NEBRASKA

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DEPT. OF HEALTH AND HUMAN SERVICES

Jim Pillen, Governor

December 31, 2024

Mr. Brandon Metzler
Clerk of the Legislature
P.O. Box 94604
Lincoln, NE 68509

Subject: Cancer and Smoking Disease Research Report

Dear Mr. Metzler:

In accordance with Neb. Rev. Stat. § 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

Creighton UNIVERSITY
Research and Scholarship

September 23, 2024

Amanda Mortensen
Program Manager
Nebraska Department of Health and Human Services
Division of Public Health – Tobacco Free Nebraska
301 Centennial Mall South
Lincoln, NE 68509

Dear Ms. Mortensen:

Enclosed please find the Creighton University Cancer and Smoking Disease Research Program Annual Progress Report for FY24. 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,

Signed by:
Juliane Strauss-Soukup
E5030E4C6FA54A9...

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

cc: Bonnie McCord

Juliane Strauss-Soukup, PhD *Associate Vice Provost*
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Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

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 Cancer Research Center, and Biorepository Infrastructure).

As documented in the program reports, the Cancer and Smoking Disease Research Program 2023-2024 has been productive for the investigators at Creighton University. Manuscripts were published in such journals as *Biomarkers and Prevention*, *Human Genetics and Genomics Advances*, *Journal of Biological Chemistry*, and *ACS Omega*.

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 29 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 $40,610,340 to Creighton University through LB595. This, coupled with Creighton's contribution of $18,071,601 through unrecovered indirect costs and $44,019,169 in internal seed grant funding, has led to $173,043,726 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, 2024)

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	516,423		99,036	10,000	800	15,591	6,938	648,788
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
Gelineau-van Waes, Janee				25,000		65,000	28,925	118,925
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	5,867,120	79,897	1,493,459		15,000	2,587,595	1,151,480	11,194,551
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	2,137,733			761,420	338,832	3,709,085
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			663,636	295,318	3,237,635
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	1,542,929		300,000	25,000	100,000	499,113	222,105	2,689,147
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					50,000	120,000	53,400	223,400
Smith, Derek	525,589			5,000	10,000	775,201	344,964	1,660,754
Strauss-Soukup, Juliane	1,841,362		361,353		5,000	665,168	296,000	3,168,883
Stessman, Holly	1,352,711		618,492			1,691,848	752,872	4,415,923
Swanson, Patrick	6,211,935	237,481	1,575,171	15,000	50,000	1,460,000	649,700	10,199,287
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	6,695,213	20,000	256,732		50,000	2,200,000	979,000	10,200,945
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	535,221		370,000			120,000	53,400	1,078,621
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	**$173,043,726**	**$23,593,067**	**$19,904,324**	**$130,000**	**$391,778**	**$40,610,340**	**$18,071,601**	**$275,744,836**

Creighton University Cancer & Smoking Disease Research Program FY23/24 Progress Report (July 1, 2023 – June 30, 2024)

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

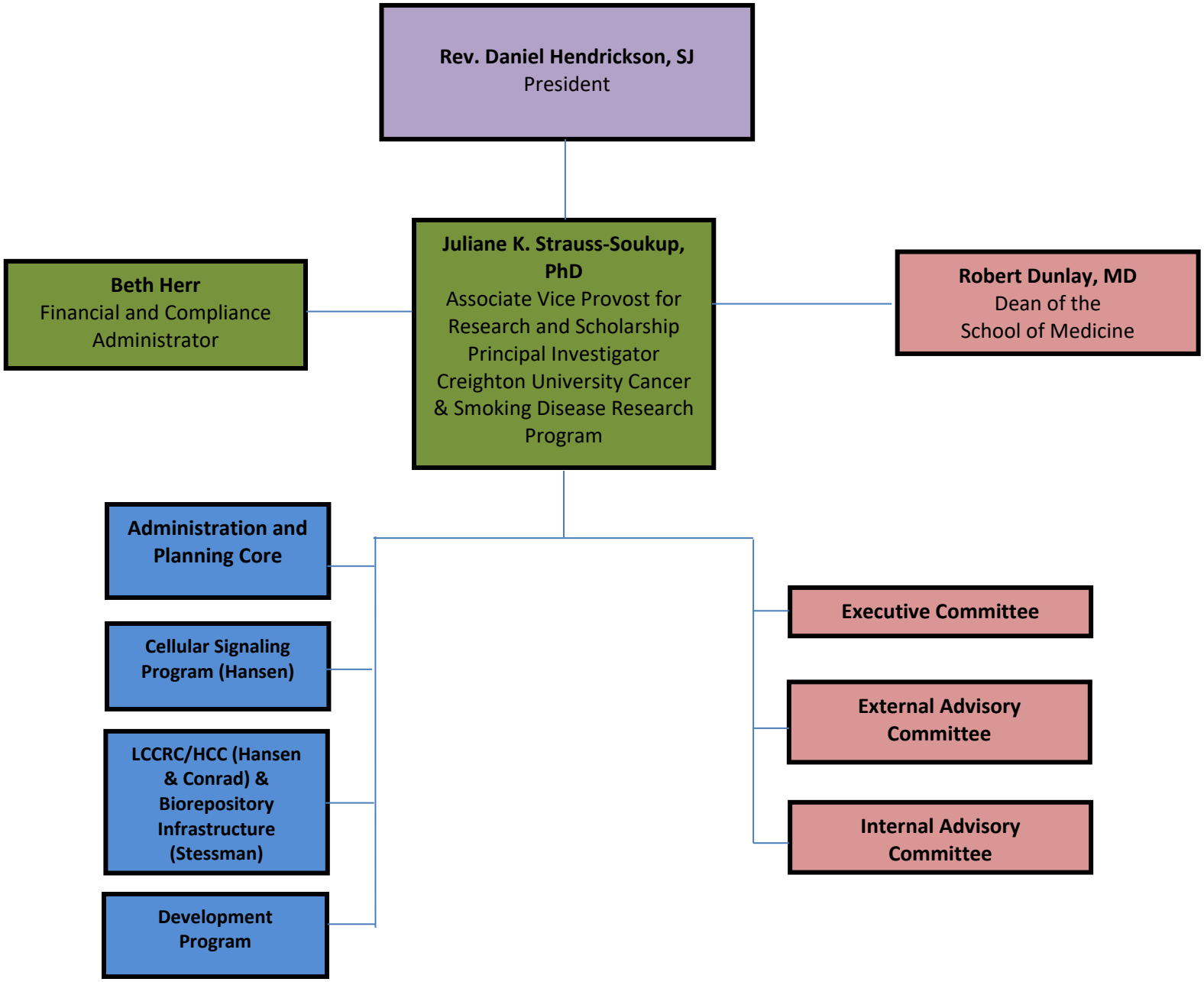
Juliane K. Strauss-Soukup, PhD
Associate Vice Provost for Research and Scholarship
Principal Investigator, Cancer and Smoking Disease Research Program

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 virtual External Advisory Committee review for the Cancer and Smoking Disease Research Program

year 2023-2024 took place on the Creighton University campus on July 26, 2024. See the agenda and External Advisory Committee report at the end of this Administration and Planning report.

Ralf Krahe, PhD, University of Texas MD Anderson Cancer Center, notified us that he was retiring from the committee after the 2023 meeting.

Members of the 2023-2024 External Advisory Committee are as follows:

- Reynold Panettieri, Jr., MD: Rutgers, The State University of New Jersey
- Christine Eichen, PhD, Thomas Jefferson University
- 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 2023-2024 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 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 enriches 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 2023-2024 year:

- David Lombard, PhD, Clinical Professor, University of Miami
  Seminar Topic: Tales of Sirtuins and Sarcoma
- Leng Han, PhD, Professor of Genomic Medicine, Indiana University School of Medicine
  Seminar Topic: Harnessing Big Data for Precision Medicine
- Reuben Harris, PhD, Professor, University of Texas Health San Antonio
  Seminar Topic: Molecular Mechanism of Cancer Mutagenesis by Antiviral APOBEC

5. Cancer Journal Access at Library

During the 2023-2024 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 these electronic journals.

EXTERNAL ADVISORY COMMITTEE REPORT
Cancer and Smoking Disease Research Program – LB595 Site Visit: Monday, July 26, 2024

External Advisory Committee: Reynold Panettieri, MD (Co-Chair), Rutgers, The State University of New Jersey; Christine M. Eischen, PhD (Co-Chair), Thomas Jefferson University; Stephen Hecht, PhD, University of Minnesota); Tomoo Iwakuma, MD, PhD, Children's Mercy Research Institute; Christy Hagen, PhD, University of Kansas Medical Center. All attended the site visit by Zoom video conferencing.

Also attending the meeting were Dr. Julie Strauss-Soukup, PhD, Beth Herr, and Barbara Bittner, all of Creighton University, Amanda Mortensen and Bonnie McCord from the State of Nebraska Department of Health and Human Services.

ADMINISTRATION & PLANNING PROGRAM – Dr. Juliane Strauss-Soukup

Dr. Juliane Strauss-Soukup, PhD (Professor of Chemistry & Biochemistry, Associate Vice Provost for Research and Scholarship) is the PI of the Creighton University LB595 Cancer and Smoking Disease Research Program since November 2020. She is highly qualified to manage multi-investigator programs. The administrative aspects of the program are shepherded under her leadership together with Beth Herr, SPA Director, who provides outstanding administrative support. Dr. Strauss-Soukup's emphasis on undergraduate education and building bridges throughout Creighton University adds an important dimension to the LB595 program. She remains 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 grow with strong institutional support. Importantly, there is a desire to recruit additional cancer-related investigators and faculty, as well as increase research infrastructure. Creighton's funding is improving with increasing NIH support over the year 2022-23 (total extramural research funding of 24.5M). The INBRE program has also been an important asset. A renewal application for the INBRE program was submitted May 2024.

This was a successful year scientifically for the LB595 program: all programs, including the infrastructure core, five individual projects, and four development projects, presented progress. There were no major weaknesses or specific areas of concern. Creighton continues to leverage LB595 into a successful seed program with a significant overall return on investment. In FY23/24, six LB595 investigators were awarded new NIH grants for $5,205,810 over 4-5 years. In the last year, there were 7 peer-reviewed publications by LB595-supported faculty. Although five LB506 grants were submitted, none were funded. Concerning LB692 New Initiative Awards, 2 of 4 applications were funded. Additionally, 3 of 6 applications were funded for the Health Science Strategic Investment Fund Faculty Development Awards. The LB692 and Strategic Fund Awards are evidence of strong institutional commitment toward LB595.

To build on recent successes and to foster growth, the administration should consider a marketing plan to advertise the LB595 program across all Creighton University Schools. To increase the awareness across their ecosystem, 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 through conventional sponsorship.

The publication productivity of the LB595 investigators remains modest. Although the projects are making progress, publishing appears to be a challenge. Only 7 publications from the LB595 program were reported for the last year. In the future, all LB595 activities (publications, grant submissions, grant awards, webinars, etc.) should be reported. It might also be helpful to count submitted manuscripts to understand the extent of the pipeline. Also, external grant funding success was modest, so additional mentoring, greater use of the program'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 should be leveraged. Additionally, with institutional financial support, researchers in specific fields outside of Creighton could be engaged for a fee to provide expert guidance on specific NIH grants to increase the chances of submitting more highly competitive, innovative cancer and smoking-related disease grants.

Concerning the Lynch Cancer Research Center (LCRC), the appointment of Co-Chairs, Dr. Laura Hansen, PhD, a basic cancer scientist, with Lesley Conrad, MD, a physician-scientist and a gynecologic oncologist, was an excellent decision. This new team made remarkable progress in providing stability, a mission, and a vision to the Center. The Lynch Cancer Research Center made excellent progress in re-imaging this resource. The ability to collaborate across the health system and to incorporate translational research with health services will serve Creighton University well, should the Institution seek expanded NCI support.

Given the impending need to renew LB595, this could be an excellent opportunity to identify key areas of cancer research for CU investment, develop liaisons with the health system, and amplify translational research approaches that impact cancer prevention, pathogenesis, treatment, and survival across Nebraska. For example, should LB595 studies include health services, community-based participatory, and population health research?

