our experimental endpoints (GPER binds xenoestrogens and phytoestrogens). Bisphenol AF (BPAF) will be purchased and provided to Envigo to formulate the (custom) experimental diets at the desired concentrations (30 ppm, 300 ppm, 900 ppm).

Current ARF charges are $48/mo for the soy-free diet ($576 per year) and $378 per year for TEK-fresh bedding ($15.75 per bag x 24). Total **$954** per year (control diet + bedding)

Custom experimental BPAF diets: 20 kg diet (in 2 kg vacuum and irradiated packaging) = $1030 each x 3 concentrations = **$3090**

Caging: Mice will be housed in (recyclable) BPA-free polyethylene static cages (with BPA-free water bottles) purchased from Innovive (San Diego, CA). Cage bottom + lid + water bottle + feed hopper = $7.99 each (sold in packs of 100). Mice will be single housed after surgery with cage changes every 10 days, so approx. 3 cages/experimental mouse/month x 3 months/mouse [AIM 1: 40 donors (10 d, 1 cage ea.) + 20 recipients (30 d, 3 cages ea.) + 20 recipients (90 d, 10 cages ea.) = 300 cages; AIM 2: 60 donors (10 d, 1 cage ea.) + 30 recipients (30 d, 3 cages ea.) + 30 recipients (90 d, 10 cages ea.) = 450 cages] = 750 experimental cages x $8 ea. = **$6000**

Per Diems: Funds are requested to support per diems for the B6.129S6-*Gper1*^{tm1Cwan}/J [Stock #030841] breeding colony and experimental animals. Mouse per diems: $0.33/day/mouse. Based on housing an average of 120 mice/day for this project (breeding pairs, weanlings, experimental females (donors and recipients) and experimental (vasectomized) males. 120 mice x 0.33/day x 365 days/yr = **$14,454** for 1 year

Genotyping Fees: (tail snip samples will be sent to Transnetyx for genotyping the *Gper1* mice) ~$50/month = **$600** for 1 year

(2) Core Facility Fees: [$4560]

● Creighton University Histology Core facility: Formalin-fixed tissues (donor + recipient uterus, ovaries, recipient lesions) will be submitted to Ms. Toni Howard in the CU histology core facility for processing, paraffin-embedding, sectioning and H&E staining (estimated cost $50/month, $600 for 1 year period)

*Core Facility fees for use of slide scanner are $10/hour; estimate 8 hrs/month x 12 mo = $960

Total = ($600 + $960) = $1560 for one year

● UNMC Tissue Sciences Facility**:** Immunohistochemical protocol optimization and staining: IHC protocol set-up fee, new Ab optimization ($37.25/slide) x 4; Stain, single client Ab, DAB reaction ($29.95/slide), estimate ~$250/mo. = $3000 for one year

(3) Publication Fees: [$1000]

$1000 is requested for the cost of publishing the research findings in a scientific journal.

Biographical Sketch

Provide the following information for all Key Personnel listed. Begin with the Principal Investigator.
Follow this format for each person. **DO NOT EXCEED TWO (2) PAGES PER SKETCH.**

NAME: Gelineau-van Waes, Janee

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

POSITION TITLE: Associate 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	Completion Date	FIELD OF STUDY
Washington State Univ., Pullman, WA	BS	05/1981	Veterinary Science
Washington State Univ., Pullman, WA	DVM	05/1983	Veterinary Medicine
Washington State Univ., Pullman, WA	PhD	05/1996	Pharmacology & Toxicology
Texas A&M Univ. College Station, TX	Post-doc	06/1999	Developmental Toxicology

A. Personal Statement

My research has focused primarily on using mouse models to understand the complex gene-nutrient-environment interactions that contribute to birth defects, with a specific focus on neural tube defects (NTDs). I have successfully competed for funding from several private foundations, as well as university, state and federal funding agencies (NIAAA, NIEHS, NCRR, NICHD and NIH Office of the Director) to investigate the role of pharmaceutical and environmental exposures during pregnancy that increase risk for birth defects. A major focus of my research has been the role of the mycotoxin fumonisin (common contaminant of corn-based food products) as a risk factor for neural tube defects. This project involved collaborations with scientists at the USDA Mycotoxin Unit (Athens, GA), Duke University and investigators in Guatemala. Another project involved investigation of the risk factors (including genetics and maternal diet) and mechanisms underlying increased risk for NTDs following gestational exposure to the HIV antiretroviral drug dolutegravir.

Of relevance to this proposal, my lab has recently developed a novel translational laparoscopic mouse model of endometriosis. The project brings together basic science researchers and clinicians to address important gaps in the translational validity of preclinical models and discovery of therapeutic targets for treating gynecological diseases. Dr. Howe and I presented our mouse model of endometriosis as a ‘late-breaking abstract’ at the Society for Reproductive Investigation (SRI) annual meeting in Denver (March 2022) and currently have a manuscript in preparation. I am excited to combine my knowledge and skills in pharmacology and reproductive biology and add ovarian endometriosis to our existing mouse model to study the role of exposure to the endocrine-disrupting chemical bisphenol AF (BPAF) and activation of G protein-coupled estrogen receptor (GPER)-mediated signaling as an environmental risk factor for progression of ovarian endometriomas to endometriosis-associated ovarian cancer (EAOC).

B. Positions and Honors

2017 NIH ZRG1 EMNR-S(02) Endocrinol, Metab, Nutr, & Repro Sci, ad hoc reviewer
2016 NIH ZRG1 F06-S(20)L Endocrinol, Metab, Nutr, & Repro Sci, ad hoc reviewer
2016-23 Chair, Creighton Institutional Animal Care and Use Committee (IACUC)
2014 NIH ZRG1 EMNR-Q(02) SEP on Endocrinol and Reproduction, ad hoc reviewer
2012-23 HESI Dev & Repro Tox (DART) Technical Committee, Scientific Advisor
2011 NIEHS Special Study Section: Role of Environmental Chemical Exposures in Development of Obesity, Type 2 Diabetes and Metabolic Syndrome ad hoc reviewer
2011-19 March of Dimes, Nebraska State Program Services Committee
2010 Creighton University School of Medicine Distinguished Research Career Award
2010-15 Journal of Reproductive Toxicology, Editorial Board
2009-23 Associate Professor, Dept. Pharmacology, Creighton Univ. School of Medicine
2009 Neural Oxidative Metabolism and Death [NIH NOMD Study Section], ad hoc reviewer
2009 Pregnancy & Neonatology [NIH PN Study Section], ad hoc reviewer
2004-18 Society of Toxicol: Repro & Dev Tox (Councilor 2009-10) & Food Safety Special Section
2001-23 Society for Birth Defects Research & Prevention (Teratology Society), member, Education Committee, Awards Committee, & Strategic Planning Committee
1999-09 Assistant Professor, Dept. Genetics & Cell Biol, Univ. of NE Medical Center (UNMC)

C. Contributions to Science within the past five (5) years – including complete references of peer-reviewed publications – *pertinent to this application only.*

Relevant to this application, ongoing and recently completed projects have included:

George F. Haddix President's Faculty Research Fund (Gelineau-van Waes, PI) [04/01/19-03/31/20] Developing a mouse model of endometriosis to test novel therapeutic targets This award was used to develop an immune competent, normally cycling (fertile) surgical (laparoscopic) mouse model of endometriosis to study signaling pathways involved in the etiology and progression of disease, and test novel therapies for prevention.

Health Sciences Strategic Investment Fund (Gelineau-van Waes, PI) [07/01/21-06/30/23] Role of G Protein-Coupled Estrogen Receptor (GPER) in Endometriosis: Pharmacological and genetic approaches are used to examine the role of the G protein-coupled estrogen receptor (GPER) on disease outcomes in a fertile, translational mouse model of endometriosis. Research findings were presented at the Society for Reproductive Investigation (SRI) 69th Annual Meeting in Denver, CO (March 15-19, 2022) and a manuscript is currently in preparation.

Although endometriosis and environmental risk factors for EAOC is a new area of research for my lab, we have long focused on exposures to environmental toxicants and pharmaceuticals during pregnancy and risk for birth defects. In the past 5 years, other recently completed projects have included investigation of environmental (fumonisin) and pharmaceutical (dolutegravir) teratogens and risk for NTDs:

LB692 New Initiatives Grant: (Gelineau-van Waes, PI) Mouse Model of Dolutegravir-Induced Neural Tube Defects [07/01/20 – 06/30/21] to test the hypothesis that gestational exposure to the antiretroviral HIV drug dolutegravir increases risk for NTDs through chelation of Mg^{2+} in maternal plasma and embryonic tissues.

*Manuscript recently published: Gelineau-van Waes, et al. (2023) Gene-Nutrient Interactions that Impact Magnesium Homeostasis Increase Risk for Neural Tube Defects in Mice Exposed to Dolutegravir. *Frontiers in Cell and Developmental Biology*. DOI: 10.3389/fcell.2023.1175917

Biographical Sketch

Provide the following information for all Key Personnel listed. Begin with the Principal Investigator.
Follow this format for each person. **DO NOT EXCEED TWO (2) PAGES PER SKETCH.**

NAME: Howe, Christina Ann

eRA COMMONS USER NAME (credential, e.g., agency login): Click or tap here to enter text.

POSITION TITLE: Attending Veterinarian

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	Completion Date MM/YYYY	FIELD OF STUDY
Texas A &M University	BS	12/1999	Biomedical Science
Texas A &M University	DVM	05/2002	Veterinary Medicine

A. Personal Statement

I am the Attending Veterinarian in the Animal Resource Facility for Creighton University. I have 20 years of clinical veterinary experience, working with various species including dogs, cats, small mammals, swine, small ruminants, horses, and cattle. I am proficient in internal medicine, anesthesia, surgery, dentistry, radiology, and ultrasonography. I routinely evaluate choice of animal model and species, experimental procedures, and technique in proposed research projects. In my position as Attending Veterinarian/IACUC member I work closely with numerous investigators on a variety of projects to maintain animal health and welfare and troubleshoot issues with their experimental technique to generate quality scientific data. In addition, I provide training on various procedures conducted on animals to research personnel. I was a co-Investigator on a Haddix grant “Developing a mouse model of endometriosis to test novel therapeutic targets”, developing and completing experimental paradigms to perfect an immunocompetent fertile mouse model of endometriosis. I successfully maintained mouse breeding colonies to generate experimental animals, and fine-tuned abdominal laparoscopic technique, endotracheal intubation, perioperative animal support, and analgesia and anesthesia in mice. As an undergraduate student I participated in two equine studies and one swine study with primary responsibilities of animal care/husbandry and data collection. As a veterinary student (maiden name Reinoehl) I was awarded a summer research position with a focus on in-vitro fertilization in horses and in-vitro oocyte maturation. After 16 years in companion animal clinical practice, I changed course in my career to focus on laboratory animal medicine and research. I am currently pursuing board certification in laboratory animal medicine which requires publication in peer-reviewed journals. With these past experiences and my current work as the facility veterinarian and co-investigator, I understand the logistics of a research study. I have the technical expertise to complete the animal procedures and experiments on this project.

B. Positions and Honors

Positions and Employment

2017-Present Attending Veterinarian, Creighton University, Omaha, NE
2014-2018 Associate Veterinarian, Fremont County Veterinary Clinic, Sidney, IA
2002-2014 Associate Veterinarian, All Creatures Veterinary Clinic, Omaha, NE

Other Experience and Professional Memberships

2020-Present Consultant, Laboratory Safety Committee, Creighton University, Omaha, NE
2017-Present Member, IACUC, Creighton University, Omaha, NE
2017- Present Member, American Association for Laboratory Animal Science
2015-2016 Member, Fremont County Board of Public Health, Sidney, IA
2002- Present Member, American Veterinary Medical Association
2002- Present Member, Iowa Veterinary Medical Association

Honors

2022 Nebraska AALAS Branch Delellis Award

C. Contributions to Science within the past five (5) years – including complete references of peer-reviewed publications – ***pertinent to this application only.***

1. Successful development of animal models of disease is a critical component to translational research. My early work in bench side research was largely related to animal husbandry and care. I provided input on animal behaviors and implemented training and acclimation techniques to improve data collection and completion of experimental procedures in horses and swine. Additionally, I have successfully enhanced Creighton University's mouse enrichment program, resulting in improved fecundity in mouse breeding colonies, directly affecting productivity of scientists working on a variety of projects.

Disorders of reproduction affect humans and animals alike. Development of assisted reproductive technology is critical to address infertility. My work in preservation of equine oocytes led to future developments in maturation of immature oocytes in vitro.

a. Katrin Hinrichs, Charles C. Love, Young Ho Choi, Dickson D. Varner, C. Nicole Wiggins and **Christina Reinoehl*(Howe)** Suppression of meiosis by inhibitors of m-phase proteins in horse oocytes with low meiotic competence. *Zygote 2002 Feb;10(1)37-45.*

Translational models of endometriosis are lacking. My work developing a translational model of endometriosis in an immunocompetent, fertile mouse can help elucidate the pathogenesis of this disease and identify therapeutic targets while allowing us to evaluate the impact on fertility.

a. Project: Developing a mouse model of endometriosis to test novel therapeutic targets Role: Co-Investigator.
    i. Optimizing a Translational Mouse Model of Endometriosis. 2022 Poster Presentation Society for Reproductive Investigation Annual Meeting
    ii. Optimizing a Translational Mouse Model of Endometriosis 2022 Presentation District 6 AALAS Annual Meeting.
b. Ongoing Project: Role of G Protein Coupled Estrogen Receptor (GPER) in Endometriosis
    i. Role: Co-investigator
    ii. 7/1/2021- Present

Biographical Sketch

Provide the following information for all Key Personnel listed. Begin with the Principal Investigator.
Follow this format for each person. **DO NOT EXCEED TWO (2) PAGES PER SKETCH.**

NAME: Ahmed Abdulrahim, MD.

eRA COMMONS USER NAME (credential, e.g., agency login): N/A

POSITION TITLE: Pathology Resident

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	Completion Date MM/YYYY	FIELD OF STUDY
Assuit University, Faculty of Medicine, Assuit, Egypt	MBBCh	03/2013	Medicine
Creighton University, Department of Pathology & Laboratory Medicine, Omaha, NE		Anticipated 06/2025	Pathology

A. Personal Statement

I am a pathology resident, and my training and research are focused on combining clinical training with laboratory expertise to contribute to medical care. Our residency program is a combined anatomic and clinical pathology program. The anatomic pathology component is focused on investigating the effect of disease on the human body via gross and microscopic examination of tissue, cells and other specimens, as well as via autopsies. The clinical pathology component is focused on laboratories and supervising testing procedures via a blend of microbiology, hematology, chemistry, immunology, molecular biology and business management. I have a good background in surgical pathology, with a training that is focused on identifying and diagnosing a wide variety of clinical conditions by looking at the gross and microscopic pictures of different tissues. I also have a good background in clinical research. I participated in multiple research projects during and after medical school as well as during my residency. As a result of this background and previous experiences both in research and clinical care, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget, and I have the expertise, leadership, training, and motivation necessary to successfully perform in the proposed research project.

B. Positions and Honors

07/2021-Present Pathology Resident Department of Pathology and Laboratory Medicine, Creighton University, Omaha, NE

02/2021-06/2021 Research Temporary Professional Mayo Clinic, Rochester, MN

10/2020-1/2021 Pathology Assistant SIParadigm Pathology Laboratory, Pine Brook, NJ

02/2015-09/2020 Primary Care Physician Medical Department of the Egyptian Town Gas Company, Cairo, Egypt

C. Contributions to Science within the past five (5) years – including complete references of peer-reviewed publications – *pertinent to this application only.*

Ongoing and recently completed projects that I would like to highlight include:

Abdulrahim (PI)

Case Series: Large B-cell Lymphoma with IRF4 Rearrangement Involving the Lung and the Tonsil in two different patients.

El-Herte (PI), Role: co-investigator

Case Report: Disseminated Coccidioidomycosis with Fungemia and Strongyloides Co-infection

Hilgers (PI), Role: co-investigator

Struma Peritonei Case Report

Citations:

Aliae A. R. Mohamed Hussein, Marwa Rashad Salem, Samar Salman, A F Abdulrahim, et al. Correlation between COVID-19 case fatality rate and percentage of BCG vaccination: is it true the vaccine is protective? Egypt J Bronchology. 2020, Sep; 14(1): 25. Cited in PubMed; PMID: PMC7479298.

Emara, Khaled M. MD, PhD; Hemida, Mohamed A. MD, Master; Abdulrahiam, A. Fayez MD; Nasr, Abdelrahman Ashraf MD, Student. The fifty most cited articles of Arab countries in the orthopaedic literature. Current Orthopaedic Practice. 2016, Jan; 27(1): p84-89.

Mahmoud AN, Amgad M, Abdelmohsen MT, Abdulrahim AF, et al. Is the Intramedullary Skeletal Kinetic Distractor a Safe Measure for Bone Lengthening? A Systematic Review: Journal of Orthopaedics, Trauma and Rehabilitation. Journal of Orthopaedics, Trauma and Rehabilitation. 2014, Dec; 18(2): 69-78.

Biographical Sketch

Provide the following information for all Key Personnel listed. Begin with the Principal Investigator.
Follow this format for each person. **DO NOT EXCEED TWO (2) PAGES PER SKETCH.**

NAME: John Joseph Coté

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

POSITION TITLE: 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	Completion Date MM/YYYY	FIELD OF STUDY
Loyola Marymount University	BS	05/1991	Biology/Biochemistry
Creighton University	MD	05/1997	Doctor of Medicine
Creighton University	Residency	07/2001	Obstetrics & Gynecology

A. Personal Statement

I have the expertise, leadership, training, and motivation necessary to successfully contribute to the proposed research project. I have a broad background in medicine, with specific training and expertise in obstetrics and gynecology. I am an Assistant Professor of Obstetrics and Gynecology, and my research has been a broad application of my specialty but has most recently focused on endometriosis. I initially started as a PI on several industry-sponsored women's health projects; I successfully administered the projects (e.g., staffing, enrollment goals, data quality), and collaborated with my OB/GYN partners. These industry-sponsored projects focused on endometriosis. As a result of these experiences, I learned how to navigate roadblocks, and appreciate the differences between industry sponsored and investigator-initiated trials. As a result of these previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. The current application builds logically on our prior work with the team and can serve as the building blocks to a more extensive research portfolio for the team, myself, the OB/GYN department, the Pharmacology and Neuroscience department and continue to foster collaboration within the University. Our mouse model is evidence of how we have succeeded in this translational collaboration and how this current application will further our ultimate goals. Ongoing and recently completed research projects that I would like to highlight include:

Creighton University Health Science Strategic Investment Fund
Coté (Co-I) Gelineau-van Waes (PI) [July 2021-June 2022]
Role of G Protein-coupled Estrogen Receptor (GPER) in Endometriosis.
Creighton University Dr. George F. Haddix President's Faculty Research Fund
Coté (Co-I) Gelineau-van Waes (PI) [March 2019-March 2020]
Developing a mouse model of endometriosis to test novel therapeutic targets.

B. Positions and Honors

Positions and Scientific Appointments

2021-Present Member Pelvic Floor and Endometriosis Steering Committee
2021-Present Member Simulator Center Steering Committee
2021-Present Endometriosis and Myfembree Advisory Board Chair Pfizer/Myovant
2021-Present Speakers Bureau Pfizer
2019-Present Site Leader and Lab director Lakeside Women's Healthcare
2019-Present, Member, Management Council, CHI Women's Healthcare
2018-Present Speakers Bureau AbbVie
2016-Present Assistant Professor, Creighton University, School of Medicine, Omaha, NE
2001-Present, Physician, CHI Health Clinic/CommonSpirit, Omaha, NE
2001-Present Fellow of the American College of Obstetrics and Gynecology
2001-Present, Diplomat of the American Board of Obstetrics and Gynecology

Honors

2021 Creighton University Department of Obstetrics and Gynecology Distinguished Service
2008 PRC top scoring physician in the nation for overall quality of doctor
2005-2006 John E. Kretiek Award for undergraduate teaching
2003 Richert Taylor Award for resident education
2001-2002 Golden Apple Finalist
2000 Winner Maurice Grier best research
1998 Winner resident in excellence award TAP pharmaceuticals
1997 Dan Cullen, MD, JD, achievement award
1997 Honors OB/GYN, Neurosurgery, Plastic Surgery, legal medicine, and ethics
1997 James Feramisco, MD. memorial scholarship

C. Contributions to Science within the past five (5) years – including complete references of peer-reviewed publications – *pertinent to this application only.*

Endometriosis is an estrogen-dependent disease associated with dysmenorrhea, non-menstrual pelvic pain, dyspareunia, infertility, and increased risk of ovarian cancer. Current medical treatments are inadequate, and surgical interventions have a high rate of recurrence This unmet need led to my participation as PI for one of the study sites in two large multi-national and multi-center industry sponsored trials examining the safety and efficacy of oral GnRH antagonists for patients with endometriosis associated pain. The data resulting from this work helped to obtain FDA approval for the first new treatment of endometriosis pain in the last ten years.

Taylor, H. S., Giudice, L. C., Lessey, B. A., Abrao, M. S., Kotarski, J., Archer, D. F. Chwalisz, K. (2017). Treatment of endometriosis-associated pain with elagolix, an oral GnRH antagonist. New England Journal of Medicine, 377(1), 28-40.

Surrey, E., Taylor, H. S., Giudice, L., Lessey, B. A., Abrao, M. S., Archer, D. F, Chwalisz, K. (2018). Long-term outcomes of elagolix in women with endometriosis: results from two extension studies. Obstetrics & Gynecology, 132(1), 147-160.

As-Sanie, S., Becker, C. M., Johnson, N., Lessey, B. A., Abrao, M. S., Brown, E. L.Giudice, L. C. (2020). Efficacy and safety of relugolix combination therapy in women with endometriosis-associated pain: Phase 3 randomized, double-blind, placebo-controlled study (spirit 2). Fertility and Sterility, 114(3), e77.

DeAngelo, C., Tarasiewicz, M.B., Strother, A., Taggart, H., Gray, C., Shanahan, M., Glowacki, C., Khandalavala, J., Talaska, E., Kinnan, A. and Coté, J.J., 2020. Endometriosis: A Malignant Fingerprint. Journal of cancer research and therapeutic oncology, 8(2).

Other Support

- Use continuous pages as needed.
- Required for the Principal Investigator only.
- Note Project Number, Source, Major Goals, Dates of Approved/Proposed Project, Annual Direct Costs, Percent Effort AND Overlap (if any) – see Application, pages 10 - 12 for more details.

ACTIVE:

Title: **Role of G Protein-Coupled Estrogen Receptor (Gper1) in Endometriosis**
Funding Period: 07/01/21-6/30/23
Funding Agency: Health Sciences Strategic Investment Fund (HSSIF)
Role: Gelineau-van Waes, PI (10% FTE)
Total Funds: $50,000 (annual direct costs $25,000 per year)
Fund: 201026 Org: 823850

Major Goals: This project uses both pharmacological and genetic approaches to examine the role of the G protein-coupled estrogen receptor (GPER) on disease outcomes in a fertile, translational mouse model of peritoneal endometriosis.

Overlap: No budgetary or commitment overlap. Funding ends June 30, 2023.
In terms of scientific overlap, this project involves examination only of peritoneal endometriosis, and does not include establishment of ovarian endometriotic lesions or evaluation of progression to endometriosis-associated ovarian cancer.
This project evaluates pharmacological agonists and antagonists of GPER available for preclinical use (G1, G36) on peritoneal endometriotic lesion endpoints and does not include evaluation of exposure to environmental endocrine-disrupting chemicals such as bisphenol AF that can bind and activate GPER.
The laparoscopic mouse model of peritoneal endometriosis was further refined on this project, and funds were used to cryo-recover *Gper* knockout mice and establish a breeding colony. *Gper* wildtype and knockout mice are used as donors and recipients to understand the role of GPER signaling in peritoneal (donor) lesions vs. recipient tissues and immune cells.

The *Gper* breeding colony established on this HSSIF project will be used to generate experimental animals for the LB506 project (if funded) to examine the role of BPAF exposure and activation of GPER signaling on risk for progression of ovarian endometriotic lesions to endometriosis-associated ovarian cancer.

PENDING:

Title: **Role of *Fam111a* in Mineral Ion Homeostasis**
Funding Period: 07/01/23 – 06/30/24
Funding Agency: LB692 Nebraska Tobacco Settlement Biomedical Research Development New Initiative Grant
Role: Gelineau-van Waes, PI (10% FTE)
Total Funds: $75,000

Major Goals: The proposed studies use a novel mouse model with 9 homozygous (predicted deleterious) variants in *Fam111a* (a gene involved in magnesium (Mg^{2+}) homeostasis), to test the hypothesis that *Fam111a* gain-of-function variants lower the homeostatic set point for plasma calcium and magnesium due to alterations in Calcium-Sensing Receptor (CaSR) and Parathyroid Hormone (PTH)-mediated signaling pathways.

Overlap: No budgetary or commitment overlap.

Creighton
UNIVERSITY

School of Medicine
Department of Pharmacology and Neuroscience

January 2, 2023
Grant Review Committee
Cancer and Smoking Disease Research Program
Department of Health and Human Services
State of Nebraska

Re: Institutional support letter for Dr. Gelineau-van Waes, DVM, PhD

Dear Committee Members,

Dr. Gelineau-van Waes, has asked that I provide a letter of support for her grant application to the LB 506 Cancer and Smoking Disease Research Program titled “Bisphenol AF Exposure and Risk for Endometriosis-Associated Ovarian Cancer”. Dr. Gelineau-van Waes is a tenured Associate Professor in the Department of Pharmacology and Neuroscience at Creighton University, School of Medicine. As her Chair, I support her research and other activities as a full-time, independent, faculty member at Creighton University. As such, she is eligible to submit federal and other grants as Principal Investigator. She has a fully functioning, dedicated research laboratory of 735 sq. ft. located in Rooms 552/553 of the Criss III Health Sciences building at Creighton. Her laboratory is fully equipped and capable of performing the studies described in her proposal. In addition, she has access to all Departmental common use spaces as well as administrative and other Departmental and University research resources.

Dr. Gelineau-van Waes is fully supported by the Department and the School of Medicine, and I believe she will make important contributions to the Cancer and Smoking Disease Research Program.

Sincerely,

Peter W. Abel, PhD
Professor and Chair
Department of Pharmacology and Neuroscience
Creighton University School of Medicine
Omaha, NE 68178
Email: pabel@creighton.edu

RESEARCH PLAN

B. SPECIFIC AIMS: Endometriosis is an estrogen-dependent gynecological disease that affects 10-15% of reproductive-age women[1]. Women with endometriosis have a higher risk of developing endometriosis-associated ovarian carcinoma (EAOC), one of the deadliest gynecological malignancies worldwide[2-5]. The most widely accepted theory for the etiology of endometriosis is retrograde menstruation into the peritoneal cavity and ectopic growth of endometrial tissue on the ovaries and other internal organs [6]. Malignant transformation of ovarian cysts or endometriomas (OE) to different types of ovarian cancer is well documented[7-10], but environmental factors that promote malignant transformation are largely unknown.

Bisphenol A (BPA) is a chemical used in polycarbonate plastics and epoxy resins commonly found in food/beverage packaging and containers[11]. Humans are ubiquitously exposed to BPA, primarily through oral ingestion, with measurable levels detected in >90% of urine samples in the U.S. population[12]. BPA is an endocrine-disrupting chemical (EDC) that alters normal hormone function through its action on estrogen receptors. At low concentrations, BPA acts preferentially on the **G protein-coupled estrogen receptor (GPER, Gpr30)**[13]. GPER signaling mediates the immune response and neoplastic transformation of endometrial and ovarian cancers[14,15] and its overexpression is associated with poor survival[16-19]. BPAF, a fluorinated BPA analog, docks effectively in the GPER ligand binding pocket, and demonstrates ~9-fold stronger binding affinity and more potent activation of GPER than BPA[20,21]. BPA exposure is linked to a wide range of adverse female reproductive health issues in humans and experimental animals[22-24], including endometriosis[25], and increased risk for endometrial and ovarian cancer[26-30]. *However, little is currently known about the potential role of exposure to BPAF, a newer 'BPA-free' substitute used in food packaging that appears to be an even more potent EDC than the parent compound*.

Better animal models are needed to study the role of exposure to EDCs and risk for EAOC. OE are thought to originate from attachment and growth of ectopic menstrual endometrium on the ovaries. However, unlike humans, rodents do not menstruate, and have a membranous bursa that encapsulates the ovaries and distal oviduct, separating it from the peritoneal cavity. Physiologically relevant models that phenocopy human disease are needed to accurately study the role of EDCs in the pathogenesis of EAOC. Our innovative translational mouse model of peritoneal and ovarian endometriosis addresses these limitations and will be used to examine the role of BPAF exposure and activation of GPER-mediated signaling in risk for EAOC.

HYPOTHESIS: *BPAF exposure and activation of GPER-mediated signaling in ectopic ovarian endometriotic (OE) lesions and peritoneal macrophages coordinate different aspects of pathogenesis that increase risk for progression to EAOC.*

AIM 1: Determine the role of dietary BPAF exposure in a physiologically relevant translational mouse model of OE on lesion pathology and risk for progression to EAOC

AIM 2: Determine the role of BPAF activation of GPER signaling in (donor) OE lesions vs. (recipient) peritoneal macrophages in the establishment and progression of OE to EAOC

The overall objectives are to (1) use a novel translational mouse model of PE/OE that accurately phenocopies human disease to examine BPAF exposure (dose-response and time) on risk for progression to EAOC, and (2) use genetically modified *Gper* wildtype and knockout mice to determine the relative role of BPAF induction of GPER-mediated signaling in OE lesions vs. peritoneal macrophages in establishment of lesions, pathology, and risk for EAOC. The long-term goal of this research is to contribute to a better understanding of the environmental factors, cell types, and signaling pathways involved in the initiation and progression of OE to EAOC.

C. SIGNIFICANCE: The FDA withdrew authorization for the use of BPA in baby bottles and sippy cups in 2012 and infant formula containers in 2014. However, BPA continues to be widely used in other products that contact food. In 2021, experts at the European Food Safety Authority (EFSA) reported that adverse health effects of BPA exposure occurred at levels significantly lower than previously acknowledged[31]. In response to public health concerns, manufacturers started replacing BPA with structurally similar "BPA-free" analogs, but relatively little is known about the potential toxicity of these substitutes, some of which (ie. BPAF) appear to be even more potent EDCs. In January 2022, several scientific and environmental organizations submitted a petition to the FDA expressing an urgent need for further research to examine potential adverse health effects and reassess BPA approval in products/packaging that contact food[32].

BPA promotes endometriosis and increased incidence of ovarian cysts in women[25,28-30], and lesion growth and atypical endometrial hyperplasia in women and mice[33,34]. BPAF is a potent activator of GPER[20,21], and GPER signaling stabilizes expression of **hypoxia-inducible factor (HIF1a)**[35-37], a key regulator of the cellular response to hypoxia and oxidative stress. Atypical endometriotic lesions and increased expression of HIF1a in ectopic OE lesions are predictive factors for progression and metastasis in ovarian carcinoma[38-40]. GPER/HIF1a signaling stimulates aerobic glycolysis in endometriomas and peritoneal fluid[41]. This metabolic shift creates a favorable niche and immune escape for cancer stem cells and promotes polarization of macrophages (Mφ) to an anti-inflammatory 'M2' phenotype[42] associated with ovarian cancer progression and malignancy[43,44]. M2φ express the hemoglobin scavenger receptor CD163 to mediate endocytic uptake of hemoglobin complexes and **heme oxygenase-1 (HO-1)** which acts as a potent antioxidant to protect cells from iron-induced toxicity and oxidative damage[45].

GPER, HIF-1α and HO-1 are widely expressed not only in OE lesions and cancer cells, but also in different innate immune cells, including M2φ[46,47]. However, the role of BPAF exposure and activation of GPER/HIF1a/HO-1 signaling in these different cell types in the pathogenesis of OE and risk for progression to EAOC is unknown. We intend to fill these *critical knowledge gaps* by using a novel translational mouse model of OE to test the hypothesis that BPAF metabolites cause DNA damage and oxidative stress, while BPAF activation of GPER/HIF1a signaling in OE creates a favorable metabolic niche for tumor stem cells, and GPER/HIF1a/HO-1 signaling in peritoneal Mφ that infiltrate OE lesions promotes M2 polarization to support immune evasion of transformed cells and facilitate progression to EAOC.

Innovation: Better animal models are needed to adequately assess environmental risk factors involved in the pathophysiology and progression of OE to EAOC. Existing models have important limitations[48]. Implantation of cells or tissues other than *menstrual* endometrium do not phenocopy the human condition or the complexity of the normal hormonal milieu and its role in pathogenesis. Rodent models that are immunocompromised or have an intact ovarian bursa are impractical for assessing the role of (infiltrating) peritoneal immune cells, and injection of cancer cells into the bursa preclude the ability to study factors involved in malignant transformation and progression. Our innovative translational mouse model of PE/OE endometriosis takes these factors into consideration. The mice are immunocompetent, both donor and recipient are 'menstruating' at the time of transplant (hormonally in sync), the ovarian bursa is opened to allow contact with the peritoneal cavity and immune cells in peritoneal fluid, and minimally invasive surgical techniques are used to implant menstrual endometrium in the peritoneal cavity and on the ovaries. In addition, the use of genetically modified *Gper* wildtype (wt) and knockout (ko) mice will enable us to examine the relative role of BPAF activation of GPER signaling in endometrial (donor) OE lesions vs. GPER signaling in infiltrating M2φ derived from (recipient) peritoneal fluid.

D. PRELIMINARY DATA

Unlike humans, the ovaries and opening to the fallopian tubes in mice are encapsulated in a membranous bursa that separates them from the peritoneal cavity. Current mouse models of EAOC utilize (1) genetically engineered strains with mutations that predispose to EAOC; (2) orthotopic intrabursal xenograft transplantation of human OC cell lines in immunocompromised mice; or (3) intrabursal injection of *in vitro* transformed ovarian surface epithelial cells in syngeneic models[48,49]. The limitation of these models is that the ovarian bursa remains intact, which prevents contact of OE with peritoneal fluid and immune cells (which we hypothesize are part of the physiologically relevant microenvironment in OE transformation/progression to EAOC). Due to these limitations, we have developed a novel physiologically relevant mouse model that better phenocopies human disease. Building on our existing laparoscopic mouse model of peritoneal endometriosis, we will add a modification of the Hayashi[50] procedure and perform a bilateral bursectomy (exposing the ovaries to peritoneal fluid and immune cells) followed by transplantation of donor menstrual endometrial tissue on the surface of each ovary to establish OE.

Peritoneal Endometriosis (PE): Mice are housed in micro-isolator cages in the Creighton Animal Resource Facility (ARF) and maintained on TEK Fresh (paper) bedding and Teklad global soy-free extruded autoclavable diet to ensure that exposure to phytoestrogens (that activate GPER) are not confounding variables. All procedures are IACUC approved. B6.129S6-*Gper1*^{tm1Cwan}/J (*Gper*) wt or ko females are used as donors and recipients for laparoscopic implantation of decidualized (menstrual) endometrial tissue. Female mice are placed overnight with vasectomized males and checked in the morning for a vaginal plug (d0.5). On (day) d4.5, decidualization is induced in time-matched pseudopregnant donor and recipient mice by infusion of 100 µl sesame oil into both uterine horns. Pseudopregnant decidualized mice display overt bleeding (visible outside the vagina) between d7-d9.5[51,52] [**Fig. 1A, B**]. On d9.5, donors are sacrificed, the uterus removed [**Fig. 1C**], and decidualized endometrium dissected away from myometrium. Recipients are anesthetized, intubated, connected to a mechanical rodent ventilator, and prepped for surgery (Sx). A small abdominal incision is made, and an endoscope with a video camera, insufflation shield and light source placed into the peritoneal cavity. A 14G catheter inserted into the left flank serves as a port for placement of ten 1.5 mm^3 'plugs' of donor endometrium into the peritoneal cavity (visualized on monitor) [**Fig 1D].** Intra-abdominal pressure created by intermittent insufflation allows biopsies to stay in place without suturing[53]. After biopsy placement, the endoscope is removed, incisions sutured, and mice extubated.

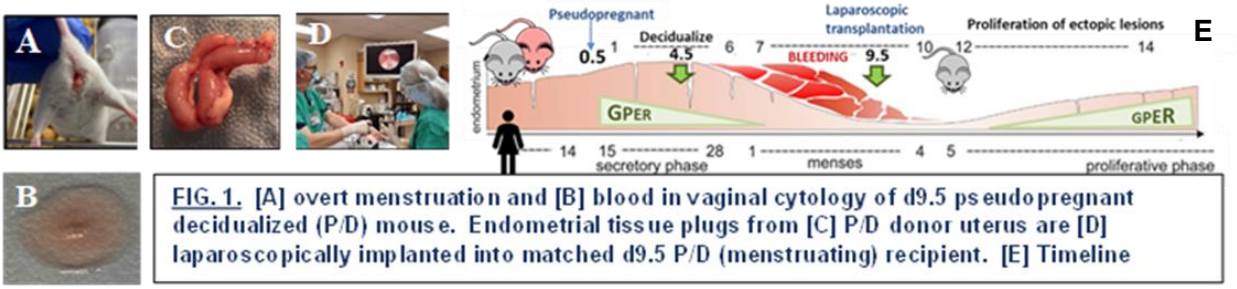


FIG. 1. [A] overt menstruation and [B] blood in vaginal cytology of d9.5 pseudopregnant decidualized (P/D) mouse. Endometrial tissue plugs from [C] P/D donor uterus are [D] laparoscopically implanted into matched d9.5 P/D (menstruating) recipient. [E] Timeline

E. EXPERIMENTAL APPROACH

AIM 1: ***Determine the role of dietary BPAF exposure in a physiologically relevant translational mouse model of OE on lesion pathology and risk for progression to EAOC***

Ovarian Endometriosis (OE): After laparoscopic implantation of peritoneal lesions, mice will be removed from the ventilator and placed in ventral recumbency. Bilateral dorsal incisions will be made to expose the ovaries, the bursal membrane incised and peeled back, and decidualized (menstrual) donor endometrial tissue plugs placed on the surface of each ovary. The ovaries will be returned to the peritoneal cavity, muscle and skin incisions sutured, and the animal recovered

from anesthesia. The bursectomy will allow contact of the ovaries with peritoneal fluid and immune cells, both of which are critical in the pathophysiology of human disease. In the Hayashi[61] protocol for establishing OE, cystic lesions with invasive margins, iron deposition, and areas of fibrosis were observed on the ovaries at 4 weeks post-implantation.

BPAF Exposure: Mice will be housed in a system with single use polyethylene (BPA-free) cages and water bottles (Innovive, San Diego, CA). Immediately following Endo (PE/OE) Sx, recipient mice will be randomly assigned to different treatment groups and placed on phytoestrogen-free control diet or diet in which BPAF has been homogeneously incorporated into the feed at 30, 300 or 900 ppm by the manufacturer (Teklad). For mice weighing 20g, this will provide an approximate daily dose of 3, 30, or 90 mg/kg/day (based on daily food consumption of ~2g/d). These BPAF doses were determined in the Jones[34] study to encompass the range of human exposures for BPA because corresponding data for BPAF are not available. In Jones et al.[34], mice on the BPAF 900 ppm diet had PE lesions significantly larger than those on control or BPA diets. Mice will be sacrificed at 30d post-op (n=5/group) to evaluate the impact of dietary exposure (dose-response) and chronic BPAF agonism of GPER on establishment and growth of OE. A second cohort (n=5) will be sacrificed at 90d to examine OE lesions for gross morphological and histopathological changes indicative of atypical endometrial hyperplasia, and progression to EAOC (clear cell carcinoma or endometrioid carcinoma). If no changes are observed at 90d, additional cohorts of animals chronically exposed to BPAF (n=5/group) will be examined at 180d post-op. As a point of reference, in a rat model of EAOC using endometrial auto-implantation coupled with DMBA induction, all rats had endometrioid ovarian carcinoma at 20 weeks (~140d post-op)[54].

Lesion Endpoints: Daily vaginal cytology (beginning at ~3 weeks post-op) will be used to determine if mice return to normal estrus cyclicity. The number ('take rate') and location of ectopic peritoneal and ovarian lesions will be recorded and photographed, and phenotype (color, size, shape, hemosiderin staining) noted [**Fig. 2A**]. Peritoneal lesions, ovaries and uterus will be removed. Ovaries will be examined for gross lesions and photographed. Tissues and lesions will be fixed in 10% formalin, paraffin-embedded, (FFPE) and sectioned for histopathology and immunohistochemistry (IHC). The model 'take rate' will be determined by dividing the total number of retrieved endometriotic lesions/mouse by the total number of implanted donor PE + OE biopsies multiplied by 100 (expressed as %). Our model of peritoneal endometriosis has produced lesions in all (100%) wildtype B6(Cg)-*Tyr*$^{c\text{-}2J}$ mice examined at 30 (n=10), 60 (n=5), and 90 (n=5) days post-op; with an average take rate of 2.6 lesions/mouse after 10 implants (26%).

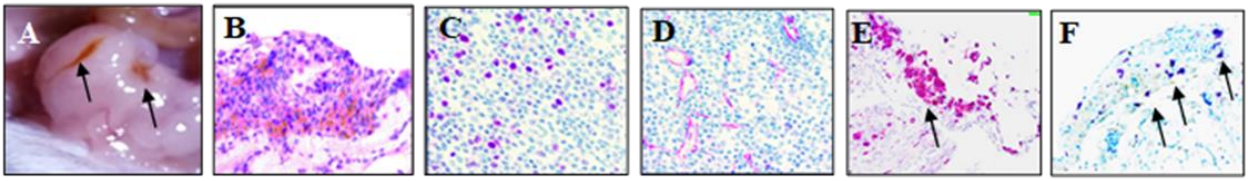


FIG. 2. [A] hemosiderin-stained lesions *in situ* (on bladder) 30d post-op. FFPE sectioned lesions (30d post-op) immunostained for [B] GPER (purple) [C] proliferation (Ki67, purple) [D] endothelial cells, angiogenesis (CD31, red) [E] CD68^{+} macrophages (red), and [F] CD163^{+} macrophages (purple)

Histopathology/Immunohistochemistry (IHC): FFPE donor endometrium and recipient tissues (eutopic uterus ectopic lesions, ovaries) will be sectioned at 5 µm, and H&E stained in the Creighton Histology Core Facility. H&E slides will be examined for histopathology (Dr. Abdulrahim). The number of confirmed PE and OE lesions/mouse, number and stage of ovarian follicles, and size of each lesion will be recorded, along with a detailed description of the findings (lesion subtype, macrophage infiltration, hemosiderin). Additional sections will be immunostained to examine GPER expression, and markers of proliferation (Ki67), angiogenesis CD31), and macrophage phenotype (**Fig. 2B-F**). Expression of HIF1a and HO-1 will be examined, and Prussian Blue used to identify iron deposition. In studies of human BPA exposure, 8-OHdG and

4-HNE are reliable biomarkers of BPA-induced oxidative stress[55]. IHC will be used to evaluate 4-HNE, an indicator of lipid-peroxidation products generated by the iron-catalyzed Fenton reaction, and 8-OHdG, a product of DNA base oxidation. Antibody concentrations will be optimized and IHC staining performed in the UNMC Tissue Science Facility. Stained sections will be photographed using the VS120 Virtual Slide Scanner or Nikon Eclipse Ci-L microscope in the CU histology core facility and staining quantitated using Image J software (https://imagej.nih.gov/ij/). *Statistical Analysis*: For histology/IHC, a minimum of 3 sections/stain (technical replicate) and lesions harvested from a minimum of 3 different animals (biological replicates) will be used for the Image J analysis. Statistical analyses for lesion, ovary and histology/IHC data will be performed using one-way ANOVA with Tukey's post-test and further comparisons between control + BPAF groups analyzed with an unpaired T-test. Differences will be considered significant when $p<.05$.

AIM 2: ***Determine the role of BPAF activation of GPER signaling in (donor) OE lesions vs. (recipient) peritoneal macrophages in the establishment and progression of OE to EAOC***

BPA activation of GPER signaling in different cell types may coordinately orchestrate OE progression to EAOC. Altered redox status is involved in malignant transformation of OE[56,57]. BPA(F) is metabolized in the liver to reactive quinone intermediates that act as DNA adducts[58] causing DNA damage, lipid peroxidation, and oxidative stress[59-61], and BPAF binds GPER to activate signaling[62]. We hypothesize that BPAF metabolites induce DNA damage, oxidative stress, and genomic instability (transformation) while BPAF activation of GPER/HIF1a/HO-1 signaling in OE and infiltrating peritoneal Mφ provide a favorable niche for tumor cell survival, and polarization of M2φ that stimulate proliferation, angiogenesis, and progression to EAOC.

Donor	Recipient	Endpoint
Gper wt	*Gper* ko	30d, 90d post-op
Gper ko	*Gper* wt	

Gper mice (JAX) have been cryorecovered and a breeding colony established. *Gper* wt and ko mice will be used to examine the relative contribution of BPAF exposure and GPER agonism in (donor) OE vs. (recipient) peritoneal fluid and infiltrating Mφ in establishment of atypical endometrial hyperplasia and OE progression to EAOC. To accomplish this goal, *Gper* wt donor menstrual endometrial tissue will be transplanted into *Gper* ko recipients, or vice versa. Surgical procedures, treatment groups (control vs. BPAF diets), lesion endpoints (30d and 90d) and histology/IHC analyses will be as described in AIM 1.

Expected Results: In **Aim 1** we anticipate a positive dose-response correlation between BPAF exposure and PE, OE lesion number and/or size, and presence of atypical endometrial hyperplasia. Blood and urine will be collected prior to sacrifice for future LCMS determination of BPAF levels. We expect to find increased expression of GPER, HIF1a, HO-1, markers of oxidative stress in OE lesions, and increased infiltration of M2φ. In **Aim 2** we expect GPER activity in both donor OE and recipient Mφ will be needed for lesion survival. GPER in donor (wt) tissue may result in BPAF-induced oxidative stress, ferroptosis (cell death), and failure to establish OE lesions if GPER is absent in (ko recipient) infiltrating M2φ to provide the anti-inflammatory microenvironment necessary for tumor cell survival. We expect the results to be informative concerning the relative importance of BPAF activated GPER signaling in donor OE/resident Mφ vs. recipient infiltrating Mφ from peritoneal fluid. No animal models have examined the role of BPAF (or BPA) exposure on OE and risk for EAOC. Our novel translational mouse model of PE/OE will fill this gap and address the critical need for further research concerning BPAF exposure and adverse health outcomes. Current pharmacokinetic studies are being used to optimize dosing and delivery of an available GPER antagonist (G36) to test effectiveness for prevention and treatment in our mouse model of PE. Data generated herein will be used to apply for NIH funding to further examine BPAF exposure (relative to BPA), underlying mechanisms associated with risk for OE/EAOC, and therapeutic efficacy of GPER inhibition.

F. LITERATURE CITATIONS:

1. Zondervan, EF, Becker, CM, Missmer, SA (2020) Endometriosis, NEJM 382(13):1244-1256.

2.Kralickova, M, Lagana, AS, Ghezzi, F, Vetvicka, V (2020) Endometriosis and risk of ovarian cancer: what do we know? Archives of Gynecology and Obstetrics 301:1-10.

3. Scarfone G, Bergamini A, Noli S, Villa A, Cipriani S, Taccagni G, Vigano' P, Candiani M, Parazzini F and Mangili G (2014) Characteristics of clear cell ovarian cancer arising from endometriosis: A two center cohort study. Gynecol Oncol 133: 480-484.

4. Sayasneh A, Tsivos D and Crawford R (2011) Endometriosis and ovarian cancer: A systematic review. ISRN Obstet Gynecol 2011: 140310.

5. Heidemann LN, Hartwell D, Heidemann CH and Jochumsen KM (2014) The relation between endometriosis and ovarian cancer-a review. Acta Obstet Gynecol Scand 93: 20-31.

6. Sampson JA. (1925) Heterotopic or misplaced endometrial tissue. American Journal of Obstetrics and Gynecology. 10(5):649–664.

7. Murakami, K, Kotani, Y, Shiro R, Takaya, H, Nakai, H, Matsumura, N (2020) Endometriosis-associated ovarian cancer occurs early during follow-up of endometrial cysts. International J of Clin Oncol 25:51-58.

8. Gaia-Oltean, AI, Braicu, C, Gulei, D, Ciortea, R, Mihu, D, Roman, H, Irimie, A, and I Berindan-Neagoe (2021) Ovarian endometriosis, a precursor of ovarian cancer: Histological aspects, gene expression and microRNA alterations (Review). Experimental and Therapeutic Medicine 21:243.

9. Hermens M, van Altena AM, Bulten J, van Vliet H, Siebers AG, Bekkers RLM (2021). Increased Incidence of Ovarian Cancer in Both Endometriosis and Adenomyosis. Gynecol Oncol 162(3):735-740.

10. Munksgaard, PS, Blaakaer, M. (2012) The association between endometriosis and ovarian cancer: a review of histological, genetic, and molecular alterations. Gynecol Oncol 124(1):164-9.

11. Siracusa JS, Yin L, Measel E, Liang S, Yu X (2018) Effects of Bisphenol A and its analogs on reproductive health: A mini review. Reprod Toxicol 79:96-123.

12. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. (2008) Exposure of the U.S. population to Bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ Health Perspect 116(1):39-44.

13. Périan S, Vanacker JM. (2020) GPER as a Receptor for Endocrine-Disrupting Chemicals (EDCs). Front Endocrinol (Lausanne). 11:545.

14.. Filardo EJ, Thomas P. (2012) Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. Endocrinology 153(7):2953-62.

15. Hernandez-Silva, CD, Villegas-Pineda, JC and AL Pereira-Suarez (2020) Expression and role of the G protein-coupled estrogen receptor (GPR30/GPER) in the development and immune response in female reproductive cancers. Frontiers in Endocrinology, Vol. 11; article 544.

16. Smith, HO, Leslie, KK, Singh, M, Qualls, CR, Revankar, CM, Joste, NE, Prossnitz, ER. (2007) GPR30: a novel indicator of poor survival for endometrial carcinoma. Am J Obstet Gynecol 196:386 e1-e9; discussion 386.e9-e11.

17. Smith, HO, Arias-Pulido, H, Kuo, DY, Howard, T, Qualls, CR, Lee SJ, Verschraegen, CE, Hathaway, HJ, Joste, NE, Prossnitz, ER (2009) GPR30 predicts poor survival for ovarian cancer. Gynecol Oncol 114:465-471.

18. Long, L, Cao, Y., Tang, LD (2012) Transmembrane estrogen receptor GPR30 is more frequently expressed in malignant than benign ovarian endometriotic cysts and correlates with MMP-9 expression. Int J Gynecol Cancer 22: 539-545.

19. Fujiwara, S, Terai, Y, Kawaguchi, H, Takai, M, Yoo, S, Tanaka, Y, Tanaka, T, Tsunetoh, S, Sasaki, H, Kanemura, M, Tanabe, A, Yamashita, Y, Ohmichi, M (2012) GPR30 regulates the EGFR Akt cascade and predicts lower survival in patients with ovarian cancer. J Ovar Res 5:35.

20. Liu X, Xue Q, Zhang H, Fu J, Zhang A (2021) Structural basis for molecular recognition of G protein-coupled estrogen receptor by selected bisphenols. Sci Total Environ 793:148558.

21. Cao LY, Ren XM, Li CH, Zhang J, Qin WP, Yang Y, Wan B, Guo LH. (2017) Bisphenol AF and Bisphenol B Exert Higher Estrogenic Effects than Bisphenol A via G Protein-Coupled Estrogen Receptor Pathway. Environ Sci Technol. 51(19):11423-11430.

22. Chapin RE, Adams J, Boekelheide K, Gray LE Jr, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenbergh JG, Woskie SR. (2008) NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res B Dev Reprod Toxicol. 83(3):157-395.

23. Rochester, J.R. (2013) Bisphenol A and human health: a review of the literature. Reprod Toxicol 42:132-55.

24. Caserta D., Di Segni N., Mallozzi M., Giovanale V., Mantovani A., Marci R., Moscarini M. (2014) Bisphenol A and the female reproductive tract: An overview of recent laboratory evidence and epidemiological studies. Reprod. Biol. Endocrinol 12:37.

25. Simonelli A, Guadagni R, De Franciscis P, Colacurci N, Pieri M, Basilicata P, Pedata P, Lamberti M, Sannolo N, Miraglia N. (2017) Environmental and occupational exposure to Bisphenol A and endometriosis: urinary and peritoneal fluid concentration levels. Int Arch Occup Environ Health 90(1):49-61.

26. Hafezi, SA., Abdel-Rahman, WM. (2019) The endocrine disruptor Bisphenol A (BPA) exerts a wide range of effects in carcinogenesis and response to therapy. Curr Mol Pharmacol 12(3):230-238.

27. Ptak A, Hoffmann M, Gruca I, Barć J. (2014) Bisphenol A induces ovarian cancer cell migration via the MAPK and PI3K/Akt signalling pathways. Toxicol Lett. 229(2):357-65.

28. Dumitrascu, M.C., Mares, C., Petca, R.C., Sandru, F., Popescu, R.I., Mehedintu, C., Petca, A. (2020) Carcinogenic effects of Bisphenol A in breast and ovarian cancers. Oncol Lett 20(6):282.

29. Shafei, A, Ramzy, MM, Hegazy, AI, Husseny, AK, El-Hadary, UG, Taha, MM, Mosa, AA (2018) The molecular mechanisms of action of the endocrine disrupting chemical Bisphenol A in the development of cancer. Gene 647:235-243.

30. Khan, NG, Correia, J, Adiga, D, Rai, PS, Dsouza, HS, Chakrabarty, S, Kabekkodu, SP (2021) A comprehensive review on the carcinogenic potential of Bisphenol A: clues and evidence. Environ Sci Pollut Res Int 16:19643-19663.

31. EFSA Panel on Food Contact Materials, Enzyme and Processing Aids (CEP), Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, November 24, 2021 at https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l1v00000E8BRD/pc0109.

32. BPA Food Additive petition to FDA at https://blogs.edf.org/health/files/2022/01/EDF-et-al-BPA-Food-Additive-Petition-FINAL-1-27-22.docx.pdf

33. Newbold RR, Jefferson WN, Padilla-Banks E (2007) Long-term adverse effects of neonatal exposure to Bisphenol A on the murine female reproductive tract. Reprod Toxicol 24(2):253-8.

34. Jones, RL, Lang, SA, Kendziorski, JA, Greene, AD, Burns, KA (2018) Use of a mouse model of experimentally induced endometriosis to evaluate and compare the effects of Bisphenol A and Bisphenol AF exposure. Environ Health Perspect 126(12):127004

35. Zhang, L, Xiong, W, Li N, Liu, H, He, H, Du, Y, Zhang, Z, Liu, Y (2017) Estrogen stabilizes hypoxia inducible factor 1 α through G protein coupled estrogen receptor 1 in eutopic endometrium of endometriosis 107(2):439-447.

36. Zhang Y, Song Y, Ren S, Zhang M, Zhang Z, Fan S, Liu X, Peng X, Qi Q, Shen X, Chen Y (2022) GPER-mediated stabilization of HIF-1α contributes to upregulated aerobic glycolysis in tamoxifen-resistant cells. Oncogene doi.org/10.1038/s41388-022-02506-4.

37. De Francesco EM, Pellegrino M, Santolla MF, Lappano R, Ricchio E, Abonante S, Maggiolini M, (2014) GPER mediates activation of HIF1α/VEGF signaling by estrogens. Cancer Res 10.1158/0008-5472.CAN-13-3590.

38. Varga, J, Reviczka, A, Hakova, H, Svajdler, P, Rabajdova, M, Ostro, A (2022) Predictive factors of endometriosis progression into ovarian cancer. J Ovarian Res 15(1):5.

39. Galanis A, Pappa A, Giannakakis A, Lanitis E, Dangaj D, Sandaltzopoulos R (2008) Reactive oxygen species and HIF-1 signalling in cancer. Cancer Letters 266(1):12-20.

40. Seeber LMS, Horree N, Vooijs MAGG, Heintz APM, van der Wall E, Verheijen RHM, van Diest PJ (2011) The role of hypoxia inducible factor-1alpha in gynecological cancer. Crit Rev Oncol Hematol 78:173-184.

41. Wendel, JRH, Wang, X, and SM Hawkins (2018) The Endometriotic Tumor Microenvironment in Ovarian Cancer. Cancers (Basel) 10(8):261.

42. Kobayashi, H and S Imanaka (2022) Understanding the molecular mechanisms of macrophage polarization and metabolic reprogramming in endometriosis: A narrative review. Reprod Med Biol 21(1):e12488.

43. Schweer D, McAtee A, Neupane K, Richards C, Ueland F, Kolesar (2022) Tumor-Associated Macrophages and Ovarian Cancer: Implications for Therapy. 14(9):2220.

44. Jammal MP, Lopes AD, Etchebehere RM, Murta EFC, Nomelini RSJ (2022) Mast cells and M2 macrophages in ovarian cancer. Obstet Gynaecol. 22:1-7.

45. Hecht JL, Janikova M, Choudhury R, Liu F, Canesin G, Janovicova L, Csizmadia E, Jorgensen EM, Esselen KM, Celec P, Swanson KD, and B Wegiel (2022) Labile Heme and Heme Oxygenase-1 Maintain Tumor-Permissive Niche for Endometriosis-Associated Ovarian Cancer. Cancers 14:2242.

46. Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, Mariani M, Brignole C, Ponzoni M, Ferrari S, Panina-Bordignon P, Manfredi AA, Rovere-Querini P (2009) Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 175(2):547-56.

47. Heublein S, Vrekoussis T, Kuhn C, Friese K, Makrigiannakis A, Mayr D, Lenhard M, Jeschke U (2013) Inducers of G-protein coupled estrogen receptor (GPER) in endometriosis: potential implications for macrophages and follicle maturation. J Reprod Immunol 97(1):95-103.

48. Karakashev, S and RG Zhang (2021) Mouse models of epithelial ovarian cancer for preclinical studies. Zoological Research 42(2):153-160.

50. Hayashi, S, Nakamura, T, Motooka, Y, Ito, F, Jiang, L, Akatsuka, S, Iwase, A, Kajiyama, H, Kikkawa, F, Toyokuni, S (2020) Novel ovarian endometriosis model causes infertility via iron-mediated oxidative stress in mice. Redox Biol 37:101726.

51. Rudolph M, Docke WD, Muller A, Menning A, Rose L, Matthias Z, Gashaw I (2012) Induction of overt menstruation in intact mice. PLoS ONE 7(3):e32922.

52. Greaves E, Cousins FL, Murray A, Esnal-Zufiaurre, A, Fassbender, A, Horne, AW, Saunders, PT (2014) A novel mouse model of endometriosis mimics human phenotype and reveals insights into the inflammatory contribution of shed endometrium. Am J Pathol 184(7):1930-1939.

53. Peterse D, Binda MM, O DF, Vanhie A, Fassbender A, Vriens J, D'Hooghe TM. (2017) Of Mice and Women: a Laparoscopic Mouse Model for Endometriosis. J Minim Invasive Gynecol pii: S1553-4650(17)31232-3.

54. Wuyung, PE, Rahadiati, FB, Tjahjadi, H, Salinah, S, Kusmardi, K, Kodariah R, Wiweko, B (2021) Histopathology and ARID1A Expression in Endometriosis-Associated Ovarian Carcinoma (EAOC) Carcinogenesis Model with Endometrial Autoimplantation and DMBA Induction. Asian Pac J Cancer Prev 22(2):553-558.

55. Steffensen IL, Dirven H, Couderq S, David A, D'Cruz SC, Fernández MF, Mustieles V, Rodríguez-Carrillo A, Hofer T. (2020) Bisphenols and Oxidative Stress Biomarkers-Associations Found in Human Studies, Evaluation of Methods Used, and Strengths and Weaknesses of the Biomarkers. Int J Environ Res Public Health 17(10):3609.

56. Fujimoto, Y, Imanaka, S, Yamada Y, Ogawa K, Ito F, Kawahara N, Yoshimoto Ch, Kobayashi H (2018) Comparison of redox parameters in ovarian endometrioma and its malignant transformation. Oncol Lett 16(4):5257-5264.

57. Kajiyama, H, Suzuki, S, Yoshihara, M, Tamauchi, S, Yoshikawa, N, Niimi, K, Shibata, K, and F Kikkawa (2019) Endometriosis and Cancer. Free Radical Biology and Medicine 133:186-192.

58. Jalal, N, Surendranath, AR, Pathak, JL, Yu, S, Chung, CY (2017) Bisphenol A (BPA) the mighty and the mutagenic. Toxicol Rep 5:76-84.

59. Hercog K, Maisanaba S, Filipič M, Sollner-Dolenc M, Kač L, Žegura B. (2019) Genotoxic activity of Bisphenol A and its analogues bisphenol S, bisphenol F and bisphenol AF and their mixtures in human hepatocellular carcinoma (HepG2) cells. Sci Total Environ 687:267-276

60. Gu J, Wang H, Zhou L, Fan D, Shi L, Ji G, Gu A (2020) Oxidative stress in Bisphenol AF-induced cardiotoxicity in zebrafish and the protective role of N-acetyl N-cysteine. Sci Total Environ 731:139190.

61. Gassman NR. (2017) Induction of oxidative stress by Bisphenol A and its pleiotropic effects. Environ Mol Mutagen 58(2):60-71.

62. Lei, B, Sun, S, Zhang, X, Feng, C, XXu, J, Wen, Y, Huang, Y, Wu, M, Yu, Y (2019) Bisphenol AF exerts estrogenic activity in MCF-7 cells through activation of Erk and Pi3k/Akt signals via GPER signaling pathway. Chemosphere 220:362-370.

Complete

Investigator and Proposal Information

Principal Investigator/Project Director/Fellowship Sponsor:
North, Brian

Email BrianNorth@creighton.edu
Phone 402-280-3855
Department Biomedical Sciences - Omaha

Personnel:

PI	Name	Department	Role	Net Effort
✔	North, Brian	Biomedical Sciences - Omaha	PD/PI	10.909

Originating Sponsor: State of Nebraska - LB595

Sponsor: State of Nebraska - LB595

Budget:

	Period 1	Total
Direct Costs	$78,187.63	**$78,187.63**
Indirect Costs	$0.00	**$0.00**
F&A Rate	0%	-
Total	**$78,187.63**	**$78,187.63**

Project Total Cost Sharing Direct Costs: 13188

Project Total Cost Sharing F&A Costs: 0

Start Date: 01-Aug-2023 End Date: 30-Jun-2024

Identification

Proposal Title
Identifying Regulators of Liver Cancer Metastasis

Brief description of project in plain language (1000 character limit).
The goal of this proposal is to carry out an in vivo CRISPR-based screen in models of hepatocellular carcinoma (HCC) to define genetic pathways that are important regulators of HCC cancer metastasis.

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

Please provide the link to the Sponsor Guidelines:
http://www.creighton.edu/researchservices/grants/internalgrantopportunities/lb595cancerandsmokingdiseaseresearchprogramdevelopmentgrant/

Protocols

Will your project involve...

☐ Yes ☑ No Human Subjects?

☑ Yes ☐ No Laboratory Animals?

Protocol Status:
Approved

☑ Yes ☐ No Protocol is listed on Protocol Approvals page?

☑ Yes ☐ No Recombinant DNA or other biological agents?

Protocol Status:
Approved

☐ 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 Statistical Core Facility CU Flow Cytometry Core Facility

- [ ] CU Histology Core Facility
- [ ] CU Integrated Biomedical Imaging Facility
- [ ] Other Non-CU Core Facility
- [ ] SOM COBRE Cell & Tissue Culture Core Facility
- [ ] SOM COBRE Auditory & Vestibular Electrophysiology Core Facility
- [ ] CU Innovative Genomics Core Facility
- [ ] CU Molecular Biology Research Core Facility
- [ ] SOM COBRE Advanced Microscopy Core Facility
- [ ] SOM COBRE Mass Spectrometry Core Facility
- [ ] SOM COBRE Drug Discovery & Delivery 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
- [ ] Neuroscience
- [ ] Science (Non-Health)
- [ ] Undergraduate Research
- [ ] Community Health
- [ ] Faith-Based
- [ ] Interdisciplinary
- [ ] Other
- [ ] Sustainability

Response to Reviewer Comments:

INTRODUCTION TO APPLICATION: We submitted this application to the Nebraska Department of Health and Human Services LB506 Cancer and Smoking Disease Research Program. We have been asked to provide and updated application for Creighton's internal LB595 Developmental Grant program funding opportunity.

Relevancy to cancer or smoking diseases: The reviewer(s) confirmed that the proposed studies was relevant to cancer or smoking diseases based on the study focuses on cancer metastasis, in particular of hepatocellular carcinoma (HCC) which continues to increase in frequency and is often at an advanced stage when diagnosed. In addition, metastasis of cancer is often considered a key driver of mortality in HCC as well as many other cancers.

Feasibility: The reviewer(s) indicated that "*The proposed experiments are presented in clear detail with expected results and alternate approaches are clearly detailed.*" However, they felt that "the amount of data to be generated will take several years and several bioinformatics personnel to analyze the data. This proposal is likely not feasible within this one-year mechanism." To address this concern, we have limited the study for this proposal to be focused on carrying out the screen as well as, in which we expect to inject mice with the three cell lines followed by isolating metastatic tumors followed up by sequencing gRNA to determine which guides are either enriched or drop out of the pool. We intend to also validate using *in vitro* models our top hits to determine which genes might be important for cell migration and invasion. We have removed the *in vivo* portion of the validation, which we anticipate would be the focus of future external grant applications. Thus, we anticipate that this reduced proposal will be achievable in the time frame anticipated.

In addition, our expectation is to utilize the Integrated Genomics & Bioinformatics Core (IGBC) here at Creighton for the carrying out the sequencing and post sequencing bioinformatics. Given the cores expertise in sequencing and analysis, as well as the fact that CRISPR guide enrichment/dropout screens have advance substantially over the past few years, we do not anticipate any issues with the sequencing and analysis aspects.

Strengths/Weaknesses: The reviewers(s) commented on the strengths of the proposal being a well-written proposal with a focus on a CRISPR knockout library approach, including appropriate models and validation of top target genes. However, as weaknesses they brought up the anticipated time frame to be longer than the 1-year proposal and the need for additional personnel (particularly bioinformatics). In addition, they were concerned that the proposal was a "*fishing expedition*" and "*there is no hypothesis*" as well as prior studies are found in the literature. To alleviate these concerns, we have reduced the amount of work attributed to this proposal as stated above, by removing *in vivo* validation of top targets identified in the screen. This will also dramatically reduce the cost associated with cloning and animals, as there will no longer be a need for subsequent cloning of cells to inject into mice as well as the number of mice needed for the validation studies. Again, we anticipate that these studies would form the basis of a subsequent external grant application. In addition, as stated above, we intend to use the IGBC to carry out the sequencing and bioinformatics associated with analysis of gRNA prevalence. We have also recently hired a new lab technician who will also be contributing to this work which will increase our overall effort on this proposal, and our personnel budget has been updated to reflect this. This has the added benefit of grant funds supporting internal cores. While we agree that this can be construed as a "fishing expedition" and lacks a hypothesis, we do believe that utilizing the latest technological advances and *in vivo* screening approaches will yield new insights into cancer metastasis. In addition, we view this as hypothesis generating research rather than hypothesis driven research, which fits well into the mission of the LB595 Developmental Grant program. Lastly, while the reviewer felt there were already numerous studies focused on this question, we disagree that there are over 300 studies performing *in vivo* screens for HCC metastasis, and we find that a majority of *in vivo* screens have set out to define drug resistance mechanisms rather than identify key regulators of cancer metastasis. In our latest literature review, we found only one study that carried out an HCC metastasis screen (PMID: 32266537). However, there are two key differences in our proposed screen compared to this study. One, this study uses one cell line, SMMC7721, which has questionable relationship with HCC as it is believed it may be contaminated with HeLa. Whereas our study is using three different lines, thus we will ideally focus on targets that show up in multiple lines representing a more rigorous approach. In addition, they used a subcutaneous injection model whereas our models include intracardiac as well as orthotopic which may provide a more physiologically relevant setting to HCC metastasis; thus, we anticipate we may identify novel regulators with greater relevance.

Abstract: List the application's specific aims and ***clearly state the project's relevancy to cancer or smoking disease***. Describe the research design and methods for achieving the aims. The abstract serves as a description of the proposed work when separated from the application. As much as possible, use non-technical language to convey intent.
Do not exceed the space provided.

Liver cancer is the third leading cause of cancer-related deaths worldwide and its incidence has more than doubled over the past decade. Hepatocellular carcinoma (HCC) is the most common type of liver cancer and comprises 75-85% of total liver cancer cases, suggesting that identifying the molecular basis driving HCC tumorigenesis is necessary to curtail its increased incidence and develop novel therapeutic approaches with increased efficacy compared to current treatment strategies.

Cancer metastasis is the spread of the tumor cells from its site of origin to distal regions of the body through a complex orchestrated process and is considered one of the driving mechanisms of cancer-related death in HCC as well as most other tumor types. Even with metastasis of HCC being a major mortality-driving mechanism our overall understanding of genetic pathways that are involved in promoting HCC metastasis remains limited. Thus, it is crucial to define genetic pathways that underly HCC metastasis. *In vivo* metastasis modeling is a significant advancement over cellular based approaches to model the complex nature of tumor metastasis. In this proposal we will carry out an *in vivo* CRISPR library screen to define genetic changes controlling metastatic potential of liver cancer.

To identify genetic pathways governing HCC metastasis, we will perform an *in vivo* whole-genome lentiviral knockout CRISPR library screen in mice using multiple cellular models of human HCC to identify the critical regulator(s) of metastatic HCC. We will then set out to validate the potential of selected genes from our *in vivo* screen in cellular settings to define the molecular basis by which identified genetic pathways are involved in regulating HCC metastatic capabilities.

Completion of this screen will provide the basis for future studies to assess the molecular basis of identified pathways in promoting HCC metastasis, as well as to achieve our goal of developing novel therapeutic strategies based on suppressing tumor metastasis as a therapeutic avenue to reduce or eliminate cancer related mortality.

This project is relevant to cancer as it sets out to define genetic pathways that enhance cancer metastasis, which is a major driver of cancer-related mortality both in HCC as well as many other cancer types. These studies will provide novel pathways to target to reduce or block the ability of HCC to metastasis thereby enhancing therapeutic efficacy and reducing cancer-related mortality.

Key Personnel

- The Principal Investigator is listed first.
- Behavioral sketches are required for ALL listed.
- See Application, page 8 for more details.

Name	**Organization**	**Role on Project**
Brian J. North, PhD	Creighton University School of Medicine	Principal Investigator

Budget Worksheet *Direct Costs Only*					From 7/1/2023		Through 6/30/2024
		Type of Appointment	% of Effort on Project	Institutional Base Salary	Amount Requested		
Personnel (Applicant Organization only)					Salary	Fringe Benefits	Totals
Name	Role in Project						
Brian J. North, PhD	Principal Investigator	Faculty	10%				
Niti Kumari, PhD	Postdoctoral Fellow	Staff	40%	$61,572	$24,629	$6,696	$31,325
Sachin Wagh	Laboratory Technician	Staff	30%	$37,000	$11,100	$3,018	$14,118
				Subtotals →	$35,729	$9,714	$45,443
Consultant Costs							$0
Equipment							$0
Supplies							$17,042
Travel							$0
Patient Costs	Inpatient						$0
	Outpatient						$0
Contractual or Third-Party							$0
Other							$2,515
Total Direct Costs for Budget Period *Also indicate at #6 on the Face Page.*							**$65,000.00**

Budget Justification

- Use continuous pages as needed.
- Explain and itemize the costs captured on the Budget Worksheet.
- See Application, pages 8-10 for more details.

Budget Justification

Personnel:

Brian J. North, Ph.D. – Principal Investigator: 10% effort. Dr. North is a tenure-track assistant professor in the Biomedical Sciences Department at Creighton University. Dr. North will be responsible for the overall administration and direction of the project, in addition to data analysis. Dr. North has been trained in studying the molecular basis of tumorigenesis using both *in vitro* and *in vivo* studies. As per guidelines, no salary and fringe support are requested.