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

Under the leadership of Dr. Hansen, Associate Dean of Research of the School of Medicine and co-Director of the LCRC, the program consists of five projects. The program is well-established and makes steady progress. Interestingly, many investigators collaborate across projects providing a sense of cohesiveness. Dr. Hansen provides strong leadership for the program. Despite these strengths, several challenges exist. The number of extramural grant submissions and publications is modest. Despite availability of a mock review service, many investigators underutilize the resource, especially for amended grant applications.

Although the program title broadly defines the content, the Director may consider alternative approaches to improve clarity for organizational purposes. All projects in this program are distinguished from the Development Awards by the level of support provided in magnitude and duration. Accordingly, if the title does not reflect a scientific or programmatic focus, potentially this program should reflect the level of support. The team may consider an Established LB595 Project vs a Propel LB595 Project (referring to early-stage, high-impact, high-risk project). Currently, Propel LB595 projects are termed developmental projects.

PI: Laura Hansen, PhD
Title: Checkpoint Signaling and Cell Survival in Normal and Tumorigenic Skin Keratinocytes

Dr. Hansen's project on function and expression of Flower (FWE) isoforms in skin keratinocytes and squamous cell carcinoma (SSC) cells is multifaceted, 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. To study the FWE isoforms *in vivo*, a CRAINBOW mouse model for lineage tracing has been developed. The central hypothesis suggests that FWE isoforms determine the fate and carcinogenesis of SSC. The project is proceeding; the new data over the past year links FWE isoforms to extracellular calcium homeostasis and handling. These findings will be explored in the next year to determine the necessity and requirement of calcium handling in modulating FWE-dependent cell differentiation. 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 *Nature Communications* manuscript is impressive, as well as her previous receipt of an LB606 for the CRAINBOW mouse studies.

PI: Brian North, PhD (Niti Kumari, PhD, Presenter)
Title: Cellular Pathways Targeting BubR1 to the Proteosome for Degradation: Implications for Skin Cancer

Dr. North's group focused on the role of over-the-counter NAD+ boosters, specifically nicotinamide mononucleotide (NMN), which, contrary to expectations, increase cell proliferation and tumor burden. The fact that NMN had the opposite results than expected for UV-induced skin cancer is interesting and can impact patients taking these supplements. Young mice (SKH-1) 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 downregulated interferon alpha and gamma expression. Despite considerable research effort, the overall effects of NMN on protection or causation of UV-induced skin cancer remain unclear. No publications or extramural grants were generated, although they submitted two NIH R01 and American Heart Society applications.

PI: Patrick Swanson, PhD
Title: Localization of RAG1 Degradation and Implication of RAG1 Stabilization on Genome Instability and Cancer

Dr. Swanson's project explores the role of RAG1 and RAG1 turnover in genome instability, with particular focus on aberrant V(D)J rearrangement in lymphoid neoplasia. The first aim is to identify the cellular localization of RAG1 degradation and factors involved. The second aim is to determine if impairing RAG1 turnover increases the frequency of 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. 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. A simpler diagram explaining the proposed mechanism and the role of each factor in B cells malignancy would be helpful. Dr. Swanson has one funded R21 based on these studies, which were originally supported by LB595 funding. Over the last 12 months, there were no publications; however, he did submit a manuscript to *J Immunology*, as

well as an R21 application and several other proposals.

PI: Yaping Tu, PhD
Title: Dysregulated Mitochondrial Dynamics and Cancer Metastasis.

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 one R01 awarded, and another submitted. 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. A new direction in studying macrophage subtypes in modulating cancer metastasis was initiated that appears to be separate and distinct from the central hypotheses of the original proposal. No manuscripts were submitted or published over the last year.

PI: Sandor Lovas, PhD
Title: Inhibition of GBM Invasion with Highly Selective and Proteolytic Stable Peptide Analogs.

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 developing this therapeutic approach. Moreover, the concentrations of the molecules to inhibit GBM survival are 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. To his credit, Dr. Lovas has developed and used both invasion and migration assays to assess the inhibitory peptide effects on GBM cell function. Despite evidence that the peptides modulate cell invasion and migration, their effects on MMP-2 function were marginal. These data suggest the inhibitory peptide effects are off target. Overall, Dr. Lovas is an excellent team scientist, and he has generated considerable data on the project. He is a co-PI on several recent grant submissions. He published in *ACS Omega* 2024 as senior author, and is a co-author on *JBC* 2023 with Dr. Hansen.

LYNCH CANCER RESEARCH CENTER – Dr. Laura Hansen

The Institute has evolved, was renamed, and reorganized. The term "Comprehensive" was removed as per EAC recommendations. Under an ELAM fellowship, Dr. Hansen developed a strategic plan and mapped outgrowth for the next 10-15 years. A vision and the beginning of a

mission statement were provided. This is remarkable and welcomed progress. The Directorship will be a partnership among clinicians and scientists. Many issues remain to be addressed. With the re-imagined Center, what is the sustainability model? What is CU's appetite for expanding the cancer research franchise? Are there partners across the state that can be leveraged collaboratively to submit a CCSG proposal to the NIH? New recruitment efforts could include cancer imaging, precision therapies, informatics, and community engagement foci. Health system engagement will be critical to broaden the impact of the Center.

BIOREPOSITORY INFRASTRUCTURE – Dr. Holly Stessman

As in the previous year, Dr. Stessman has continued to make significant progress with the conversion, update, and restructuring of the existing LCRC database and specimen collection and tracking system. Dr. Stessman has effectively completed the lion's share of the aims of auditing and modernizing the tremendous resource that Creighton University has in this biorepository, which was started by Dr. Lynch. Dr. Stessman has made exemplary progress. With samples from approximately 9,000 individuals audited, deidentified, and all but 1% with IRB approval, the biorepository team was able to complete two studies for publication, establish collaborations to utilize these samples within and outside Creighton for funded projects, and is currently working on a multi-institutional R01 grant for submission to the NIH. These are important steps to bring more funding and gravitas to Creighton for genetic studies. This unique biorepository should be capitalized on as the cancer programs grow at Creighton, and is now positioned to be a national resource that should attract faculty from across the country. Currently, there are biorepository established procedures for gathering additional samples and distribution of samples, but it is recommended that a small committee be established for transparency and to ensure fair distribution of the precious samples moving forward. Dr. Stessman continues to work on the patient/participant outreach tool/app (LIMS, Lab Vantage) to ensure a secure digital connection to participants and re-consenting participants for future research. Also, some additional work is needed for rendering digital pedigrees and to finalize the "orphan" specimens. The biorepository offers an assortment of expertise and skilled techniques for proper storage and analysis of samples that are necessary to run an effective biorepository and meet the needs of its users. Although geneticists in the U.S. are learning of this biorepository from Dr. Stessman at national/international meetings, a plan to more broadly market it outside of Creighton would be helpful. Some extramural funding has already occurred that offsets a portion of the costs to run this Core, but a more long-term sustainability plan should be developed to ensure this unique and valuable resource continues. The EAC has no concerns regarding this Core and fully supports the improvements that have been made by Dr. Stessman. However, Dr. Stessman needs to be assured of protected effort for the management of such a complex and valuable Creighton asset.

DEVELOPMENT PROGRAM – Dr. Juliane Strauss-Soukup

For the LB595 grant proposals, a statement is required regarding the project's relevance to cancer or smoking-related 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 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. The

EAC has reviewed all projects in the Development Program and all are cancer-specific or cancer-focused.

Development Awards

PI: Dr. Jun Xia
Title: Mechanism of Lung Cancer Risk Gene FUBP1-Induced DNA Damage

The goal of this pilot grant, led by Dr. Xia, is to understand the pathogenesis of lung cancer via DNA repair processes. Dr. Xia is focusing on a gene called FUBP1, which is overexpressed in lung and other cancers, and its role in DNA damage. FUBP1 is a transcription factor protein that, as Dr. Xia's data shows, causes DNA damage when it is overexpressed. The use of domain mutants of FUBP1 and approaches that allow for a genome-wide analysis of DNA binding (CUT&RUN) and cutting-edge, high-resolution RNA-sequencing approaches (SHERRY and Duplex-sequencing) revealed a FUBP1-binding motif that was unexpected and initially verified, as well as an increased mutational burden with elevated levels of FUBP1. The approaches utilize advanced sequencing technologies to uncover the mechanism of FUBP1's effects on DNA damage, which contributes to cancer development and progression. Additional sequencing technologies (NanoSeq) are proposed. Overall, significant progress has been made on this project. Dr. Xia published three manuscripts, including one as senior author and two as co-author. He submitted several grant applications as PI and co-I, including three applications submitted with Drs. Hansen (co-I), Nandipati (co-PI), and Fu (co-I) to the NIH, Department of Defense, and Mary Kay Foundation, respectively. He also submitted and obtained a Kicks for a Cure grant this year as PI, in collaboration with Dr. Fu. Dr. Xia is also collaborating with Creighton clinicians, which will increase translational research at Creighton. The EAC recommends Dr. Xia submit at least one NIH grant application this year to support his novel research into lung cancer.

PI: Dr. Brian North; Dr. Niti Kumari (postdoctoral fellow) presented
Title: Identifying Regulators of Liver Cancer Metastasis

Dr. Kumari described the CRISPR screen that was performed to identify genes whose loss contribute to liver cancer metastasis once injected into mice. One liver cancer cell line that already has metastatic capabilities was used for the CRISPR screen, but it was not clear why; however, other liver cancer lines will be screened as well. Although the liver cancer cell line expressed fluorescent proteins, equipment at Creighton was not functional, so only larger tumors that could be seen by eye were isolated and sequenced. Multiple pathways were identified from the sequencing and there are plans to pursue these data. Overall, there is an unclear direction for this project and how the results compare to others in the liver cancer field that have already identified metastasis regulators. The EAC recommends additional guidance by Dr. North for this pilot project and that Creighton either repair the equipment that can read fluorescence or arrange an agreement with UNMC to use their equipment. No publications or grants have been yet generated, but the PI did submit four grant applications, including two NIH grants, on unrelated topics.

PI: Dr. Holly Stessman
Title: Deep Mutational Scanning to Resolve Variants of Undetermined Significance in Lynch Syndrome

This pilot project focuses on studying mutations in a highly mutated gene (PMS2) in mismatch DNA repair for which their effects are not yet associated to pathogenesis of Lynch syndrome-associated or other cancers. Development and testing of assays and cell lines to test PMS2 variants, including high-throughput methods, are being done and should yield productive results that can be further validated. A figure in a breast cancer study published this year by Dr. Stessman showed part of their approach is valid. Also, the engineered cell lines are a valuable model system and will be able to be licensed by Creighton for use by others in the field for future studies. Dr. Stessman submitted four grant applications unrelated to this project, of which one was awarded with the others under review. The EAC recommends that Dr. Stessman continue excelling at her research and seek avenues to obtain more funding to further support her outstanding project.

PI: Dr. Janee Gelineau-van Waes
Title: Bisphenol AF Exposure and Risk for Endometriosis-Associated Ovarian Cancer

Dr. Gelineau-van Waes described the breeding of the mice used in this pilot project studying endocrine-disrupting chemicals and their impact on endometrial cancers. The experimental approach was presented: it requires inducing peritoneal and ovarian endometriosis, measurement of bisphenol A (BPA) and bisphenol AF (BPAF) in murine fluids, and evaluation of the pathology. The mouse model is a challenging one to use and is quite time-consuming, but there are no other mouse models to test the hypothesis proposed. Multiple technical challenges were encountered and solutions were implemented, as possible. BPA and BPAF both bind to the G protein-coupled estrogen receptor (GPER), so they have utilized mice in which GPER is knockout to test its requirement in the effects of the chemicals. Because this pilot project requires breeding mice and complicated time approaches, it will take longer to complete. No manuscripts or grants have been submitted or funded this year. The EAC recommends continuing with the approaches taken and seeking avenues of funding, including NIH, as soon as data are available.

Creighton University Cancer & Smoking Disease Research Program FY23/24 Progress Report

(July 1, 2023 – June 30, 2024)

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

Following are the final reports for the one-year Development projects awarded in 2023-2024:

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

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

PI: Holly Stessman, PhD, Department of Pharmacology and Neuroscience
Title: Deep Mutational Scanning to Resolve Variants of Undetermined Significance in Lynch Syndrome

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

This is the year two report for a Development project that was 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

The full reports follow this page.

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

DEVELOPMENT PROGRAM
Director: Juliane Strauss-Soukup, PhD

Project Title: Identifying Regulators of Liver Cancer Metastasis
Principal Investigator: Brian J. North, PhD

I. Progress Report Summary

A. Specific Aims

Due to time constraints, the final outcomes of the grant will be focused on Aim 1.