Niti Kumari, Ph.D. - Postdoctoral Fellow: 40% effort. Dr. Kumari has previous experience in molecular and cellular biology of cancer, as well as mouse handling experience. She will be responsible for carrying out the proposed studies under the guidance of Dr. North. Commensurate salary and fringe support are requested.

Sachin Wagh, M.S. - Technician: 30% effort. Mr. Wagh has previous experience in molecular and cellular biology. He will be responsible for carrying out the proposed studies under the guidance of Drs. Kumari and North. Commensurate salary and fringe support are requested.

External Funding Personnel Fringe Rate: 27.19%

Non-Personnel:

Total Non-Personnel Costs: $19,557

Supplies:

Lab Chemicals:	$1,500
Tissue Culture Supplies:	$5,200
Molecular Biology:	$2,000
DNA Oligos/shRNAs/siRNAs:	$500
Tumor tissue sequencing:	$6,000
Animal Order*:	$1,842

Other:

Animal per diems**:	$2,515

* We will order two male and six female NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice from the Jackson Laboratories for establishing our experimental cohorts (Based on Jax.org and recent orders, an eight week male costs ~$181.75, an eight week female costs ~$222.75, crate charges are ~$30.00, and shipping charges are ~$112.00). This animal model is chosen as it provides an ideal host when transplanting human cancer cell lines as xenografts for these studies.

** We expect to use 60 mice in our studies with an anticipated lifespan of 84 days at $0.33 per day *per diem* charge for a total of $1,663.20 for our experimental studies. We expect that it will costs $851.80 in *per diem* charges for breeding the initial eight animals acquired from the Jackson laboratories to reach our required cohort numbers for a total expected budget of $2,515 in *per diem* animal charges.

OMB No. 0925-0001 & 0925-0002 - Modified for NE 506 Application (Rev. 09/17 Approved Through 03/31/2020)

Biographical Sketch

Provide the following information for all Key Personnel listed. Begin with the Principal Investigator. Follow this format for each person. **DO NOT EXCEED TWO (2) PAGES PER SKETCH.**

NAME: Brian J. North, PhD

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

POSITION TITLE: 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	Completion Date MM/YYYY	FIELD OF STUDY
Gustavus Adolphus College, St. Peter, MN	B.A.	09/1999	Biomedical Sciences
University of California, San Francisco, CA	Ph.D.	12/2005	Biomedical Sciences
Harvard Medical School, Boston, MA	Postdoc		Aging and Cancer

A. Personal Statement

The major focus of my research laboratory is to understand the molecular and cellular basis of aging and its interrelationship with the development of age-related diseases, with a major focus on tumorigenesis. In particular, I am interested in protein homeostasis pathways that control the stability of tumor suppressors and oncogenes, and where dysregulation of these protein homeostasis pathways leads to tumorigenesis. We recently identified regulation of CHK1 ubiquitination by the E3 ubiquitin ligase $SCF^{\beta\text{-}TrCP}$ in response to glucose deprivation which may promote further genome instability in centrally located cells within a tumor mass (***Molecular Oncology,*** 2018). In addition, we also identified is targeted by the E3 ubiquitin ligase SCF^{Fbw7} in an Akt-dependent manner (***Oncotarget,*** 2014). We have also contributed to studies identifying a role for $SCF^{\beta\text{-}TrCP}$ in targeting Lipin1 for degradation in the liver to control lipogenesis (***Science Signaling,*** 2017), and deubiquitinase-mediated regulation of tumorigenesis through controlling the mTOR pathway (***Nature,*** 2017). In particular, we identified a regulatory circuit where Smurf1-mediated ubiquitination and destabilization of the tumor suppressor DAB2IP plays a role in metastatic potential including cellular proliferation and migration (***Oncotarget,*** 2016).
The current proposed studies are intimately tied to our goals of understanding molecular basis of tumorigenesis with a particular interest in the metastatic process in liver cancer, a major driver of liver cancer associated death. We are well positioned to carry out these studies by screening for genes that are key regulators of hepatocellular carcinoma (HCC) growth and metastasis, elucidate the mechanisms that candidate factors regulate HCC metastatic potential, and in the long-term, identify and developing novel treatment strategies aimed at HCC growth and metastasis.

B. Positions and Honors

Employment:

2006-2013 Research Fellow in Pathology – Department of Genetics, Harvard Medical School, Boston, MA. (Mentor: Dr. David A. Sinclair).
2013-2019 Instructor – Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.
2019-Pres Assistant Professor – Department of Biomedical Sciences, Creighton School of Medicine, Omaha, NE

Honors/Awards:

1999 Beta Beta Beta Student Research Award.
1999 Gustavus Adolphus College Department of Biology Student Research Award.
2006-2008 BIDMC/National Institutes of Aging T32 Translational Research in Aging Award.
2009 Gustavus Adolphus College Decade Award
2012 Guest Editor, Special Issue "Cardiovascular Aging Review Series", *Circulation Research.*
2016-2021 National Institutes of Health K01 Career Development Award (NIH/NIA AG052627)

C. Contributions to Science within the past five (5) years – including complete references of peer-reviewed publications – pertinent to this application only.

I have had a longstanding interest in mechanisms regulating tumorigenesis with a particular emphasis on post-translational modifications and their roles in tumor suppressor and oncogenic pathways. In particular, I have spent a majority of my scientific career studying acetylation as a mechanism to control pathways involved in tumorigenesis. More recently, I have developed an interest in E3 ubiquitin ligases and their role in regulating stability of tumor suppressors and oncogenes to cancer relevant pathways. Finally, in the past few years we have begun to focus our attention cell stress responses relevant to cancer with a focus on glucose homeostasis pathways.

1. Ci, Y., Li, X., Chen, M., Zhong, J., **North, B.J.**, Inuzuka, H., He, X., Li, Y., Guo, J., and Dai, X. (2018) SCFβ-TRCP E3 ubiquitin ligase targets the tumor suppressor ZNRF3 for ubiquitination and degradation. *Protein & Cell*. *doi: 10.1007/s13238-018-0510-2*.
2. Ma, Y., Cui, C., Xiong, X., Inuzuka, H., Wei, W., Sun, Yi., **North, B.J.,**[#] and Zhao, Y.[#] (2018) $SCF^{\beta\text{-}TrCP}$ Ubiquitinates CHK1 in an AMPK-Dependent Manner in Response to Glucose Deprivation. *Molecular Oncology*. *13*:307-321.
3. Yang Gao, Naoe T. Nihira, Xia Bu, Chen Chu, Jinfang Zhang, Aleksandra Kolodziejczyk, Yizeng Fan, Ngai Ting Chan, Leina Ma, Jing Liu, Dong Wang, Xiaoming Dai, Huadong Liu, Masaya Ono, Akira Nakanishi, Hiroyuki Inuzuka, Brian J. North, Yu-Han Huang, Samanta Sharma, Yan Geng, Wei Xu, X Shirley Lui, Lei Li, Yoshio Miki, Piotr Sicinski, Gordon J. Freeman, and Wenyi Wei. (2020) Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy. **Nature Cell Biology**. *22*:1064-1075.
4. Kouhei Shimizu, Brian J. North, Satoshi Fukumoto, Wenyi Wei, Hiroyuki Inuzuka. (2021) Interplay between protein acetylation and ubiquitination controls MCL1 protein stability. **Cell Reports**. *37*:109988.
5. Jing Liu, Collin Tokheim, Jonathan D. Lee, Wenjian Gan, Brian J. North, X Shirley Liu, Pier Paolo Pandolfi, and Wenyi Wei. (2021) Genetic fusions favor tumorigenesis through degron loss in oncogenes. **Nature Communications**. *12*:6704.

North, Brian – CU LB595 (LB506) – 2024

Other Support

- Use continuous pages as needed.
- Required for the Principal Investigator only.
- Note Project Number, Source, Major Goals, Dates of Approved/Proposed Project, Annual Direct Costs, Percent Effort AND Overlap (if any) – see Application, pages 10 - 12 for more details.

Active:

1. Project Number: NIH R01 AG077574
Source: National Institute of Aging/NIH
PI: Brian J. North
Title: Regulatory Mechanisms Governing BubR1 Protein Stability During Stress and Aging
Project Goal: The goal of this proposal is to elucidate the molecular basis of BubR1 decline with age and under conditions of cellular stress.
Dates of Project: 06/01/2022 - 03/31/2027
Annual Direct Costs: $205,000
Percent Effort: 16.6%
Overlap: None

2. Project Number: LB595 Program Project Grant
Source: Nebraska Health and Human Services
PI: Brian J. North (PD: Laura Hansen)
Title: Cellular Pathways Targeting BubR1 to the Proteasome for Degradation: Implications for Skin Cancer
Major Goals: The overall goal of this program project grant is to study: "*Cellular Signaling and Molecular Trafficking in Cancer."* The goal of this specific project is to define the role of the NAD^+/SIRT2/β-TRCP/BubR1 signaling pathway in the age-related increase in susceptibility to carcinogen-induced skin tumorigenesis and its involvement in the protective effect of calorie restriction on skin cancer.
Dates of Project: 07/01/2019 - 06/30/2024
Annual Direct Costs: $75,000
Percent Effort: 2.5%
Overlap: None

3. Project Number: Health Science Strategic Investment Fund Faculty Development grant
Source: Creighton University
PI: Brian J. North
Title: Regulation of cardiac development and function through BubR1 control of the potassium channel adaptor *Kcne1*
Major Goals: The goal of this research proposal is to uncover the role for BubR1 in regulating the cardiac development and conduction through upregulating the potassium channel adaptor *Kcne1*.
Dates of Project: 07/01/2022 - 06/30/2024
Annual Direct Costs: $25,000
Percent Effort: 8.3%
Overlap: None

Pending: None

Creighton UNIVERSITY School of Medicine

Jian Zuo, Ph.D.
Chairman | Department of Biomedical Sciences
Creighton University School of Medicine
2500 California Plaza | Omaha, NE 68178
phone: 402-280-4033 | fax: 402-280-2690
e-mail: jianzuo@creighton.edu

January 8, 2023

Re: Institutional letter of commitment for LB506 application being submitted by Brian North, Ph.D.

Dear Review Committee Members,

I am writing as Chair of the Biomedical Sciences Department at Creighton University School of Medicine, to express our strongest support for Dr. Brian North, who is submitting an LB506 grant application to the Nebraska Department of Health and Human Services.

Dr. North is a tenure-track Assistant Professor in my department, where he has established a research lab with a primary focus on the molecular biology of aging and cancer. As Chair of the Biomedical Sciences Department at CU, I am fully committed to facilitating Dr. North's research and continued career development by providing all necessary resources for the establishment of his research program at CU. Dr. North devotes over 75% of his time for research during the LB506 funding period. In addition to dedicated lab (~1,000 sq. ft.) and office space, he will have full access to institutional facilities and laboratory infrastructure at CU. All required equipment and support personnel, and a fully accredited and professionally staffed animal facility, are available for the proposed studies.

Dr. North's research group has developed a proposal set out to utilize a CRISPR library to carry out an *in vivo* screen to define genetic factors involved in liver cancer metastasis. Successful completion of this proposal will both provide significant findings for publication as well as serve as a foundation for future NIH grant applications. Dr. North's lab will benefit from close interactions with cancer research programs already established at CU, where his laboratory is situated next to the labs of Dr. Laura Hansen, who is an expert at carcinogen-induced tumorigenesis, Dr. Jun Xia who performs genetic screens to define DNA damage pathways, and Dr. Patrick Swanson who studies pathways involved in lymphoid malignancies. Therefore, I believe that Dr. North is well positioned to complete this novel and innovative project that will lead to a significant advancement in our understanding of genetic pathways promoting liver cancer metastasis but will also provide the preliminary data necessary for future NIH grant applications.

Sincerely yours,

Jian Zuo, PhD
Professor and Chair, Department of Biomedical Sciences

Specific Aims

Liver cancer is the third leading cause of cancer-related deaths worldwide, accounting for more than 800,000 deaths each year (Figure 1a), and its incidence has more than doubled over the past decade[1]. Hepatocellular carcinoma (HCC) is the most common type of liver cancer and constitute 75-85% of total liver cancer cases[2], suggesting that identifying the molecular basis of this increase in tumor incidence as well as novel therapeutic strategies are desperately needed.

Cancer metastasis is the spread of the tumor cells from its site of origin to distal regions of the body through a complex orchestrated process, as is considered one of the driving mechanisms of cancer related death in HCC as well as most other tumor types. Metastatic HCC can be either intrahepatic (tumor migrates to other regions of the liver) or extrahepatic (tumor migrates to distal organs/tissues, or both. Extrahepatic HCC metastasis is the major contributing factor leading to poor survival in patients[3].

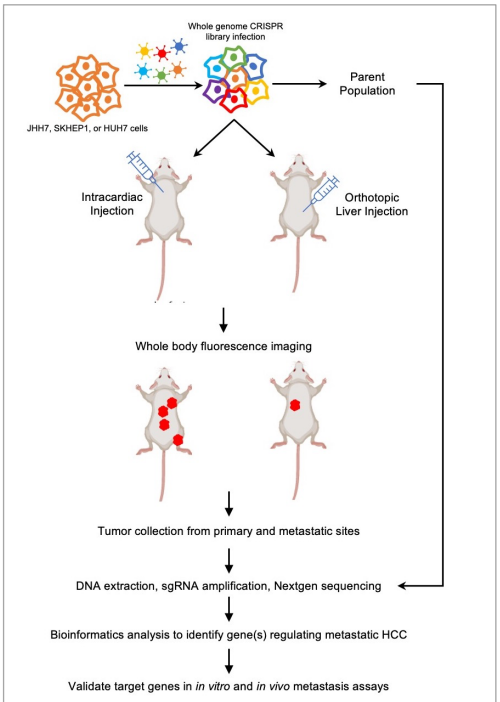


Figure 1: Schematic of CRISPR-Cas9 screening approach to identify novel regulators of liver cancer metastasis.

Our overall understanding of genetic pathways that are involved in promoting HCC metastasis is limited, therefore it is crucial to define genetic pathways involved in promoting HCC metastasis. While there are established cell culture models of cancer metastasis, *in vivo* metastasis modeling has become a significant advancement to model the complex nature of tumor metastasis. In this proposal we will carry out a CRISPR library screen to define genetic changes controlling metastatic potential of liver cancer (Figure 1).

We have developed two specific aims centered around an *in vivo* metastasis CRISPR screen. **Specific Aim 1: Screening of regulator(s) of metastatic hepatocellular carcinoma.** In this aim, we will perform an *in vivo* whole-genome lentiviral knockout CRISPR library screen in mice using multiple cellular models of human HCC to identify the critical regulator(s) of metastatic HCC. **Specific Aim 2**: **Validation of selected hit(s) as potential therapeutic target(s) for metastatic HCC.** In this aim, we will validate the potential of selected genes from our *in vivo* screen using *in vitro* models of metastatic potential.

Completion of this screen will provide the basis for future studies to assess the molecular basis of identified pathways in promoting HCC metastasis, as well as to achieve our goal of developing novel therapeutic strategies based on suppressing tumor metastasis as a therapeutic avenue to reduce or eliminate cancer related mortality.

Significance

Liver cancer is the third leading cause of cancer-related deaths worldwide, accounting for more than 800,000 deaths each year (Figure 2A) [1]. It is also the sixth most lethal cancer in the United States (Figure 2B). The incident and mortality rates of liver and intrahepatic bile duct cancers have more than doubled from 2000-2019 in United States (Figure 2C-D). This increased incidence suggests that the liver cancer associated deaths will continue to climb unless novel therapeutic strategies can be identified and brought to the clinic. Hepatocellular carcinoma (HCC) is the most common type of liver cancer and constitute 75-85% of total liver cancer cases[2]. HCC is more often associated with poor prognosis, as more than 80% cases are diagnosed at an advanced or metastatic stage, for which very limited effective treatment options are available. For example,

Sorafenib and Regorafenib are currently used for treatment of advanced stage HCC but have low response rate and provides only a modest increase in median survival of ~10-14 months[4]. Toxicity and loss of efficacy are also associated with these drugs with the development of drug resistance often occurring within six months of treatment initiation.

Importantly, HCC is often associated with metastasis, and metastatic disease is the major driver of cancer morbidity and mortality accounting for nearly 90% of cancer-related deaths. Given that diagnosis of HCC occurs most often at an advanced stage of disease and metastasis is a key driver of HCC related deaths, it is critically important to identify novel pathways and drug targets that can provide the impetus to develop therapeutics with greater efficacy, safety, and better survival benefits to these patients.

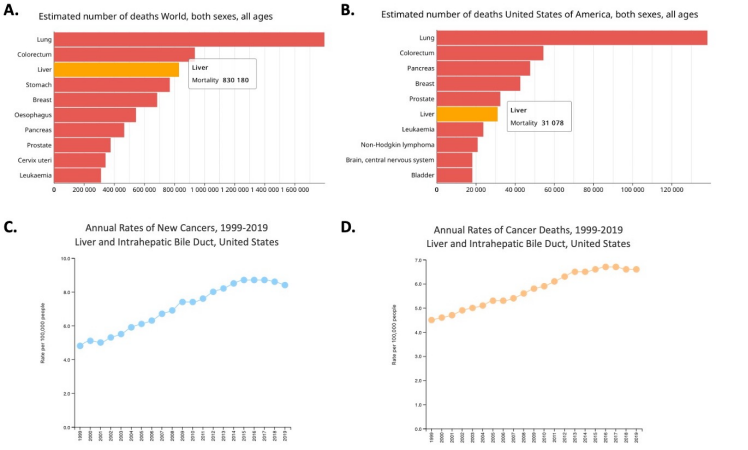


Figure 2: Liver cancer incidence and mortality statistics. Bar charts of (**A**) the estimated number of deaths worldwide, and (**B**) in United States of America due to Liver cancer. Line graphs showing increasing trends of (**C**) new liver cancer diagnosis and, (**D**) cancer-related deaths from 2000-2019. Source: (**A**) and (**B**) GLOBOCAN 2020, (**C**) and (**D**) Cancer Statistics Working Group. U.S. Cancer Statistics Data Visualizations Tool, based on 2021 submission data (1999-2019): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute.

Metastasis is the spread of tumor cells from their primary site of the origin to various organs/tissues in the body. The process of metastasis is a complicated series of events regulated by both intrinsic (genetic or epigenetic makeup) and several extrinsic factors (microenvironmental cellular and non-cellular components). Cells in a tumor over the time gain heterogeneity due to ongoing genetic changes and evolve in response to stresses within the tumor microenvironment. During this process, a subset of cancer cells will acquire metastatic potential in the process, a multistep process which involves dissemination of tumor cells from the primary site, extravasation, entrance into the circulatory system, intravasation, colonization and growth at secondary distant organ sites in the body. Metastatic HCC can be characterized as intrahepatic or extrahepatic or both. Intrahepatic metastasis may be due to dissemination of a primary tumor into multiple sites in the liver or development of multiple independent hepatocellular carcinomas and is a major contributor to disease relapse[5]. Extrahepatic metastasis to lungs, colon, bones, brain, lymph nodes, and adrenal glands is the major contributing factor leading to poor survival in patients. Patients with advanced intrahepatic tumor stages are at higher risk of developing extrahepatic HCC[3].

Metastasis greatly reduce patient survival as was observed in the SORAMIC Trial where extrahepatic metastases to lungs significantly reduced the median survival (7.6 vs. 15.0 months, $p = 0.0060$) [6]. Similarly, in a recent case report study, metastases to colon reduced median survival of patients to 2.5 months[7]. Therefore, therapies that target the mechanisms of metastatic disease may enhance therapeutic outcomes in HCC. Our overall understanding of the interaction among intrinsic and extrinsic factors, including the genetic pathways that are involved in promoting HCC metastasis is limited, therefore it is of utmost important to define these interactions and their impact on metastatic potential so that effective therapeutic approaches can be devised. While cell culture models of metastasis exist, *in vivo* metastasis modeling is the best approach to simulate the complex nature of the metastatic processes of human cancers and their progression. In this proposal we will carry out a CRISPR library screen to define genetic changes controlling metastatic potential of liver cancer.

Experimental Approach

Specific Aim 1: Screening of regulator(s) of metastatic hepatocellular carcinoma.

Rationale: Metastasis is a major cause for the death of HCC patient, and in some cases the patient presents with metastatic carcinoma prior to diagnosis of a primary liver tumor. Due to the fact that metastasis is a key driver of cancer mortality, the ability to target the metastatic potential of cancer cells would be a breakthrough in cancer treatment. To achieve this goal, it is necessary to understand what molecular changes impart metastatic potential to tumor cells, allowing them the capacity to migrate to a secondary organ/tissue in new microenvironment distant from its primary location. Multiple genes with diverse functions, such as SLFN11[8], chromatin remodeling factor ARID2[9], circular RNA cSMARCA5[10], MiR-4310[11], NXN[12] have been studied as negative regulators of HCC employing subcutaneous xenograft, orthotopic HCC and tail vein injection as model of lung metastasis. On the other hand, metabolic checkpoint regulator, STIM1[13], STAT3/lncRNA HOXD-AS1[14], YTHDF2[15], NET1[16], PRMT1[17], CAV1[18], ARHGEF37[19], have been shown to promote metastasis. Similarly, studies have been performed comparing differential gene expression of primary tumors with secondary tumors from metastatic sites from patients to delineate the modulators of metastasis have been carried out, these studies are limited in their ability to determine whether these differentially expressed gene are drivers of metastasis or provide benefits of survival at new location after establishment of metastatic event. Though, single gene-based studies are critical for analysis of importance of a given gene in a particular process/phenotype, how prominent the gene is in the presence of other genetic perturbations cannot be known. Previously, studies have exploited the use of CRISPR technology in HCC cell line models to identify regulators of drug resistance or targets for combination therapy[20-26] or drivers of liver cancer[27-29]. In Aim I, we intend to utilize an unbiased CRISPR screen to identify the gene(s) playing a critical role in supporting metastatic colonization.

We have selected three cell lines to carry out CRISPR-mediated genetic screens in to define pathways involved in liver cancer metastasis. The JHH7 and SKHEP1 liver cancer cell lines (HCC and adenocarcinoma, respectively) are potent in metastatic colonization to multiple organs (Figure 2)[30]. Hence, we will utilize these cell lines to determine genes that when deleted will prevent these cells from undergoing metastasis. In addition, we selected Huh7 cells, which show limited metastatic potential, do define pathways that when suppressed will enhance metastatic potential.

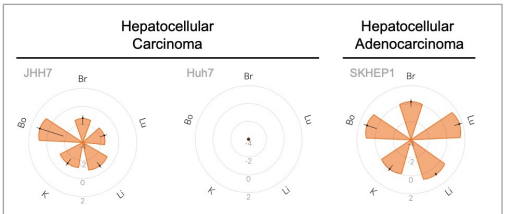


Figure 2: Metastatic localization map of JHH7, Huh7, and SKHEP1 cell lines. Br (Brain), Lu (Lung), Li (Liver), K (Kidney), B (Bones). Source: Broad Institute.

Aim 1.A: Generation of RFP positive cells: *In vivo* studies are useful in understanding the tumor growth and metastatic spread, particularly with the recent advancement in fluorescent protein imaging. Fluorescent tagging of cancer cells provides the advantage of longitudinally assessing cancer growth and migration *in vivo* and therefore the capacity to monitor of the metastatic colonization and growth in multiple organs simultaneously. Therefore JHH7, SKHEP1, and Huh7 liver cancer models will be engineered to stably express the red fluorescent protein (RFP) using a lentiviral vector (LeGO-dKatushka2). Single cell clones expressing moderate levels of RFP will be purified by Fluorescence Activated Cell Sorting (FACS) for subsequent experiments.

Aim 1.B: Infection of RFP-cells with whole-genome lentiviral-based CRISPR knockout library: JHH7, SKHEP1, and Huh7 cells stably expressing RFP will be infected with a pooled genome-scale CRISPR-Cas9 Knock-out library (Toronto KnockOut library v3 (TKOv3) at an MOI of 0.3. The TKOv3 CRISPR pooled library consists of specific guide RNAs (gRNAs) for knocking out each gene in the human genome (specifically the library contains 70,948 gRNAs targeting 18,053 protein coding genes (4 gRNAs/gene) with 142 control non-targeting guides against EGFP, LacZ and luciferase for a total library size of 71,090 guides). After 48-72 h of puromycin selection, a pooled population of cells consisting of a minimum of 500-fold coverage will be

pelleted and stored in -80^{0}C, which will represent our parent population, T_0. Remaining cells representing at least 100-fold coverage will be transplanted into mice as described below in Aim 1.C.

Aim 1.C: *In vivo* transplantation of cells: Approximately 4 to 6-week-old NSG mice (Stock No: 005557) obtained from The Jackson laboratory will serve as recipient animals. The mice will receive intracardiac injections as model of metastasis[30]. Orthotopic liver injections will be used as control group and allow us to differentiate genes involved in promoting metastasis from those that are essential for tumor growth at the primary site. The whole genome TKOv3 CRISPR library that will be used has approximately 71,000 gRNA. Given it is recommended to have a 100-fold representation of each gRNA[31], we will need to inject 7.1 x 10^6 (71,000*100) cells for each cell line and each injection route (intracardiac and orthotopic injection). We intend to inject 0.7 x 10^6 cells per mouse which will require a minimum of 10 mice per group per cell line will be utilized in our screen. Injections will be performed as previously described[32,33].

Aim 1.D: Monitoring cancer cell spread and lesion formation via whole-body fluorescence imaging: *In vivo* metastasis progression in real time will be carried out via tracking the spread and lesion formation of RFP expressing at metastatic sites. Mice will be anesthetized with oxygen/isoflurane mixture to perform whole-body fluorescent imaging using an *in vivo* fluorescence imaging system IVIS Lumina XR (Caliper life sciences). The excitation wavelength used will be 570 and 605 nm. Imaging will be performed 5 time per week until lesions are observed and then daily for 21 days until tumors at metastatic sites are harvested.

Aim 1.E: Endpoint and tumor collection: Endpoints will be determined based on fluorescence imaging up to six weeks post transplantation. Mice will be euthanized, and tumors collected from the primary site (orthotopic liver injection model) and metastatic sites including lymph nodes, lungs, and organs identified via imaging.

Aim 1.F: Processing for deep sgRNA sequencing: Sequencing of sgRNAs represented in each sample will be performed as described by the TKOv3 CRISPR library instructions[34]. Briefly, the parent cell population T_0 (Aim 1.B), primary tumors, and tumors pooled by organ will be subjected to DNA extraction (Wizard Genomic DNA Purification Kit) as per manufacturer's instructions. Sequencing of samples will be performed in a two-step PCR procedure: (1) enrich gRNA regions in the genome and (2) amplify sgRNAs with Illumina TruSeq adapters with i5 and i7 indices. PCR will be performed using primers and protocol as previously described[34]. The amplified gRNAs will be deep sequenced using on Illumina HiSeq 2500 or NextSeq 500.

Aim 1.G: Data analysis of CRISPR screen: sgRNA sequence reads will be mapped to the reference sgRNA sequence library using standard sequence alignment tools such as Bowtie with the following parameters: -v2 (allowing two mismatches) and – m1 (discarding any read that mapped to more than one sequence in the library). Read counts will be normalized to ten million reads per sample. The log2 fold change of each sgRNA for each replicate at each timepoint (T) will be calculated and compared to the T_0 sample (T/T_0). A pseudo count of 0.5 reads will be added to all read counts to prevent discontinuities from zeros. sgRNAs with < 30 raw reads in the T_0 sample will be excluded from fold- change calculation and downstream analysis. Screen performance will be assessed by calculating the fold change values for the gold-standard reference essentials and non-essentials gene sets. Also, enrichment of sgRNA of known negative regulators (e.g., ARID2, SLFN11) of HCC or depletion of sgRNA of known positive regulators (e.g., STAT3, NET1) will be analyzed as a criterion for screen performance. Fold changes will be analyzed with the Bayesian Analysis of Gene Essentiality (BAGEL) algorithm [35] using the essential and non-essential training sets defined in Hart et al. [34] or MAGeCK[36].

Anticipated results: We anticipate that sequencing data generated following the *in vivo* CRISPR screen will reveal sgRNAs that are either enriched and depleted in either primary or secondary tumors from metastatic sites when compared to the parental T_0 population. Enriched sgRNAs will represent the loss of critical tumor suppressors in HCC and depleted sgRNAs will represent loss of potential oncogenes. The differential sgRNA expression in parent cell population

vs primary tumors will reveal the gene(s) involved in tumor growth at primary sites (Liver). On the other hand, comparison between sgRNA profiles of primary tumor versus metastatic site will uncover genes which are responsible for promoting metastatic colonization at secondary locations. In addition, comparing the sgRNA profiles of secondary tumors from different locations will illustrate gene(s) that may impart organ-specific capability for metastatic colonization.

Potential problems and alternative strategies: Intracardiac injection vs. orthotopic injections will be our preference for modelling *in vivo* metastasis. Intracardiac injection (IC) involves injecting cancer cells into the left ventricle to disseminate them to the whole-body via the arterial bloodstream, which eventually develop into metastatic colonies in other organs. IC injection recapitulates the metastasis process, including survival of cancer cells in the bloodstream, extravasation, microcolony formation, and metastatic progression. Importantly, this technique is capable of delivering cancer cells to different organs in an unbiased manner, as depicted in Figure 2, and hence more informative[30]. While we are cognizant of the fact that that this methodology is ignoring early stages of metastasis, such as detaching from the primary tumor site and migration to the bloodstream, mice will usually die from an established orthotopic tumor prior to metastasis occurring, and subcutaneous xenografts lack proper modeling of organ-specific tumor microenvironments. While the IC method is preferred, if we find difficulty in establishing *in vivo* metastatic lesions, we will use tail-vein injections as an alternative delivery method to compare with orthotopic injection model.

Specific Aim 2: **Validation of selected hit(s) as potential therapeutic target(s) for metastatic HCC.**

Rationale: As described above, we anticipate that identifying three classes of genes in our *in vivo* metastasis screen, those indispensable for: tumor growth in primary liver site; for metastatic tumor growth; and metastatic colonization in different organs. While some genes may be unique to a particular class, there may be genes that are common between the three classes. In Aim 2 we will validate the top novel enriched and depleted genes from each cell line for *in vitro* and *in vivo* metastatic potential. The most promising candidate(s) in Aim 2 will be further characterized in future studies to define their role in promoting HCC metastasis and their potential as a novel therapeutic target for suppressing HCC metastasis relevance.

Aim 2: *In vitro* validation of selected candidates for metastatic potential.

The top novel hit genes identified from the *in vivo* metastasis CRISPR screen will be depleted/inhibited using shRNA, siRNA or by inhibitor treatment (if available) in JHH7, SKHEP1, and/or Huh7 liver cancer, to define their role in detachment, migration, and/or invasion as described below.

Aim 2.A: Cell migration (transwell) assays will be conducted in 12-well plate format using transwell inserts with a 0.8 μM membrane. Approximately $2x10^4$ control (shGFP/siGFP) or cells depleted of the top novel target genes identified in Aim 1 will be seeded into the inserts containing low (0-1%) serum. The inserts will be placed in 12-well plates containing complete media. Control and experimental cells will be compared for migration from low serum media to complete media after 24 and 48 hrs. At each time point, the membrane will be fixed and stained with crystal violet. Migrated cells will be image and quantified. If specific inhibitors of our target genes are available, cells will be pretreated with a range of concentrations for 24 and 48 hrs, or vehicle as control, and will be subjected to migration assay as described above. Similarly, cell migration will be evaluated in HCC cells with overexpression of candidate genes that are shown to be depleted in our screen comparisons.

Aim 2.B: To determine invasion capacity of cells either depleted or overexpressing our metastasis targets invasion chambers will be coated with Matrigel in a 24-well format. Matrigel inserts will be seeded with $3x10^4$ cells in 0-1% serum as attractant which will then be then placed in 24-well plates containing complete medium as attractant. Non-coated inserts will be used as control to assess the percentage of cell invasion. After 16-24 h, cells will be stained with crystal

violet, imaged, and counted. The assay will be performed to assess the consequence on invasive properties of cells after deletion of selected CRISPR screen targets via shRNA, siRNA or inhibitors as described above. Cell invasion assays will also be performed in HCC cells with overexpression of the candidate genes.

Anticipated results: We anticipate that our *in vitro* assays will guide our secondary assessment of hits identified in Aim 1 of gene involved in regulating metastatic potential which would by evident by a significant decrease in the migration and invasion capability upon depletion/knockout.

Potential problems and alternative strategies: It is possible that JHH7, SKHEP, or Huh7 cells may not be good models for our *in vitro* migration and invasion assays. If this appears to be the case, we will utilize additional cellular models of liver cancer such as HEP3B.

Impact of this study on future publications and grant applications: We expect that using an *in vivo* metastasis screen will provide valuable information as to genes and pathways involved in regulating liver cancer metastasis in a pathophysiologically relevant setting, a major driver of liver cancer associated deaths. We anticipate that targets identified and validated in this study, and the data generated in the aims described above will provide the data for an initial publication on the screen results as well as a foundation for a future grant to understand the molecular mechanism behind HCC metastasis and the therapeutic potential of targeting identified genes and genetic pathways in HCC. In addition, we may also identify genes that provide unique attributes that enhance metastasis to specific organs, shedding light on the molecular basis for why certain tumors have preferential secondary sites for metastatic disease. Therefore, outcomes from these studies will provide the basis of significant publications as well as funding future studies into translating novel genes/genetic pathways identified in these studies to novel therapeutic avenues for treating liver cancer.

Literature Citations

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49. PubMed PMID: 33538338.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. PubMed PMID: 30207593.
3. Becker AK, Tso DK, Harris AC, Malfair D, Chang SD. Extrahepatic metastases of hepatocellular carcinoma: A spectrum of imaging findings. Can Assoc Radiol J. 2014;65(1):60-6. PubMed PMID: 24239313.
4. Aggarwal M, Arain A, Jin Z. Systemic treatment for hepatocellular carcinoma. Chronic Dis Transl Med. 2018;4(3):148-55. PubMed PMID: 30276361; PMCID: PMC6160617.
5. Feo F, Pascale RM. Multifocal hepatocellular carcinoma: intrahepatic metastasis or multicentric carcinogenesis? Ann Transl Med. 2015;3(1):4. PubMed PMID: 25705636; PMCID: PMC4293477.
6. Schutte K, Schinner R, Fabritius MP, Moller M, Kuhl C, Iezzi R, Ocal O, Pech M, Peynircioglu B, Seidensticker M, Sharma R, Palmer D, Bronowicki JP, Reimer P, Malfertheiner P, Ricke J. Impact of Extrahepatic Metastases on Overall Survival in Patients with Advanced Liver Dominant Hepatocellular Carcinoma: A Subanalysis of the SORAMIC Trial. Liver Cancer. 2020;9(6):771-86. PubMed PMID: 33442545; PMCID: PMC7768116.
7. Yu YM, Cao YS, Wu Z, Huang R, Shen ZL. Colon metastasis from hepatocellular carcinoma: a case report and literature review. World J Surg Oncol. 2020;18(1):189. PubMed PMID: 32723336; PMCID: PMC7389379.
8. Zhou C, Liu C, Liu W, Chen W, Yin Y, Li CW, Hsu JL, Sun J, Zhou Q, Li H, Hu B, Fu P, Atyah M, Ma Q, Xu Y, Dong Q, Hung MC, Ren N. SLFN11 inhibits hepatocellular carcinoma tumorigenesis and metastasis by targeting RPS4X via mTOR pathway. Theranostics. 2020;10(10):4627-43. PubMed PMID: 32292519; PMCID: PMC7150495.
9. Jiang H, Cao HJ, Ma N, Bao WD, Wang JJ, Chen TW, Zhang EB, Yuan YM, Ni QZ, Zhang FK, Ding XF, Zheng QW, Wang YK, Zhu M, Wang X, Feng J, Zhang XL, Cheng SQ, Ma DJ, Qiu L, Li JJ, Xie D. Chromatin remodeling factor ARID2 suppresses hepatocellular carcinoma metastasis via DNMT1-Snail axis. Proc Natl Acad Sci U S A. 2020;117(9):4770-80. PubMed PMID: 32071245; PMCID: PMC7060681.
10. Yu J, Xu QG, Wang ZG, Yang Y, Zhang L, Ma JZ, Sun SH, Yang F, Zhou WP. Circular RNA cSMARCA5 inhibits growth and metastasis in hepatocellular carcinoma. J Hepatol. 2018;68(6):1214-27. PubMed PMID: 29378234.
11. Li H, Chen Z, Zhang Y, Yuan P, Liu J, Ding L, Ye Q. MiR-4310 regulates hepatocellular carcinoma growth and metastasis through lipid synthesis. Cancer Lett. 2021;519:161-71. PubMed PMID: 34303763.
12. Zhang Y, Zuo D, Qiu J, Li K, Niu Y, Yuan Y, Qiu Y, Qiao L, He W, Wang C, Yuan Y, Li B. NXN suppresses metastasis of hepatocellular carcinoma by promoting degradation of Snail through binding to DUB3. Cell Death Dis. 2022;13(8):676. PubMed PMID: 35927236; PMCID: PMC9352874.
13. Zhao H, Yan G, Zheng L, Zhou Y, Sheng H, Wu L, Zhang Q, Lei J, Zhang J, Xin R, Jiang L, Zhang X, Chen Y, Wang J, Xu Y, Li D, Li Y. STIM1 is a metabolic checkpoint regulating the invasion and metastasis of hepatocellular carcinoma. Theranostics. 2020;10(14):6483-99. PubMed PMID: 32483465; PMCID: PMC7255033.
14. Wang H, Huo X, Yang XR, He J, Cheng L, Wang N, Deng X, Jin H, Wang N, Wang C, Zhao F, Fang J, Yao M, Fan J, Qin W. STAT3-mediated upregulation of lncRNA HOXD-AS1 as a ceRNA facilitates liver cancer metastasis by regulating SOX4. Mol Cancer. 2017;16(1):136. PubMed PMID: 28810927; PMCID: PMC5558651.