B. Studies and Results

During this budget year, we will be able to carry out an *in vivo* screen using two models, cardiac injection, and intra-liver injection. These studies are currently ongoing with the expectation that we will have sequencing of gRNAs from isolated tumors by the end of July. We encountered a number of non-technical and technical hurdles over the course of this project. These included challenges faced during standardization of the CRISPR library, ultimately leading us to switch to a library from another source, and the need for controlled substances and the DEA taking longer than anticipated to complete the review for the PI's DEA license. Once these issues were cleared, we tested the IVIS *in vivo* imaging system, which is not working and is likely past its life expectancy to make it feasible for repair. Due to this, we have needed to adjust our protocol to isolate tumor tissues after a set time (five weeks post-cell injection) rather than rely on *in vivo* imaging to follow tumor metastasis. While this may offer modest challenges in our experimental program, based on prior studies in the literature, we anticipate that this will not negatively influence our progress.

We have injected mice (either intrahepatic or intracardiac) with cells infected with our CRISPR library and have allowed tumors to develop over approximately 4 weeks, with our time point based on weight loss that we anticipated was due to tumor growth (which was necessary as the IVIS system is not functional). Tumors were observed and have been extracted from these mice and are currently stored for subsequent analysis.

Our immediate future plans are to isolate DNA from these tumors and carry out gRNA sequencing followed by bioinformatic analysis to determine gRNAs that are either enriched or depleted in the tumor tissues compared to the input cells. Thus, when this analysis is complete, we anticipate defining genes that when knocked out will either enhance or reduce cancer cell metastasis in each of the two models.

C. Significance

It is our belief that defining novel regulators of liver metastasis will provide novel therapeutic avenues for the treatment of liver cancer. The short-term significance of these studies is that a dataset of novel regulators that either promote or suppress liver cancer cell metastasis will form the basis of a grant submission to further define key players in metastatic cancer, as

well as an initial publication. Long-term significance of this data will be generation of novel methods to target proteins encoded by the genes targeted by the gRNAs to test in preclinical models with the hope to identify hits to further develop into clinically relevant modalities.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

None.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

National Institute of Diabetes and Digestive and Kidney Diseases/NIH
4/01/2025 - 3/31/2030
Project Number: Not available yet
PI: Brian J. North
Title: BubR1 as a Novel Regulator of Intestinal Homeostasis: Implications in Disease and Aging
Major Goals: The goals of these studies are to elucidate the cellular, molecular, and tissue consequences of BubR1 loss on intestinal epithelial tissue homeostasis, disease states such as ulcerative colitis, and during intestinal aging.

National Heart, Lung, and Blood Institute/NIH
4/01/2025 - 3/31/2030
Project Number: R01 HL178792-01
PI: Brian J. North
Title: The Role of BubR1 in Cardiac Development
Major Goals: The goals of these studies are to elucidate the molecular and cellular basis for why heart specific BubR1 knockout leads to embryonic lethality with failure of cardiac maturation and features similar to congenital heart defects.

Nebraska Health and Human Services
07/01/2024 - 06/30/2025
Project Number: NHHS LB606 Stem Cell Research Program
PI: Brian J. North
Title: Intestinal Stem Cell Maintenance by BubR1
Major Goals: The goal of this research proposal is to utilize single-cell RNA-sequencing and cellular characterization to define the role for BubR1 in maintenance of intestinal stem cells.

American Heart Association
7/01/2024 - 6/30/2027
Project Number: N/A
PI: Brian J. North
Title: Regulation of Heart Development by the Mitotic Checkpoint Factor BubR1
Major Goals: The goal of these studies is to define the role of BubR1 in regulating proper cardiac maturation during embryonic development.

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

None

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

DEVELOPMENT PROGRAM
Program Director: Juliane Strauss-Soukup, PhD

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

I. Progress Report Summary

A. Specific Aims

The original aims have not been modified.

Aim 1: 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

In the last funding period, we have achieved significant progress to advance the proposed research. More specifically:

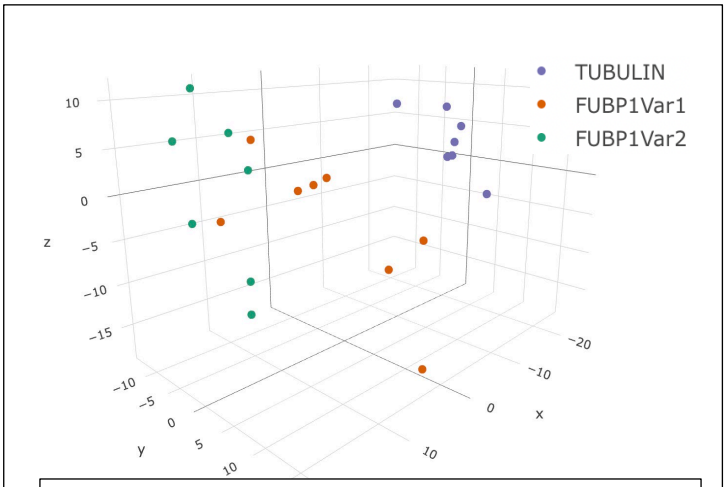


Fig1. Principal component analysis (PCA) reveals distinct clusters of transcriptional patterns of FUBP1 overproduced, and Tubulin overproduced cells.

1. **SHERRY single-cell analysis of FUBP1 var 1 and var 2 in MRC5 cells.** Sequencing HEteRo RNA-DNA-hYbrid (SHERRY) is a single-cell technique that is 5-10 times more sensitive than traditional 10x-based single-cell approaches. We have performed SHERRY to uncover the transcriptome heterogeneity of both FUBP1var1 and var2 overproduction-induced gene regulations. FUBP1var1 and var2 form different clusters than the Tubulin-overproducing cells. And between FUBP1var1 and var2 they are noticeably different in the PCA grouping (Fig 1).

2. **Binding maps of FUBP1 var 1 and var 2**. The peaks generated from the FUBP1 CUT&Tag experiments were superimposed, and enrichment near the transcription start site (TSS) was observed (Fig 2). There is enrichment near the TSS for many transcription factors and other regulatory proteins. CUT&Tag and ChIP-seq peaks are often enriched

near the TSS of genes because these proteins play roles in regulating gene expression, often by binding to promoter regions located immediately upstream of the TSS. The enrichment of ChIP-seq peaks near the TSS indicates active regulatory regions where the binding of transcription factors can directly influence gene expression. This is crucial for understanding gene regulation mechanisms, identifying target genes of transcription factors, and exploring the functional impacts of genetic variations within or near these peaks. The binding maps for both FUBP1 variants 1 and 2 are similar, with most peaks overlapping the promoter regions, indicating that the CUT&Tag approaches effectively identify the FUBP1 binding regions.

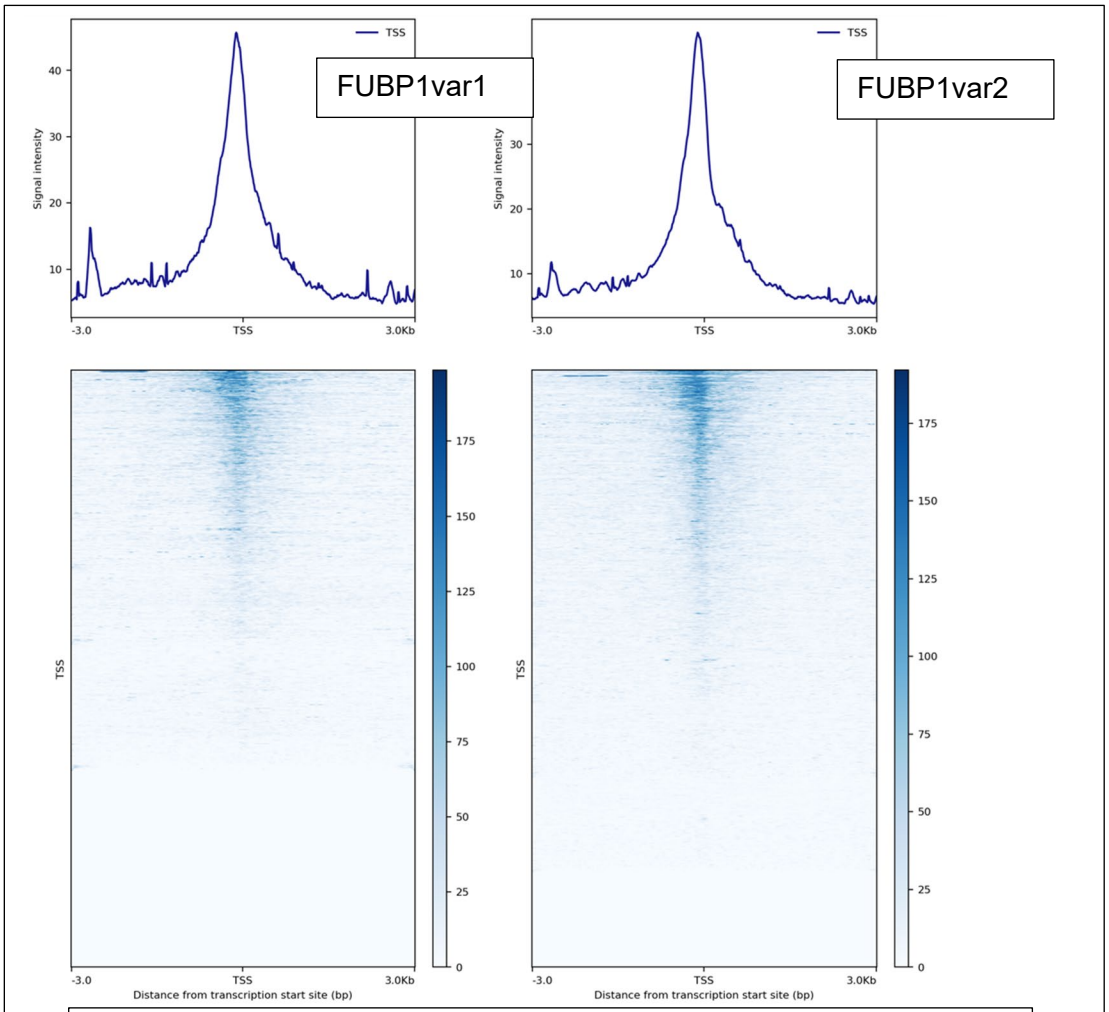


Fig2. FUBP1 binds to Transcription start sites. Both variants of FUBP1 prefer binding to TSS.

3. **The discovery of AP1-like binding motifs from FUBP1 CUT&Tag results**. Interestingly, the motifs we discovered from CUT&Tag of FUBP1 variants 1 and 2 (Fig 3) showed a striking similarity to those of the AP1 transcription factors. The Activator Protein-1 (AP-1) transcription factor family, composed of various members including c-JUN, c-FOS, and ATF, is involved in mediating many biological processes, including cancer. This discovery leads us to consider that all or most of the FUBP1 overproduction-induced DNA damage and mutagenesis effects might be mediated through the upregulation of AP1 transcription factors.

Fig3. FUBP1 binding motifs.

FUBP1 V2

AP-1(bZIP)/ThioMac-PU.1-ChIP-

FUBP1 V1

Jun-AP1(bZIP)/K562-cJun-ChIP-Seq/Homer

4. **Overproduction of FUBP1 variants 1 and 2 induces the upregulation of AP1 component genes**. The usual suspect in FUBP1 overproduction, c-Myc, is not differentially expressed. The lack of c-Myc regulation is uncommon but has been observed in certain cancers. Nevertheless, we have observed binding motifs similar to those of the AP1 transcription factor. AP1 transcription factors are dimers; AP-1 can be a homo/heterodimer composed of proteins from the Jun, Fos, Maf, and ATF families. Jun proteins can form homo or heterodimers with themselves, while Fos proteins can only form dimers with proteins from other families. We have screened the expression levels for all major AP1 partner proteins, and most of them are upregulated during FUBP1 overproduction conditions (Fig 4).