15. Zhang C, Huang S, Zhuang H, Ruan S, Zhou Z, Huang K, Ji F, Ma Z, Hou B, He X. YTHDF2 promotes the liver cancer stem cell phenotype and cancer metastasis by regulating OCT4 expression via m6A RNA methylation. Oncogene. 2020;39(23):4507-18. PubMed PMID: 32366907.
16. Chen ZH, Ni QZ, Zhang XP, Ma N, Feng JK, Wang K, Li JJ, Xie D, Ma XY, Cheng SQ. NET1 promotes HCC growth and metastasis in vitro and in vivo via activating the Akt signaling pathway. Aging (Albany NY). 2021;13(7):10672-87. PubMed PMID: 33839702; PMCID: PMC8064201.
17. Zhang XP, Jiang YB, Zhong CQ, Ma N, Zhang EB, Zhang F, Li JJ, Deng YZ, Wang K, Xie D, Cheng SQ. PRMT1 Promoted HCC Growth and Metastasis In Vitro and In Vivo via Activating the STAT3 Signalling Pathway. Cell Physiol Biochem. 2018;47(4):1643-54. PubMed PMID: 29945155.
18. Yu H, Shen H, Zhang Y, Zhong F, Liu Y, Qin L, Yang P. CAV1 promotes HCC cell progression and metastasis through Wnt/beta-catenin pathway. PLoS One. 2014;9(9):e106451. PubMed PMID: 25180681; PMCID: PMC4152279.
19. Zhang X, Ren L, Wu J, Feng R, Chen Y, Li R, Wu M, Zheng M, Wu XG, Luo W, He H, Huang Y, Tang M, Li J. ARHGEF37 overexpression promotes extravasation and metastasis of hepatocellular carcinoma via directly activating Cdc42. J Exp Clin Cancer Res. 2022;41(1):230. PubMed PMID: 35869555; PMCID: PMC9308268.
20. Pan J, Zhang M, Dong L, Ji S, Zhang J, Zhang S, Lin Y, Wang X, Ding Z, Qiu S, Gao D, Zhou J, Fan J, Gao Q. Genome-Scale CRISPR screen identifies LAPTM5 driving lenvatinib resistance in hepatocellular carcinoma. Autophagy. 2022:1-15. PubMed PMID: 36037300.
21. Feng J, Lu H, Ma W, Tian W, Lu Z, Yang H, Cai Y, Cai P, Sun Y, Zhou Z, Feng J, Deng J, Shu Y, Qu K, Jia W, Gao P, Zhang H. Genome-wide CRISPR screen identifies synthetic lethality between DOCK1 inhibition and metformin in liver cancer. Protein Cell. 2022;13(11):825-41. PubMed PMID: 35217990; PMCID: PMC9237198.
22. Chen Y, Li L, Lan J, Cui Y, Rao X, Zhao J, Xing T, Ju G, Song G, Lou J, Liang J. CRISPR screens uncover protective effect of PSTK as a regulator of chemotherapy-induced ferroptosis in hepatocellular carcinoma. Mol Cancer. 2022;21(1):11. PubMed PMID: 34983546; PMCID: PMC8725338.
23. Lu Y, Shen H, Huang W, He S, Chen J, Zhang D, Shen Y, Sun Y. Genome-scale CRISPR-Cas9 knockout screening in hepatocellular carcinoma with lenvatinib resistance. Cell Death Discov. 2021;7(1):359. PubMed PMID: 34795217; PMCID: PMC8602346.
24. Xu F, Tong M, Tong CSW, Chan BKC, Chu HY, Wong TL, Fong JHC, Cheung MSH, Mak KH, Pardeshi L, Huang Y, Wong KH, Choi GCG, Ma S, Wong ASL. A Combinatorial CRISPR-Cas9 Screen Identifies Ifenprodil as an Adjunct to Sorafenib for Liver Cancer Treatment. Cancer Res. 2021;81(24):6219-32. PubMed PMID: 34666996.
25. Wei L, Lee D, Law CT, Zhang MS, Shen J, Chin DW, Zhang A, Tsang FH, Wong CL, Ng IO, Wong CC, Wong CM. Genome-wide CRISPR/Cas9 library screening identified PHGDH as a critical driver for Sorafenib resistance in HCC. Nat Commun. 2019;10(1):4681. PubMed PMID: 31615983; PMCID: PMC6794322.
26. Wang C, Wang H, Lieftink C, du Chatinier A, Gao D, Jin G, Jin H, Beijersbergen RL, Qin W, Bernards R. CDK12 inhibition mediates DNA damage and is synergistic with sorafenib treatment in hepatocellular carcinoma. Gut. 2020;69(4):727-36. PubMed PMID: 31519701.
27. Guo Y, Wang J, Benedict B, Yang C, van Gemert F, Ma X, Gao D, Wang H, Zhang S, Lieftink C, Beijersbergen RL, Te Riele H, Qiao X, Gao Q, Sun C, Qin W, Bernards R, Wang C. Targeting CDC7 potentiates ATR-CHK1 signaling inhibition through induction of DNA replication stress in liver cancer. Genome Med. 2021;13(1):166. PubMed PMID: 34663432; PMCID: PMC8524847.
28. Niu Y, Lin Z, Wan A, Sun L, Yan S, Liang H, Zhan S, Chen D, Bu X, Liu P, Chen C, He W, Lu X, Wan G. Loss-of-Function Genetic Screening Identifies Aldolase A as an Essential

Driver for Liver Cancer Cell Growth Under Hypoxia. Hepatology. 2021;74(3):1461-79. PubMed PMID: 33813748; PMCID: PMC8518375.
29. Bao MH, Yang C, Tse AP, Wei L, Lee D, Zhang MS, Goh CC, Chiu DK, Yuen VW, Law CT, Chin WC, Chui NN, Wong BP, Chan CY, Ng IO, Chung CY, Wong CM, Wong CC. Genome-wide CRISPR-Cas9 knockout library screening identified PTPMT1 in cardiolipin synthesis is crucial to survival in hypoxia in liver cancer. Cell Rep. 2021;34(4):108676. PubMed PMID: 33503428.
30. Jin X, Demere Z, Nair K, Ali A, Ferraro GB, Natoli T, Deik A, Petronio L, Tang AA, Zhu C, Wang L, Rosenberg D, Mangena V, Roth J, Chung K, Jain RK, Clish CB, Vander Heiden MG, Golub TR. A metastasis map of human cancer cell lines. Nature. 2020;588(7837):331-6. PubMed PMID: 33299191.
31. Bock C, Datlinger P, Chardon F, Coelho MA, Dong MB, Lawson KA, Lu T, Maroc L, Norman TM, Song B, Stanley G, Chen S, Garnett M, Li W, Moffat J, Qi LS, Shapiro RS, Shendure J, Weissman JS, Zhuang X. High-content CRISPR screening. Nature Reviews Methods Primers. 2022;2(1):8.
32. Brown ZJ, Heinrich B, Greten TF. Establishment of Orthotopic Liver Tumors by Surgical Intrahepatic Tumor Injection in Mice with Underlying Non-Alcoholic Fatty Liver Disease. Methods Protoc. 2018;1(2). PubMed PMID: 31164564; PMCID: PMC6526445.
33. Campbell JP, Merkel AR, Masood-Campbell SK, Elefteriou F, Sterling JA. Models of bone metastasis. J Vis Exp. 2012(67):e4260. PubMed PMID: 22972196; PMCID: PMC3490264.
34. Hart T, Tong AHY, Chan K, Van Leeuwen J, Seetharaman A, Aregger M, Chandrashekhar M, Hustedt N, Seth S, Noonan A, Habsid A, Sizova O, Nedyalkova L, Climie R, Tworzyanski L, Lawson K, Sartori MA, Alibeh S, Tieu D, Masud S, Mero P, Weiss A, Brown KR, Usaj M, Billmann M, Rahman M, Constanzo M, Myers CL, Andrews BJ, Boone C, Durocher D, Moffat J. Evaluation and Design of Genome-Wide CRISPR/SpCas9 Knockout Screens. G3 (Bethesda). 2017;7(8):2719-27. PubMed PMID: 28655737; PMCID: PMC5555476.
35. Hart T, Moffat J. BAGEL: a computational framework for identifying essential genes from pooled library screens. BMC Bioinformatics. 2016;17:164. PubMed PMID: 27083490; PMCID: PMC4833918.
36. Li W, Xu H, Xiao T, Cong L, Love MI, Zhang F, Irizarry RA, Liu JS, Brown M, Liu XS. MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biol. 2014;15(12):554. PubMed PMID: 25476604; PMCID: PMC4290824.

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

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
FY 23/24	4	0

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

Cellular Signaling and Molecular Trafficking in Cancer
Laura Hansen, PhD

Checkpoint signaling and cell survival in normal and tumorigenic skin keratinocytes

Principal Investigator: Laura Hansen, PhD

I. Progress Report Summary

A. Specific Aims

As described in last year's progress report, we expanded the scope of the work to assess FWE localization and membrane topology in order to understand how FWE isoforms interact and impact cell cycle checkpoints and carcinogenesis. Results described below have led us to further refine the direction of the project to assess the role of Flower isoforms in differentiation pathways in cutaneous squamous cell carcinoma (cSCC).

B. Studies and Results

In the past year, we submitted a manuscript documenting the membrane topology of hFWE3 and hFWE4 and the role of hFWE4 in endocytic trafficking (Rudd et al., 2023, attached). Several figures in this manuscript were reported in prior LB595 progress reports. After minor revisions, that manuscript is now published online.

We performed subcutaneous xenografts of cSCC cells expressing a green fluorescent protein (EGFP) alone or EGFP and hFWE2, hFWE3, or hFWE4. hFWE2 and hFWE4 tumors were nonsignificantly reduced in size while hFWE3 grafts grew similarly to the control tumors (Fig. 1A). Using a newly acquired FWE antibody, we showed that the most intense hFWE expression occurred in the subrabasal cells of control cSCC xenografts (Fig. 1B). This is significant because in epidermis and cSCC, the more basal cells are the proliferative population while the terminal differentiation pathway is engaged as cells move suprabasally. Immunoblotting with hFWE2, 3, or 4 overexpressing cells demonstrated that this antibody recognizes hFWE3 and 4 (not shown), which are also the most highly expressed isoforms in

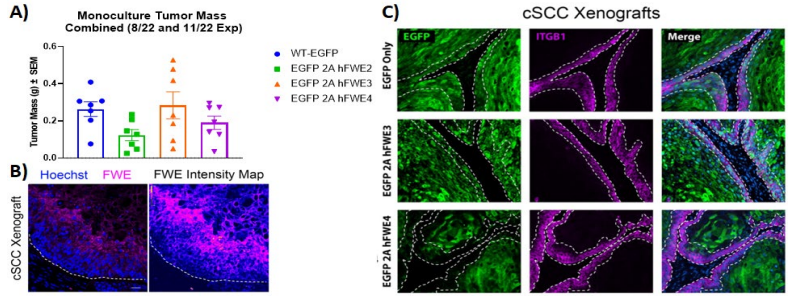


Fig 1. hFWE expression promotes terminal differentiation in cSCC cells. (A) Weight of control or hFWE2, 3, or 4 overexpressing SCC13 xenograft tumors (N= 6). B) Immunofluorescence for endogenous hFWE using anti-mFWE2 polyclonal antibody in control SCC13 xenografts. (C) Double immunofluorescence of xenograft tumors for EGFP and ITGB1 to evaluate representation of hFWE-expressing cells in the basal keratinocyte population. hFWE4-expressing cells were significantly depleted from the ITGB1 population, while hFWE3 and EGFP control cells were maintained in this layer.

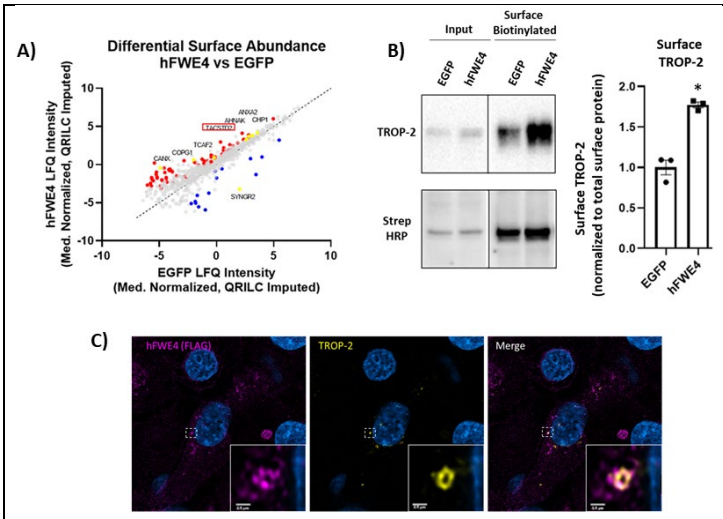


Fig. 2. hFWE4-positive vesicles traffic proteins to the cell surface in SCC cells. (A) SCC13 cells expressing EGFP or EGFP 2A hFWE4 were surface biotinylated and total surface protein was used for label-free MS proteomic analysis. Plot depicts average normalized label-free intensity values for each identified protein. Proteins exhibiting enrichment (red) or depletion (blue) are color coded (q <0.05, N=5) (Fig. 2A). Among these, several were proteins involved in calcium-dependent biological processes (labeled in text and colored yellow). (B) Confirmatory surface biotinylation-IB analysis of TACSTD2 (TROP-2) enrichment at the cell surface in SCC13 expressing EGFP 2A hFWE4 and densitometry (right)(N = 3). (C) SCC13 cells expressing EGFP 2A hFWE4-3xFLAG were double immunostained for FLAG and TROP-2. Images show representative Airyscan (63x oil, 2.3x zoom) following Airyscan joint deconvolution. Inset shows colocalization of hFWE4 and TROP-2 on a single vesicle expressing EGFP 2A hFWE4.

human skin and skin tumors (data included in a previous progress report). Interestingly, while double immunofluorescence for EGFP and the basal keratinocyte marker integrin β1 (IGFB1) showed EGFP signal in the basal and suprabasal keratinocytes, the hFWE4 tumors were largely negative for basal hFWE4 (EGFP)-positive cells (Fig. 1C). Taken together, these data suggest exclusion of highly hFWE4 expressing cells in the basal compartment and that there may be a role for hFWE4 in differentiating cells.

Upon our finding that hFWE4 was localized in part to the plasma membrane (Rudd et al., 2023), we conducted cell surface biotinylation and mass spectrometry (MS) analysis in control and hFWE4-overexpressing cSCC cells. This analysis revealed a significant alteration in the surface proteome of hFWE4-expressing cells, with 75 proteins exhibiting enrichment (red) and 15 exhibiting depletion (blue) (q <0.05, N=5) (Fig. 2A). Among these, several were proteins involved in calcium-dependent biological processes (labeled in text and colored yellow). Confirmatory surface biotinylation immunoblotting analysis of TROP-2 revealed significant enrichment at the cell surface (Fig. 2B, left). Densitometry of multiple immunoblots (N = 3) showed ~1.7x increase in surface abundance of TROP-2 after normalization to total surface protein (strep-HRP) (Fig. 2B, right). Double immunofluorescence of hFWE4 and TROP-2 revealed colocalization of these proteins (Fig. 2C).

Because of the key role of an increased calcium gradient in driving differentiation in cutaneous keratinocytes, the frequency of identification of calcium-dependent proteins in these analyses and the suprabasal localization of hFWE4 in the tumors; we hypothesized that hFWE4 may regulate keratinocyte differentiation. As shown in Fig. 3, endogenous hFWE is primarily localized to differentiating stratum granulosum cells, consistent with colocalization with the stratum granulosum marker involucrin (IVL) in the epidermis. hFWE levels were increased approximately 4.5-fold during differentiation using two nontumorigenic keratinocyte cell line (N/TERT-2G and HaCaT) models, where extended time post-confluence induces terminal differentiation, as shown by increased levels of differentiation markers (Fig. 4A-B, Fig. 5A). hFWE transcripts were significantly increased along with differentiation marker transcripts, while the proliferation marker Ki67 was decreased (Fig. 4C). Additional organotypic cultures of the HaCaT hFWE4 or control cells in a 1:1 ratio with control cells suggested a partial depletion of

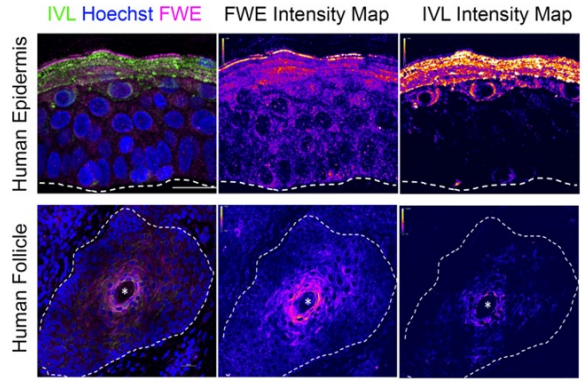


Fig 3. Endogenous hFWE is localized to terminally differentiated layers of human interfollicular epidermis and hair follicles. Immunostaining for endogenous hFWE and IVL reveals that most robust hFWE expression is found in suprabasal keratinocyte layers of normal human interfollicular epidermis and hair follicle.

hFWE4-overexpressing cells from the basal compartment in the hFWE4 cocultures (Fig. 5B).

Last year, we reported the generation of a Crainbow transgenic mouse. The system was 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. Our development of this line and initial experiments documenting transgene expression were used as preliminary data in a grant application to the State of Nebraska Stem Cell Research Program, LB606. That grant was awarded with funding scheduled to start July 1, 2023.

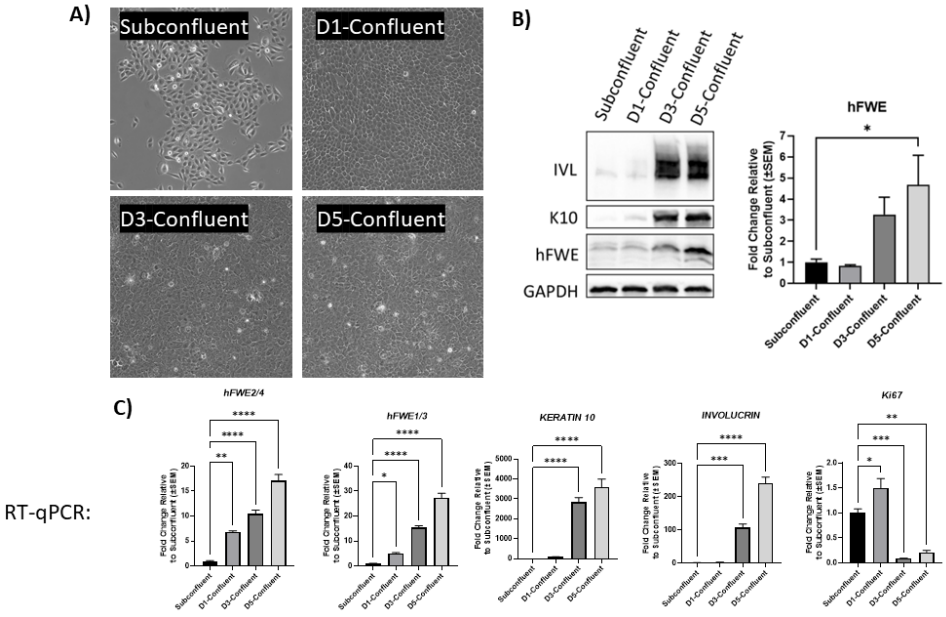


Fig 4. hFWE expression is induced upon terminal differentiation in epidermal keratinocytes. (A) Representative phase contrast images of TERT-immortalized normal keratinocyte cell line (N/TERT-2G) that were terminally differentiated by switching to high calcium (1.4 mM) at confluence and maintaining for 5 days. (B) Immunoblotting for endogenous hFWE revealed a significant increase in protein quantity during terminal differentiation (*p<0.05). (C) RT-qPCR demonstrated a significant increase in transcript quantity for all hFWE isoforms during terminal differentiation.

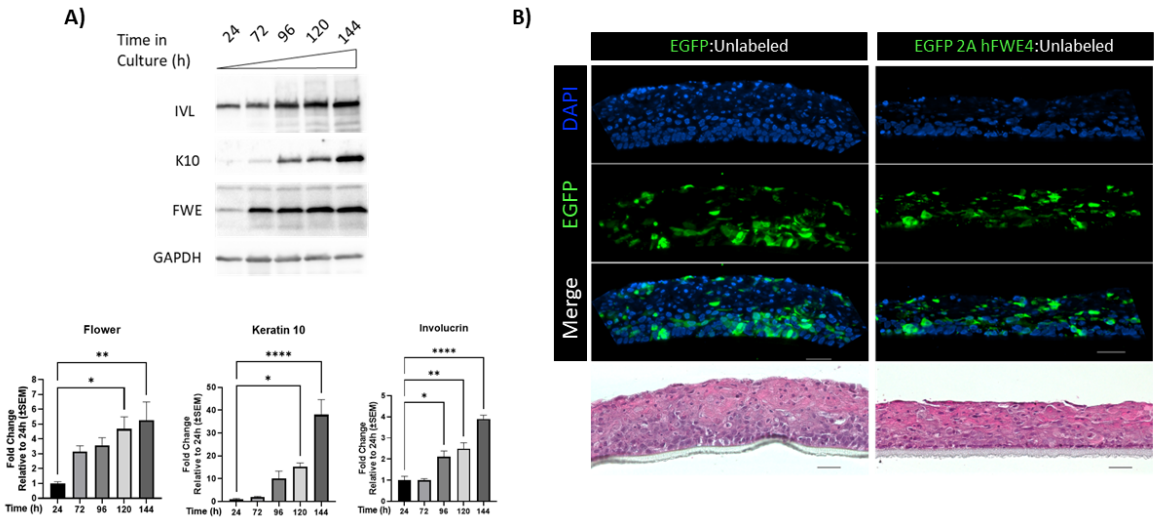


Fig 5. hFWE4 promotes terminal differentiation in HaCaT keratinocytes. (A) Immunoblotting for endogenous hFWE revealed a significant increase in protein quantity during terminal differentiation of HaCaT keratinocytes induced by extended time in culture (*p<0.05). (B) Organotypic raft cultures were generated from HaCaT keratinocytes expressing EGFP or EGFP 2A hFWE4 in a 1:1 ratio with unlabeled parental cells.

C. Significance

In the Flower literature, there have been competing lines of investigation and interpretation of Flower isoform interactions, activities, and function. Flower isoforms appear to regulate a diverse set of biological processes, either fitness sensing during cell competition or calcium channeling that drives endocytic events in different cell types. Correspondingly disparate hypotheses have been proposed to mechanistically define Flower function. Evaluation of these hypotheses relies on specific isoform membrane topologies and subcellular localization, characterization of which had not been previously reported for human isoforms. Our newly published manuscript now provides this information for hFWE3 and hFWE4 isoforms. In our study, we utilized several complementary techniques that together provide strong evidence that the canonical human FWE isoform hFWE4 assumes a four transmembrane structure with cytosolic termini that was trafficked between endocytic vesicles and the plasma membrane. Additionally, we showed that the non-canonical hFWE3 isoform was not trafficked to the plasma membrane but rather remained in the ER membrane. Our structural and subcellular localization data for human hFWE isoforms appear incompatible with a cell competition mechanism reliant on extracellular display of isoform-specific C-terminal tails. Instead, our data are consistent with a mechanism of hFWE4 action involving regulation of cell surface protein trafficking.

There have also been discordant reports of calcium channeling by FWE proteins from different species. Our data suggest a role for hFWE isoforms in keratinocytes differentiation, a process driven by increased calcium gradient in the differentiating cell layers, and in calcium signaling in keratinocytes as well. Our cell surface biotinylation experiments and newly published data (Rudd et al., 2023) will guide our next experiments in testing this hypothesis. Results generated from our LB595 work will focus on further revealing Flower mechanisms and function in skin cancer in the coming year.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

Rudd, J., Maity, S., Grunkemeyer, J., Snyder, J.C., Lovas, S., and Hansen, L.A. Membrane structure and internalization dynamics of human Flower isoforms, *Journal of Biological Chemistry*, 2023, doi: 10.1016/j.jbc.2023.104945.

III. List of extramural grants submitted from 7/1/2022–6/30/2023

Agency: Nebraska Dept. of HHS Stem Cell Research Grant
PI: Laura Hansen
Submitted: March 2023
Title: Flower lineage tracing in CRAINBOW mice stem cells
Amount: $109,125
Status: Active

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

Agency: Nebraska Dept. of HHS Stem Cell Research Grant
PI: Laura Hansen
Submitted: March 2023
Title: Flower lineage tracing in CRAINBOW mice stem cells
Amount: $109,125
Status: Active as of 7/1/2023

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

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 continued on from our prior progress report to make significant strides in understanding how BubR1 is lost following UV exposure. In particular, we have completed studies showing that BubR1 loss following UV exposure is due largely to induced degradation of BubR1 through the 26S proteosome, as blocking the activity of the protosome by treating with MG132 abrogates the loss of BubR1 following exposure to UV. In addition, we have assessed what class of E3 ubiquitin ligases target BubR1 in this setting and have found that treating cells with Pevonedistat (MLN4924), which is a neddylation inhibitor that blocks activity of Cullen-RING E3 ubiquitin ligases, also blocked the loss of BubR1 following UV treatment. β-TRCP, our proposed E3 ubiquitin ligase that targets BubR1 for degradation, is a Cullen-RING E3 ubiquitin ligase. To define specifically whether β-TRCP is responsible for the degradation of BubR1 following UV exposure, we depleted *β-TRCP* from HaCaT (an immortalized keratinocyte line) and SSC13 (a squamous cell carcinoma line) cells. These, and control cells expressing shRNA directed against *GFP*, were treated with UV and assessed for loss of BubR1, where we observed in both lines that the loss of BubR1 was substantially abrogated in the *β-TRCP*-depleted cells compared to the controls, suggesting that *β-TRCP* is a key regulator of BubR1 protein levels under genotoxic stress. These results continue to be 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. Next, given that BubR1 loss was blocked when β-TRCP-mediated degradation pathways were suppressed, we assessed whether *BubR1* mRNA levels were reduced following UV exposure. Contrary to our expectation, we found that transcript levels of *BubR1* were also reduced, suggesting that the loss of BubR1 may occur via multiple mechanisms, both at the transcript and post-translational level. Finally, we assessed whether depletion of *β-TRCP* influenced cell survival following UV exposure. To this end, we assessed the viability of *shGFP*- and *shβ-TRCP*-expressing cells following UV exposure using an MTT assay. We found that cells depleted of *β-TRCP* showed enhanced survival compared to control cells. Our goal this upcoming reporting period is to complete these studies and submit for publication.

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." In our prior reporting period, we had completed an *in vivo* UV induced skin tumorigenesis study where we treated SKH1 mice with nicotinamide mononucleotide (NMN), which promotes NAD^+ generation through the NAD^+ salvage pathway, to assess whether boosting NAD^+ would suppress tumorigenesis. Contrary to our hypothesis, we found that NMN treatment enhanced tumor burden. In this reporting period, we have focused our attention on understanding how long-term NMN treatment enhances UV-induced skin tumor burden.

Long-term NMN treatment was not toxic to the mice, and the increase in skin fold thickness in response to UV treatment was not altered between the water and NMN groups. To assess the mechanistic basis for enhanced tumor burden in NMN-treated mice, we carried out transcriptomic analysis and identified a number of pro-tumorigenic pathways that are enhanced following NMN treatment. These include upregulation of the epithelial to mesenchymal transition, angiogenesis, and KRAS signaling. In addition, pathways that were suppressed by NMN include interferon alpha and gamma responses, fatty acid metabolism, oxidative phosphorylation, and p53 signaling. We are currently assessing normal and tumor skin samples for these pathways being upregulated, as well as measuring markers of tumorigenesis, including proliferation and DNA damage response in tissue sections, as well as assessing the effects of modulating the NAD^+ salvage pathway in normal and skin cancer lines. Our goal this reporting period is to complete our assessment of regulation of UV-induced skin tumorigenesis by NMN and submit this study for publication.

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 that previously planned. Given the cost and effort for carrying out studies related to Aims 1 and 2, we have been forced to delay this aim. However, with our studies in Aim 1 close to completion, as well as its results showing targeting of BubR1 by β-TRCP following UV exposure, we will embark on studies to define the interaction between these two proteins in response to UV treatment to help define this interaction further to reach our ultimate goal of defining small molecules and/or peptides to block this interaction.

C. Significance

NAD^+ boosters have gained notoriety lately due to their potential at delaying 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.

In addition, our work shows that BubR1 downregulation by the E3 ubiquitin ligase β-TRCP may alter the cellular response to UV-induced DNA damage. There are limited studies demonstrating a role for β-TRCP and BubR1 in UV, and none to date showing an interrelationship between these two factors. Therefor our work will shed new light on both β-TRCP-mediated degradation of key regulators of genomic stability and the role of BubR1 loss has in the cellular response to UV irradiation and how we may target these pathways for therapeutic intervention into skin cancer.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

None.

III. List of extramural grants submitted from 7/1/2022–6/30/2023

Nebraska Health and Human Services Cancer and Smoking Disease Research Program (LB 506)
Dates: 07/01/2023 - 06/30/2024
Project Number: Cancer and Smoking Disease Research Program One-Year (LB 506)
PI: Brian J. North
Title: Identifying Regulators of Liver Cancer Metastasis
Major Goals: The goal of this research proposal is to perform a CRISPR-based *in vivo* screen to identify key regulators of hepatocellular carcinoma metastasis.

National Institute of Allergy and Infectious Disease
Dates: 12/1/2023 – 11/30/2028
Project Number: R01 AI179878
PI: Patrick Swanson (North Co-I)
Title: RACK1 in B Cell Development and V(D)J Recombination
Major Goals: The proposed research will investigate whether a protein called RACK1 facilitates degradation of a pro-apoptotic factor called Bim in B cells to suppress cell death during V(D)J recombination and support key signaling pathways during B cell development.

National Institute of Arthritis, Musculoskeletal and Skin Diseases/NIH
Dates: 7/01/2023 - 6/30/2028
Project Number: R01 AR082862
PI: Brian J. North
Title: Upstream regulators of connexin 43-dependent intercellular communication to promote wound healing.
Major Goals: The goals of these studies are to elucidate the molecular basis of NMN in promoting BubR1 function to regulate intercellular communication and wound healing in cellular and *in vivo* settings.

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

None

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

CELLULAR SIGNALING AND MOLECULAR TRAFFICKING IN CANCER
Program Director: Laura A. Hanson, PhD

Localization of RAG1 degradation and implications of RAG1 stabilization on genome instability and cancer
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

Specific aim 1.

As mentioned in the previous progress report, progress on Aim I was hindered somewhat by the departure of my graduate student/post-doc, Dr. Nathan Schabla (now directing a Flow Cytometry Core Lab at Shoreline Biosciences in San Diego), so I hired a technician to learn the RAG1 degradation assays and had two new MS students join my laboratory in 2021/22 academic year to learn these assays, as well.

My technician's progress was delayed by a problem with a new lot of fetal bovine serum which we use to culture cells, but we eventually overcame that obstacle. Unfortunately for my lab, after having worked to support my animal colony, she decided to apply to veterinary school and was accepted. She departed at the beginning of 2022 to start that program before she was able to make substantive progress on the project.

My MS student, who had been working to identify determinants of RAG1 required for mediating RAG1 degradation, shifted focus to understand how RACK1 altered RAG protein levels, a project that my technician had started. She was able to make progress on that project, providing convincing evidence that ectopic RACK1 expression suppressed levels of full-length RAG1 but not core RAG1, in a dose-dependent manner. She has been working on determining whether this effect is associated with a direct association with full-length RAG1, but during this period, she applied to medical school and was accepted at Western Michigan. They did not allow her to defer enrollment, so she decided to leave our program at the end of June 2023 to pursue this opportunity.

My PharmD-MS student was interested in developing 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. He has made considerable progress developing and optimizing the microscopy portion of the project and is currently working on best practices for data analysis. He is now well-positioned to make important progress toward the ultimate goal of the project, including understanding how inhibition with cullin and ubiquitin inhibitors alter RAG1 localization.

In the previous progress report, I also indicated that we are pursuing two additional subaims, which extended from preliminary studies of RACK1-BKO mice. The first new subaim was to determine whether loss of RACK1 affected levels of other putative targets of RACK1-dependent degradation, including HIF1α and Bim. During this period we have obtained data showing loss of RACK1 in B cells significantly increases levels of Bim, but not HIF1α in B cells. Furthermore, given RACK1's role in mediating signaling pathways, we have used flow cytometry to investigate the activation status of several signaling nodes in response to cell treatment with pervanadate. Together with new RNAseq datasets, we now have a plausible hypothesis for how RACK1 alters key pathways required for normal B cell development. Some of this preliminary data was used to support a new R01 application involving LB595 investigators Dr. Yusi Fu and Brian North. Based on reviewer feedback from the first R01 submission, future studies would focus on experiments necessary to improve competitiveness of the resubmission.

A second new subaim, partly related to the new subaim 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 ran into some unanticipated challenges with the mouse colony because the RAG mutant mice were found to harbor *Helicobacter*. We have successfully treated these animals and the first group of mice should be ready to analyze during the next reporting period.

For specific aim 2, as noted in the previous progress report, effort was shifted to a NIH R21 grant, entitled "A novel form of light chain gene replacement" awarded in January 2021.

C. Significance

The findings in specific aim 1 are significant because they suggest that RACK1 plays a central role in regulating RAG1 protein expression and specific signal transduction cascades in B cells. As RACK1 has been implicated in various pathways integral to cancer etiology and progression (e.g. Li and Xie, Oncogene (2015) 34, 1890–1898), insights gained through studies of primary B cells may have important implicants for understanding RACK1 in B cell malignancy. Data and reagents obtained with LB595 support were used to support the submission of a new R01 proposal focused on RACK1, with LB595 co-investigators Dr. Yusi Fu and Brian North.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

None

III. List of extramural grants submitted from 7/1/2022–6/30/2023

Agency: NIH/NIAID 1R01AI179878-01, submitted February 2023
Role: PI
Title: RACK1 in B cell development and V(D)J Recombination
Dates: 12/01/2023 to 11/30/2028
Amount: $1,837,500 total (20% FTE).

Agency: Creighton University, Health Sciences Strategic Investment Fund program
Role: PI
Title: Role of RACK1 in Regulating RAG Protein Translation
Dates: 07/01/2023 to 06/30/2025
Amount: $50,000

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

None

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

CELLULAR SIGNALING AND MOLECULAR TRAFFICKING IN CANCER
Program 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) that endows epithelial cancer cells with enhanced motility and invasiveness (3). 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). In addition, we expanded our research to investigate genetic mechanisms of apoptosis resistance in CRC. The preliminary data we obtained laid the foundation for our recent research award from the Lynch Comprehensive Cancer Research Center.