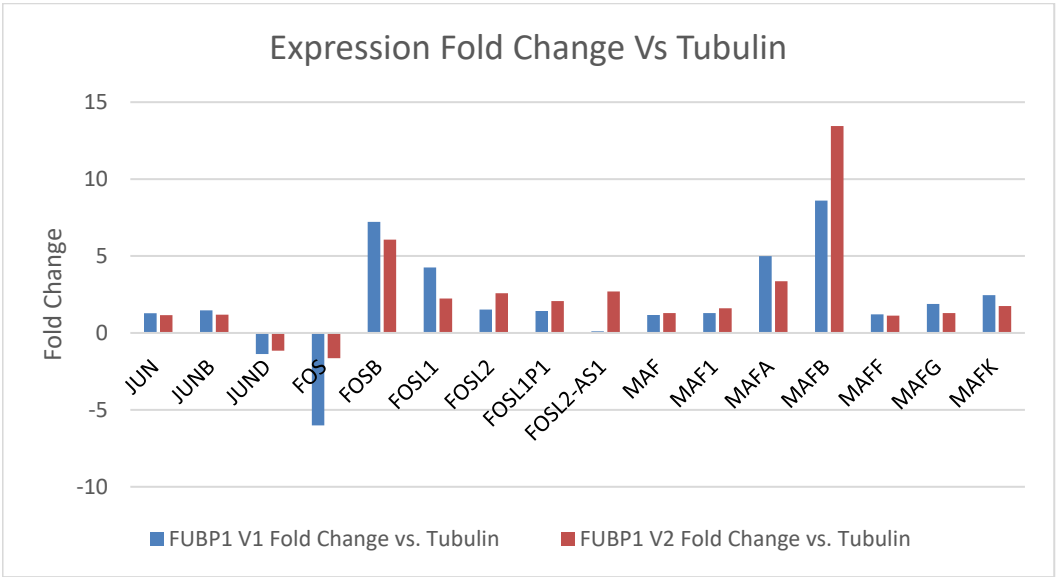


Fig 4. FUBP1 overproduction induces upregulation of AP1 partner genes.

5. **Duplex-sequencing approaches for deep mutational mapping for FUBP1-overproduced cells.** We have employed duplex-sequencing approaches for deep mutational mapping in cells overproducing FUBP1. Using a capture panel, we targeted the 48kb evolution-neutral regions of the genome and the 200kb encompassing 21 cancer driver genes that we designed to estimate the mutational frequency and signature of FUBP1. Overall, cells overproducing FUBP1 exhibited slightly higher mutation frequencies than the mock samples, with an increase in C>A mutations. The analysis of mutational signatures indicates a higher similarity to SBS10a, SBS10c, SBS10d, and SBS56. More detailed mapping will require the design of FUBP1-binding-specific panels for further mutagenesis studies.

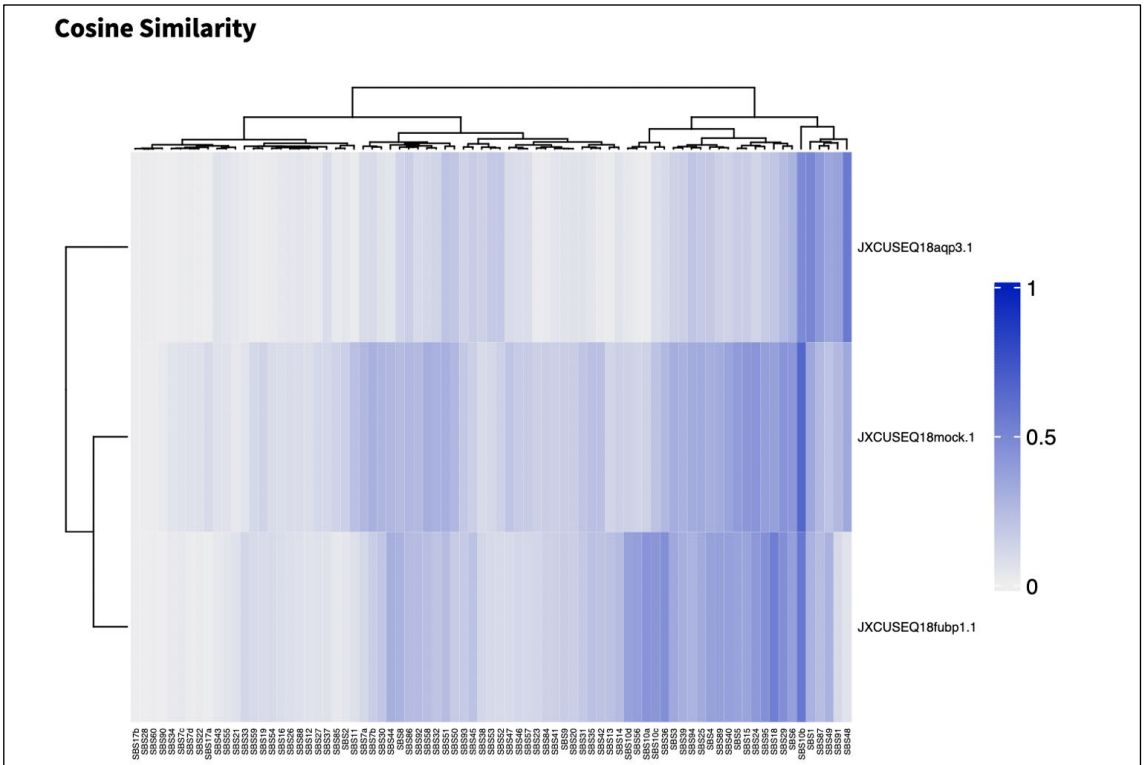


Fig 5. FUBP1 overproduction induces mutational signatures.

6. **A NanoSeq approach has been implemented for additional mutational mapping.** NanoSeq is an error-corrected sequencing approach similar to duplex-sequencing but eliminates the end-repair step, which is responsible for most artifacts seen in duplex-sequencing. Instead, NanoSeq degrades the ends of DNA molecules before ligating them to UMI adapters. The subsequent steps are similar to those in duplex-sequencing. Like duplex-sequencing, NanoSeq is particularly sensitive to detecting low-frequency mutations, crucial for understanding genetic variations and disease mechanisms. Its error rate is 100-1,000 times lower than that of duplex-sequencing. We have successfully implemented this technique, initially developed by the Sanger Institute, and will use it for future FUBP1-induced mutational studies.

7. We will test some additional hypotheses regarding the DNA damage-inducing abilities of the following AP1 partner proteins, which will be fused with either EmGFP or RFP. Additional genes have been synthesized: JUN (NM_002228.4), JUNB (NM_002229.3), FOSB (Variant 1, NM_006732.3), FOSL1 (Variant 1, NM_005438.5), FOSL2 (NM_005253.4), and MAFB (NM_005461.5). We will mix and match different AP1 binding partners and test which combinations are most effective at promoting DNA damage.

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. The study will provide new mechanistic insights into how FUBP1 promotes DNA damage and genome instability.

3. This study generates single-base resolution maps for FUBP1 mapping and reveals the involvement of additional transcription factors, AP1, in FUBP1 overproduction-induced effects. This highlights the complexity and importance of studying a network of TFs when it comes to the effectors in terms of genome stability and cancer.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

Fu Y[#], Agrawal S, Snyder D, Yin S, Zhong N, Grunkemeyer J, Hansen L, Waddah A, Nandipati K[#], **Xia J[#]**. Transcriptomic and gene fusion changes during the Progression from Barrett's Esophagus to Esophageal Adenocarcinoma. Accepted for publication at ***Biomarker Research***, [#]Corresponding

Li Y, Xiao X, Li J, Han Y, Cheng C, Fernandes GF, Slewitzke SE, Rosenberg SM, Zhu M, Byun J, Bossé Y. Lung cancer in ever-and never-smokers: findings from multi-population GWAS studies. ***Cancer epidemiology, biomarkers & prevention***. 2024 Mar 1;33(3):389-99.

Long E, Yin J, Shin JH, Li Y, Kane A, Patel H, Luong T, Xia J, Han Y, Byun J, Zhang T. Context-aware single-cell multiome approach identified cell-type specific lung cancer susceptibility genes. bioRxiv (minor revision at ***Nature Communications***). 2023 Sep 26.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

Kicks for a Cure, Inc.
Lineage Tracking of Copy Numbers: From Barrett's Esophagus to Esophageal Adenocarcinoma
Role: PI (Xia)
Total Award Amount (including Indirect Costs): $40,000

NIH/NIGMS (CoBRE team science project)
The Translational Hearing Center
3P20GM139762-04S2
Role: Co-PI (Xia)
Total Award Amount (including Indirect Costs): $145,660.69 for Xia, total $440,259

DOD (CA240133) (Pre-Proposal)
Single-Cell Genomics of Esophageal Adenocarcinoma in Service Members: Impact of Burn Pit Exposure and Potential for Immunotherapy
Role: Co-PI (Xia), PI (Nandipati)
Total Award Amount (including Indirect Costs): $400,000

NIH/NIAMS R01AR084834
Flower Regulates Late-Stage Epidermal Differentiation and Barrier Function
Role: Co-I (Xia), PI (Hansen)
Total Award Amount (including Indirect Costs): $ 3,117,352

Kay (Mary) Foundation
Genomic Characterization of Endometrial Serous Carcinoma with Ultra-Sensitive and Accurate Duplex DNA Sequencing
Role: Co-I (Xia), PI (Fu)
Total Award Amount (including Indirect Costs): $100,000

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

Kicks for a Cure, Inc.
Lineage Tracking of Copy Numbers: From Barrett's Esophagus to Esophageal Adenocarcinoma
Role: PI (Xia)
Total Award Amount (including Indirect Costs): $40,000

NIH/NIGMS (CoBRE team science project)
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Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

DEVELOPMENT PROGRAM
Dr. Julie Strauss-Soukup

Deep Mutational Scanning to Resolve
Variants of Undetermined Significance in Lynch Syndrome
Principal Investigator: Holly A. Feser Stessman

I. Progress Report Summary

A. Specific Aims

Specific Aim. Identify likely-pathogenic variants in the *PMS2*.

a) We will use a random mutagenesis strategy to generate all possible mutations in the *PMS2* coding sequence on an inducible lentiviral vector. This library will be used to identify which mutants are resistant versus sensitive to mismatch repair (MMR) selection *in vitro* using a high-throughput next-generation sequencing approach.
b) Likely-pathogenic variants identified through screening will be individually validated.

Aims have not been changed.

B. Studies and Results

This was a mid-cycle award that started in January 2024 and will complete in June 2025. However, some progress (noted below) has been made and includes a publication.

Specific Aim. Identify likely-pathogenic variants in the *PMS2*.

a) We will use a random mutagenesis strategy to generate all possible mutations in the *PMS2* coding sequence on an inducible lentiviral vector. This library will be used to identify which mutants are resistant versus sensitive to mismatch repair (MMR) selection *in vitro* using a high-throughput next-generation sequencing approach.

   We are currently developing and validating this mutagenized *PMS2* library.

b) Likely-pathogenic variants identified through screening will be individually validated.

   We began with this sub-Aim to test specific variants in *PMS2* that we identified through targeted screening of the Lynch legacy collection. We were able to establish that known "benign" and "pathogenic" ClinVar variants are working as predicted in our assay and that a variant of undetermined significance (VUS) identified in a family suspected of Lynch Syndrome is likely benign (see publication below).

C. Significance

Lynch syndrome (LS) is one of the most common hereditary cancer syndromes affecting an estimated 1 in 279 people. LS cases are known to be caused by germline mutations in the genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*, which encode proteins that function in DNA mismatch repair (MMR). In healthy cells, when DNA damage is acquired during replication, the MMR

pathway halts the cell cycle until the damage can be repaired or signals the damaged cell to die. Individuals with loss-of-function mutations in the LS genes fail to cull damaged cells, resulting in the accumulation of excess somatic mutations over time, which increases cancer risk. While genetic testing has become more common among individuals with a family history of cancer, the interpretation of identified variants remains difficult. Variants in *PMS2* are the most common among LS cases; however, they are the least well understood. Disruptive *PMS2* variants (nonsense, frameshifting, and canonical splice-site) are often confidently classified as pathogenic, resulting in an LS diagnosis.