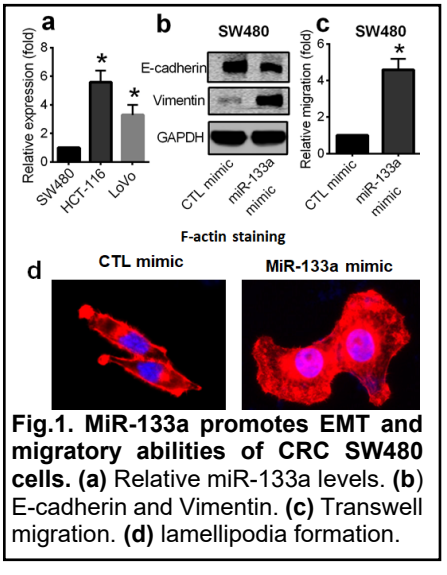


Fig.1. MiR-133a promotes EMT and migratory abilities of CRC SW480 cells. (a) Relative miR-133a levels. **(b)** E-cadherin and Vimentin. **(c)** Transwell migration. **(d)** lamellipodia formation.

1) Upregulated miR-133a promotes EMT and migratory abilities of CRC cells. MiR-133a is highly expressed in skeletal and cardiac muscle but very low in epithelial cells. Surprisingly, miR-133a was reported to be reduced in cancers and its overexpression inhibits cancer cell proliferation. However, the higher expression of miR-133a correlates with metastases and poor prognosis in CRC patients (2). Indeed, expression levels of miR-133a were higher in HCT-116 and LoVo cells as compared to SW480 cells (**Fig.1a**). Transfection of miR-133a mimic caused changes in EMT markers (loss of E-cadherin and increased Vimentin) (**1b**) and enhanced migratory potential of SW480 cells (**1c**). Importantly, miR-133a triggers actin cytoskeleton reorganization to form lamellipodia, a flattened F-actin–rich leading edge of migrating cells, which is critical for cancer cell migration and invasion.

2) MiR-133a upregulates Drp1 and promotes mitochondrial fission. Mitochondria are more fragmented in cells transfected with miR-133a mimic compared to CTL mimic (**Fig.2a**). Western blot assay showed that miR-133a increased Drp1 expression without effects on mitochondrial fusion proteins Mfn1 and Mfn2 (**2b**).

3) MiR-133a enhances mitochondrial respiration in CRC cells. We further studied the effects of miR-133a on mitochondrial oxidative phosphorylation (OXPHOS) levels. *In situ* analysis of oxygen consumption by Seahorse XF24 confirmed that miR-133a mimic elevated both the basal and the maximal respiratory rate in SW480 cells (**Fig.3**), suggesting a significant elevation of mitochondrial respiration upon miR-133a mimic treatment.

4) Parkin is a target of miR-133a in CRC cells. We searched for 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). 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.

We found that HCT-29 and SW-480 cells express higher levels of Parkin as 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.

5) Silencing endogenous Parkin increases Drp1 expression (**Fig.5a**) and mitochondrial respiration (**5b**) in SW480 cells.

6) Effects of overexpression of Parkin on HCT-116 cell. HCT-116 cells express lower levels of endogenous Parkin. Expression of recombinant Park1 decreases Drp1 expression (**Fig.6a**), promotes mitochondrial elongation (**6b**), and reduces mitochondrial respiration (**6c**) and HCT-116 cell migration (**6d**).

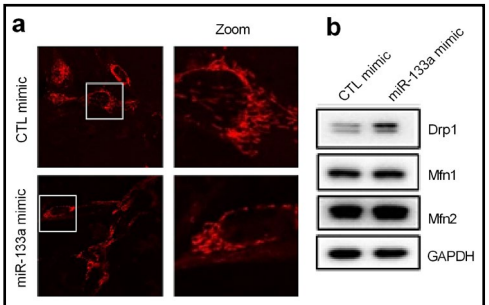


Fig.2. MiR-133a increases Drp1 and mitochondrial fission.

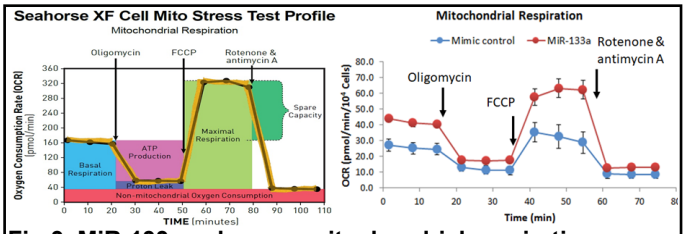


Fig.3. MiR-133a enhances mitochondrial respiration.

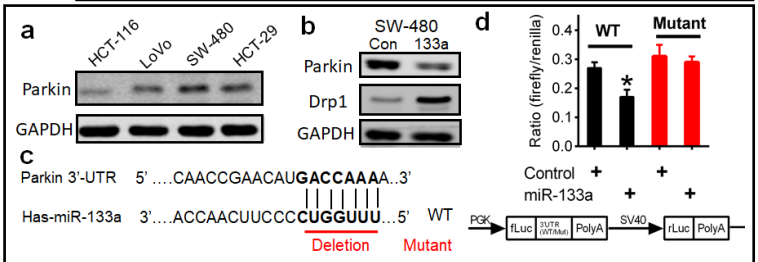


Fig.4. Parkin is a target of miR-133a. (a) Decreased Parkin expression in metastatic CRC cells**. (b)** miR-133a reduced Parkin expression with increased Drp1 expression. **(c)** The putative miR-133a binding site in the 3′-UTR of the *Parkin* and its deletion mutant at the seeding region. **(d)** Firefly 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.

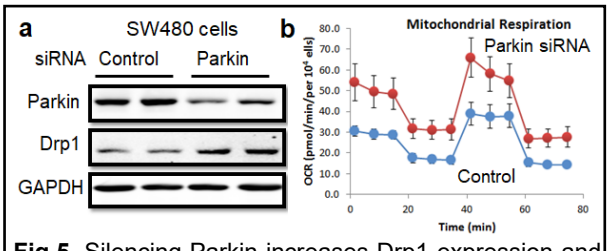


Fig.5. Silencing Parkin increases Drp1 expression and mitochondrial respiration in SW480 cells. N=3.

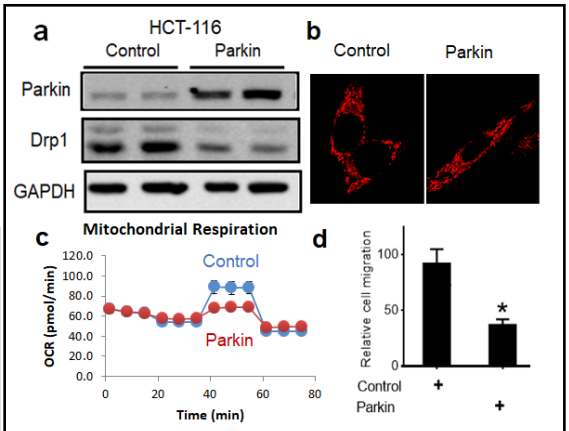


Fig.6. Parkin regulates Drp1, mitochondrial fission, respiration and HCT-116 cell migration**.** N=3-5, **p*<0.05.

EXPANSION OF THE PROJECT: Genetic Mechanisms of Apoptosis Resistance in Colorectal Cancer

Dysregulation of apoptosis is a hallmark of cancer, contributing to uncontrolled cancer cell growth. Numerous cancer therapeutics exert their activity by promoting apoptosis. However, most cancer cells eventually develop drug resistance, in large part by altering the apoptotic pathways (6). Finding critical molecular targets and designing therapeutics to effectively kill cancer cells is urgently needed. The Bcl-2 family proteins, including anti-apoptotic members, the BH3-only proteins, and effectors Bax and Bak, have long been recognized as the major regulators of apoptosis and promising therapeutic targets. While anti-apoptotic members Bcl-2, Bcl-xL, and Mcl-1 inhibit Bax/Bak, the pro-apoptotic BH3-only proteins, such as Bim, Puma, Bid, and Bad, promote Bax/Bak activation. Through collaboration with Dr. Xu Luo, an expert studying cell apoptosis at the University of Nebraska Medical Center, we found that knockdown of Bcl-xL and inhibition of Mcl-1 together is sufficient to induce apoptosis in CRC cells. Thus, Bcl-xL and Mcl-1 provide new molecular targets for therapeutic agents against CRC.

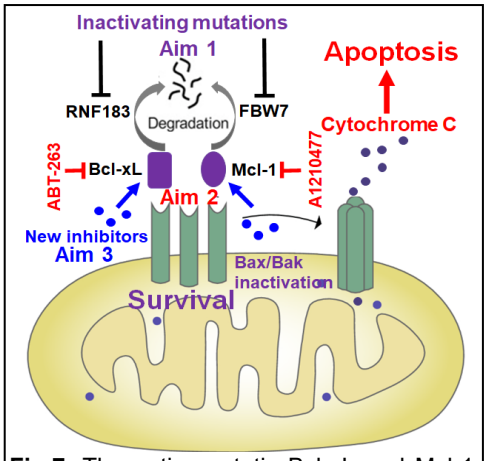


Fig.7. The anti-apoptotic Bcl-xL and Mcl-1 inhibit Bax/Bak-dependent cell apoptosis. Inactivating mutations of *FBW7* and *RNF183* contribute to apoptosis resistance in CRC via defective Mcl-1 and Bcl-xL degradation. **Aim 1** will identify and characterize the inactive FBW7 and RNF183 mutants on Mcl-1 and Bcl-xL degradation and apoptosis resistance in CRC; **Aim 2** will determine *in vivo* effects of pharmacologically targeting Mcl-1 and Bcl-xL on apoptosis-resistant CRC; **Aim 3** will screen for small molecule drugs that kill CRC cells via targeting Mcl-1 and/or Bcl-xL.

Bcl-xL and Mcl-1 are frequently overexpressed in human cancers (7). Twenty percent of CRC have Bcl-xL amplification, whereas increased Mcl-1 protein leads to drug resistance in CRC. Distinctive from other Bcl-2 members, Mcl-1 and Bcl-xL are unstable and their degradation can be triggered by various stresses. Previous studies identified the tumor suppressor FBW7 as an E3 ubiquitin ligase that targets Mcl-1 for degradation, and its inactivating mutations cause drug-resistance in CRC cells due to defective Mcl-1 degradation (8,9). A recent study also showed that the E3 ligase RNF183 ubiquitinates Bcl-xL for degradation (10), and inhibition of Cathepsin D enhances anticancer drug-induced apoptosis via RNF183-mediated destabilization of Bcl-xL in cancer cells (11). Although RNF183 was implicated in CRC and may confer resistance to anti-cancer drugs in CRC cells (12,13), its mutational status and roles in Bcl-xL stability and apoptosis resistance in CRC are totally unknown. Our preliminary studies identified defective Mcl-1 and Bcl-xL degradation in apoptosis-resistant CRC cells. Thus, **we hypothesize** that genetic inactivating mutations of *FBW7* and *RNF183* contribute to apoptosis-resistant CRC development via defective Mcl-1 and Bcl-xL degradation (**Fig.7**).

1) Mcl-1 and Bcl-xL are two major determinants of CRC cell survival. The CRISPR/Cas9 technique was used to generate human CRC HCT116 cells deficient for *Bax and Bak* (DKO), or Bid, Bim, and Puma (TKO), and TKO/*Bax/Bak* KO cells (**Fig.8a**) (14). Three TKO clones were examined. Cells were then transfected with siRNAs against Bcl-xL and Mcl-1, singly or in combination. Surprisingly, the double knockdown of Mcl-1 and Bcl-xL induced robust apoptosis as evidenced by PARP cleavage in both WT and TKO cells, but not in DKO or TKO/*Bax/Bak* KO cells (**8b**).

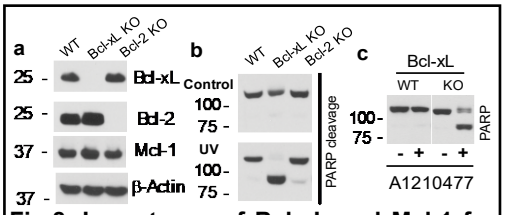


Fig.8. Importance of Bcl-xL and Mcl-1 for suppression of HCT116 cell apoptosis. (a) Generation of Bcl-xL or Bcl-2 KO cells. UV- **(b)** or A1210477-induced **(c)** apoptosis.

2) Mcl-1 and Bcl-xL requirement for suppression of CRC cell apoptosis. The strong sensitization to UV treatment (eliminating Mcl-1)-induced apoptosis by the loss of Bcl-xL, but not that of Bcl-2, indicates that Bcl-xL is involved in protecting cells against apoptosis (**Fig.8a, 8b**). Consistent with this, the Mcl-1 inhibitor A1210477 was able to induce apoptosis in Bcl-xL KO HCT116 cells (**8c**).

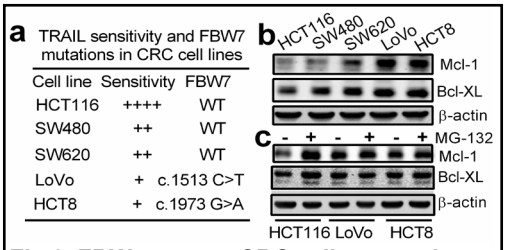


Fig.9. FBW7-mutant CRC cells are resistant to TRAIL-induced apoptosis. **(a)** TRAIL sensitivity and *FBW7* mutations. **(b)** Western blot showing Mcl-1 and Bcl-xL expression. **(c)** Effects of MG132 on Mcl-1 and Bcl-xL levels.

3) Defective Mcl-1 and Bcl-xL degradation and *FBW7* mutations in apoptosis-resistant CRC cells: TRAIL (TNF-related apoptosis-inducing ligand) is a promising anti-cancer agent due to its minimal toxicity to normal tissues and remarkable apoptotic activity in tumors. However, TRAIL is less effective in certain cancer types, including CRC, due to the presence of an intrinsic resistance mechanism. We previously

showed that the mitochondrial pathway is required for TRAIL-induced apoptosis in CRC cells (15). Interestingly, HCT116 cells are sensitive to TRAIL-induced apoptosis (TRAIL EC_{50}: 10.6 ± 4.5 ng/ml). In contrast, LoVo and HCT8 cells are more resistant to TRAIL-induced apoptosis (**Fig.9a**) (LoVo 87.5 ± 5.0 ng/ml, HCT8: 67.5 ± 5.6 ng/ml), correlating with their higher Mcl-1 and Bcl-xL levels (**9b**). Treatment with proteasome inhibitor MG132 markedly increased Mcl-1 and Bcl-xL levels in HCT116 cells but not in LoVo and HCT8 cells (**3c**). We also found *FBW7* heterozygous missense mutation R505C (c.1513 C>T) in LoVo cells and R658Q (c.1973C>A) in HCT8 cells (**9a**). These inactivating mutations cause drug-resistance in CRC cells due to defective Mcl-1 degradation (9).

Table 1 Tumor charcteristics of CRC patients

mxlnv	Numbers
0 (Benign)	30
1 (Proliferative without atypia)	53
3 (In-situ)	3
4 (Invasive)	147

Column mxlnv is the highest invasiveness code of any block or slide tumor for the individual.

4) Identification of *FBW7* and *RNF183* mutations in human CRC specimens. We have identified 233 tissue blocks from Creighton-banked CRC specimens (**Table 1**). DNA has been extracted from paraffin-embedded formalin-fixed tumor tissue. Samples will be evaluated using a next-generation sequencing platform for the detection of frequently reported mutations in cancers at **GENEWIZ Global Headquarters**. The lower limit of detection for single nucleotide variations is 5% (one mutant allele in 19 wild type alleles). Dr. Holly Stessman is a geneticist who investigates genetic “drivers” of complex human diseases to find new drug targets. We are collaborating with Dr. Stessman to identify *FBW7 and RNF183* mutations, and their associations with pathologic/clinical indices of CRC specimens will be evaluated. *FBW7* R505C and R658Q mutations are used as controls.

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, which has considerable potential as 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 that endows epithelial cancer cells with enhanced motility and invasiveness, **we hypothesize** that miR-133a-dependent upregulation of Drp1 promotes mitochondrial fission, which in turn promotes CRC metastasis. Our studies address the following two issues: 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.

Furthermore, dysregulation of apoptosis is a hallmark of cancer, contributing to uncontrolled cancer cell growth. Numerous cancer therapeutics exert their activity by promoting apoptosis. However, most cancer cells eventually develop drug resistance, in large part by altering the apoptotic pathways. The anti-apoptotic Bcl-2 family members Bcl-xL and Mcl-1 have long been recognized as the major regulators of apoptosis and promising therapeutic targets. Bcl-xL and Mcl-1 are frequently overexpressed in human cancers. Distinctive from other Bcl-2 members, Mcl-1 and Bcl-xL are unstable and their degradation can be triggered by various stresses. Based on the literature and our preliminary data, **we hypothesize** that genetic inactivating mutations of E3 ubiquitin ligase *FBW7* and *RNF183* contribute to apoptosis-resistant CRC development via defective Mcl-1 and Bcl-xL degradation. We will first utilize existing Creighton CRC specimens to identify *FBW7* and *RNF183* mutations. The importance of these mutations in Mcl-1 and Bcl-xL stability and apoptosis resistance of CRC cells will be determined (**Aim 1**). We will also test whether pharmacological inhibition of Mcl-1 and Bcl-xL can cause tumor regression *in vivo* (**Aim 2**). Finally, we will screen for new small molecule drugs that target Mcl-1 and Bcl-xL to induce apoptosis in CRC (**Aim 3**). Completion of these studies will not only establish mutational status of *FBW7* and *RNF183* and defective Mcl-1 and Bcl-xL degradation as key determinants of apoptosis resistance in CRCs, but also provide a rationale for effective combinations of drugs targeting Mcl-1 and Bcl-xL in apoptosis-resistant CRC. In the long term, our results could be useful for rational design of more effective strategies and agents to overcome therapeutic resistance caused by genomic instability in CRC. Thus, our studies will have a significant impact on both basic and clinical cancer research and eventually on patient survival.

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.
6. Johnstone RW, Ruefli AA, and Lowe SW. (2002) Apoptosis: a link between cancer genetics and chemotherapy. *Cell.*108:153-164.
7. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. (2010) The landscape of somatic copy-number alteration across human cancers. *Nature.* 463:899-905.
8. Inuzuka H, Shaik S, Onoyama I, Gao D, Tseng A, Maser RS, Zhai B, Wan L, Gutierrez A, Lau AW, Xiao Y, Christie AL, Aster J, Settleman J, Gygi SP, Kung AL, Look T, Nakayama KI, DePinho RA, Wei W. (2011) SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. *Nature.* 471:104-9.
9. Tong J, Tan S, Zou F, Yu J, Zhang L. (2017) FBW7 mutations mediate resistance of colorectal cancer to targeted therapies by blocking Mcl-1 degradation. *Oncogene.* 36:787-96.
10. Wu Y, Li X, Jia J, Zhang Y, Li J, Zhu Z, Wang H, Tang J, Hu J. (2018) Transmembrane E3 ligase RNF183 mediates ER stress-induced apoptosis by degrading Bcl-xL. *Proc Natl Acad Sci U S A.* 115:E2762-71.
11. Seo SU, Woo SM, Im SS, Jang Y, Han E, Kim SH, Lee H, Lee HS, Nam JO, Gabrielson E, Min KJ, Kwon TK. (2022) Cathepsin D as a potential therapeutic target to enhance anticancer drug-induced apoptosis via RNF183-mediated destabilization of Bcl-xL in cancer cells. *Cell Death Dis.* 13:115.
12. Geng R, Tan X, Wu J, Pan Z, Yi M, Shi W, Liu R, Yao C, Wang G, Lin J, Qiu L, Huang W, Chen S. (2017) RNF183 promotes proliferation and metastasis of colorectal cancer cells via activation of NF-κB-IL-8 axis. *Cell Death Dis.* 8:e2994.
13. Geng R, Tan X, Zuo Z, Wu J, Pan Z, Shi W, Liu R, Yao C, Wang G, Lin J, Qiu L, Huang W, Chen S. (2017) Synthetic lethal short hairpin RNA screening reveals that ring finger protein 183 confers resistance to trametinib in colorectal cancer cells. *Chin J Cancer.* 36:63.
14. Zhang J, Huang K, O'Neill KL, Pang X, Luo X. (2016) Bax/Bak activation in the absence of Bid, Bim, Puma, and p53. *Cell Death & Disease*. 7:e2266.
15. Huang K, Zhang J, O'Neill KL, Gurumurthy CB, Quadros RM, Tu Y**,** Luo X. (2016) Cleavage by caspase 8 and mitochondrial membrane association activate the BH3-only protein Bid during TRAIL-induced apoptosis. *J Biol Chem*. 291:11843-51.

II. List of publications (7/1/2022– 6/30/2023)

Xie Y, Abel PW, Casale TB, Tu Y. (2022) TH17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149(2):467-479. PMCID: PMC8821175.

III. List of extramural grants submitted from 7/1/2022 – 6/30/2023

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
R01 HL164593-01A1
Title: A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-Resistant (Refractory) Asthma
Dates: 4/2023 - 3/2028

Role: Tu (PI)
Total funds requested: $2,092,677
Impact Score: 29; Percentile: 15 (**Will be awarded**)

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
R01 HL171202-01
Title: Targeting Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Refractory Asthma
Dates: 12/2023 - 11/2028
Role: Tu (PI)
Total funds requested: $2,236,132
Impact Score: 29; Percentile: 13 (Current payline: 14)

National Institutes of Health – NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
R01HL170466
Title: Impact of genetic variation in the RGS pathway on airway smooth muscle mechanophenotypes and asthma severity measures
Dates: 9/2023 - 8/2027
Role: Co-Investigator; Juan Carlos Cardet (PI)
Total funds requested: $42,337; **Unfunded**

The Lynch Comprehensive Cancer Research Center
Kicks for a Cure Cancer Research Grant
Title: Genetic Mechanisms of Apoptosis Resistance in Colorectal Cancer
Dates: 1/2023 - 12/2023
Role: Tu (PI)
Total funds requested: $40,000
Impact Score: 20 (**Awarded**)

Health Sciences Strategic Investment Fund
Title: Molecular mechanisms and new targets of refractory asthma
Dates: 7/1/2023-06/30/2025
Role: Tu (PI)
Total funds requested: $50,000

IV. List of extramural grants awarded from 7/1/2022 – 6/30/2023

Nebraska Department of Health and Human Services Cancer and Smoking Disease Research (LB506)
Title: New therapeutic agents for cigarette smoke-related pulmonary fibrosis
$50,000 (7/2022 - 6/2023)
Role: Tu (PI)

The Lynch Comprehensive Cancer Research Center
Kicks for a Cure Cancer Research Grant
Title: Genetic Mechanisms of Apoptosis Resistance in Colorectal Cancer
$40,000 (1/2023 - 12/2023)
Role: Tu (PI)

Creighton University Cancer & Smoking Disease Research Program
FY20/21 Progress Report
(July 1, 2022 – June 30, 2023)

CELLULAR SIGNALING AND MOLECULAR TRAFFICKING IN CANCER
Program Director: Laura A. Hansen, PhD

Inhibition of cancer growth with highly selective and proteolytically stabile peptide analogs
Principal Investigator: Sándor Lovas, PhD

I. Progress Report Summary

A. Specific Aims

The aims have not been modified.

B. Studies and Results

Aim 1.

1. Following our original design, we further modified the thirteen residue C-terminal fragment of chlorotoxin (CTX), which is a 36-amino acid peptide and has high-binding selectivity for glioblastoma cells: Ac-[Cys^{26},$Ser^{28,33}$]CTX(24-36)-NH_2 (P76). The sequence of P76: Ac-Gly^{24}-Arg-(Cys^{26}-Lys-Ser^{28}-Tyr-Gly-Pro-Gln-Ser^{33}-Leu-Cys^{35})-Arg-NH_2; the peptide has Cys^{26}-Cys^{36} disulfide bridge. At 1 μM concentration P76 and the full length CTX had similar MMP-2 inhibitory efficacy.

We have also designed a shorter analog of CTX: Ac-CTX(27-34)-NH_2, Ac-Lys-(Cys-Tyr-Gly-Pro-Gln-Cys)-Leu-NH_2, the peptide has Cys^{28}-Cys^{33} disulfide bridge.

2. In order to obtain a clear picture about where our designed peptides bind and interact with MMP-2, we have initiated blind docking simulations of the peptide analogs to the molecular dynamics (MD) simulations refined X-ray structure of MPP-2. The docking poses were refined by MD simulation and currently re-ranked by MM-PBSA calculations.

3. Structure of β-turn stabilized P76 analogs were studied by extensive molecular MD simulations and electronic circular dichroism (ECD) sprectropolarimetry. Follwing the previous P75 design, five analogs were synthesized in which the central -Gly-Pro- sequence was replaced with turn-stabilizing dipeptides. Markov state modeling of the MD trajectories showed that the peptide analogs lost their native antiparallel hairpin structure (β-sheet-loop-β-sheet) but a β-bend structure at residues Tyr^{29} – Gln^{32} was stabilized. The simulation results are supported by ECD measurements.

Aim 2.

1. To determine experimentally the interaction between our synthetic peptide analogs and the active form of recombinant MMP-2, we are developing a methodology by using a differential scanning fluorometry (DSF) technique. Initial data indicate that we can detect binding of P75

and P76 to MMP-2. This methodology would be an alternative/additional methodology to the direct enzyme inhibition assay.

2. We have expanded U-87 glioblastoma cells migration inhibitory testing using the scratch assay methodology. With this assay, we have showed that P75 and our new P76 analog have the same migration inhibitory effect as does the parent CTX. Testing of the other synthetic analogs is in progress.

C. Significance

In the previous budget year, we established that the C-terminal fragment analog of CTX (P75) has the same MMP-2 inhibitory activity as the full, native CTX. By modifying the disulfide bridge pattern, we have designed a new peptide (P76) This peptide also inhibits the cell migratory activity of U-87 glioblastoma cells. From MD and ECD studies, the structural preferences of free peptides are revealed. Blind docking studies indicate that the peptide analogs are binding not only to the catalytic site of MMP-2, but also to neighboring sites indicating possible allosteric mechanism of action of the peptides.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

Rudd, J., Maity, S., Grunkemeyer, J., Snyder, J.C., Lovas, S., and Hansen, L.A. Membrane structure and internalization dynamics of human Flower isoforms, *Journal of Biological Chemistry*, 2023, doi: 10.1016/j.jbc.2023.104945.

III. List of extramural grants submitted from 7/1/2022–6/30/2023

NIH 1R01NS133338-01 PI: S. Dravid
Dates: 7/1/2023 – 6/30/2028
Project title: Striatal Trans-Synaptic Signaling Mechanism in Parkinsonism
Role: Co-I

NIH 1R01MH134953-01 PI: S. Dravid
Dates: 12/01/23 – 11/30/2028
Project title: Novel molecular tools to modulate the function of glutamate delta 1 receptor
Role: co-I

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

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-PI
Award: $50,000

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

Lynch Comprehensive Cancer Research Center
Principal Investigators: Laura Hansen, PhD; Lesley Conrad, MD

I. Progress Report Summary

A. Specific Aims

The overall goal of this funding is to further the growth of the Lynch Comprehensive Cancer Research Center (LCCRC) by providing funds for the recruitment and support of new faculty whose research is focused on cancer research. This aim has not changed.

B. Studies and Results

Research Progress

This has been a transitional year for the LCCRC with the departure of the previous director, Dr. Robin Farias-Eisner, who was replaced by Dr. Laura Hansen as LCCRC basic sciences director and Dr. Lesley Conrad as the interim clinical director by the dean. Under their direction, the LCCRC is restructuring to maximize current strengths in basic science and translational research, while also planning for the future. The LCCRC website has been updated to include several recent faculty hires and LCCRC additions, including Yusi Fu, PhD; Jun Xia, PhD; Brian North, PhD; Gopal Jadhav, PhD; Tal Teitz, PhD; and Waddah Al-Refaie, MD. Dr. Hansen has coordinated many meetings with LCCRC faculty, including multiple series of monthly meetings focused on developing and communicating cancer research. Dr. Hansen has monthly meetings with Dr. Al-Refaie, newly hired as the chair of the Department of Surgery. Dr. Al-Refaie is an established clinician-scientist and cancer researcher who recently received a notice of grant award from the NIH for his R01 application entitled “REmote symptom COllection to improVE postopeRative care (RECOVER).” In his role as chair, he is currently recruiting a thoracic surgery chief, who will have the responsibility of building research strength in that division, along with other surgery faculty with cancer research interests. Other significant current recruiting activities include a new chair for the Department of Biomedical Sciences (who will be a cancer researcher), a medical oncology chief, and an ENT chair.

The LCCRC directors convened monthly LCCRC oversight committee meetings; the focus in the past year has been on developing several clinician-basic scientist collaborations in the area of women’s cancers. As a result, the Creighton Women’s Health Initiative Haddix grant was submitted and subsequently awarded (see below). This collaborative project will collect samples from OB/GYN patients to be provided to Dr. Fu for genomic sequencing and analysis. A collaborative project between Dr. Fu and Dr. Conrad entitled “Genomic Characterization of Endometrial Serous Carcinoma with Ultra-Sensitive and Accurate Duplex DNA Sequencing” was also developed and submitted to the Mary Kay Ash Foundation for funding.

Dr. Hansen's LB595 program also holds regular joint lab meetings in which trainees of LB595-funded faculty present their recent research results and the faculty provide their expertise and advice. The LCCRC also hosted the LB595-funded cancer research seminar series to bring several leading cancer researchers to Creighton to present their research to our local cancer research community.

Salary support was provided in the past year to two recent faculty hires, Dr. Yusi Fu and Dr. Jim Grunkemeyer, who were recruited and hired by the previous LCCRC director. Dr. Grunkemeyer has been instrumental over the past year in facilitating the success of Dr. Hansen's research, as well as developing his own cancer research project (see grant submissions below). Dr. Grunkemeyer is a coauthor on one manuscript published in the *Journal of Biological Chemistry* and developed and submitted a proposal for funding through the LB595 Development program. Dr. Fu, an emerging faculty member, had a very productive year, coauthoring two publications

Basic Science	**Clinical Researchers**
Laura Hansen, PhD	Waddah Al-Refaie, MD
Sandor Lovas, PhD	Peter Silberstein, MD
Jun Xia, PhD	Kalyana Nandipati, MD
Patrick Swanson, PhD	*Medical Oncology Chief (recruitment in progress)*
Yaping Tu, PhD	*ENT Chair (recruitment in progress)*
Brian North, PhD	*Thoracic Surgery Chief (recruitment in progress)*
Yusi Fu, PhD	
Holly Stessman, PhD	
Gopal Jadhav, PhD	
Tal Tietz, PhD	
BMS Department Chair, successful applicant will be a cancer researcher (recruitment in progress)	
Tumor immunology faculty member in MMI (recruitment in progress)	

and submitting eight grant applications to a mix of internal, foundation, and federal research opportunities.

LCCRC Membership
Building for the future of the LCCRC

The LCCRC mission builds upon the legacy of Henry Lynch, MD, whose persistence and insight produced seminal advances in our understanding of the molecular underpinnings of cancer and improved outcomes for hereditary cancer patients.

The current year of funding will be pivotal in determining the future of the LCCRC. The dean has stated a goal of ambitious growth in cancer research and for the LCCRC in the next five to ten years. Development of a strategic plan in support of this growth will be a major focus of Dr. Hansen's work this year. Dr. Hansen was recently accepted as a fellow in the prestigious Hedwig van Ameringen Executive Leadership in Academic Medicine (ELAM) program. This program is an intensive one-year fellowship with extensive coaching, networking, and mentoring opportunities dedicated to developing leaders in academic medicine. A key component of the program is the yearlong development of an individual project related to the fellow's professional responsibilities. Over this year, Dr. Hansen will leverage the resources of the ELAM program to

develop a roadmap for the growth of the LCCRC. In the Lynch tradition, Dr. Hansen will focus on strategies by which the LCCRC can produce cutting-edge cancer research that yields improvements in patient care. This project will be completed in April of 2024 and presented to the dean at the ELAM Leaders Forum in Philadelphia. We anticipate that next year's progress report will incorporate aspects of this plan.

II. List of refereed publications germane to this project from 7/1/2022–6/30/2023

Rudd, J., Maity, S., **Grunkemeyer, J.**, Snyder, J.C., Lovas, S., and Hansen, L.A. Membrane structure and internalization dynamics of human Flower isoforms, In press in *the Journal of Biological Chemistry*, 2023.

Zhenhang Xu, Shu Tu, Caroline Pass, Yan Zhang, Huizhan Liu, **Yusi Fu**, David Z. Z. He and Jian Zuo. Profiling mouse cochlear cell maturation using 10x Genomics single-cell transcriptomics, *Frontiers in Cellular Neuroscience,* 2023.

Shihong Max Gao, Yanyan Qi, Qinghao Zhang, Aaron S. Mohammed, Yi-Tang Lee, Youchen Guan, Hongjie Li, **Yusi Fu**, Meng C. Wang. Aging Atlas Reveals Cell-Type-Specific Effects of Pro-longevity Strategies Submitted to *Nature Aging.* Available on bioRxiv, 2023.

III. List of extramural grants submitted from 7/1/2022–6/30/2023

LB692 New Initiative
Title: Molecular classification of endometrial serous carcinoma with error-corrected sequencing
PI: Yusi Fu

National Institutes of Health: NCI R21 (1R21CA286359-01)
Title: Evaluation of uterine blood as a liquid biopsy for endometrial carcinoma early diagnosis with single-cell RNA-seq
PI: Yusi Fu

Mary Kay Ash Foundation
Title: Genomic characterization of endometrial serous carcinoma with ultra-sensitive and accurate duplex DNA sequencing
PI: Yusi Fu

COBRE NIGMS Pilot Project
Title: WGS to identify candidate genetic variants associated with susceptibility to AIHL
COBRE PI: Peter Steyger; Pilot project PI: Yusi Fu

Kicks for a Cure Cancer Research Program
Title: Genomic characterization of endometrial serous carcinoma with ultra-sensitive and accurate duplex DNA sequencing.
PI: Yusi Fu

Creighton University – Dr. George F. Haddix President's Faculty Research Fund
Title: Creighton Women's Health Initiative
PI: Lesley Conrad
Co-I: Yusi Fu

National Institutes of Health: NIEHS R01 (1R01ES035884-01)
Title: The arsenic-aquaglyceroporin interactome in genome and transcriptomic evolution at single-cell level
PI: Jun Xia
Co-I: Yusi Fu

National Institutes of Health: NIAID R01 (1R01AI179878-01)
Title: RACK1 in B cell development and V(D)J recombination
PI: Patrick Swanson
Co-I: Yusi Fu

NE Dept of HHS LB595 Cancer Research Grant Application
Title: S100 Signaling as a Driver of Cutaneous Squamous Cell Carcinoma
PI: James A. Grunkemeyer

III. List of extramural grants awarded from 7/1/2022–6/30/2023

Nebraska Stem Cell Research Project – LB606
Title: ALDH1A1^{+} cancer stem cells abundance in uterine blood as a potential diagnostic marker for endometrial cancer
PI: Yusi Fu

Creighton University – Dr. Dr. George F. Haddix President's Faculty Research Fund
Title: Creighton Women’s Health Initiative
PI: Lesley Conrad
Co-I: Yusi Fu

National Institutes of Health
Title: REmote symptom COllection to improVE postopeRative care (RECOVER)
PI: Waddah Al-Refaie

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

Biorepository Infrastructure
Principal Investigator: 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. Efforts over the last year have worked to implement the 2021 decision to deidentify all samples in the former Lynch collection, allowing for the preservation of 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 ~8,996 (up from 2,700 individuals in the last report) and identified that ~7,667 (85%) of these are okay for future use under a deidentified structure; 1,217 (14%) are not approved for future use, and 112 (~1%) require additional IRB guidance/ruling, which is forthcoming. For all reapproved participants, a high-quality DNA sample has been identified, quantified, and stored for request. All blocks and slides in storage have been inventoried. Associated data files associated with specimens are being redacted as they are requested for a de-identified format and attached to the LIMS. An updated biobanking consent form allowing for ongoing identifiable contact with past research participants was approved by the local IRB in March 2023. This consent form can now be widely used as part of other studies for ongoing identifiable biobanking of any specimens and/or data collected. This consent form is currently being sent to all former Lynch biorepository participants requesting ongoing identifiable contact (aligned with the original vision of the collection). Ongoing biorepository staff efforts continue with the manual audit of remaining legacy data and specimens, including the transfer of approved data to the updated LIMS system, the deidentification of data documents (e.g., pathology reports), and the extraction, quantification, normalization, and modernized storage of DNA specimens.