Individuals who carry known pathogenic variants can significantly reduce their cancer risk through early and frequent surveillance and prophylactic measures. However, most *PMS2* protein-coding variants are classified as variants of undetermined significance (VUSs). Certainly, a portion of these variants carry an increased cancer risk, but which ones? Not only are VUSs clinically unactionable, they also are unclear to patients and clinicians. Thus, there is a critical need to better classify the genetic risk associated with *PMS2* VUSs. The long-term goal of our work is to understand the contribution of genetic variation to LS risk. Our overall objective in this proposal is to utilize an *in vitro* system to test *PMS2* variants for MMR activity in a high-throughput way. Once the underlying genetic architecture of LS is fully understood, better diagnostic, management, and family planning approaches can be developed that will significantly decrease LS-associated cancer risk.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

Plowman JN, Matoy EJ, Uppala LV, Draves SB, Watson CJ, Sefranek BA, Stacey ML, Anderson SP, Belshan MA, Blue EE, Huff CD, Fu Y, Stessman HAF. Targeted sequencing for hereditary breast and ovarian cancer in BRCA1/2-negative families reveals complex genetic architecture and phenocopies. HGG Adv. 2024 May 10;5(3):100306. doi: 10.1016/j.xhgg.2024.100306. Epub ahead of print. PMID: 38734904.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

COBRE Pilot Project (PI: Stessman)
TREM1 as a Novel Therapeutic Target for Global Ischemia

1 R21 HD114018-01A1 NIH/NICHD (R21; PI/PD: Stessman)
KMT5B Contribution to Genomic Imprinting Maintenance

1 R01 MH133600-01A1 NIH/NIMH (R01; PI/PD: Stessman)
Epigenetic Regulation of the Neuroendocrine Axis in Autism with Global Delays

COBRE Research Project Leader (PI/RPL: Stessman)
TREM1 as a Novel Therapeutic Target for Global Ischemia

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

COBRE Pilot Project (PI: Stessman)
TREM1 as a Novel Therapeutic Target for Global Ischemia

Other 3 grants pending review/council.

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

DEVELOPMENT PROGRAM
Director: Juliane Strauss-Soukup, PhD

Project Title: *Bisphenol AF Exposure and Risk for Endometriosis-Associated Ovarian Cancer*
Principal Investigator: Janee Gelineau-van Waes, PhD, DVM

I. Progress Report Summary

A. Specific Aims

Hypothesis: *Bisphenol AF (BPAF) exposure and activation of G protein-coupled estrogen receptor (GPER)-mediated signaling in ectopic ovarian endometriotic (OE) lesions and peritoneal macrophages coordinate different aspects of pathogenesis that increase risk for progression to endometriosis-associated ovarian cancer (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

The original aims have not been modified.

B. Studies and Results

Notification of funding was received on August 14, 2023, at which time breeding to generate experimental animals was initiated in our *Gper1* mouse colony. Experimental females were born in Sept. – Oct. and reached the appropriate size and maturity to begin performing surgeries in Nov. – Dec. 2023. In the interim, BPA-free caging and water bottles were ordered, and we worked with a nutritionist at Innotiv to formulate custom rodent diets containing defined concentrations of bisphenol AF (BPAF) that span the range of predicted human exposures. Based on the age and weight of our mice at the time of surgery, and their estimated daily food consumption, diets were formulated to contain 0 ppm (control), 25 ppm, 250 ppm, or 750 ppm BPAF. Human exposure data for BPAF are limited; for this reason, we selected the 25 ppm concentration to approximate the NOAEL (No Observed Adverse Effect Level, 5 mg/kg/day) and the 250 ppm concentration to approximate the LOAEL (Low Observed Adverse Effect Level, 50 mg/kg/day) advised by the Environmental Protection Agency for human exposure to the parent compound bisphenol A (BPA). The highest BPAF concentration selected (750 ppm) approximates the high end of human exposures to BPA. An IACUC protocol for the proposed experiments was submitted and approved, and practice surgeries were performed to optimize the approach and placement of decidualized endometrium under the ovarian bursa.

Due to the timing (need to reach endpoints by June 2024), mice that had surgery in Nov. – Dec. 2023 were randomly assigned to the 180-day control or high BPAF (750 ppm) exposure groups (n = 5 BPAF, n = 4 Control). In 2024, mice that had surgery were placed on either control or high BPAF diet to fill the 90-day and 30-day endpoints. Mice and feed were weighed weekly to calculate BPAF exposure (external measure), and daily vaginal cytology was performed to

determine the number of days post-op before the mice resumed normal estrus cyclicity. All mice returned to normal estrus cyclicity after surgical induction of peritoneal and ovarian endometriosis, but females maintained on the high BPAF diet started to exhibit prolonged estrus cycles by ≥ 30 days post-op.

The concentration of BPAF in the diet was calculated based on an (estimated) average mouse weight of 23 g and average food consumption of 3 g/day. However, for all mice on high BPAF diet (750 ppm), the average mouse weight was 22.2 g and average food consumption was 3.75 g/day. This resulted in slightly higher BPAF consumption (127.5 ± 9.1 mg/kg/d) than originally estimated, but this approximated high-end exposure estimates for human BPA consumption (120 mg/kg/d; WHO, 2009).

At the time of sacrifice, the stage of estrus was recorded and blood and urine were collected for mass spectrometry analysis of BPAF (internal measure of exposure). Dr. Molly McDevitt in the CU mass spectrometry core facility developed and validated an LC-MS/MS assay for determination of BPAF in mouse plasma and urine. Final samples were collected the week of June 17, 2024, and submitted for LC-MS/MS analysis. Results were presented at the External Advisory Committee meeting on July 26, 2024. No BPA was detected in the plasma or urine of mice on control or high BPAF diet, and BPAF levels in plasma and urine of mice on control diet were below the limit-of-detection (LOD) of 0.25 ng/ml. Detectable levels of BPAF (within the standard curve) were present at all post-op time points in the plasma and urine of mice on high BPAF diet (750 ppm). The concentration range of (unconjugated) BPAF detected in urine samples from mice maintained on high BPAF diet spanned the range of BPA concentrations (200 – 700 ng/ml) detected in urine samples collected from humans exposed to high levels of BPA. Levels of (unconjugated) BPAF detected in plasma samples from mice maintained on high BPAF diet were higher than levels of BPA detected in plasma from humans exposed to high levels of BPA. However, unconjugated BPA (and therefore possibly BPAF) has been shown to undergo enterohepatic recirculation in mice, but not in humans. Interestingly, although plasma levels of BPAF at 30- and 90-days post-op in mice maintained on high BPAF diet were similar, plasma BPAF levels at 180-days post-op were significantly elevated compared to both earlier time points, suggesting the possibility of bioaccumulation with chronic high exposure to BPAF.

Induction of Peritoneal and Ovarian Endometriosis: The surgeries went smoothly, and peritoneal endometriotic lesions were observed in 100% of control and BPAF exposed mice. The average model 'take rate' (total number of retrieved endometriotic lesions/mouse divided by the total number of implanted donor PE biopsies (10) multiplied by 100 (expressed as %)) was 4/10 (40%) across all treatment groups and endpoints. Suspected ovarian lesions (on one or both ovaries) were observed in 93% of BPAF-exposed mice and in 80% of mice maintained on control diet. Gross lesions (peritoneal and ovarian) were photographed, harvested, and fixed in 10% formalin for histology. Tissues were sectioned and every other slide was H&E stained (Toni Howard, CU histology core facility); the unstained slides were reserved for immunohistochemistry. Slides with sectioned ovaries (n = 36 ovaries, ~10 slides each) were submitted for histopathology. Drs. Abdulrahim, Fnu, and Groh (CUMC pathologists) are performing a blinded histopathological examination of the (suspected) ovarian lesions to look for treatment and/or timepoint-related changes indicative of atypical endometrial hyperplasia, endometriomas (cysts), and/or progression to EAOC (clear cell carcinoma or endometrioid carcinoma). Endometrial stroma and hemosiderin-laden macrophages have been detected in all ovarian lesions examined to date. Slides with sectioned and stained peritoneal lesions have also been submitted for histopathology to confirm endometriosis.

Technical Issues: An unanticipated complication experienced was difficulty breeding *Gper1* wildtype (WT) mice to produce enough experimental females for all proposed treatment groups.

The *Gper1* WT x WT matings generated very small litters, and neonatal pups frequently died due to maternal neglect. We had better success with pup survival when we used WT x het matings, but with this breeding scheme, only 50% of the pups were of the correct genotype for the proposed studies. All available *Gper1* wildtype females old enough for surgery were placed in weekly timed matings with a vasectomized male. Following observation of a vaginal plug, pseudo-pregnant females were decidualized on day 4.5 and surgery performed on day 9.5. To perform surgery, at least two females need to be plugged on the same night (one donor and one recipient). However, on several occasions, only one female at a time got plugged (and therefore had to be recycled in the timed mating pool), and we were unable to perform surgeries as often as scheduled. Due to breeding complications, lack of available experimental female mice, and the timeframe needed to reach endpoints, we were unable to start the proposed experiments for Aim 2.

Mass Spectrometry Determination of BPA and BPAF in Plasma and Urine: An LC-MS/MS method was developed to measure BPA and BPAF levels in mouse urine and plasma. Plasma and urine samples from control mice (no surgical intervention) maintained on control (0 ppm), low (25 ppm), or high BPAF (750 ppm) diets were provided to Dr. Molly McDevitt for optimization and validation of the LC=MS/MS assay. After the addition of an internal standard (d16-BPA), samples were extracted with a mixture of hexane:ethyl acetate (3:2, v/v), dried in a vacuum concentrator and resuspended in methanol:water (1:1, v/v). LC-MS/MS analysis was performed on a Vanquish UHPLC coupled to a Q Exactive mass spectrometer (Thermo Scientific). BPA and BPAF eluted over a gradient of 65-80% mobile phase B over four minutes. Mobile phases A and B were made up of water and methanol, respectively. BPA (1 – 100 ng/mL) and BPAF (0.25 – 30 ng/mL) standards were prepared in the same manner as the samples. MS/MS analysis was done using electrospray ionization in negative mode with normalized collision energy of 40. Compounds were quantified based on fragment ions unique to the compound.

Technical Issues: During method development, it was discovered that BPA and BPAF contamination was coming from the extraction process. After several experiments, it was determined that most of the contamination was coming from the glass tubes used for extraction, though a small amount was also coming from the microcentrifuge tubes used during the final centrifugation step. This issue was resolved by washing all glassware three times with LCMS grade water and methanol. Microcentrifuge tubes were switched to Eppendorf brand as they exhibited the least amount of contamination. Although not entirely eliminated, contamination peaks were < 25% of the lowest standard following these procedures. Following these changes, limit of detection, limit of quantitation, sensitivity, linearity, precision, accuracy, and matrix effects were determined.

C. Significance

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. 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 endocrine disruptors than BPA. 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.

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 atypical endometrial hyperplasia, endometriosis, ovarian cysts (endometriomas), and increased risk for endometrial and ovarian cancer. BPAF has greater

binding affinity and is a more potent activator of GPER than the parent compound BPA; however, little is known about BPAF toxicity and its role in female reproductive health, including gynecological cancers. Our innovative translational mouse model of PE/OE endometriosis and the use of genetically modified *Gper* wildtype (wt) and knockout (ko) mice will enable us to examine the potential impact of BPAF exposure on endometriosis and progression to EAOC. The significance of our findings could have important implications not only for management of these adverse health consequences, but also for informing future regulatory policy concerning use of BPAF in products and packaging materials that contact food and personal care products.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

None

III. List of extramural grants submitted from 7/1/2023–6/30/2024

None

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

None

Creighton University Cancer & Smoking Disease Research Program
FY22/24 Progress Report
(July 1, 2022 – Dec 31, 2023)

DEVELOPMENT PROGRAM
Program Director: Juliane Strauss-Soukup, PhD

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

I. Progress Report Summary

This proposal seeks to explore new pathways governing plasticity at the synapses between the parabrachial nucleus (PB) and the central laterocapsular amygdala (CeLC) in cisplatin-induced neuropathic condition. Cisplatin, a commonly used anti-neoplastic agent associated with severe side effects like neuropathic pain and anorexia which often lead to treatment discontinuation and reduce its therapeutic effectiveness. The primary objective is to investigate the role of the therapeutically targetable GluD1-Cerebellin 1 (Cbln1) signaling pathway in pain-related plasticity within the central amygdala (CeA). Understanding this mechanism could offer insights into potential therapeutic interventions to restore synaptic function in cisplatin-induced neuropathic pain and anorexia.

A. Specific Aims: (No modifications were done in 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:

Experiment 1: To address whether systemic cisplatin treatment leads to changes in expression and localization of GluD1 and Cbln1 at PB-CeLC synapses.