Specific Aim 2. Data migration from paper to digital records. (1) All paper records were converted to digital copy in 2020-2021. Spot checking of original files identified no missing scanned documents. All paper records were securely disposed of in May 2023. 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 final auditing 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 currently undergoing a security audit through a local vendor, RedBerry. Data collection will be performed going forward using this interface after audit and IRB approval (expected to be complete in the next year).

Specific Aim 3. Modernize laboratory techniques. To support other LB595-funded work on campus, we added the following techniques/services to the Biorepository over the past year: 10X single cell genomics library preparation, RNA-seq, whole genome sequencing, whole exome sequencing, targeted sequencing, basic cell culture training and services (adherent and suspension), ELISA, DNA shearing (Covaris), custom cloning, and Sanger sequencing preparation/variant validation. These techniques continue to allow us to partially offset the cost of employing core staff. For example, 50% of personnel/benefits were covered by CDC contract work (PI: Belshan) for July 2022, and 5% of the total annual salary/benefits were covered by an IDeA grant (PI: Cote) in 2022-2023.

Two new projects were awarded in this fiscal year that will utilize the Biorepository. First, a Kicks for a Cure award (PI: Stessman) was awarded to perform targeted sequencing for high-risk hereditary cancer risk genes on all biobanked (former Lynch collection) samples. The goals of this study are to better genetically characterize the collection, to identify families carrying variants of undetermined significance (VOUSs) for additional family and functional work-up, and to identify families at high risk for novel and/or modifier mutations that would benefit from additional whole genome sequencing. In addition to these local plans for the Lynch Legacy collection, we have also begun discussion with two other potential external collaborators (Baylor College of Medicine and University of Michigan) who may seek to utilize these samples/data. Second, a Dr. George F. Haddix President's Faculty Research Fund Interprofessional Award (PI: Conrad; Co-Is: Stessman and Fu) was awarded to collect DNA from 1,000 female patients at local CHI OB-GYN clinics over the next year, with the purposes of targeted sequencing for known hereditary cancer risk variation. The goals of this study are to define local genetic carrier rates, identify those at risk for developing cancer ahead of diagnosis, and to biobank local samples with associated health data for future identifiable use.

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 7/1/2022–6/30/2023

None.

III. List of extramural grants submitted from 7/1/2022–6/30/2023

1 R01 MH133600-01NIH/NIMH (R01; PI/PD: Stessman)
“KMT5B regulation of brain development and autism”

1 R21 CA284165-01 NIH/NCI (R21; PI/PD: Stessman)
“KMT5B loss as a novel mutator phenotype in cancer”

1 R21 MH133714-01 NIH/NIMH (R21; PI/PD: Stessman)
“KMT5B regulation of IGF2 expression”

State of NE DHHS (LB506; PI/PD: Stessman)
“Tumor Suppressive Effects of KMT5B Expression”

American Lung Association (PI/PD: Belshan; Co-I: Stessman)
“Multidimensional Modelling of SARS-CoV-2 Evolution and Variant Emergence”

1 R21 HD114018-01 NIH/NICHD (R21; PI/PD: Stessman)
“KMT5B Maintenance of Genomic Imprinting”

1 R21 AR083622-01 NIH/NIAMS (R21; PI/PD: Stessman)
“KMT5B as an Epigenetic Regulator of Skeletal Muscle Development”

1 R01 NS134993-01 NIH (R01; PI/PD: Jee-Yeon Hwang; Co-I: Stessman)
“A Novel Therapeutic Strategy Targeting Neuroinflammation for Global Cerebral Ischemia Associated with Cardiac Arrest”

The Dr. George F. Haddix Interprofessional Award (Co-I: Stessman)
“Creighton Women’s Health Initiative”

Kicks for a Cure (PI: Stessman)
“Resolving Effects of Familial PMS2 Mutations in Endometrial Cancer”

Kicks for a Cure (PI: Yaping Tu; Co-I: Stessman)
“Genetic Mechanisms of Apoptosis Resistance in Colorectal Cancer”

COBRE Administrative Supplement (Project Leader: Stessman)
“TREM1 as a Novel Therapeutic Target for Global Ischemia”

State of Nebraska – LB692 New Initiative (PI: Gelineau-van Waes; Co-I: Stessman)
“Role of Fam111a in Mineral Ion Homeostasis”

State of Nebraska – LB692 New Initiative (PI: Stessman)
“KMT5B Regulation of the IGF Neurotrophic Axis”

IV. List of extramural grants awarded from 7/1/2022–6/30/2023

The Dr. George F. Haddix Interprofessional Award (Co-I: Stessman)
“Creighton Women’s Health Initiative”

Kicks for a Cure (PI: Stessman)

“Resolving Effects of Familial PMS2 Mutations in Endometrial Cancer”

Patient-Centered Outcomes Research Institute (PCORI) (Co-I: Stessman)
“Midwest Autism Patient-Centered Research Consortium (MARC)”

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

Investigator	Submitted FY 22/23	# Submitted
Juliane Strauss-Soukup	$510,219	2
Holly Feser Stessman	$3,922,396	10
Laura Hansen	$182,641	3
Brian North	$2,755,444	2
Patrick Swanson	$1,887,500	2
Yaping Tu	$4,499,339	6
Sandor Lovas	$0	0
John Cote	$201,375	1
Yusi Fu	$579,249	3
Peter Abel	$0	0
Jun Xia	$2,881,500	2
Gajanan Shelkar	$474,250	2
TOTAL SUBMISSIONS	$17,893,913	33

Investigator	Awarded FY 22/23	# Awarded
Juliane Strauss-Soukup	$410,810	4
Holly Feser Stessman	$64,493	3
Laura Hansen	$393,788	3
Brian North	$323,275	2
Patrick Swanson	$0	0
Yaping Tu	$80,340	3
Sandor Lovas	$0	0
John Cote	$50,000	1
Yusi Fu	$81,652	1
Peter Abel	$0	0
Jun Xia	$249,000	1
Gajanan Shelkar	$24,911	1
TOTAL AWARDS	$1,678,269	19

LB595 Investigator Awards FY22/23

Principal Investigator	Sponsor Name	Project Title	Awarded Project Period Start Date	Awarded Project Period End Date	Directs	Indirects	Total
John Cote	National Institutes of Health/Great Plains IDeA-CTR/State of NE - LB692	Great Plains 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	$50,000.00	$0.00	$50,000.00
					Total: $50,000.00	Total: $0.00	Total: $50,000.00
Holly Feser Stessman	State of NE - LB692	Laboratory Equipment	27-Apr-2023	30-Jun-2023	$20,000.00	$0.00	$20,000.00
	Patient-Centered Outcomes Research Institute (PCORI)	Midwest Autism Patient-Centered Research Consortium (MARC)	01-Jun-2021	31-May-2023	$3,209.00	$1,284.00	$4,493.00
	Kicks for a Cure, Inc.	Resolving Effects of Familial PMS2 Mutations in Endometrial Cancer	01-Jul-2021	30-Jun-2022	$40,000.00	$0.00	$40,000.00
					Total: $63,209.00	Total: $1,284.00	Total: $64,493.00
Yusi Fu	State of Nebraska Stem Cell Research Project - LB606	ALDH1A1+ Cancer Stem Cells Abundance in Uterine Blood as a Potential Diagnostic Marker for Endometrial Cancer	01-Aug-2022	30-Jun-2023	$81,652.00	$0.00	$81,652.00
					Total: $81,652.00	Total: $0.00	Total: $81,652.00
Laura Hansen	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
	State of NE - LB692	Laboratory Equipment	27-Apr-2023	30-Jun-2023	$3,287.00	$0.00	$3,287.00
	State of NE - LB692	Research Salary Support	01-Jul-2022	30-Jun-2023	$64,326.34	$0.00	$64,326.34
					Total: $291,788.34	Total: $102,000.00	Total: $393,788.34
Brian North	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	$25,000.00	$0.00	$25,000.00
	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
					Total: $230,000.00	Total: $93,275.00	Total: $323,275.00
Gajanan Shelkar	Health Sciences Strategic Investment Fund	Role of GluN2C-Containing NMDA Receptor in Cocaine Addiction	01-Jul-2022	30-Jun-2024	$24,911.00	$0.00	$24,911.00
					Total: $24,911.00	Total: $0.00	Total: $24,911.00
Juliane Strauss-Soukup	National Institutes of Health/University of NE Medical Center	Nebraska Research Network in Functional Genomics	01-May-2020	30-Apr-2025	$152,075.00	$69,194.00	$221,269.00
	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	$33,500.00	$15,243.00	$48,743.00

Principal Investigator	Sponsor Name	Project Title	Awarded Project Period Start Date	Awarded Project Period End Date	Directs	Indirects	Total
	State of NE - LB692	Bridge Funding for NIH project: Examination of Ornithine Decarboxylase Antizyme RNA Structure and Function for the Development of Antibiological Agents	01-Jul-2022	30-Jun-2023	$75,000.00	$0.00	$75,000.00
	State of NE - LB692	Research Salary Support	01-Jul-2022	30-Jun-2023	$65,798.00	$0.00	$65,798.00
					Total: $326,373.00	Total: $84,437.00	Total: $410,810.00
Yaping Tu	Kicks for a Cure, Inc.	Genetic Mechanisms of Apoptosis Resistance in Colorectal Cancer	03-Jan-2023	31-Dec-2023	$40,000.00	$0.00	$40,000.00
	State of NE - LB692	Laboratory Equipment	27-Apr-2023	30-Jun-2023	$38,194.00	$0.00	$38,194.00
	American Lung Association/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: $80,340.00	Total: $0.00	Total: $80,340.00
Jun Xia	National Institutes of Health	The Role of Aquaporin 3 in Arsenic-Induced DNA Damage and Mutagenesis	15-Aug-2022	31-Jul-2025	$192,876.00	$56,124.00	$249,000.00
					Total: $192,876.00	Total: $56,124.00	Total: $249,000.00
							Total: $1,678,269.34

LB595 Investigator Submissions FY22/23

Principal Investigator	Sponsor Name	Project Title	Requested Project Period Start Date	Requested Project Period End Date	Directs	Indirects	Total
John Cote	March of Dimes	3D Printed Models and Psychological Constructs in Pregnancies With and Without Facial Clefts	01-Mar-2023	28-Feb-2025	$183,068.43	$18,306.84	$201,375.27
					Total: $183,068.43	Total: $18,306.84	Total: $201,375.27
Holly Feser Stessman	National Institutes of Health	KMT5B Regulation of Brain Development and Autism	01-Jul-2023	30-Jun-2028	$1,250,000.00	$587,500.00	$1,837,500.00
	State of Nebraska - LB506	Tumor Suppressive Effects of KMT5B Expression	01-Jul-2023	30-Jun-2024	$65,000.00	$0.00	$65,000.00
	National Institutes of Health	KMT5B Loss as a Novel Mutator Phenotype in Cancer	01-Jul-2023	30-Jun-2025	$275,000.00	$129,250.00	$404,250.00
	State of Nebraska - LB692	KMT5B Regulation of the IGF Neurotrophic Axis	01-Jul-2023	30-Jun-2024	$75,000.00	$0.00	$75,000.00
	Kicks for a Cure, Inc.	Resolving Effects of Familial PMS2 Mutations in Endometrial Cancer	03-Jan-2023	31-Dec-2023	$40,000.00	$0.00	$40,000.00
	National Institutes of Health	KMT5B as an Epigenetic Regulator of Skeletal Muscle Development	01-Sep-2023	31-Aug-2025	$275,000.00	$129,250.00	$404,250.00
	National Institutes of Health	KMT5B Maintenance of Genomic Imprinting	01-Sep-2023	31-Aug-2025	$275,000.00	$129,250.00	$404,250.00
	National Institutes of Health	KMT5B Regulation of IGF2 Expression	01-Jul-2023	30-Jun-2025	$275,000.00	$129,250.00	$404,250.00
	State of Nebraska - LB692	Laboratory Equipment	27-Apr-2023	30-Jun-2023	$20,000.00	$0.00	$20,000.00
	National Institutes of Health/University of Nebraska Medical Center	COBRE Administrative Supplement: TREM1 as a Novel Therapeutic Target for Global Ischemia (Stessman Project)	01-Aug-2023	31-Jan-2024	$182,242.00	$85,654.00	$267,896.00
					Total: $2,732,242.00	Total: $1,190,154.00	Total: $3,922,396.00
Yusi Fu	State of Nebraska - LB692	Molecular Classification of Endometrial Serous Carcinoma with Error-Corrected Sequencing	01-Jul-2023	30-Jun-2024	$75,000.00	$0.00	$75,000.00
	National Institutes of Health	Evaluation of Uterine Blood as a Liquid Biopsy for Endometrial Carcinoma Early Diagnosis with Single-Cell RNA-seq	01-Dec-2023	30-Nov-2025	$275,000.00	$129,250.00	$404,250.00
	Mary Kay Foundation	Genomic Characterization of Endometrial Serous Carcinoma with Ultra-Sensitive and Accurate Duplex DNA Sequencing	01-Jul-2023	30-Jun-2025	$86,955.75	$13,043.36	$99,999.11
					Total: $436,955.75	Total: $142,293.36	Total: $579,249.11
Laura Hansen	State of Nebraska - LB692	Laboratory Equipment	27-Apr-2023	30-Jun-2023	$3,287.38	$0.00	$3,287.38
	State of Nebraska - LB692	Research Salary Support	01-Jul-2023	30-Jun-2024	$69,353.18	$0.00	$69,353.18

Principal Investigator	Sponsor Name	Project Title	Requested Project Period Start Date	Requested Project Period End Date	Directs	Indirects	Total
	State of Nebraska Stem Cell Research Project - LB606	Flower Lineage Tracing in CRAINBOW Mice Stem Cells	01-Jul-2023	30-Jun-2024	$110,000.59	$0.00	$110,000.59
					Total: $182,641.15	Total: $0.00	Total: $182,641.15
Brian North	National Institutes of Health	Upstream Regulators of Connexin 43-Dependent Intercellular Communication to Promote Wound Healing	01-Jul-2023	30-Jun-2028	$1,830,234.08	$860,210.02	$2,690,444.10
	State of Nebraska - LB506	Identifying Regulators of Liver Caner Metastasis	01-Jul-2023	30-Jun-2024	$65,000.00	$0.00	$65,000.00
					Total: $1,895,234.08	Total: $860,210.02	Total: $2,755,444.10
Gajanan Shelkar	Brain and Behavior Research Foundation	Astrocytic NMDA Receptor Plasticity in Cocaine Addiction	15-Jan-2024	14-Jan-2026	$70,000.00	$0.00	$70,000.00
	National Institutes of Health	Astrocytic NMDA Receptors in Cocaine-Induced Neuroadaptations: Relevance to Addiction	01-Dec-2023	30-Nov-2025	$275,000.00	$129,250.00	$404,250.00
					Total: $345,000.00	Total: $129,250.00	Total: $474,250.00
Juliane Strauss-Soukup	National Institutes of Health	Examination of Ornithine Decarboxylase Antizyme RNA Structure and Function from Various Organisms for the Development of Antibiological Agents	01-Jul-2023	30-Jun-2026	$299,999.06	$140,999.56	$440,998.62
	State of Nebraska - LB692	Research Salary Support	01-Jul-2023	30-Jun-2024	$69,220.35	$0.00	$69,220.35
					Total: $369,219.41	Total: $140,999.56	Total: $510,218.97
Patrick Swanson	Health Sciences Strategic Investment Fund	Role of RACK1 in Regulating RAG Protein Translation	01-Jul-2023	30-Jun-2025	$50,000.00	$0.00	$50,000.00
	National Institutes of Health	RACK1 in B Cell Development and V(D)J Recombination	01-Dec-2023	30-Nov-2028	$1,250,000.00	$587,500.00	$1,837,500.00
					Total: $1,300,000.00	Total: $587,500.00	Total: $1,887,500.00
Yaping Tu	National Institutes of Health	A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-Resistant (Refractory) Asthma	01-Apr-2023	31-Mar-2028	$1,495,493.00	$597,184.00	$2,092,677.00
	Kicks for a Cure, Inc.	Genetic Mechanisms of Apoptosis Resistance in Colorectal Cancer	03-Jan-2023	31-Dec-2023	$40,000.00	$0.00	$40,000.00
	National Institutes of Health	Targeting Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Refractory Asthma	01-Dec-2023	30-Nov-2028	$1,580,912.00	$655,220.00	$2,236,132.00
	National Institutes of Health	Impact of Genetic Variation in the RGS Pathway on Airway Smooth Muscle Mechanophenotypes and Asthma Severity Measures	01-Sep-2023	31-Aug-2027	$28,800.00	$13,536.00	$42,336.00

Principal Investigator	Sponsor Name	Project Title	Requested Project Period Start Date	Requested Project Period End Date	Directs	Indirects	Total
	State of Nebraska - LB692	Laboratory Equipment	27-Apr-2023	30-Jun-2023	$38,193.50	$0.00	$38,193.50
	Health Sciences Strategic Investment Fund	Molecular Mechanisms and New Targets of Refractory Asthma	01-Jul-2023	30-Jun-2025	$50,000.00	$0.00	$50,000.00
					Total: $3,233,398.50	Total: $1,265,940.00	Total: $4,499,338.50
Jun Xia	National Institutes of Health	The Arsenic-Aquaglyceroporin Interactome in Genome and Transcriptomic Evolution at Single-Cell Level	01-Dec-2023	30-Nov-2028	$1,500,000.00	$634,500.00	$2,134,500.00
	National Institutes of Health	The Role of Aquaporin 3 in Arsenic-Induced DNA Damage and Mutagenesis	01-Aug-2022	31-Jul-2026	$546,089.00	$200,911.00	$747,000.00
					Total: $2,046,089.00	Total: $835,411.00	Total: $2,881,500.00
							Total: $17,893,913.10

Creighton University Cancer & Smoking Disease Research Program
FY22/23 Progress Report
(July 1, 2022 – June 30, 2023)

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

Cellular Signaling and Molecular Trafficking in Cancer Program

Publications for Hansen:

1. Rudd, J., Maity, S., Grunkemeyer, J., Snyder, J.C., Lovas, S., and Hansen, L.A. Membrane structure and internalization dynamics of human Flower isoforms, *Journal of Biological Chemistry*, 2023, doi: 10.1016/j.jbc.2023.104945. https://www.jbc.org/article/S0021-9258(23)01973-7/fulltext

Publications for North:

None in this cycle.

Publications for Swanson:

None in this cycle.

Publications for Tu:

1. Xie Y, Abel PW, Casale TB, Tu Y. (2022) TH17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149(2):467-479. PMCID: PMC8821175. https://www.jacionline.org/article/S0091-6749(21)02672-5/pdf

Publications for Lovas:

1. Rudd, J., Maity, S., Grunkemeyer, J., Snyder, J.C., Lovas, S., and Hansen, L.A. Membrane structure and internalization dynamics of human Flower isoforms, *Journal of Biological Chemistry*, 2023, doi: 10.1016/j.jbc.2023.104945. https://www.jbc.org/article/S0021-9258(23)01973-7/fulltext

Biorepository Infrastructure

Publications for Stessman Project:

None in this cycle.

Development Program

Publications for Coté project:

1. Coté, J.J., Côté-Arsenault, D., Handelzalts, J., Badura-Brack, A., Kalata, M., Walters, R.W., Kasinath, P., Herbig, K., Kump, D.A., Tampi, R. (2023). The effects of 3D printed models and 3D printed pictures on maternal and paternal-fetal attachment, anxiety, and depression. Journal of Obstetric, Gynecologic & Neonatal Nursing https://doi.org/10.1016/j.jogn.2023.02.002. https://www.sciencedirect.com/science/article/abs/pii/S0884217523000205

2. Coté, J. J., Coté, B. P., & Badura-Brack, A. S. (2022). 3D printed models in pregnancy and its utility in improving psychological constructs: A case series. 3D Printing in Medicine, *8*(1), 1-6. doi 10.1186/s41205-022-00144-w. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9178798/

Publications for Abel project:

1. Hulen J, Kenny D, Black R, Hallgren J, Hammond KG, Bredahl EC, Wickramasekara RN, Abel PW, Stessman HAF. KMT5B is required for early motor development. Front Genet. 2022;13:901228. doi: 10.3389/fgene.2022.901228. eCollection 2022. PubMed PMID: 36035149; PubMed Central PMCID: PMC9411648. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9411648/

2. Xie Y, Abel PW, Casale TB, Tu Y. (2022) TH17 cells and corticosteroid insensitivity in severe asthma. J Allergy Clin Immunol. 149(2):467-479. PMCID: PMC8821175. https://www.jacionline.org/article/S0091-6749(21)02672-5/pdf
2.

Publications for Fu project:

1. *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 Z. Z. He and Jian Zuo. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9434313/

2. Submitted to *Nature Aging.* Available on bioRxiv, Aging Atlas Reveals Cell-Type-Specific Effects of Pro-longevity Strategies, Authors: Shihong Max Gao, Yanyan Qi, Qinghao Zhang, Aaron S. Mohammed, Yi-Tang Lee, Youchen Guan, Hongjie Li, Yusi Fu, Meng C. Wang. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10002668/

Publications for Shelkar project:

None for this cycle.

Publications for Xia project:

1. Ashour ME, Byrum AK, Meroni A, **Xia J**, Singh S, Galletto R, Rosenberg SM, Vindigni A, Mosammaparast N. Rapid profiling of DNA replication dynamics using mass spectrometry–based analysis of nascent DNA. ***Journal of Cell Biology***. 2023 Feb 16;222(4):e202207121. https://rupress.org/jcb/article-abstract/222/4/e202207121/213875/Rapid-profiling-of-DNA-replication-dynamics-using?redirectedFrom=fulltext

2. Zhai Y, Pribis JP, Dooling SW, Garcia-Villada L, Minnick PJ, Xia J, Liu JJ, Mei Q, Fitzgerald DM, Herman C, Hastings, PJ, Costa-Mattoli M, Rosenberg SM. Drugging evolution of antibiotic resistance at a regulatory network hub. ***Science Advances***. 9, eadg0188 (2023). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10289659/

Creighton University
Cancer & Smoking Disease Research Program
Report of Expenditures
July 1, 2022 - June 30, 2023

	Approved Budget	Total Expenses	Remaining Budget
Personnel	$812,621.00	$727,485.99	$85,135.01
Consultant	26,000.00	12,020.94	13,979.06
Equipment	220,771.00	325,914.29	(105,143.29)
Supplies	28,247.00	51,318.58	(23,071.58)
Other Expenses	212,361.00	109,567.61	102,793.39
Total	$1,300,000.00	$1,226,307.41	$73,692.59

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 2022 – June 2023

TABLE OF CONTENTS

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

Dr. Cowan plans to step down as Director during the coming funding period but will remain at UNMC for some time to help successfully facilitate the leadership transition. A search process has been undertaken by UNMC leadership, guided by a national firm and a search committee formed with key stakeholders from across UNMC, along with patient and community representatives to provide critical input from those groups. A new director is anticipated to take the helm at BCC in late 2023.

DIRECTOR'S OVERVIEW AND SIX ESSENTIAL CHARACTERISTICS

Physical Space

The BCC integrated cancer research and cancer care facility celebrated its grand opening in June 2017, with now-President Biden (then-former Vice President Biden) delivering the keynote address. The 615,000 sq ft

BCC 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 reviewers made note of the fact that the new facility providing the BCC Director with direct 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. In the coming reporting periods, the BCC will continue to build on its existing strengths in space and facilities as aligns with the vision of a new director once Dr. Cowan has completed his tenure.

Organizational Capabilities

The Cancer Center continues to rely primarily on two principal advisory boards to guide center organization, planning, and evaluation: the BCC External Advisory Board, comprised of nationally recognized leaders from NCI-designated centers with expertise in basic, translational, clinical, and population research, as well as cancer center administration; and the BCC Senior Leadership Council, made up of key cancer research leaders from across various units at UNMC. (A listing of the current SLC roster is shown below.) These groups meet regularly with Center leadership to review and develop various research, outreach and engagement, as well as training and education activities. The BCC Senior Leadership Council includes the Associate Directors, program leaders, clinical oncology leaders, translational working group leaders, and key department chairs. It meets at least monthly to review Cancer Center activities and shape strategic initiatives. In the coming project periods, the BCC will continue improving on clarifying the roles of the leadership committees in the planning process to help delineate how the individual committees function and interact and redefine the processes and procedures surrounding decision-making.

FRED & PAMELA 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
Benjamin Teply, MD: Medical Director, BCC Clinical Trials Office
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

Transdisciplinary Collaboration and Coordination

The BCC's transdisciplinary collaboration and coordination have been evaluated by the National Cancer Institute to be "Oustanding". The reviewers appreciated that 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. The BCC was assessed 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 many multi-member peer-reviewed publications and several multi-investigator grants.

As previously noted, 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 highlighted again here:

- **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.

- **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.

- **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.

- **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.

- **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.

As discussed, the reviewers noted that a primary mechanism to facilitate collaboration is the successful pilot grant program involving BCC leadership at all levels, with each area getting to prioritize pilot funding directives. In 2022, the BCC funded 21 collaborative pilot projects totaling more than $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 through a key partnership with 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.

In the coming reporting periods, the BCC will continue working to improve the translation of basic science

discoveries into clinical trial success, as the reviewers suggested. Dr. Benjamin Teply was named as Medical Director of the BCC Clinical Trials Office to support Dr. Apar Ganti, the Associate Director for Clinical Research. The BCC also hosted a clinical research review in April 2023, inviting five successful clinical research administrators from various NCI-designated cancer centers to evaluate and advise on our clinical trials infrastructure and operations. The valuable insight and guidance they provided will assist us in improving upon our transdisciplinary success in the next funding periods.

Cancer Focus

Highlights of the BCC's commitment to cancer focus include the Center's established history of outstanding cancer research, which it continues to maintain and expand. Grants awarded by the NCI to BCC members remain a significant source of extramural funding to support and stimulate growth in cancer research at UNMC. In coming project periods, the BCC will continue to work to further increase cancer-focused funding and also on facilitating the publishing of cancer research papers in high-impact journals.

Institutional Commitment

The level of institutional commitment afforded to the BCC remains very strong. This is evidenced by the recent improved integration of cancer research efforts and clinical activities, via the BCC Director's direct reporting relationship to the UNMC Chancellor and also the Nebraska Medicine CEO. Of particular note are the key authorities ascribed to the BCC Director, including:
- Being a member of the major leadership cabinets for UNMC (Chancellor's Council) and Nebraska Medicine (Senior Leadership Team);
- Having the authority to confer BCC membership status to faculty from across all University of Nebraska academic units;
- Having authority over the state budget for the Eppley Institute, an independent college-level academic unit at UNMC, and for the BCC, as well as over all fundraising and philanthropic funds for EI/BCC;
- Having complete authority over all BCC-controlled space for research and administration, approximately 400,000 net square feet, which is a 67% increase since 2015; and
- Having authority to recruit faculty with primary academic appointments into the Eppley Institute and oversight of promotion and tenure in the Institute.

Taken together, these authorities allow for better consolidation of the matrix responsibilities of the Director and linking of efforts in cancer research and clinical care. These increased levels of administrative linkage, oversight, and integration are an important demonstration of the commitment from UNMC to the mission and success of BCC. A focus in coming reporting periods will be to leverage these critical institutional commitments to help successfully transition leadership to a new BCC director, once Dr. Cowan has finalized his tenure and stepped down as BCC director.

Center Director

Dr. Cowan, has had an extraordinary cancer career in research, practice, and as a center director. He, along with the BCC leadership team and membership he built over his 20-plus-year tenure at UNMC, were directly responsible for significant progress made 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
- Improvements in clinical trial functionality and enrollment
- Growth of the BCC leadership team via adding roles such as Deputy Director, Associate Directors for Basic Research, Translational Research, Clinical Research, Cancer Research Training and Education Coordination, Community Outreach and Engagement, and Diversity, Equity and Inclusion, as well as several key Assistant Director positions.

Dr. Cowan will step down as Director during the coming funding period, but he plans to remain at UNMC for

some time to help successfully facilitate the leadership transition. As mentioned, the search process for Dr. Cowan's replacement has been underway throughout the summer of 2023, and the next BCC director is anticipated to begin at UNMC later this year.

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.

Leadership

BCC senior leaders are tasked with implementing the Cancer Center's research mission, under the guidance of internal and external advisors and related evaluation systems. The BCC senior leadership team continues to meet regularly to discuss and shape the Cancer Center's research priorities and strategic priorities.

In the previous reporting period, the following key roles on the BCC senior leadership were created and filled to further develop the strategic areas of diversity, equity and inclusion and shared resources.

Quan Ly, MD, was named as Associate Director for Diversity, Equity and Inclusion, emphasizing the importance of developing DEI-focused initiatives at the Buffett Cancer Center. (BCC funds are not being requested to support Dr. Ly at this time, since there is not an existing DEI component to support that as of yet; however, they will be requested in the next competitive renewal application.) Dr. Ly is a professor in the UNMC Department of Surgical Oncology. She has an experienced surgical oncologist specializing in the management of gastrointestinal, soft tissue and endocrine neoplasms. In addition to caring for patients with GI cancers, she has actively pursued a career in translational research collaborating with other basic science researchers with the goal of bringing bench research to bedside. Since being appointed as the AD of DEI in May 2022, Dr. Ly has been meeting monthly with the ADs of CRTEC and COE to define areas of overlap and ways that each program can support the other. She has also attended the monthly Cancer Center DEI Network Group meeting to ascertain the current best practices being used by cancer centers. Specifically, Dr. Ly is currently in the process of developing DEI-focused IAB and EABs for the BCC.

Heather Jensen-Smith, PhD, was named as Assistant Director for Shared Resources, in recognition of the value the BCC places on developing quality, cutting-edge research resources for our member investigators. Dr. Jensen-Smith is a research assistant professor in the Eppley Institute for Research in Cancer, and she also serves as Director of both the Preclinical Imaging and Advanced Microscopy Shared Resources. Her scientific contributions have focused on the development, and use of, various imaging methodologies to generate

images accurately representing various biological phenomena during normal and disease states. Dr. Jensen-Smith actively supports numerous basic and translational studies by developing and implementing minimally invasive imaging techniques for observing a broad spectrum of endogenous and exogenous fluorescent indicators used to characterize various physiological and biological changes in individual organs, tissues, and cells. As Assistant Director for Shared Resources in the Fred & Pamela Buffett Cancer Center, she will play an important role in aligning current and emerging research resource needs with state-of-the-art instrumentation for Cancer Center researchers.

Benjamin Teply, MD, was recently named as the Medical Director of the Clinical Trials Office for the Buffett Cancer Center. Dr. Teply is an associate professor in the UNMC Department of Internal Medicine, Division of Oncology & Hematology, focusing on genitourinary malignancies. His research interests are centered on prostate cancer and include novel hormonal and non-hormonal approaches, optimal use of approved therapies, and clinical trials. He was recognized with UNMC Internal Medicine's 2021 award for excellence in clinical research. Dr. Teply's experience as a physician and his research activities made him an excellent choice to step into this newly created position.

Dr. Teply will work with the Associate Director for Clinical Research, Dr. Apar Ganti, to oversee the clinical operations in the Clinical Trials Office. This will include strategic planning and ensuring that trial participants, investigators, regulatory authorities, and trial sponsors are provided with quality service. As Medical Director of the BCC CTO, Dr. Teply's responsibilities include:

· Reviewing and resolving or escalating clinical, regulatory, or budget issues with investigators, service line, other departments, and internal/external partners.
· Overseeing the CTO operations and administrative directors with respect to the operations of the CTO, including management of staff job descriptions, workload assessment, hiring, and performance evaluations.
· Working with CTO operations/administrative directors and ADCR to optimize timely trial activation and high-quality study conduct.
· Reviewing internal and external audit/monitoring visit reports and working with the CTO staff to develop, implement, and monitor corrective action plans with specific focus on physician oversight and compliance; determining best methods for disseminating communications regarding corrective action plans with faculty and clinical staff.
· Providing oversight of the development and review of standard operating procedures (SOPs) for the CTO.
· Providing the annual performance evaluation of the CTO operations and administrative director.

Planning and Evaluation

The Buffett Cancer Center continues to utilize two principal Advisory Boards for planning and evaluation purposes: the BCC External Advisory Board and the BCC 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 typically annually with Center leadership to review research programs, clinical research, outreach and engagement, training and education, and progress made on DEI goal setting, metric measuring, and initiative developments. BCC senior leadership are currently discussing plans for future EAB meetings. A table with the current EAB roster is included below.

Fred & Pamela Buffett Cancer Center External Advisory Board	
Kerry L. Burnstein, Ph.D.	Professor and Chair, Department of Molecular and Cellular Pharmacology, University of Miami Miller School of Medicine Associate Director for Education and Training, Sylvester Comprehensive Cancer Center
Victoria L. Champion, Ph.D., R.N., F.A.A.N.	Mary Margaret Walther Professor of Nursing, Distinguished Professor, and Edward & Sarah Stam Cullipher Endowed Chair, Indiana University School of Nursing Associate Director of Community Outreach and Population Science Research, IU Simon Comprehensive Cancer Center

Robert B. Diasio, M.D.	Professor, Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine Director Emeritus, Mayo Clinic Cancer Center
Eric R. Fearon, M.D., Ph.D.	Professor, Departments of Human Genetics, Internal Medicine, and Pathology, and Emanuel N. Maisel Professor of Oncology, University of Michigan Michigan Medicine Director, University of Michigan Rogel Cancer Center
Robert W. Gerlach, M.P.A.	Associate Director for Administration and Scientific Affairs, Dartmouth-Hitchcock Norris Cotton Cancer Center
Stanton L. Gerson, M.D.	Professor, Departments of Medicine and Environmental Health Sciences; Asa and Patricia Shiverick-Jane Shiverick (Tripp) Professor of Hematological Oncology; and Case Western Reserve University Distinguished University Professor Dean and Senior Vice President for Medical Affairs, School of Medicine Director, National Center for Regenerative Medicine Case Western Reserve University Acting Director, Case Comprehensive Cancer Center
I. David Goldman, M.D. **EAB Chair**	Professor, Departments of Medicine (Division of Oncology) and Molecular Pharmacology, and Susan Resnick Fisher Chair in Brain Cancer Research, Albert Einstein College of Medicine Former Director, Albert Einstein Cancer Center
Stanley R. Hamilton, M.D.	Professor and Chair, Department of Pathology, City of Hope Comprehensive Cancer Center
Ernest T. Hawk, M.D., M.P.H.	Vice President and Head, Division of Cancer Prevention and Population Sciences, Department of Clinical Cancer Prevention, and T. Boone Pickens Distinguished Chair for Early Prevention of Cancer, The University of Texas MD Anderson Cancer Center
Patrick J. Loehrer, M.D.	Professor, Division of Hematology/Oncology, Department of Internal Medicine; Joseph W. and Jackie J. Cusick Professor of Oncology; and IU Distinguished Professor, Indiana University School of Medicine Former Director, Indiana University Simon Cancer Center
Linda Malkas, Ph.D.	Dean of Translational Science, External Affairs, City of Hope Deputy Director for Basic Research, City of Hope Comprehensive Cancer Center
James J. Mulé, Ph.D.	Associate Center Director for Translational Science, Interim Associate Director for Basic Science, Michael McGillicuddy Endowed Chair for Melanoma Research and Treatment, and Scientific Director of Cell-Based Therapies, Moffitt Cancer Center
Douglas Yee, M.D.	Professor, Departments of Medicine (Division of Hematology, Oncology and Transplantation) and Pharmacology, University of Minnesota Medical School Director, Masonic Cancer Center

The Senior Leadership Council includes the BCC Associate Directors, Program Leaders, clinical oncology leaders, Translational Working Group Leaders, and key UNMC department chairs. It continues to meet monthly and during other ad hoc meetings as needed to review Cancer Center activities and update, revise, and evaluate the progress of strategic initiatives. A table with the current SLC roster is available on page 4 of this report.

BCC senior leadership is looking forward to working with the next director of the Cancer Center, once named, to further develop its strategic planning systems and initiatives. A BCC external advisory board meeting will likely be held sometime in 2023 after the leadership transition to the future director once Dr. Cowan's tenure has been completed. Anticipated leadership developments include identifying a new Co-Leader for the Cancer Biology Program, after the previous co-leader, Pankaj Singh, PhD, recently departed UNMC. This process is being led by the BCC Director, Deputy Director, and other senior leaders, in coordination with Program Co-Leader, Dr. Hamid Band.

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 BCC senior leadership has assessed the best use of developmental funds to be supporting the strategic recruitment of cancer research positions at the University of Nebraska. Recent and planned recipients of BCC developmental funds and outlines of their existing and future individual research programs are highlighted here.

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*, *Scientific Reports*, *Diagnostics*, *Journal of Central Nervous System Disease*, *Immunotherapy*, *Clinical Lymphoma, Myeloma and Leukmia*, *The Journal for ImmunoTherapy of Cancer*, and *Radiation Oncology Journal*. Dr. Baine currently has active funding from the Otis Glebe Medical Foundation through a partnership with the University of Nebraska Foundation.

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", and she has a pending R01 under consideration at NCI titled "Sit Less, Exercise More: A Self-Managed Behavioral Intervention to Improve Physical Function in Older Cancer Survivors". She has also contributed to the *Journal of the National Comprehensive Cancer Network*.

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*, *FEBS Journal*, *Leukemia*, *Molecular Cancer Research*, *Communications Biology*, *bioRxiv*, *Current Protocols*, and *Methods in Molecular Biology*. She was recently awarded two R01 grants, one from the National Cancer Institute ("Mechanisms Underlying USP1-Mediated Bypass of EWS-FLI1 Oncogene-Induced Senescence in Ewing Sarcoma") and one from the National Institute of General Medical Sciences ("Regulation of SPRTN Protease and SPRTN-Mediated DNA-Protein Crosslink Repair").

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", along with papers in *Experimental Hematology*, *Bioessays*, and *Blood Advances*. His is actively funded via an R01 grant from the National Heart, Lung, and Blood Institute titled "GATA Factor Mechanisms in Erythroid Regeneration". He also serves as a Co-Core Leader for the Nebraska Center for Molecular Target Discovery and Development, an NIGMS Center of Biomedical Research Excellence.

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*, *International Journal of Molecular Sciences*, *Cancers (Basel)*, *Science Advances*, *American Journal of Reproductive Immunology*, *Journal of Assisted Reproduction and Genetics*, *Frontiers in Endocrinology (Lausanne)*, *BMC Public Health*, *JNCI Monographs*, and *Advanced Science*. She has active R01 funding from the National Institute of Child Health and Human Development to examine "Development of Mechanism-Based Ovarian Reserve Protecting Adjuvant Therapies Against Gonadotoxic Therapeutic Agents", along with ongoing funding from the Granulosa Cell Tumor Research Foundation to support an "Investigation of the Role of PPARalpha in Growth and Metabolism of Granulosa Cell Tumor".

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". Dr. Lally has recently published in the journals *Indian Journal of Cancer* and *Cancer Nursing*. 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".

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*, *Molecular Oncology*, and *NPJ Precision Oncology*. He has two active R01 grants from the National Cancer Institute ("Novel Approach to Attenuate Small-Cell Lung Cancer Growth and Metastasis" and "Targeting MUC5AC Mucin in Breast Cacner Brain Metastasis") and collaborates on an NCI P01 award ("Pancreatic Cancer Metastasis") 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*, *American Journal of Physiology – Gastrointestinal and Liver Physiology*, and *Molecular Cancer Research*. He has an 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*, *Molecular Oncology*, *Stem Cells*, *Breast Cancer Research*, *NPJ Precision Oncology*. He has an active R01 award ("Truncated O-Glycan-Dependent Mechanisms Inducing Metastatic Dissemination in Pancreatic Cancer") from the National Cancer Institute and collaborates on an NCI P01 award in "Pancreatic Cancer Metastasis" led by Principal Investigator Dr. Surinder Batra (BCC Associate Director for Translational Research). Dr. Ponnusamy also has LB 606 funding from NE DHHS to look at "Unique Stemness Potentiate Organ-Specific Metastasis".

Satyanarayana Rachagani, PhD: Dr. Rachagani was previously 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*, *Molecular Oncology*, *NPJ Precision Oncology*, and *bioRxiv*. His active R01 grant from the NCI is titled "Targeting Tumor and Its Microenvironment Using Nanotherapeutics for Pancreatic Cancer". Dr. Rachagani recently accepted a position as Associate Professor in the Department of Veterinary Medicine & Surgery at the University of Missouri.

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*, *Autophagy*, *Gastroenterology*, *Journal of Biological Chemistry*, and *Endocrinology*. He was awarded an NIAAA R00 that investigated "Synergy of Lipolysis and Lipophagy in Alcoholic Liver Disease", and he served as a Research Project Leader on a Phase 2 NIGMS COBRE (P20GM121316) led by Principal Investigator Dr. Robert Lewis (Co-Leader, BCC Targets, Modulators and Delivery Program). Dr. Schott recently received an NCI R21 to look into "Mechanisms of Lipid Droplet Trafficking in Hepatocellular Carcinoma", as well as an R35 from NIGMS evaluating "Mechanisms of Endosomal Trafficking in Lipid Droplet Catabolism".

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*, *Cancer Letters*, and *Frontiers in Immunology*. 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 currently has an active Merit award from the U.S. Veterans Administration examining "Claudin-3, Gut Dysbiosis, and Inflammatory Bowel Disease".

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 ("AKR1C3 Inhibitors as Chemotherapeutic Potentiators"), along with an NINDS R01 ("Development and Characterization of Peptidomimetic Small Molecule Activators of Peptidase Neurolysin for Stroke Therapy") and an NICHD R01 ("Small-Molecule Drug Discovery for CLN3 and CLN6 Disease"), as well as ongoing funding from the U.S. Department of Defense Congressionally Directed Medical Research Programs.

The BCC continues its pursuit of diverse discretionary investment approaches. Because of the availability of significant charitable, institutional, and endowment discretionary funds with which to support pilot project activities, we plan to continue focusing developmental funds expenditures on faculty recruitment. Historically, the Buffett Cancer Center has a strong track record of recruitment of high-quality, well-funded investigators, and we look to continue in this vein. We aim to facilitate BCC faculty additions during the coming funding periods through recruitment in the Eppley Institute (under the direct authority of the Buffett Cancer Center

Director), as well as via collaboration with leadership of the UNMC Colleges of Medicine, Dentistry, Nursing, Pharmacy, and Public Health. The BCC will continue its ongoing work with the Research Program Co-Leaders and the disease-focused translational Working Groups to identify areas of recruitment that would strengthen interdisciplinary research and promote clinical translational research opportunities.

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. 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 a regular review of Shared Resources with each Manager (and associated personnel that conduct cancer related activities). Also on a regular 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 aids 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:
Pilot Projects Program: In 2022, the BCC funded 21 collaborative pilot projects totaling more than $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 through a key partnership with 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.

Principal Investigator: **Cowan, Kenneth H.**

BCC Clinical Trials Office: The BCC has recently 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 continues to play a significant role during this ongoing process.

Major administrative plans for the coming funding periods include to effectively support the transition to a new BCC director after Dr. Cowan steps down, as well as to work with that director to initiate and carryout new director activities such as conducting a thorough membership review. BCC administration also plans to work with the Associate Director for Basic Research and the new Assistant Director for Shared Resources to conduct a shared resource satisfaction survey in order to gauge how well the BCC-supported resources are serving the Cancer Center members and to obtain input regarding potential enhancements for the facilities and services currently being offered.

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*, *Journal of Surgical Research*, *Oncology Nursing Forum*, and *JCO Oncology Practice*.

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).

Recent BCC clinical research updates include appointing Dr. Ben Teply has been appointed the Medical Director of the Clinical Trials Office. In this new role, he reports to Dr. Apar Kishor Ganti, the Associate Director for Clinical Research. Further, in a continued effort to streamline regulatory requirements and decrease timelines, we have adopted additional strategies. As part of continuing institutional commitment four additional regulatory positions were approved and funded within the clinical trials office. Three of these individuals have been hired and this has helped decrease some of the timelines for activation of NCTN clinical trials.

Plans for the coming reporting period include the CTO administrator working with the Medical Director of the CTO and the Associate Director for Clinical Research to develop metrics for each disease focused team (DFT). This will enable the individual team to keep track of their portfolio and identify under occurring trials sooner than what is currently practical. Additionally, the CTO is working with the UNMC Associate Vice Chancellor for Clinical Research to identify ways in which the electronic medical records system can be leveraged to identify potential candidates for clinical trials. This will also enable us to identify potential patient population for a given trial thereby giving the individual DFT information which will help them decide on selection of trials to open. Finally, the CTO in conjunction with the UNMC Clinical Research Center, is working on further streamlining the regulatory process which will help shorten the timelines for opening clinical trials.

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 have been no new personnel changes in 2022. The membership of the SRC, DSMC and Audit committees continues to be multidisciplinary.

Michelle Desler, M.S., is 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, B.S., B.A., 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 Data and Safety Monitoring Committee (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.

Laurel Ahlman, B.Med.Sc., is the Scientific Review Committee (SRC) Coordinator. Her primary function is to serve as the SRC coordinator for Cancer Center trials. She collaborates with the PI's and their representatives to assist in the development of protocols to ensure SRC requirements are met prior to submission and ensures that all cancer therapeutic trials sponsored by the institution are monitored by the DSMC and Audit committees. She also maintains and tracks low accrual waivers, reviews, and covenants. Ms. Ahlman also monitors all non-compliance issues with NCI guidelines, PRMS committee policies, and/or the IRB.
Monica Williams-Mason is the PRMS Regulatory Data specialist and is responsible for providing administrative support of all functions of the PRMS office. This includes the coordination of functions related to the PRMS SRC, DSMC, and Audit Committees (AC).

Future plans for the PRMS office include a regular review of the policies and procedures. We have now instituted a process where-in these will be reviewed and updated on a biannual basis. Individual topics are discussed during the quarterly meetings between the PRMS staff and the Associate Director for Clinical Research. If there is felt to be a need to modify these policies, that will be worked on by the PRMS staff and be incorporated into the next version after concordance from all the stakeholders.

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*, *Oncogene*, *Elife*, *Advances in Biological Regulation*, and *bioRxiv*.

Principal Investigator: **Cowan, Kenneth H.**

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.

Dr. David Kelly oversees the MB/HTS Facility located in the BCC, and Dr. James Eudy oversees the Genomics Facility located in the Durham Research Center II, both on the UNMC campus. The Genomics facility continues to provide access to next-generation DNA sequencing (NGS) services, Sanger sequencing, single cell sequencing, and targeted gene expression assays to the University of Nebraska system researchers. During FY22, the major enhancements of the core focused on single cell applications as opposed to instrumentation. The core worked to establish multiplexing strategies and use of frozen tissue samples by optimizing single nuclei RNAseq protocols. In addition, new cell fixation techniques have been implemented to help with single cell applications.

The MB/HTS facility continues to provide small molecule siRNA and chemical HTS screening, high-content imaging and analysis, and multi-analyte profiling technologies. During FY22, a significant enhancement to the high-content imaging capabilities of the Core was achieved with the purchase of an Agilent BioTek Cytation C10 Confocal Imaging Reader featuring automated 40 and 60 uM spinning disk confocal and widefield fluorescent microscopy, Hamamatsu sCMOS camera, combined with conventional monochromatic multimode (fluorescence, luminescence, absorbance) microplate reading. The reader is integrated with an Agilent BioTek BioSpa 8 Automated Incubator with 8-plate capacity for simultaneous profiling assays using confocal/widefield fluorescence and phase imaging, plasticware/formats, image acquisition frequencies, or magnifications for quantitative measurements of cell health, viability, apoptosis, chemotaxis, cell migration, 3D cell culture, slide scanning, and other applications. This instrument provides higher-throughput capacity, more live-cell and tissue based imaging and analysis applications, and the use of a broader range of fluorescent wavelengths than current instrumentation. The instrument was purchased through funds from the FPBCC and is currently only available to Cancer Center members.

In conjunction with the MBSR, Dr. Eudy has most recently published in *Molecular and Cellular Endocrinology*, *Human Genomics*, *Frontiers in Immunology*, *Human Reproduction*, and *Genome Biology*. Dr. Kelly's efforts have contributed to publications in the journals *Fetal Pediatric Pathology*, *Scientific Data*, and *PLoS One*. The Genomics Core plans to continue to provide NGS consultation and services and to continue to develop the scRNAseq component of the lab. As the core recently upgraded the 10x Genomics instrument the core is well equipped in that context. As the area of spatial transcriptomics is gaining momentum, the core has been collaborating with investigators and has plans to continue to interface with other Core directors in imaging and bioinformatics in order to provide this service. The MB/HTS facility will continue to identify and expand unique screening libraries and instrumentation for its users. The Genomics Core sponsored 3 Tech Talk presentations focused on single cell genomics (10x Genomics) and for targeted gene expression technologies (Nanostring Inc). Similarly, the MB/HTS facility hosted three training seminars on the Cytation 10 system and Gen5 imaging and analysis system. All of these presentations were hybrid live / ZOOM webinars that were advertised widely. They were also recorded and are available for viewing through both the FPBCC and VCR office at UNMC.

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.

Principal Investigator: **Cowan, Kenneth H.**

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. Foster has most recently published in *BMC Infectious Diseases*, *Nanomedicine*, *Case Reports in Rheumatology*, *Biomaterials*, *Journal of Controlled Release*, and *Molecular Pharmaceutics*. Dr. Swanson's efforts have contributed to recent collaborative publications in: *International Journal of Surgical Pathology*, *Cancer Biomarkers*, *Journal of the National Comprehensive Cancer Network*, *International Journal of Surgical Pathology*, and *AACE Clinical Case Reports*. The TSF has experienced increased interest in advanced multiplex IHC services. To continue to meet the unique project needs FPBCC investigators and provide financially feasible custom IHC assays, the facility is developing modifiable multiplex protocols to characterize the tumor microenvironment, including innate and adaptive immune responses, apoptosis, and proliferation. The Ventana Discovery Ultra automated IHC/ISH slide staining system enables the investigator to customize base multiplex protocols to their unique interests and run distinctly different protocols with up to 7 chromogenic or 5 fluorescent markers, and archive protocols to maximize reproducibility and eliminate run-to-run variability.

The facility does not use a laboratory information management system, laboratory information is tracked manually with paper requisitions and Microsoft Excel. Equipment needs to modernize the TSF shared resource are a laboratory information management system (LIMS) and related equipment; including touch screen monitors, barcode readers and slide labelers to create a paperless sample management system with comprehensive specimen barcoding and tracking. Addition of the LIMS system would increase workflow efficiency and personnel productivity, reduce turn-around time, and permit facility personnel accommodate a significant increase in order volume.

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*, *Acta Crystallographica Section F Structural Biology Communications*, *Molecular Cancer Therapeutics*, *NPJ Microgravity*, *Vaccines (Basel)*, *Journal of Biological Chemistry*, and *Bioorganic & Medicinal Chemistry Letters*. Plans for the coming reporting period for the Structural Biology Shared Resource include soon-to-be-required replacements for the conductivity monitors on the two AKTA Pure purification systems. The facility intends to try and adapt the RI-54 to function in a cold room to test additional crystallization conditions. The university is discussing a transition to Windows 11 (fast approaching Windows 10 EOL) for all networked equipment which will put financial stress on research equipment that may have an expensive but upgradeable path or require investment in new equipment.

Principal Investigator: **Cowan, Kenneth H.**

Efforts are being made to secure funding from the College of Pharmacy, the College of Medicine, the Eppley Institute, and from the Vice-Chancellor of Research Office from excess year-end funds for the purchase of a 24-position robotic sample changer for the 600 NMR from Bruker.

Research equipment is beginning to show its age and will need to be replaced. It is becoming more difficult to find spare parts due to obsolescence as well as dealing with locating people with the knowledge to repair those systems because the original people who had this knowledge are beginning to retire. We are investigating ways to get this equipment upgraded.

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*, *RSC Medicinal Chemistry*, *Biomedicine & Pharmacotherapy*, *Scientific Reports*, *Cell Reports*, 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 for whom developmental funds were budgeted and/or expended during the previous reporting period. BCC members are shown in **bold**.

1. McDonald R, Larsen M, Liu Z, Southekal S, **Eudy J**, **Guda C**, Kumar TR. RNA-seq analysis identifies age-dependent changes in expression of mRNAs – encoding N-glycosylation pathway enzymes in mouse gonadotropes. Mol Cell Endocrinol. 2023 Aug 20;574:111971. doi: 10.1016/j.mce.2023.111971. Epub 2023 Jun 8. PMID: 37301504; PMCID: PMC10528389.

2. Ray S, **Hewitt K**. Sticky, Adaptable, and Many-sided: SAM protein versatility in normal and pathological hematopoietic states. Bioessays. 2023 Aug;45(8):e2300022. doi: 10.1002/bies.202300022. Epub 2023 Jun 15. PMID: 37318311; PMCID: PMC10527593.

3. **Siddiqui JA**, **Nasser MW**. Editorial: Role of chemokines in tumor heterogeneity. Semin Cancer Biol. 2023 Jul;92:128-129. doi: 10.1016/j.semcancer.2023.03.011. Epub 2023 Apr 5. PMID: 37028577.

4. Khan MA, Khan P, Ahmad A, Fatima M, **Nasser MW**. FOXM1: A small fox that makes more tracks for cancer progression and metastasis. Semin Cancer Biol. 2023 Jul;92:1-15. doi: 10.1016/j.semcancer.2023.03.007. Epub 2023 Mar 22. PMID: 36958703; PMCID: PMC10199453.

5. Blanco MJ, Bryant-Friedrich A, Georg G, Ali A, Ornstein PL, Ferrins L, **Trippier PC**. Excellence in Medicinal Chemistry: Celebrating ACS Medicinal Chemistry Division (MEDI) Awards. A Call for Nominations. ACS Med Chem Lett. 2023 May 18;14(6):682-684. doi: 10.1021/acsmedchemlett.3c00190. PMID: 37312854; PMCID: PMC10258899.

6. Xie S, **Naslavsky N**, **Caplan S**. EHD1 promotes CP110 ubiquitination by centriolar satellite delivery of HERC2 to the mother centriole. EMBO Rep. 2023 Jun 5;24(6):e56317. doi: 10.15252/embr.202256317. Epub 2023 Apr 19. PMID: 37074924; PMCID: PMC10240189.

7. Shahbazi Nia S, Hossain MA, Ji G, Jonnalagadda SK, Obeng S, Rahman MA, Sifat AE, Nozohouri S, Blackwell C, Patel D, Thompson J, Runyon S, Hiranita T, McCurdy CR, McMahon L, Abbruscato TJ, **Trippier PC**, Neugebauer V, German NA. Studies on diketopiperazine and dipeptide analogs as opioid receptor ligands. Eur J Med Chem. 2023 Jun 5;254:115309. doi: 10.1016/j.ejmech.2023.115309. Epub 2023 Mar 29. PMID: 37054561.

8. Pecka CJ, Thapa I, **Singh A**, **Bastola D**. A computational approach to demonstrate the control of gene expression via chromosomal access in colorectal cancer. Res Sq [Preprint]. 2023 Jun 1:rs.3.rs-2981903. doi: 10.21203/rs.3.rs-2981903/v1. PMID: 37398471; PMCID: PMC10312914.

9. Yu SY, Luan Y, Tang S, Abazarikia A, Dong R, Caffrey TC, **Hollingsworth MA**, **Oupicky D**, **Kim SY**. Uncovering Tumor-Promoting Roles of Activin A in Pancreatic Ductal Adenocarcinoma. Adv Sci (Weinh). 2023 Jun;10(16):e2207010. doi: 10.1002/advs.202207010. Epub 2023 Apr 21. PMID: 37083240; PMCID: PMC10238186.

10. Prajapati DR, Molczyk C, Purohit A, Saxena S, Sturgeon R, **Dave BJ**, **Kumar S**, **Batra SK**, **Singh RK**. Small molecule antagonist of CXCR2 and CXCR1 inhibits tumor growth, angiogenesis, and metastasis in pancreatic cancer. Cancer Lett. 2023 Jun 1;563:216185. doi: 10.1016/j.canlet.2023.216185. Epub 2023 Apr 14. PMID: 37062329; PMCID: PMC10218365.

11. Huwaimel BI, Jonnalagadda SK, Jonnalagadda S, Kumari S, Nocentini A, Supuran CT, **Trippier PC**. Selective carbonic anhydrase IX and XII inhibitors based around a functionalized coumarin scaffold. Drug Dev Res. 2023 Jun;84(4):681-702. doi: 10.1002/ddr.22049. Epub 2023 Mar 5. PMID: 36872587; PMCID: PMC10257758.

12. Ogunleye AO, Nimmakayala RK, **Batra SK**, **Ponnusamy MP**. Metabolic Rewiring and Stemness: A Critical Attribute of Pancreatic Cancer Progression. Stem Cells. 2023 May 15;41(5):417-430. doi: 10.1093/stmcls/sxad017. PMID: 36869789; PMCID: PMC10183971.

13. Kung CP, Skiba MB, Crosby EJ, Gorzelitz J, Kennedy MA, Kerr BA, Li YR, Nash S, Potiaumpai M, Kleckner AS, James DL, Coleman MF, Fairman CM, Galván GC, Garcia DO, Gordon MJ, His M, Hornbuckle LM, **Kim SY**, Kim TH, Kumar A, Mahé M, McDonnell KK, Moore J, Oh S, Sun X, Irwin ML. Key takeaways for knowledge expansion of early-career scientists conducting Transdisciplinary Research in Energetics and Cancer (TREC): a report from the TREC Training Workshop 2022. J Natl Cancer Inst Monogr. 2023 May 4;2023(61):149-157. doi: 10.1093/jncimonographs/lgad005. PMID: 37139978; PMCID: PMC10157760.

14. Kumar N, **Rachagani S**, Natarajan G, Crook A, Gopal T, Rajamanickam V, Kaushal JB, Nagabhishek SN, **Powers R**, **Batra SK**, **Saraswathi V**. Histidine Enhances the Anticancer Effect of Gemcitabine against Pancreatic Cancer via Disruption of Amino Acid Homeostasis and Oxidant-Antioxidant Balance. Cancers (Basel). 2023 May 3;15(9):2593. doi: 10.3390/cancers15092593. PMID: 37174059; PMCID: PMC10177467.

15. Sane S, Srinivasan R, Potts RA, Eikanger M, Zagirova D, Freeling J, Reihe CA, Antony RM, Gupta BK, Lynch D, Bleeker J, Turaihi H, Pillatzki A, Zhou W, **Luo X**, Linnebacher M, Agany D, Zohim EG, Humphrey LE, **Black AR**, Rezvani K. UBXN2A suppresses the Rictor-mTORC2 signaling pathway, an established tumorigenic pathway in human colorectal cancer. Oncogene. 2023 May;42(21):1763-1776. doi: 10.1038/s41388-023-02686-7. Epub 2023 Apr 10. PMID: 37037900; PMCID: PMC10287065.

16. Muilenburg KM, Isder CC, **Radhakrishnan P**, **Batra SK**, **Ly QP**, **Carlson MA**, Bouvet M, **Hollingsworth MA**, **Mohs AM**. Mucins as contrast agent targets for fluorescence-guided surgery of pancreatic cancer. Cancer Lett. 2023 May 1;561:216150. doi: 10.1016/j.canlet.2023.216150. Epub 2023 Mar 29. PMID: 36997106; PMCID: PMC10150776.

17. **Lockridge O**, Schopfer LM. Review: Organophosphorus toxicants, in addition to inhibiting acetylcholinesterase activity, make covalent adducts on multiple proteins and promote protein crosslinking into high molecular weight aggregates. Chem Biol Interact. 2023 May 1;376:110460. doi: 10.1016/j.cbi.2023.110460. Epub 2023 Mar 23. PMID: 36963650; PMCID: PMC10100150.

18. Ren B, Burkovetskaya M, Jung Y, Bergdolt L, Totusek S, Martinez-Cerdeno V, Stauch K, Korade Z, Dunaevsky A. Dysregulated cholesterol metabolism, aberrant excitability and altered cell cycle of astrocytes in fragile X syndrome. Glia. 2023 May;71(5):1176-1196. doi: 10.1002/glia.24331. Epub 2023 Jan 3. PMID: 36594399; PMCID: PMC10023374.

19. Salomon JD, Qiu H, Feng D, Owens J, Khailova L, Osorio Lujan S, Iguidbashian J, Chhonker YS, **Murry DJ**, Riethoven JJ, Lindsey ML, **Singh AB**, Davidson JA. Piglet cardiopulmonary bypass induces intestinal dysbiosis and barrier dysfunction associated with systemic inflammation. Dis Model Mech. 2023 May 1;16(5):dmm049742. doi: 10.1242/dmm.049742. Epub 2023 Jan 12. PMID: 36426663; PMCID: PMC9844230.

20. Rohrer KA, Song H, Akbar A, Chen Y, Pramanik S, Wilder PJ, McIntyre EM, **Chaturvedi NK**, **Bhakat KK**, **Rizzino A**, **Coulter DW**, **Ray S**. STAT3 Inhibition Attenuates MYC Expression by Modulating Co-Activator Recruitment and Suppresses Medulloblastoma Tumor Growth by

Augmenting Cisplatin Efficacy In Vivo. Cancers (Basel). 2023 Apr 11;15(8):2239. doi: 10.3390/cancers15082239. PMID: 37190167; PMCID: PMC10136921.

21. Southekal S, Shakyawar SK, Bajpai P, Elkholy A, Manne U, Mishra NK, **Guda C**. Molecular Subtyping and Survival Analysis of Osteosarcoma Reveals Prognostic Biomarkers and Key Canonical Pathways. Cancers (Basel). 2023 Apr 4;15(7):2134. doi: 10.3390/cancers15072134. PMID: 37046795; PMCID: PMC10093233.

22. 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. 2023 Apr 3;11(2):2077069. doi: 10.1080/21688370.2022.2077069. Epub 2022 May 21. PMID: 35603609; PMCID: PMC10161950.

23. Obeidat RF, **Lally RM**. Determinants of breast cancer screening mammography uptake: Results from Jordan population and family health survey. Indian J Cancer. 2023 Apr-Jun;60(2):266-274. doi: 10.4103/ijc.IJC_622_20. PMID: 37530252.

24. Halder S, Basu S, Lall SP, **Ganti AK**, **Batra SK**, **Seshacharyulu P**. Targeting the EGFR signaling pathway in cancer therapy: What's new in 2023? Expert Opin Ther Targets. 2023 Apr-May;27(4-5):305-324. doi: 10.1080/14728222.2023.2218613. Epub 2023 Jun 2. PMID: 37243489; PMCID: PMC10330690.

25. Thomes PG, Strupp MS, Donohue TM Jr, Kubik JL, Sweeney S, Mahmud R, **Schott MB**, Schulze RJ, McNiven MA, **Casey CA**. Hydroxysteroid 17β-dehydrogenase 11 accumulation on lipid droplets promotes ethanol-induced cellular steatosis. J Biol Chem. 2023 Apr;299(4):103071. doi: 10.1016/j.jbc.2023.103071. Epub 2023 Feb 25. PMID: 36849008; PMCID: PMC10060109.

26. Cole K, **Al-Kadhimi Z**, **Talmadge JE**. Highlights into historical and current immune interventions for cancer. Int Immunopharmacol. 2023 Apr;117:109882. doi: 10.1016/j.intimp.2023.109882. Epub 2023 Feb 27. PMID: 36848790; PMCID: PMC10355273.

27. **Ratnapradipa KL**, Napit K, Ranta J, Luma LB, Dinkel D, Robinson T, Schabloske L, **Watanabe-Galloway S**. Qualitative Analysis of Colorectal Cancer Screening in Rural Nebraska. J Cancer Educ. 2023 Apr;38(2):652-663. doi: 10.1007/s13187-022-02170-y. Epub 2022 Apr 18. PMID: 35437633; PMCID: PMC9015281.

28. Chakraborty S, Bhat AM, Mushtaq I, Luan H, Kalluchi A, **Mirza S**, Storck MD, **Chaturvedi N**, Lopez-Guerrero JA, Llombart-Bosch A, Machado I, Scotlandi K, **Meza JL**, **Ghosal G**, **Coulter DW**, **Rowley JM**, **Band V**, **Mohapatra BC**, **Band H**. EHD1-dependent traffic of IGF-1 receptor to the cell surface is essential for Ewing sarcoma tumorigenesis and metastasis. bioRxiv [Preprint]. 2023 Mar 22:2023.01.15.524130. doi: 10.1101/2023.01.15.524130. Update in: Commun Biol. 2023 Jul 20;6(1):758. PMID: 36711452; PMCID: PMC9882098.

29. Huynh LM, Hwang Y, Taylor O, **Baine MJ**. The Use of MRI-Derived Radiomic Models in Prostate Cancer Risk Stratification: A Critical Review of Contemporary Literature. Diagnostics (Basel). 2023 Mar 16;13(6):1128. doi: 10.3390/diagnostics13061128. PMID: 36980436; PMCID: PMC10047271.

30. Liu C, Carmicheal J, **Baine MJ**, **Zhang C**. Radiation therapy for pineal parenchymal tumor of intermediate differentiation: A case series and literature review. J Cent Nerv Syst Dis. 2023 Mar 16;15:11795735231160036. doi: 10.1177/11795735231160036. PMID: 36949932; PMCID: PMC10026104.

31. Chaudhary S, Appadurai MI, Maurya SK, Nallasamy P, Marimuthu S, Shah A, Atri P, Ramakanth CV, **Lele SM**, **Seshacharyulu P**, **Ponnusamy MP**, **Nasser MW**, **Ganti AK**, **Batra SK**,

Lakshmanan I. MUC16 promotes triple-negative breast cancer lung metastasis by modulating RNA-binding protein ELAVL1/HUR. Breast Cancer Res. 2023 Mar 14;25(1):25. doi: 10.1186/s13058-023-01630-7. PMID: 36918912; PMCID: PMC10012760.

32. Chhonker YS, Ahmed N, Johnston CM, Barnabas RV, **Murry DJ**. A Simultaneous Extraction/Derivatization Strategy for Quantitation of Vitamin D in Dried Blood Spots Using LC-MS/MS: Application to Biomarker Study in Subjects Tested for SARS-CoV-2. Int J Mol Sci. 2023 Mar 13;24(6):5489. doi: 10.3390/ijms24065489. PMID: 36982565; PMCID: PMC10054002.

33. **Gautam SK**, Khan P, Natarajan G, Atri P, Aithal A, **Ganti AK**, **Batra SK**, **Nasser MW**, **Jain M**. Mucins as Potential Biomarkers for Early Detection of Cancer. Cancers (Basel). 2023 Mar 7;15(6):1640. doi: 10.3390/cancers15061640. PMID: 36980526; PMCID: PMC10046558.

34. **Kim SY**, Xu J, Mayerhofer A, Bishop CV. Editorial: Small molecules and peptides in paracrine/autocrine regulation of ovarian folliculogenesis. Front Endocrinol (Lausanne). 2023 Mar 3;14:1168701. doi: 10.3389/fendo.2023.1168701. PMID: 36942025; PMCID: PMC10023699.

35. Iwahata H, **Kim SY**, Iwahata Y, Suzuki N, Woodruff TK. Thyroid hormone triiodothyronine does not protect ovarian reserve from DNA damage induced by X-ray and cisplatin. J Assist Reprod Genet. 2023 Mar;40(3):481-490. doi: 10.1007/s10815-023-02740-z. Epub 2023 Feb 21. PMID: 36805842; PMCID: PMC10033774.

36. Fatima I, Uppada JP, Chhonker YS, Gowrikumar S, Barman S, Roy S, Tolentino KT, **Palermo N**, **Natarajan A**, Beauchamp DR, Vecchio A, **Murry DJ**, **Singh AB**, **Hopkins CR**, **Dhawan P**. Identification and characterization of a first-generation inhibitor of claudin-1 in colon cancer progression and metastasis. Biomed Pharmacother. 2023 Mar;159:114255. doi: 10.1016/j.biopha.2023.114255. Epub 2023 Jan 23. PMID: 36696800.

37. Bhatia R, **Siddiqui JA**, Ganguly K, Thompson CM, Cannon A, Aithal A, Perumal N, Maurya SK, Li X, **Cox JL**, **Gurumurthy CB**, **Rachagani S**, **Jain M**, **Nasser MW**, **Batra SK**, **Kumar S**. Muc4 loss mitigates epidermal growth factor receptor activity essential for PDAC tumorigenesis. Oncogene. 2023 Mar;42(10):759-770. doi: 10.1038/s41388-022-02587-1. Epub 2023 Jan 9. PMID: 36624189; PMCID: PMC10198580.

38. Shahjin F, Patel M, Machhi J, Cohen JD, Nayan MU, Yeapuri P, Zhang C, Waight E, Hasan M, Abdelmoaty MM, Dash PK, Zhou Y, Andreu I, **Gendelman HE**, Kevadiya BD. Multipolymer microsphere delivery of SARS-CoV-2 antigens. Acta Biomater. 2023 Mar 1;158:493-509. doi: 10.1016/j.actbio.2022.12.043. Epub 2022 Dec 26. PMID: 36581007; PMCID: PMC9791794.

39. Cooley ME, Biedrzycki B, Brant JM, Hammer MJ, **Lally RM**, Tucker S, Ginex PK. Translation of Evidence-Based Interventions Into Oncology Care Settings: An Integrative Review. Cancer Nurs. 2023 Mar-Apr 01;46(2):E110-E121. doi: 10.1097/NCC.0000000000001109. Epub 2022 Dec 11. PMID: 36480276.

40. Johnson J, **Kim SY**, Sam PK, Asokan R, Cari EL, Bales ES, Luu TH, Perez L, Kallen AN, Nel-Themaat L, Polotsky AJ, Post MD, Orlicky DJ, Jordan KR, Bitler BG. Expression and T cell regulatory action of the PD-1 immune checkpoint in the ovary and fallopian tube. Am J Reprod Immunol. 2023 Mar;89(3):e13649. doi: 10.1111/aji.13649. Epub 2022 Dec 26. PMID: 36394352.

41. Rauth S, Ganguly K, Atri P, Parte S, Nimmakayala RK, Varadharaj V, Nallasamy P, Vengoji R, Ogunleye AO, **Lakshmanan I**, Chirravuri R, Bessho M, **Cox JL**, **Foster JM**, **Talmon GA**, **Bessho T**, **Ganti AK**, **Batra SK**, **Ponnusamy MP**. Elevated PAF1-RAD52 axis confers chemoresistance to human cancers. Cell Rep. 2023 Feb 28;42(2):112043. doi: 10.1016/j.celrep.2023.112043. Epub 2023 Jan 28. PMID: 36709426; PMCID: PMC10374878.

42. Caven LT, Brinkworth AJ, **Carabeo RA**. Chlamydia trachomatis induces the transcriptional activity of host YAP in a Hippo-independent fashion. Front Cell Infect Microbiol. 2023 Feb 27;13:1098420. doi: 10.3389/fcimb.2023.1098420. PMID: 36923592; PMCID: PMC10008951.

43. Otte ML, Lama Tamang R, Papapanagiotou J, Ahmad R, **Dhawan P**, **Singh AB**. Mucosal healing and inflammatory bowel disease: Therapeutic implications and new targets. World J Gastroenterol. 2023 Feb 21;29(7):1157-1172. doi: 10.3748/wjg.v29.i7.1157. PMID: 36926666; PMCID: PMC10011951.

44. Zhou Y, Dogiparthi VR, Ray S, Schaefer M, Harris HL, **Rowley MJ**, **Hewitt KJ**. Defining A Cohort of Anemia-Activated Cis-Elements Reveals a Mechanism Promoting Erythroid Precursor Function. Blood Adv. 2023 Feb 21:bloodadvances.2022009163. doi: 10.1182/bloodadvances.2022009163. Epub ahead of print. PMID: 36809789.