To address this question, we conducted immunohistochemical (IHC) studies. We adopted global approach to locate key brain regions regulating cisplatin-induced neuropathic pain, accordingly in the first-year studies Cbln1 was administered via intra-cerebroventricular route. 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.

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 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 intraperitoneal route and tested for mechanical hypersensitivity in 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 increased 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 suggest that the restoration of GluD1-Cbln1 signaling reversed the increased excitatory neurotransmission observed in cisplatin-induced neuropathic pain conditions within CeA neurons.

Molecular assessments in CeA: Next, knowing the pivotal role of CeA in cisplatin-induced neuropathic pain, we conducted our investigation in the CeA to understand the molecular mechanisms regulating cisplatin-induced neuropathic pain. A separate cohort of mice were cannulated into CeA. Following recovery period these mice were tested for baseline mechanical sensitivity using 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 intraperitoneal route and tested for mechanical hypersensitivity in von-fry 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 collected for further immunohistochemical investigations. However, due to change of research place we were unable to continue further investigations at Creighton University. Thus, future investigations to study molecular mechanisms regulation cisplatin's effect will be conducted at Texas A&M University.

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.

Firstly, we have conducted preliminary studies to test the therapeutic effect of Cbln1 injection in mitigating the 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.

Consecutively to substantiate our preliminary results, we conducted additional experiments in the separate cohort of mice. Animals were prepared and treated as described in experiment 2. We found that cisplatin injection resulted in significantly heightened mechanical hypersensitivity. Notably, intra-CeA Cbln1 injection significantly reduced cisplatin-induced mechanical hypersensitivity. These results confirmed therapeutic potential of Cbln1 in chemotherapy-induced neuropathic pain.

C. Significance

Together, our research findings provide support for the hypothesis that cisplatin treatment reduces GluD1 expression, leading to increased excitatory neurotransmission in CeLC neurons and resulting in mechanical hypersensitivity and symptoms akin to neuropathic pain. Importantly, the administration of recombinant Cbln1 effectively reinstates GluD1 expression, normalize excitatory neurotransmission, and improves the pain-like condition, as evidenced by reduced mechanical hypersensitivity. These results underscore the pivotal role of GluD1-Cbln1 signaling in the CeA region in regulating the effects of cisplatin. The implications of our findings are substantial for the development of potential therapeutic interventions for cisplatin-induced neuropathic pain. By uncovering the underlying mechanisms and identifying a therapeutic target in the GluD1-Cbln1 signaling pathway, our research provides insights into strategies that could potentially mitigate the severe side effects associated with cisplatin treatment. The proposed interventions, centered around restoring GluD1 expression and normalizing excitatory neurotransmission, may enhance the effectiveness of cisplatin therapy while reducing the occurrence of neuropathic pain, ultimately improving patient outcomes.

II. List of refereed publications germane to this project from 7/1/2022–12/31/2023
-Under preparation

III. List of extramural grants submitted from 7/1/2022–12/31/2023
-None

IV. List of extramural grants awarded from 7/1/2022–12/31/2023
-None

Creighton University Cancer & Smoking Disease Research Program FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

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 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.

The call for proposals for the FY25 awards was revised since this will be the final year of the Cancer & Smoking Disease Research Program's six-year award cycle. We decided to request one-year project applications with a budget of $100,000 to allow sufficient funding to complete the work in the one-year timeframe. One application was received. All members of the External Advisory Committee reviewed the application and unanimously decided it was an excellent application to fund.

PI: Waddah Al-Refaie, MD, Department of Surgery
Title: Medicaid Expansion, Hospital Closure, and Rural Cancer Surgery Disparities in the Midwest

Complete

Investigator and Proposal Information

Principal Investigator/Project Director/Fellowship Sponsor:
Al-Refaie, Waddah

Email waddahalrefaie@creighton.edu
Phone
Department Surgery - Omaha

Personnel:

PI	Name	Department	Role	Net Effort
✔	Al-Refaie, Waddah	Surgery - Omaha	PD/PI	0.000
	Timperley, Jillian	Creighton University	Co-Investigator	0.000
	Gillaspie, Erin	Surgery - Omaha	Co-Investigator	0.000
	Singh, Awinder	Surgery - Omaha	Co-Investigator	0.000
	Walters, Ryan	Clinical Research & Public Health - Omaha	Co-Investigator	0.000

Originating Sponsor: State of Nebraska - LB595

Sponsor: State of Nebraska - LB595

Budget:

	Period 1	Total
Direct Costs	$99,590.00	**$99,590.00**
Indirect Costs	$0.00	**$0.00**
F&A Rate	0%	-
Total	**$99,590.00**	**$99,590.00**

Project Total Cost Sharing Direct Costs: 0

Project Total Cost Sharing F&A Costs: 0

Start Date: 01-Jul-2024 End Date: 30-Jun-2025

Identification

Proposal Title
Medicaid Expansion, Hospital Closure, and Rural Cancer Surgery Disparities in the Midwest

Brief description of project in plain language (1000 character limit).
Using a ten-Midwest state inpatient and emergency departments datasets, the study team will examine the impact of Medicaid expansion on disparities in cancer surgery access and outcomes among vulnerable rural populations in expansion vs. non-expansion states

Sponsor Funding Opportunity Announcement: Please provide a link or upload the funding opportunity announcement here.
Upload Guidelines

Please upload the sponsor Funding Opportunity Announcement:

Protocols

Will your project involve...

☐ Yes ☑ No Human Subjects?

☐ Yes ☑ No Laboratory Animals?

☐ 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 Will this project utilize any core facilities?

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

If Other, please specify:
Rural care, surgery, colorectal cancer, pancreatic cancer, lung cancer

RESEARCH PLAN

SPECIFIC AIMS

Nearly 15 million rural people live in Nebraska and its surrounding states in the Midwest, representing nearly 30% and 25% of the entire Midwest and US rural populations, respectively.[1] They also face significant cancer mortality relative to urban residents.[2] Rural residents also include vulnerable populations, defined herein as Medicaid beneficiaries, the uninsured, the poor, and ethno-racial minorities, who experience disproportionately higher rates of cancer deaths, in part, due their complex medical and social determinants of health. The rising rates of rural hospital closures may be detrimental. Whether these disparities were improved by the enactment of the Affordable Care Act (ACA) Medicaid Expansion is largely unknown. As such, rural health has become a national priority evidenced by The Executive Order to support underserved communities and The National Institute of Minority Health and Disparities priorities for rural populations.[3,4] Inspired by the mission of Creighton University, the Department of Surgery is committed to building a robust agenda to promote equity in rural surgery. To gain a deeper understanding of these issues in Nebraska, the overarching goal of this proposal is to rigorously evaluate the impact of ACA's Medicaid Expansion on cancer surgery inequalities in rural residents living in the Midwest, and how rural hospital closures may have contributed to these disparities.

ACA's Medicaid Expansion dramatically changed the landscape for U.S. healthcare coverage, improving healthcare access for many low-income Americans.[5] Surgery remains the most critical part of care for many solid cancers, and nearly 100,000 operations are performed each year.[6] However, little is known about Medicaid Expansion's impact on inequities in cancer surgery access and outcomes for rural residents in the Midwest overall and its vulnerable populations. Surgical resection of lung (smoking-related cancer), pancreas, and colorectal cancers are common.[7,8,9] Most evaluations heavily focused on urban non-Midwest states or on the pre-COVID-19 era.[10-13] A deeper understanding of the impact of a major health policy also requires evaluating this impact in different contexts. Specifically, rural hospital closures may yield different effects on these cancer surgery inequities. It remains unknown whether, and how, continued rural hospital closures affect rural surgical cancer care in Midwest states that opted to (or not to) expand their Medicaid under the ACA.

This study will provide a rigorous regional assessment of these research gaps. Using inpatient data from 10 Midwest states, the study team will examine the impact of Medicaid Expansion on disparities in cancer surgery access and outcomes among rural residents overall and among vulnerable rural residents (with Medicaid, low income, and ethno-racial minorities) in expansion vs. non-expansion states. We will examine pre/post-ACA changes on lung, pancreatic, or colorectal cancer surgeries (representing 29% and 38% new US cancer cases and deaths respectively).[14] We will also examine vulnerable rural residents who represent 40% of our cohort and are among the most medically and socially complex patients. The potential impact of state-level rural hospital closures on surgical cancer care will also be examined in expansion vs. non-expansion states in the Midwest. Building on our prior work in ACA's Medicaid Expansion on surgical disparities, our specific aims are:

Aim 1: Examine the impact of Medicaid Expansion on the utilization rates of cancer surgery among rural residents in expansion vs. non-expansion states and whether this impact differed by income or race/ethnicity.
H1a: Utilization rates of cancer surgery improved in Medicaid Expansion relative to non-expansion states.
H1b: Disparities by income and race/ethnicity in the utilization rates of cancer surgery decreased more among rural surgical patients in Medicaid Expansion relative to non-expansion states.

Aim 2: Examine how Medicaid Expansion affected cancer surgery outcomes (operative mortality, length of stay, and readmission) and how outcomes differ by income and race/ethnicity.
H2a: Cancer surgery outcomes improved for rural residents in expansion vs. non-expansion states.
H2b: Outcome disparities, by income or race/ethnicity, are smaller in expansion vs. non-expansion states.

Aim 3 (Exploratory): Evaluate the impact of state-level rural hospital closures on disparities in access to cancer surgery and whether these disparities changed in ACA expansion vs. non-expansion states.
H3a: Cancer surgery rates decreased among states with higher rates of rural hospital closures.
H3b: Cancer surgery rates in states with high rural hospital closures were smaller in ACA expansion states.

Results from the current study represent one of the largest Midwest state evaluations that have policy, healthcare delivery, and research implications for the State of Nebraska. Findings from this study will be leveraged to solicit a successor NIH (NIMHD or NCI) R01 grant in 2026.

RESEARCH STRATEGY

a. Significance

The burden of cancer in rural America

Nearly 15 million rural people live in Nebraska and its surrounding states in the Midwest, representing nearly 30% and 25% of the entire Midwest and US rural populations, respectively.[1] This large demographic faces significant health disparities due to geographic barriers, limited healthcare access, and socioeconomic challenges.[15,16] Rural residents are typically older and less educated, and exhibit higher rates of risky health behaviors such as smoking, physical inactivity, obesity, and lower screening rates, which may contribute to elevated rates of lung and colorectal cancers in rural communities.[2,17,18] These disparities impact the entire cancer care continuum (prevention, diagnosis, and treatment) for rural patients, and are further exacerbated by limited access to surgical services and greater travel distances, further delaying diagnosis and timely care. Rural residents have higher death rates for lung, colorectal, and pancreatic cancers, with the highest rates among ethnically and racially underrepresented populations.[19,20,21,22] Relevant to the current proposal, Nebraska is 98% rural by land area, with minorities, poor, and older adults representing a sizable fraction of the entire state.[1]

Rural health equity is a policy priority!

Rural health equity has become an important policy focus on the federal and state levels. A 2023 Presidential Executive Order was released which aims to advance racial equity and support underserved communities, and specifically provides strategies to address economic disparities in rural communities.[3] Also, the U.S. Department of Health and Human Services Healthy People 2030 and Centers for Medicare and Medicaid Frameworks acknowledge the challenges faced by rural communities and provide strategies to improve access.[23,24] Furthermore, The National Institute of Minority Health and Disparities have prioritized research which addresses rural health disparities, particularly among ethnically and racially underrepresented populations.[4] Several state-level policies echo the federal-level objectives, initiatives, and research priorities, and can address the unique needs of rural communities. Several state-level policies demonstrate efforts to improve access, such as Nebraska's recent legislative proposals which aim to provide requirements and change provisions relating to insurance coverage of screening for colorectal and lung cancers.[25,26] The prioritization of rural health equity policies is indicative of a pressing need within rural America to address health disparities. As such, this proposal aims to provide the stepping stones of a funded research agenda to promote equitable quality rural surgical cancer care in Nebraska.

Importance to the strategic goals of the Department of Surgery at Creighton and CHI Health

The 2023 inaugural Creighton-CHI Health Equity in Rural Surgery Symposium brought together a wide range of healthcare leadership stakeholders and had an overarching goal to promote equity in rural surgery with emphasis on Nebraska and the Midwest. The symposium sought to develop a consensus around how to better deliver surgical care for patients at rural hospitals or who reside in rural settings. A major topic of discussion centered on Nebraska's prioritization of policies to address rural disparities, which is a matter of significance to Creighton's Department of Surgery and CHI Health, given their dedication to serving the state's large rural population. In response to the research implications for rural access identified during the event, seeking grant funding emerges as a direct outcome of the symposium and serves as an actionable step toward promoting surgical equity.