45. Chirravuri-Venkata R, Dam V, Nimmakayala RK, Alsafwani ZW, Bhyravbhatla N, **Lakshmanan I**, **Ponnusamy MP**, **Kumar S**, **Jain M**, Ghersi D, **Batra SK**. MUC16 and TP53 family co-regulate tumor-stromal heterogeneity in pancreatic adenocarcinoma. Front Oncol. 2023 Feb 3;13:1073820. doi: 10.3389/fonc.2023.1073820. PMID: 36816942; PMCID: PMC9936860.

46. **Lockridge O**. Overview of Adductomics in Toxicology. Curr Protoc. 2023 Feb;3(2):e672. doi: 10.1002/cpz1.672. PMID: 36799690; PMCID: PMC9942099.

47. Dasgupta A, Kurenbekova L, Patel TD, Rajapakshe K, **Ghosal G**, Nirala B, Coarfa C, Yustein J. Modeling Ewing Sarcoma Lung Metastasis. Curr Protoc. 2023 Feb;3(2):e670. doi: 10.1002/cpz1.670. PMID: 36799651; PMCID: PMC9942121.

48. Amin S, **Baine M**, **Lin C**. Immunotherapy plus stereotactic body radiation therapy or whole-brain radiation therapy in brain metastases. Immunotherapy. 2023 Feb;15(3):163-174. doi: 10.2217/imt-2022-0051. Epub 2023 Feb 7. PMID: 36748364.

49. Trinh JQ, Ellithi M, **Shonka N**, **Bergan RC**. FDA Approval Summary: Belzutifan for VHL Disease Tumors-Letter. Clin Cancer Res. 2023 Feb 1;29(3):684. doi: 10.1158/1078-0432.CCR-22-2968. PMID: 36722140; PMCID: PMC10124993.

50. Liu C, **Baine MJ**, **Fisher KW**, **Armitage JO**, **Enke CA**. Recurrent Mantle Cell Lymphoma Isolated to the Testis After 19-Year Remission: A Case Report and Review of the Literature. Clin Lymphoma Myeloma Leuk. 2023 Feb;23(2):e125-e129. doi: 10.1016/j.clml.2022.12.005. Epub 2022 Dec 6. PMID: 36572584.

51. Haney SL, Feng D, Chhonker YS, Varney ML, Williams JT, **Smith LM**, **Ford JB**, **Murry DJ**, **Holstein SA**. Evaluation of geranylgeranyl diphosphate synthase inhibition as a novel strategy for the treatment of osteosarcoma and Ewing sarcoma. Drug Dev Res. 2023 Feb;84(1):62-74. doi: 10.1002/ddr.22012. Epub 2022 Nov 25. PMID: 36433690; PMCID: PMC9931648.

52. Ramos GP, Bamidele AO, Klatt EE, Sagstetter MR, Kurdi AT, Hamdan FH, Kosinsky RL, Gaballa JM, Nair A, Sun Z, Dasari S, Lanza IR, Rozeveld CN, **Schott MB**, Urrutia G, Westphal MS, Clarkson BD, Howe CL, Marietta EV, Luckey DH, Murray JA, Gonzalez M, Braga Neto MB, Gibbons HR, Smyrk TC, Johnsen S, Lomberk G, Faubion WA. G9a Modulates Lipid Metabolism in CD4 T Cells to Regulate Intestinal Inflammation. Gastroenterology. 2023 Feb;164(2):256-271.e10. doi: 10.1053/j.gastro.2022.10.011. Epub 2022 Oct 20. PMID: 36272457; PMCID: PMC9892272.

53. Sharma G, Dal Col J, **Siddiqui JA**. Editorial: Rising stars in molecular and cellular oncology 2022. Front Oncol. 2023 Jan 23;13:1136335. doi: 10.3389/fonc.2023.1136335. PMID: 36761974; PMCID: PMC9903174.

54. Schopfer LM, David E, **Hinrichs SH**, **Lockridge O**. Human butyrylcholinesterase in Cohn fraction IV-4 purified in a single chromatography step on Hupresin. PLoS One. 2023 Jan 13;18(1):e0280380. doi: 10.1371/journal.pone.0280380. PMID: 36638134; PMCID: PMC9838835.

55. Luan H, Bielecki TA, **Mohapatra BC**, Islam N, Mushtaq I, Bhat AM, **Mirza S**, Chakraborty S, Raza M, Storck MD, Toss MS, **Meza JL**, Thoreson WB, **Coulter DW**, Rakha EA, **Band V**, **Band H**. EHD2 overexpression promotes tumorigenesis and metastasis in triple-negative breast cancer by regulating store-operated calcium entry. Elife. 2023 Jan 10;12:e81288. doi: 10.7554/eLife.81288. PMID: 36625722; PMCID: PMC9988264.

56. Nehme Z, Roehlen N, **Dhawan P**, Baumert TF. Tight Junction Protein Signaling and Cancer Biology. Cells. 2023 Jan 6;12(2):243. doi: 10.3390/cells12020243. PMID: 36672179; PMCID: PMC9857217.

57. Khan P, **Siddiqui JA**, Kshirsagar PG, Venkata RC, Maurya SK, Mirzapoiazova T, Perumal N, Chaudhary S, **Kanchan RK**, Fatima M, Khan MA, Rehman AU, **Lakshmanan I**, **Mahapatra S**, **Talmon GA**, Kulkarni P, **Ganti AK**, **Jain M**, Salgia R, **Batra SK**, **Nasser MW**. MicroRNA-1 attenuates the growth and metastasis of small cell lung cancer through CXCR4/FOXM1/RRM2 axis. Mol Cancer. 2023 Jan 4;22(1):1. doi: 10.1186/s12943-022-01695-6. PMID: 36597126; PMCID: PMC9811802.

58. Tarpley M, Chen Y, **Bhakat KK**. Genome-Wide Binding Analysis of DNA Repair Protein APE1 in Tumor Cells by ChIP-Seq. Methods Mol Biol. 2023;2701:243-252. doi: 10.1007/978-1-0716-3373-1_16. PMID: 37574487.

59. Perry M, **Ghosal G**. Isolation and Immunodetection of Enzymatic DNA-Protein Crosslinks by RADAR Assay. Methods Mol Biol. 2023;2701:135-148. doi: 10.1007/978-1-0716-3373-1_8. PMID: 37574479.

60. Sharma T, Sirpu Natesh N, Pothuraju R, **Batra SK**, **Rachagani S**. Gut microbiota: a non-target victim of pesticide-induced toxicity. Gut Microbes. 2023 Jan-Dec;15(1):2187578. doi: 10.1080/19490976.2023.2187578. PMID: 36919486; PMCID: PMC10026936.

61. Muehlebach ME, **Holstein SA**. Geranylgeranyl diphosphate synthase: Role in human health, disease and potential therapeutic target. Clin Transl Med. 2023 Jan;13(1):e1167. doi: 10.1002/ctm2.1167. PMID: 36650113; PMCID: PMC9845123.

62. Raut P, Nimmakayala RK, **Batra SK**, **Ponnusamy MP**. Clinical and Molecular Attributes and Evaluation of Pancreatic Cystic Neoplasm. Biochim Biophys Acta Rev Cancer. 2023 Jan;1878(1):188851. doi: 10.1016/j.bbcan.2022.188851. Epub 2022 Dec 16. PMID: 36535512; PMCID: PMC9898173.

63. Azadmanesh J, Seleem MA, Struble L, Wood NA, Fisher DJ, Lovelace JJ, Artigues A, Fenton AW, **Borgstahl GEO**, **Ouellette SP**, **Conda-Sheridan M**. The structure of caseinolytic protease subunit ClpP2 reveals a functional model of the caseinolytic protease system from Chlamydia trachomatis. J Biol Chem. 2023 Jan;299(1):102762. doi: 10.1016/j.jbc.2022.102762. Epub 2022 Dec 1. PMID: 36463962; PMCID: PMC9823225.

64. Shakyawar SK, Mishra NK, **Vellichirammal NN**, Cary L, Helikar T, **Powers R**, **Oberley-Deegan RE**, **Berkowitz DB**, **Bayles KW**, Singh VK, **Guda C**. A Review of Radiation-Induced Alterations of

Multi-Omic Profiles, Radiation Injury Biomarkers, and Countermeasures. Radiat Res. 2023 Jan 1;199(1):89-111. doi: 10.1667/RADE-21-00187.1. PMID: 36368026; PMCID: PMC10279411.

65. Kumari N, Siddhanta K, Panja S, Joshi V, Jogdeo C, Kapoor E, Khan R, Kollala SS, Kumar B, Sil D, **Singh AB**, **Murry DJ**, **Oupický D**. Oral Delivery of Nucleic Acid Therapies for Local and Systemic Action. Pharm Res. 2023 Jan;40(1):107-122. doi: 10.1007/s11095-022-03415-7. Epub 2022 Oct 21. PMID: 36271204; PMCID: PMC9589866.

66. Amin SA, **Baine MJ**, Rahman I, **Lin C**. The Association of Immunotherapy With the Overall Survival of Inoperable Stage III Non-small Cell Lung Cancer Patients Who Do Not Receive Chemoradiation. J Immunother. 2023 Jan 1;46(1):14-21. doi: 10.1097/CJI.0000000000000443. Epub 2022 Oct 17. PMID: 36256124.

67. **Foster JM**, **Zhang C**, Rehman S, Sharma P, Alexander HR. The contemporary management of peritoneal metastasis: A journey from the cold past of treatment futility to a warm present and a bright future. CA Cancer J Clin. 2023 Jan;73(1):49-71. doi: 10.3322/caac.21749. Epub 2022 Aug 15. PMID: 35969103.

68. Ayayee PA, Wesner JS, **Ouellette SP**. Geography, taxonomy, and ecological guild: Factors impacting freshwater macroinvertebrate gut microbiomes. Ecol Evol. 2022 Dec 23;12(12):e9663. doi: 10.1002/ece3.9663. PMID: 36582772; PMCID: PMC9789321.

69. Nallasamy P, Nimmakayala RK, Parte S, Are AC, **Batra SK**, **Ponnusamy MP**. Tumor microenvironment enriches the stemness features: the architectural event of therapy resistance and metastasis. Mol Cancer. 2022 Dec 22;21(1):225. doi: 10.1186/s12943-022-01682-x. PMID: 36550571; PMCID: PMC9773588.

70. Haney SL, **Holstein SA**. Targeting the Isoprenoid Biosynthetic Pathway in Multiple Myeloma. Int J Mol Sci. 2022 Dec 21;24(1):111. doi: 10.3390/ijms24010111. PMID: 36613550; PMCID: PMC9820492.

71. Luan Y, Yu SY, Abazarikia A, Dong R, **Kim SY**. TAp63 determines the fate of oocytes against DNA damage. Sci Adv. 2022 Dec 21;8(51):eade1846. doi: 10.1126/sciadv.ade1846. Epub 2022 Dec 21. PMID: 36542718; PMCID: PMC9770984.

72. Pokorzynski ND, Alla MR, **Carabeo RA**. Host Cell Amplification of Nutritional Stress Contributes To Persistence in Chlamydia trachomatis. mBio. 2022 Dec 20;13(6):e0271922. doi: 10.1128/mbio.02719-22. Epub 2022 Nov 15. PMID: 36377897; PMCID: PMC9765610.

73. Amador C, Bouska A, Wright G, **Weisenburger DD**, Feldman AL, **Greiner TC**, Lone W, Heavican T, **Smith L**, Pileri S, Tabanelli V, Ott G, Rosenwald A, Savage KJ, Slack G, Kim WS, Hyeh Y, Li Y, Dong G, Song J, Ondrejka S, Cook JR, Barrionuevo C, Lim ST, Ong CK, Chapman J, Inghirami G, Raess PW, Bhagavathi S, Gould C, Blombery P, Jaffe E, Morris SW, Rimsza LM, **Vose JM**, Staudt L, Chan WC, **Iqbal J**. Gene Expression Signatures for the Accurate Diagnosis of Peripheral T-Cell Lymphoma Entities in the Routine Clinical Practice. J Clin Oncol. 2022 Dec 20;40(36):4261-4275. doi: 10.1200/JCO.21.02707. Epub 2022 Jul 15. Erratum in: J Clin Oncol. 2023 May 20;41(15):2866. PMID: 35839444; PMCID: PMC9916147.

74. Bennett SJ, Yalcin D, Privatt SR, Ngalamika O, Lidenge SJ, West JT, **Wood C**. Antibody epitope profiling of the KSHV LANA protein using VirScan. PLoS Pathog. 2022 Dec 19;18(12):e1011033. doi: 10.1371/journal.ppat.1011033. PMID: 36534707; PMCID: PMC9810164.

75. Sharma G, Barbieri I, Blandino G, Poli V, **Siddiqui JA**. Editorial: Roles of non-coding RNAs in tumor growth and development. Front Oncol. 2022 Dec 1;12:1098315. doi: 10.3389/fonc.2022.1098315. PMID: 36530997; PMCID: PMC9753936.

76. Khan P, Fatima M, Khan MA, **Batra SK**, **Nasser MW**. Emerging role of chemokines in small cell lung cancer: Road signs for metastasis, heterogeneity, and immune response. Semin Cancer Biol. 2022 Dec;87:117-126. doi: 10.1016/j.semcancer.2022.11.005. Epub 2022 Nov 9. PMID: 36371025; PMCID: PMC10199458.

77. Miller DR, Ingersoll MA, Chou YW, Kosmacek EA, **Oberley-Deegan RE**, **Lin MF**. Dynamics of antioxidant heme oxygenase-1 and pro-oxidant p66Shc in promoting advanced prostate cancer progression. Free Radic Biol Med. 2022 Nov 20;193(Pt 1):274-291. doi: 10.1016/j.freeradbiomed.2022.10.269. Epub 2022 Oct 17. PMID: 36265795; PMCID: PMC10335728.

78. Bhat AA, Nisar S, Mukherjee S, Saha N, Yarravarapu N, Lone SN, Masoodi T, Chauhan R, Maacha S, Bagga P, **Dhawan P**, Akil AA, El-Rifai W, Uddin S, Reddy R, Singh M, Macha MA, Haris M. Integration of CRISPR/Cas9 with artificial intelligence for improved cancer therapeutics. J Transl Med. 2022 Nov 18;20(1):534. doi: 10.1186/s12967-022-03765-1. PMID: 36401282; PMCID: PMC9673220.

79. Buhlke EG, Hobbs AM, Rajput S, Rokusek B, Carlson DJ, Gillan C, Carlson KA. Characterization of Cross-Species Transmission of Drosophila melanogaster Nora Virus. Life (Basel). 2022 Nov 17;12(11):1913. doi: 10.3390/life12111913. PMID: 36431048; PMCID: PMC9697521.

80. Bertrand BP, Heim CE, West SC, Chaudhari SS, Ali H, Thomas VC, **Kielian T**. Role of Staphylococcus aureus Formate Metabolism during Prosthetic Joint Infection. Infect Immun. 2022 Nov 17;90(11):e0042822. doi: 10.1128/iai.00428-22. Epub 2022 Oct 26. PMID: 36286525; PMCID: PMC9670962.

81. **Fox HS**, Niu M, Morsey BM, Lamberty BG, Emanuel K, Periyasamy P, Callen S, Acharya A, Kubik G, **Eudy J**, **Guda C**, Dyavar SR, **Fletcher CV**, **Byrareddy SN**, **Buch S**. Morphine suppresses peripheral responses and transforms brain myeloid gene expression to favor neuropathogenesis in SIV infection. Front Immunol. 2022 Nov 16;13:1012884. doi: 10.3389/fimmu.2022.1012884. PMID: 36466814; PMCID: PMC9709286.

82. Menousek J, Horn CM, Heim CE, Van Roy Z, Korshoj LE, **Kielian T**. Transcriptional Profiling of Phagocytic Leukocytes and Microglia Reveals a Critical Role for Reactive Oxygen Species in Biofilm Containment during Staphylococcus aureus Craniotomy Infection. J Immunol. 2022 Nov 15;209(10):1973-1986. doi: 10.4049/jimmunol.2200503. PMID: 36426943; PMCID: PMC9643635.

83. Sharma G, Sultana A, Abdullah KM, Pothuraju R, **Nasser MW**, **Batra SK**, **Siddiqui JA**. Epigenetic regulation of bone remodeling and bone metastasis. Semin Cell Dev Biol. 2022 Nov 12:S1084-9521(22)00294-4. doi: 10.1016/j.semcdb.2022.11.002. Epub ahead of print. PMID: 36379849; PMCID: PMC10175516.

84. Maurya SK, Rehman AU, Zaidi MAA, Khan P, **Gautam SK**, **Santamaria-Barria JA**, **Siddiqui JA**, **Batra SK**, **Nasser MW**. Epigenetic alterations fuel brain metastasis via regulating inflammatory cascade. Semin Cell Dev Biol. 2022 Nov 12:S1084-9521(22)00293-2. doi: 10.1016/j.semcdb.2022.11.001. Epub ahead of print. PMID: 36379848; PMCID: PMC10198579.

85. **Lakshmanan I**, Marimuthu S, Chaudhary S, **Seshacharyulu P**, **Rachagani S**, **Muniyan S**, Chirravuri-Venkata R, Atri P, Rauth S, Nimmakayala RK, **Siddiqui JA**, **Gautam SK**, Shah A, Natarajan G, Parte S, Bhyravbhatla N, Mallya K, Haridas D, **Talmon GA**, **Smith LM**, **Kumar S**,

Ganti AK, **Jain M**, **Ponnusamy MP**, **Batra SK**. Muc16 depletion diminishes KRAS-induced tumorigenesis and metastasis by altering tumor microenvironment factors in pancreatic ductal adenocarcinoma. Oncogene. 2022 Nov;41(48):5147-5159. doi: 10.1038/s41388-022-02493-6. Epub 2022 Oct 21. PMID: 36271032; PMCID: PMC9841597.

86. Li X, Gulati M, Larson AC, **Solheim JC**, **Jain M**, **Kumar S**, **Batra SK**. Immune checkpoint blockade in pancreatic cancer: Trudging through the immune desert. Semin Cancer Biol. 2022 Nov;86(Pt 2):14-27. doi: 10.1016/j.semcancer.2022.08.009. Epub 2022 Aug 27. PMID: 36041672; PMCID: PMC9713834.

87. Trease AJ, Niu M, Morsey B, **Guda C**, **Byrareddy SN**, **Buch S**, **Fox HS**. Antiretroviral therapy restores the homeostatic state of microglia in SIV- infected rhesus macaques. J Leukoc Biol. 2022 Nov;112(5):969-981. doi: 10.1002/JLB.3HI0422-635R. Epub 2022 Jun 10. PMID: 35686500; PMCID: PMC9796061.

88. 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 Nov;86(Pt 2):511-520. doi: 10.1016/j.semcancer.2022.03.022. Epub 2022 Mar 26. PMID: 35346803; PMCID: PMC9793394.

89. 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 Nov;86(Pt 3):497-512. doi: 10.1016/j.semcancer.2022.02.017. Epub 2022 Feb 16. PMID: 35181473; PMCID: PMC9793433.

90. Rodman SN, Kluz PN, Hines MR, **Oberley-Deegan RE**, Coleman MC. Sex-based differences in the severity of radiation-induced arthrofibrosis. J Orthop Res. 2022 Nov;40(11):2586-2596. doi: 10.1002/jor.25297. Epub 2022 Feb 21. PMID: 35148568; PMCID: PMC9365890.

91. 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 Nov;86(Pt 3):457-472. doi: 10.1016/j.semcancer.2022.02.003. Epub 2022 Feb 3. PMID: 35124194; PMCID: PMC9744380.

92. 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. 2022 Nov;86(Pt 3):914-930. doi: 10.1016/j.semcancer.2021.12.009. Epub 2021 Dec 27. PMID: 34968667; PMCID: PMC9234104.

93. Rasmussen Mandolfo N, **Berger AM**, Struwe LA, Shade MY, **Goldner W**, **Klute K**, **Langenfeld SJ**, Hammer MJ. Glycemic Variability in Patients With Stage II-III Colon Cancer Treated With Surgery and Adjuvant Chemotherapy. Oncol Nurs Forum. 2022 Oct 20;49(6):571-584. doi: 10.1188/22.ONF.571-584. PMID: 36413736.

94. Lisova AE, **Baranovskiy AG**, Morstadt LM, Babayeva ND, Stepchenkova EI, **Tahirov TH**. The iron-sulfur cluster is essential for DNA binding by human DNA polymerase ε. Sci Rep. 2022 Oct 19;12(1):17436. doi: 10.1038/s41598-022-21550-4. PMID: 36261579; PMCID: PMC9581978.

95. Poole JA, Gaurav R, Schwab A, Nelson AJ, Gleason A, **Romberger DJ**, **Wyatt TA**. Post-endotoxin exposure-induced lung inflammation and resolution consequences beneficially impacted by lung-delivered IL-10 therapy. Sci Rep. 2022 Oct 15;12(1):17338. doi: 10.1038/s41598-022-22346-2. PMID: 36243830; PMCID: PMC9569365.

96. Schopfer LM, **Lockridge O**. Dichlorvos-induced formation of isopeptide crosslinks between proteins in SH-SY5Y cells. Anal Biochem. 2022 Oct 15;655:114844. doi: 10.1016/j.ab.2022.114844. Epub 2022 Aug 9. PMID: 35961399; PMCID: PMC10368009.

97. Lee FS, Ney KE, Richardson AN, **Oberley-Deegan RE**, Wachs RA. Encapsulation of Manganese Porphyrin in Chondroitin Sulfate-A Microparticles for Long Term Reactive Oxygen Species Scavenging. Cell Mol Bioeng. 2022 Oct 14;15(5):391-407. doi: 10.1007/s12195-022-00744-w. PMID: 36444349; PMCID: PMC9700555.

98. Shafieifini M, Sun Y, Staley ZR, Riethoven JJ, Li X. Effects of Nutrient Level and Growth Rate on the Conjugation Process That Transfers Mobile Antibiotic Resistance Genes in Continuous Cultures. Appl Environ Microbiol. 2022 Oct 11;88(19):e0112122. doi: 10.1128/aem.01121-22. Epub 2022 Sep 12. PMID: 36094214; PMCID: PMC9552606.

99. Frodyma DE, Troia TC, Rao C, Svoboda RA, Berg JA, Shinde DD, Thomas VC, **Lewis RE**, **Fisher KW**. PGC-1β and ERRα Promote Glutamine Metabolism and Colorectal Cancer Survival via Transcriptional Upregulation of PCK2. Cancers (Basel). 2022 Oct 5;14(19):4879. doi: 10.3390/cancers14194879. PMID: 36230802; PMCID: PMC9562873.

100. Huynh LM, Bonebrake BT, **Enke C**, **Baine MJ**. Survival Outcomes After Radiotherapy for the Treatment of Synchronous Oligometastatic Prostate Cancer. JAMA Netw Open. 2022 Oct 3;5(10):e2235345. doi: 10.1001/jamanetworkopen.2022.35345. PMID: 36206000; PMCID: PMC9547319.

101. 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; PMCID: PMC9864431.

102. Obuya S, Elkholy A, Avuthu N, Behring M, Bajpai P, Agarwal S, Kim HG, El-Nikhely N, Akinyi P, Orwa J, Afaq F, Abdalla M, Michael A, Farouk M, Bateman LB, Fouad M, Saleh M, **Guda C**, Manne U, Arafat W. A signature of Prevotella copri and Faecalibacterium prausnitzii depletion, and a link with bacterial glutamate degradation in the Kenyan colorectal cancer patients. J Gastrointest Oncol. 2022 Oct;13(5):2282-2292. doi: 10.21037/jgo-22-116. PMID: 36388691; PMCID: PMC9660062.

103. Sanft T, Day A, Peterson L, Rodriguez MA, Ansbaugh S, Armenian S, Baker KS, Ballinger T, Broderick G, Demark-Wahnefried W, **Dickinson K,** Fairman NP, Friedman DL, Goldman M, Henry NL, Hill-Kayser C, Hudson M, Khakpour N, Koura D, McDonough AL, Melisko M, Mooney K, Moore HCF, Moryl N, Neuman H, O'Connor T, Overholser L, Paskett ED, Patel C, Pirl W, Porpiglia A, Ruddy KJ, Schapira L, Shockney L, Smith S, Syrjala KL, Tevaarwerk A, Yang EH, Zee P, McMillian NR, Freedman-Cass DA. NCCN Guidelines® Insights: Survivorship, Version 1.2022. J Natl Compr Canc Netw. 2022 Oct;20(10):1080-1090. doi: 10.6004/jnccn.2022.0052. PMID: 36240847.

104. 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.

105. 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.

106. 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; PMCID: PMC9796922.

107. Lookian PP, Chen EX, Elhers LD, Ellis DG, Juneau P, Wagoner J, **Aizenberg MR**. The Association of Fractal Dimension with Vascularity and Clinical Outcomes in Glioblastoma. World Neurosurg. 2022 Oct;166:e44-e51. doi: 10.1016/j.wneu.2022.06.073. Epub 2022 Jun 27. PMID: 35772703.

108. Yu SY, Luan Y, Dong R, Abazarikia A, **Kim SY**. Adipose Tissue Wasting as a Determinant of Pancreatic Cancer-Related Cachexia. Cancers (Basel). 2022 Sep 29;14(19):4754. doi: 10.3390/cancers14194754. PMID: 36230682; PMCID: PMC9563866.

109. **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; PMCID: PMC10198578.

110. Buttari B, Arese M, **Oberley-Deegan RE**, Saso L, Chatterjee A. NRF2: A crucial regulator for mitochondrial metabolic shift and prostate cancer progression. Front Physiol. 2022 Sep 23;13:989793. doi: 10.3389/fphys.2022.989793. PMID: 36213236; PMCID: PMC9540504.

111. Verma AK, McKelvey M, Uddin MB, Palani S, Niu M, Bauer C, Shao S, Sun K. IFN-γ transforms the transcriptomic landscape and triggers myeloid cell hyperresponsiveness to cause lethal lung injury. Front Immunol. 2022 Sep 20;13:1011132. doi: 10.3389/fimmu.2022.1011132. PMID: 36203588; PMCID: PMC9530332.

112. Knoche SM, Brumfield GL, Goetz BT, Sliker BH, Larson AC, Olson MT, Poelaert BJ, Bavari A, **Yan Y**, **Black JD**, **Solheim JC**. The histone deacetylase inhibitor M344 as a multifaceted therapy for pancreatic cancer. PLoS One. 2022 Sep 20;17(9):e0273518. doi: 10.1371/journal.pone.0273518. PMID: 36126055; PMCID: PMC9488834.

113. Zhang C, Atri P, Nallasamy P, Parte S, Rauth S, Nimmakayala RK, Marimuthu S, Chirravuri-Venkata R, Bhatia R, Halder S, Shah A, **Cox JL**, **Smith L**, **Kumar S**, **Foster JM**, Kukreja RC, **Seshacharyulu P**, **Ponnusamy MP**, **Batra SK**. Small molecule inhibitor against onco-mucins disrupts Src/FosL1 axis to enhance gemcitabine efficacy in pancreatic ductal adenocarcinoma. Cancer Lett. 2022 Sep 19;551:215922. doi: 10.1016/j.canlet.2022.215922. Epub ahead of print. PMID: 36285687; PMCID: PMC10124158.

114. Schopfer LM, Onder S, **Lockridge O**. Organophosphorus Pesticides Promote Protein Cross-Linking. Chem Res Toxicol. 2022 Sep 19;35(9):1570-1578. doi: 10.1021/acs.chemrestox.2c00194. Epub 2022 Sep 1. PMID: 36048166; PMCID: PMC9490787.

115. Ayayee PA, Currie A, Peterson JA. Different Gut Microbiomes of Developmental Stages of Field-Collected Native and Invasive Western Bean Cutworm, Striacosta albicosta, in Western Nebraska. Microorganisms. 2022 Sep 14;10(9):1828. doi: 10.3390/microorganisms10091828. PMID: 36144430; PMCID: PMC9505167.

116. 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.

117. **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 Sep;1877(5):188783. doi: 10.1016/j.bbcan.2022.188783. Epub 2022 Aug 24. PMID: 36028149.

118. 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.

119. **Ratnapradipa KL**, Ranta J, Napit K, Luma LB, Robinson T, Dinkel D, Schabloske L, **Watanabe-Galloway S**. Qualitative analysis of cancer care experiences among rural cancer survivors and caregivers. J Rural Health. 2022 Sep;38(4):876-885. doi: 10.1111/jrh.12665. Epub 2022 Apr 5. PMID: 35381622; PMCID: PMC9492624.

120. **Watanabe-Galloway S**, Kim J, LaCrete F, Samson K, **Foster J**, **Farazi PA**, **LeVan T**, Napit K. Cross-sectional survey study of primary care clinics on evidence-based colorectal cancer screening intervention use. J Rural Health. 2022 Sep;38(4):845-854. doi: 10.1111/jrh.12631. Epub 2021 Nov 16. PMID: 34784067; PMCID: PMC9108125.

121. Shakyawar S, Southekal S, **Guda C**. mintRULS: Prediction of miRNA-mRNA Target Site Interactions Using Regularized Least Square Method. Genes (Basel). 2022 Aug 25;13(9):1528. doi: 10.3390/genes13091528. PMID: 36140696; PMCID: PMC9498445.

122. He D, Feng H, Sundberg B, Yang J, Powers J, Christian AH, Wilkinson JE, Monnin C, Avizonis D, Thomas CJ, Friedman RA, Kluger MD, **Hollingsworth MA**, **Grandgenett PM**, **Klute KA**, Toste FD, Chang CJ, Chio IIC. Methionine oxidation activates pyruvate kinase M2 to promote pancreatic cancer metastasis. Mol Cell. 2022 Aug 18;82(16):3045-3060.e11. doi: 10.1016/j.molcel.2022.06.005. Epub 2022 Jun 24. PMID: 35752173; PMCID: PMC9391305.

123. Manevski M, Devadoss D, Long C, Singh SP, **Nasser MW**, Borchert GM, Nair MN, Rahman I, Sopori M, Chand HS. Corrigendum: Increased expression of LASI LncRNA regulates the cigarette smoke and COPD associated airway inflammation and mucous cell hyperplasia. Front Immunol. 2022 Aug 8;13:988069. doi: 10.3389/fimmu.2022.988069. Erratum for: Front Immunol. 2022 Jun 14;13:803362. PMID: 36003371; PMCID: PMC9393848.

124. 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; PMCID: PMC9635595.

125. Joshi V, Chhonker YS, Soni D, Cunningham KC, Samuelson DR, **Murry DJ**. A Selective and Sensitive LC-MS/MS Method for Quantitation of Indole in Mouse Serum and Tissues. Metabolites. 2022 Aug 2;12(8):716. doi: 10.3390/metabo12080716. PMID: 36005588; PMCID: PMC9416675.

126. 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.

127. 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.

128. **Black JD**, Affandi T, **Black AR**, Reyland ME. PKCα and PKCδ: Friends and Rivals. J Biol Chem. 2022 Aug;298(8):102194. doi: 10.1016/j.jbc.2022.102194. Epub 2022 Jun 24. PMID: 35760100; PMCID: PMC9352922.

129. Yeapuri P, Olson KE, Lu Y, Abdelmoaty MM, Namminga KL, Markovic M, Machhi J, **Mosley RL**, **Gendelman HE**. Development of an extended half-life GM-CSF fusion protein for Parkinson's disease. J Control Release. 2022 Aug;348:951-965. doi: 10.1016/j.jconrel.2022.06.024. Epub 2022 Jun 30. PMID: 35738463; PMCID: PMC10350880.

130. Huwaimel BI, Jonnalagadda S, Jonnalagadda S, Zahra FT, Nocentini A, Supuran CT, Mikelis CM, **Trippier PC**. Chlorinated benzothiadiazines inhibit angiogenesis through suppression of VEGFR2 phosphorylation. Bioorg Med Chem. 2022 Aug 1;67:116805. doi: 10.1016/j.bmc.2022.116805. Epub 2022 May 15. PMID: 35635929; PMCID: PMC9888588.

131. Costanzo-Garvey DL, Case AJ, Watson GF, Alsamraae M, Chatterjee A, **Oberley-Deegan RE**, **Dutta S**, **Abdalla MY**, **Kielian T**, Lindsey ML, **Cook LM**. Prostate cancer addiction to oxidative stress defines sensitivity to anti-tumor neutrophils. Clin Exp Metastasis. 2022 Aug;39(4):641-659. doi: 10.1007/s10585-022-10170-x. Epub 2022 May 23. PMID: 35604506; PMCID: PMC9338904.

132. 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.

133. Tan YD, **Guda C**. NBBt-test: a versatile method for differential analysis of multiple types of RNA-seq data. Sci Rep. 2022 Jul 27;12(1):12833. doi: 10.1038/s41598-022-15762-x. PMID: 35896555; PMCID: PMC9329447.

134. 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.

135. Bulock LL, Ahn J, Shinde D, Pandey S, Sarmiento C, Thomas VC, **Guda C**, **Bayles KW**, Sadykov MR. Interplay of CodY and CcpA in Regulating Central Metabolism and Biofilm Formation in Staphylococcus aureus. J Bacteriol. 2022 Jul 19;204(7):e0061721. doi: 10.1128/jb.00617-21. Epub 2022 Jun 23. PMID: 35735992; PMCID: PMC9295537.

136. Mir S, Golden BDO, Griess BJ, Vengoji R, Tom E, Kosmacek EA, **Oberley-Deegan RE**, **Talmon GA**, **Band V**, **Teoh-Fitzgerald ML**. Upregulation of Nox4 induces a pro-survival Nrf2 response in cancer-associated fibroblasts that promotes tumorigenesis and metastasis, in part via Birc5 induction. Breast Cancer Res. 2022 Jul 14;24(1):48. doi: 10.1186/s13058-022-01548-6. PMID: 35836253; PMCID: PMC9281082.

137. Zeng Y, Yin L, Zhou J, Zeng R, Xiao Y, **Black AR**, Hu T, **Singh PK**, Yin F, **Batra SK**, Yu F, Chen Y, **Dong J**. MARK2 regulates chemotherapeutic responses through class IIa HDAC-YAP axis in pancreatic cancer. Oncogene. 2022 Jul;41(31):3859-3875. doi: 10.1038/s41388-022-02399-3. Epub 2022 Jul 2. PMID: 35780183; PMCID: PMC9339507.

138. Kaur N, Lum MA, **Lewis RE**, **Black AR**, **Black JD**. A novel antiproliferative PKCα-Ras-ERK signaling axis in intestinal epithelial cells. J Biol Chem. 2022 Jul;298(7):102121. doi: 10.1016/j.jbc.2022.102121. Epub 2022 Jun 10. PMID: 35697074; PMCID: PMC9270260.

139. Halder S, Parte S, Kshirsagar P, **Muniyan S**, Nair HB, **Batra SK**, **Seshacharyulu P**. The Pleiotropic role, functions and targeted therapies of LIF/LIFR axis in cancer: Old spectacles with new insights. Biochim Biophys Acta Rev Cancer. 2022 Jul;1877(4):188737. doi: 10.1016/j.bbcan.2022.188737. Epub 2022 Jun 6. PMID: 35680099; PMCID: PMC9793423.

140. **Bhatt VR**, Wichman C, **Al-Kadhimi ZS**, **Koll TT**, Fisher AL, **Mahato RI**, **Hyde RK**, **Berger A**, **Armitage JO**, **Holstein SA**, **Maness LJ**, **Gundabolu K**. Integrating geriatric assessment and genetic profiling to personalize therapy selection in older adults with acute myeloid leukemia. J Geriatr Oncol. 2022 Jul;13(6):871-874. doi: 10.1016/j.jgo.2022.04.005. Epub 2022 Apr 18. PMID: 35450817; PMCID: PMC9482687.

141. 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.

142. 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.

143. 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; PMCID: PMC10042269.

Principal Investigator: **Cowan, Kenneth H.**

SUMMARY OF EXPENDITURES

The following summary table includes the original Proposed Budget, approved Revised Budget, and Actual Expenditures from July 1, 2022, to June 30, 2023.

Budget Category	Revised LB 595 Budget	LB 595 Expenditures
Salaries and Fringe Benefits	$991,827	$1,003,292
Equipment	$25,787	$25,787
Supplies	$69,416	$71,323
Other Expenses	$212,970	$199,598
Total	**$1,300,000**	**$1,300,000**