Effects of Medicaid Expansion on Health Care Access, Outcomes, and Disparities

The Affordable Care Act's (ACA) Medicaid Expansion provision aimed to increase healthcare coverage and access for low-income Americans. As rural residents are more likely to have lower socioeconomic status (SES), the influence of insurance status and policies such as Medicaid Expansion become critical to accessing healthcare.[16,27-29] States that have implemented Expansion have seen large increases of rural residents covered by Medicaid, and therefore lower uninsurance rates.[5,30] Medicaid Expansion has been associated with increased screening, earlier detection, and improved access to timely surgical cancer care.[31-40] Expansion further improved mortality in patients with lung and colorectal cancers.[41,42] Non-expansion states experience populations that fall into the "coverage gap," which disproportionately impacts ethnically and racially underrepresented populations.[43] As several non-expansion states also have large rural populations (Figure 1), it becomes imperative to understand the impact of Medicaid Expansion on access to cancer surgery and

outcomes. Since most evaluations heavily focused on urban non-Midwest states or on the pre-COVID-19 era, the applicability of these studies to rural residents deserves further investigation.

Rising hospital closures in America

	Year Medicaid Expansion	Total Population	Rural Population	% Population Rural	% Population Non- White	% Covered by Medicaid/CHIP (2021)	% of Uninsured Population (2022)	% of Population Low Income (2021)	% Rural Hospitals Closed (since 2005)
Iowa	2014	3,190,369	1,175,538	36.8	15.6	20	4.1	26	1.1
Colorado	2014	5,773,714	806,778	14	33.2	18.5	7	23	-
Michigan	2014	10,037,261	2,673,073	26.5	21.2	24	4.6	30	4.8
Minnesota	2014	5,737,915	1,604,740	28.1	18	18	4.3	22	6.3
Nebraska	2020	1,961,504	529,501	27	22.3	14.9	6.8	26	2.8
Missouri	2021	6,154,913	1,879,250	30.5	21.8	14.7	8.4	29	17
Indiana	2015	6,862,199	1,995,842	28.8	16	20	6.8	29	7.7
South Dakota	2023*	886,667	379,320	42.8	19.2	13.8	8.0	27	6.3
Wisconsin	-	5,910,955	1,940,027	32.9	13.4	18	5.4	25	1.3
Kansas	-	2,937,880	813,821	27.7	25.2	15.2	8.7	29	9.8

*South Dakota expanded in 2023, which is outside of available study data, therefore treated as Non- expansion.

Non-Expansion States
Late Expansion States
Early Expansion States

Kaiser Family Foundation. "Medicaid State Fact Sheets." KFF, June 30, 2023.
U.S. Census Bureau. "QuickFacts." Census.gov, October 8, 2021.
Kaiser Family Foundation. Total Population, by State (2019). Accessed May 3, 2024.
America Counts. (2017, August 9). What is Rural America? U.S. Census Bureau. Retrieved January 30, 2024
Becker's Hospital Review. States with the most rural hospital closures. Beckers Hospital Rev. 2019

As shown in Figure 1, recent years have seen an alarming growth of rural hospital closures. Since 2005, 104 rural hospitals have closed, with 37 of those closures taking place since 2020 alone.[44] Rural hospital closures are evident in states with large rural populations. With 30% of all rural hospitals at risk of closing,[45] it is important to understand the impact of Medicaid Expansion and rural hospital closures on rural access to and outcomes after cancer surgery. While there are many factors that cause a rural hospital to close, changes in payor's mix play a large role in a hospital's viability.[45] Rural hospital closures will negatively impact cancer surgery access and outcomes, especially in non-expansion states. These disparities further complicate critical shortages of healthcare professionals including general and specialty surgeons.[46-50] Given that Medicaid Expansion has been associated with better hospital financial performance and lower likelihoods of closure, especially in rural areas,[51] further investigation is warranted.

b. Innovation

As a deliverable of the 2023 Inaugural Creighton-CHI Equity in Rural Surgery symposium, the Department of Surgery Creighton University School of Medicine and CHI Health seeks to build a robust clinical, research, and educational agenda in understanding and mitigating rural surgical disparities. Rural vs. urban America has suffered one of the worst cancer mortalities. The impact of Medicaid Expansion on disparities in access to and outcomes of cancer surgeries in rural America are not, to date, fully understood. Our proposal is innovative in three areas. First, the current study will be one of the largest multi-rural state evaluations of Medicaid Expansion in surgical cancer care. The quasi-experimental study design will generate timely knowledge on how this important policy change will affect disparities in access to, and quality of, surgical cancer care within both expanded vs. non-expanded states. Evaluations of the impact of Medicaid Expansion have typically focused on urban states or from the pre-COVID-19 pandemic era.[10-13] Second, the proposal is innovative in its focus solely on rural residents with surgical cancers overall and particularly vulnerable rural surgical patients (e.g., Medicaid, uninsured, the poor, or ethno-racial minorities). Despite the access to care post-ACA overall, the impact of Medicaid Expansion on these vulnerable rural patients remains largely unknown. Third, we will examine the interaction between Medicaid Expansion with rural hospital closure on the utilization of cancer surgery. Aside from the availability of health coverage, the availability of hospitals is crucial in the delivery of

surgical cancer care. However, it is unclear how key geographic issues such as rural hospital closures can affect rural cancer surgery access and outcomes. A better understanding of this relationship will inform future federal- and state-level policy interventions.

c. Approach

Research Team and Contribution

Our team members are experienced in surgical oncology, health equity, health services research, and biostatistics. Waddah Al-Refaie (PI) is the Chair of Surgery at Creighton and CHI Health. Al-Refaie is a federally funded surgical oncologist ($3.3M) in surgical disparities in vulnerable populations and experienced in secondary analysis of data sets used in this study. Ryan Walters, PhD (Co-I) is the Vice Chair of Clinical Research with expertise in observational study designs, modeling longitudinal data, and management and analysis of large administrative databases. Dr. Erin Gillaspie (Co-I) is the Founding Chief of Thoracic Surgery at Creighton University-CHI Health with extensive clinical and research expertise in lung cancer. Dr. Awinder Singh (Co-I) is an experienced gastrointestinal and pancreatic surgeon. Finally, Jillian Timperley (Co-I) is a research assistant in the Department of Surgery with active surgical equity projects. Together, our team provides the relevant complementary experience and skills in a highly supportive environment that optimizes our ability to successfully complete the proposed project.

Study Population

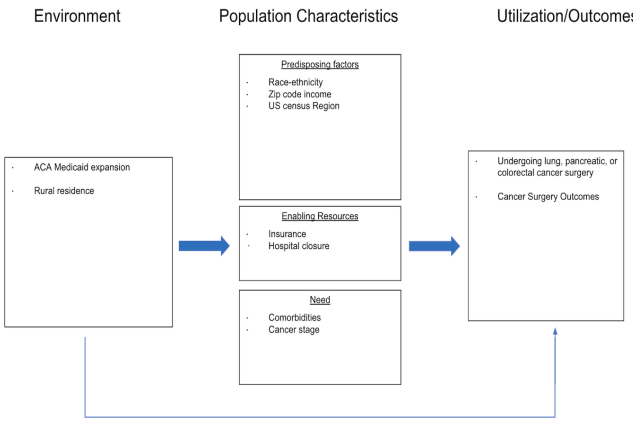
To accurately test the central hypotheses of the proposed study, we will include only rural residents who are of Medicaid-eligible age groups between 18 and 64 who underwent lung, pancreatic, or colorectal cancer surgery. Given the low prevalence of dual Medicare and Medicaid insurance coverage (in this age group) and to reduce the unmeasured confounding of dual insurance coverage, we will exclude these patients from this study. Lung, pancreatic, and colorectal cancer resections were selected as the three common or complex cancer procedures for the study cohort with procedures identified using ICD-9 and ICD-10 diagnostic and procedural coding. These three cancers represent nearly one third of new cancer cases and half of cancer-related deaths in the US.[52] Rurality of patient residence will be measured using the Rural-Urban Continuum Codes (RUCC) from the Economic Research Service from the U.S. Department of Agriculture.[53] The RUCC is a classification scheme with 9 categories that distinguish metropolitan (urban) counties by population size and non-metropolitan (rural) counties by the degree of urbanization and geographic proximity to metro area(s). Categories include metropolitan counties with populations of >1 million, 250,000 to 1 million, or <250,000, whereas categories for non-metropolitan counties include urban populations of >20,000, 2,500 to 19,999, or completely rural with <2,500 urban population; non-metropolitan countries are further distinguished by adjacency to metropolitan area. We will define rural residents as those living in non-metropolitan counties.

Data Sources

State in-patient Datasets (SIDs)[54]: Drawing on the study team's expertise with large administrative datasets to perform population-level analyses using 10 SIDs from Midwestern states;[55,56] the data will span from 2010 through 2021. SIDs include all discharge records from community hospitals in a given state for a particular year, as well as important structure- and process-of-care measures and patient outcome measures. While ensuring socio-demographic diversity across states within each group, states are selected to enhance balance in population characteristics between expansion (treatment) and non-expansion (control) states. To date, SIDs collects information from 36 states representing approximately 97% of community hospital discharges in the United States. In addition to Nebraska, we have selected 9 states from the Midwest to maximize rural geographic representation similar to Nebraska. Specifically, the 7 expansion states include Nebraska, Iowa, Colorado, Indiana, Michigan, Minnesota, Missouri. All of these expansion states opted in for Medicaid Expansion in 2014 except for Indiana (2015), Nebraska (2020), and Missouri (2021). In contrast, the three non-expansion states include Kansas, Wisconsin, and South Dakota (opted in beginning in 2023, which is outside current data availability).

Conceptual Framework

This study's conceptual framework is adapted from the Andersen Behavioral Model, which has been widely used to examine health care access and utilization, including in surgical research (Figure 2).[57,58] Prior research has generally focused on individual predisposing factors (e.g., race/ethnicity, income), enabling resources (e.g., insurance, hospital availability vs. closure) and need factors (e.g., comorbidities, cancer stage) on health care use and outcomes with focus on income and race/ethnic minority among rural patients. However, our project will also specifically focus on aspects of the environment, including the ACA's Medicaid Expansion (policy change) and hospital closures (environment) in access to and outcomes after cancer surgery.



Analytical Framework

Our longitudinal study design is quasi-experimental allowing us to compare outcomes before and after expansion for states that expanded as well as compare states that did or did not expand during the study period.[59] For all study aims, and in line with prior literature, generalized linear mixed models will be used to account for the repeated observations from the same state. Separate models will be estimated for each cancer surgery. Hospitalization counts and time will be quantified using quarter-level epochs (four per year). The primary fixed effects include a fixed effect of time and a fixed effect of time-varying Medicaid Expansion to accurately represent states that adopted with early or late expansion. In addition to studying the secular time trend of undergoing major cancer surgery, the impact of Medicaid Expansion on vulnerable rural population, previously defined as low-income persons and ethno-racial minorities, will be separately tested via three-way interactions between time, Medicaid Expansion, and patient characteristics that include income quartile and ethno-racial indicators (non-Hispanic white, non-Hispanic Black, Hispanic, other). Estimates of the combined secular effect with the Medicaid Expansion and income and ethno-racial indicators will be used to determine if utilization and outcomes of the targeted three cancer surgeries have changed after institution of Medicaid Expansion and if there are disparities in the rate of change in regionalized surgery across ethno-racial groups or income quartiles; differences by ethno-racial indicators or income quartile will be examined separately.

Measures

Dependent Variables: **cancer surgery utilization rates and operative outcomes**.

Cancer surgery utilization rates will be defined as the count of hospitalized patients with a cancer diagnosis who live in a rural county who underwent surgery for their cancer with one of the following insurances: Medicaid, private, or uninsured; to account for state population size differences, the number of hospitalized patients meeting inclusion criteria will be used as an offset. Operative outcomes are provided directly by the SIDs and include in-hospital mortality, length of stay, as well as all-cause and cause-specific 30- and 90-day hospital readmission (available for Nebraska, Iowa, Colorado, Indiana, Missouri, and Wisconsin).

Primary Independent Variable: Medicaid Expansion status

Medicaid Expansion status will be defined at the quarter of the calendar year of its date of adoption. For analysis, Medicaid Expansion will be modeled as a time-varying predictor as some states had adopted expansion later in the study period. For states that did not adopt expansion at the start of a quarter, expansion will be specified at the start of the subsequent quarter (e.g., Indiana adopted Medicaid Expansion on February 1, 2015, so Indiana will be considered as expanding Medicaid beginning with 2015Q2).

Independent Variables: ethno-racial indicators, income quartiles, and rural hospital closures

Ethno-racial indicators will include non-Hispanic white, non-Hispanic Black, Hispanic, or other. Income quartile will be defined at the level of the county within which the patient resides. Rural hospital closures will be defined yearly for each state.

Covariates:

Patient-level covariates include age at the time of procedure, biological sex, primary payer, Elixhauser comorbidity index, geographic region, whereas **hospital-level covariates include** bed size, location and teaching status, payer mix, proportion of non-Hispanic white, non-Hispanic African American, Hispanic, and patients of other races. Hospital-level payer mix and race mix will be calculated from all inpatient admissions for any reason for each hospital in the SID.

Analysis Plan

Aim 1: Utilization rates will be modeled using the log link and Poisson conditional response distribution with an offset of the number of hospitalized patients meeting inclusion criteria. To test the hypothesis that Medicaid Expansion is associated with greater cancer surgery utilization rates, we will estimate a time-by-Medicaid Expansion interaction effect to evaluate whether Medicaid Expansion was associated with differential increases during the study period; the (simple) main effects of time and Medicaid Expansion will also be included in the model. To test our second hypothesis specific to disparities in temporal effects of Medicaid Expansion, we will estimate three-way interaction effects between time, time-varying Medicaid Expansion status, and ethno-racial indicator or income quartile. To minimize confounding due to patient and hospital-level characteristics, we will then include as covariates the patient and hospital level variables listed previously in the Covariates section.

Aim 2: Operative mortality and readmission (for states that include readmission data) will be modeled using the log link and binomial conditional response distribution; providing relative risks. Length of stay will be modeled using log link and Poisson or negative binomial response distribution with response distribution dictated by likelihood ratio test; given the cancer surgeries under study, we expect very few zero-day lengths of stay, so we are not expected to require zero-inflated or hurdle models. Both hypotheses for Aim 2 will be tested using the modeling approach detailed for Aim 1 - we will include time-by-Medicaid Expansion interaction effect (hypothesis 1) and subsequently three-way interaction between time, Medicaid Expansion, and ethno-racial indictor or income quartile (hypothesis 2).

Aim 3: In this exploratory study aim, utilization rates will be modeled using the log link and Poisson conditional response distribution with an offset of the number of hospitalized patients meeting inclusion criteria. To test the hypothesis that rural hospital closures are associated with lower cancer surgery utilization rates, we will estimate the fixed effect of rural hospital closures; we will also test a time-by-rural hospital closure interaction to determine whether this effect differed across the study period. To test the second hypothesis that Medicaid Expansion was associated with a smaller effect of rural hospital closure, we will estimate an interaction effect between rural hospital closure and Medicaid Expansion; should the time-by-rural hospital closure interaction effect in the first hypothesis be significant, we will estimate a three-way time-by-rural hospital closure-by-Medicaid Expansion interaction.

Strategies to Overcome Study Limitations

The study team offers two proposed strategies for study limitations. First, difficulty in accounting for cancer stage in the selected datasets: While the importance of stage is recognized, nearly all patients undergo curative cancer surgery in the absence of advanced/stage IV disease. Second, difficulty in accounting for “undocumented” patients who are categorized as “Uninsured”: To date, estimated unauthorized “undocumented” populations in the US have been relatively stable at 3.5% of US population since 2005. Given that ACA Medicaid Expansion will not cover “undocumented” persons, unmeasured expansion coverage differences are assumed to be minimal. The team will reassess and factor in any statistically significant changes in “undocumented” rates at the time of conducting Aim 1 analyses.

Study Strengths

The proposed study has a number of strengths including 1) state selection strategy that maximizes relevance to the State of Nebraska, 2) comprehensiveness of our study evaluating measures of access and outcomes across common surgical cancers, and 3) selection of surgical cancer procedures that are either linked to prevention/screening programs, a key problem in rural America.

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Budget for: Medicaid Expansion, Hospital Closure, and Rural Cancer Surgery Disparities in the Midwest

Sponsor:	State of Nebraska - LB595
Investigator:	Waddah Al-Refaie
Project Period:	7/1/2024-6/30/2025

Category	**Item**	**Total**
Salary	Waddah Al-Refaie	0.00
	Jillian Timperley	0.00
	Erin Gillaspie	0.00
	Awinder Singh	0.00
	Ryan Walters	0.00
	TBN Statistician	1,857.94
	Alexander Hall	10,828.36
	Danielle Dilsaver	8,662.69
	Subtotal Personnel:	**21,348.99**
Supplies	Supplies	77,241.00
	Subtotal Non-Personnel:	**77,241.00**
	Total Sponsor Direct Costs:	98,590.00
	Sponsor F&A:	0.00
	Total Sponsor Costs:	**98,590.00**

Budget Justification
Total requested = **$98,590**

Personnel:

Waddah Al-Refaie, MD Principal Investigator (0.06 FTE/0.72 calendar mos)
Dr. Al-Refaie is a Professor and Chair of the Department of Surgery at Creighton University School of Medicine and CHI Health. Dr. Al-Refaie is a federally funded surgical oncologist ($3.3M) in surgical disparities in vulnerable populations and experienced in secondary analysis of data sets used in this study. He will be responsible for the overall direction of the project and will determine research priorities to be successful. Along with Dr. Walters and the other investigators, he will be responsible for the analysis of the data and subsequent writing and submission of manuscripts/publications. His salary support is not allowed by the grant.

Ryan Walters, PhD Co-Investigator (0.05 FTE/0.6 calendar mos)
Dr. Walters is an Associate Professor and Vice Chair of the Department of Clinical Research and Public Health at Creighton University School of Medicine and Director of Statistics and Analytics. He has expertise in observational study designs, modeling longitudinal data, and management and analysis of large administrative databases He will be responsible for overseeing the statistical methodology and analysis for this project.

Erin Gillaspie, MD Co-Investigator (0.02 FTE/0.24 calendar mos)
Dr. Gillaspie is an Associate Professor and the Founding Chief of Thoracic Surgery in the Department of Surgery at Creighton University School of Medicine and CHI Health. Dr. Gillaspie has extensive clinical and research expertise in lung cancer. She will work closely with the investigators and assist to prepare any subsequent submission of manuscripts, publications and/or research grants. Her salary support for this project will be an in-kind investment.

Awinder Singh, MD Co-Investigator (0.02 FTE/0.24 calendar mos)
Dr. Singh is an Associate Professor in the Department of Surgery at Creighton University School of Medicine. He is an experienced gastrointestinal and pancreatic surgeon. He will work closely with the investigators and assist to prepare any subsequent submission of manuscripts, publications and/or research grants. Her salary support for this project will be an in-kind investment.

Jillian Timperley Co-Investigator (0.05 FTE/0.6 calendar mos)
Ms. Timperley is a research assistant in the Department of Surgery at Creighton University School of Medicine with active surgical equity projects. She will work closely with the investigators and assist to prepare any subsequent submission of manuscripts, publications and/or research grants. We are not requesting salary support for Ms. Timperley.

Alexander Hall, MS, MA Statistician (11% FTE/1.32 calendar mos)
Mr. Hall is a member of the Department of Clinical Research and Public Health in the Division of Statistics and Informatics in the Creighton University School of Medicine. Along with Ms. Dilsaver, he will be responsible for the statistical methodology and analysis for this project. He will be supervised by Ryan Walters, PhD, Associate Professor in the School of Medicine, Director of Statistics and Informatics and Vice Chair of the Department of Clinical Research and Public Health – Omaha.

Danielle Dilsaver, MS Statistician (11% FTE/1.32 calendar mos)
Ms. Dilsaver is a member of the Department of Clinical Research and Public Health in the Division of Statistics and Informatics in the Creighton University School of Medicine. Along with Mr. Hall, she will be responsible for the statistical methodology and analysis for this project. She will be supervised by Ryan Walters, PhD, Associate Professor in the School of Medicine, Director of Statistics and Informatics and Vice Chair of the Department of Clinical Research and Public Health – Omaha.

Supplies:

State in-patient Datasets (SIDs) will be purchased from the Healthcare Cost and Utilization Project (HCUP). **We are requesting $77,241** for the following state databases and years of data:

Colorado 2010-2021 - 12 years at $950 per year = $11,400

Indiana 2017-2020 – 4 years at $1300 = $5,200

Iowa 2010-2016 – 4 years at $585 + 3 years at $600 = $4,140

Kansas 2010-2021 – 8 years at $1,050 + 4 years at $1,100 = $12,800

Michigan 2010-2021 – 4 years at $585 +4 years at $600 + 4 years at $650 = $7,340

Minnesota 2010-2021 – 8 years at $900 + 4 years at $950 = $11,000

Missouri 2017-2021 – 5 years at $1,550 = $7,750

Nebraska 2010-2015 – 4 years at $535 + 2 years at $550 = $3.240

South Dakota 2010-2016 – 4 years at $808 + 3 years at $823 = $5,701

Wisconsin 2012-2021 – 2 years at $835 + 4 years at $850 + 4 years at $900 = $8,670

BIOGRAPHICAL SKETCH

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

NAME: Al-Refaie, Waddah Badir

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

POSITION TITLE: Professor and Chair of Surgery at Creighton University School of Medicine, Chair of Surgery at CHI Health

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Royal College of Surgeons in Ireland, Dublin, Ireland	MD	05/1997	Medicine (Honors Degree)
University of California, San Diego		05/1999	Postdoctoral Fellow
University of California, San Diego		07/2024	Residency in General Surgery
The University of Texas M. D. Anderson Cancer Center, Houston, TX		07/2006	Fellowship in Surgical Oncology

A. Personal Statement

I serve as the Professor and Chair of Surgery at Creighton University School of Medicine and CHI Health. Inspired by my previous leadership experience in Minnesota, I created and now lead the new MedStar-Georgetown Surgical Outcomes Research Center (MG-SORC). The mission of MG-SORC is to ameliorate the burden of surgical disease through effective care and research informed by the priorities of stakeholders of health. MG-SORC's central research focus is to uncover and mitigate surgical care disparities among vulnerable populations including Medicaid patients. Prior to Georgetown, I served as the Co-director of the University of Minnesota Surgical Outcomes Research Center where I co-lead a group of research analysts and mentored several surgical residents. I have over 115 peer-reviewed manuscripts uncovering various aspects of variations and disparities in surgical care. Since 2011 I have contributed to most of these publications in a senior author capacity. In 2015, I was a member of the NIH-American College of Surgeons symposium for identification of priorities in surgical disparities research. Leveraging ongoing collaborations with my Co-PI Dr. Nawar Shara, whom we have 22 peer-reviewed manuscripts in surgical cancer outcomes. Together, we will work with an ideal research team with published expertise in artificial intelligence, surgical cancer outcomes, cancer disparities, and biostatistics. For this project "**REmote symptom COllection to improVE postopeRative care (RECOVER)"** I will serve as the Co-Principal Investigator and support the research team by providing leadership and guidance on the patient recruitment and symptom design and test of the proposed patient centered as an innovative tool for adults who undergo cancer surgery of the esophagus, stomach, liver, pancreas, colon, or rectum across MedStar Health and CHI Health.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2023 - Chair and Professor of Surgery, Creighton University School of Medicine and CHI Health, Omaha, NE
2018-2023 Vice-Chair for Research of Surgery, Regional Department of Surgery
2018-2023 Regional Chief of Surgical Oncology, MedStar Health

2015–2023 John S. Dillon Professor of Surgical Oncology. Georgetown University Medical Center. Washington, DC
2012–2023 Founding Chief of Surgical Oncology and Surgeon in Chief, Associate Professor in Surgery, Georgetown University Hospital and Lombardi Comprehensive Cancer Center, Washington, DC
2012–2023 Director of MedStar-Georgetown Surgical Outcomes Research Center, Washington, DC
2011–2012 Associate Professor and Staff Surgeon, Department of Surgery, Co-Director Minnesota Surgical Outcomes Workgroup, University of Minnesota and Minneapolis VAMC, Minneapolis, MN
2006–2011 Assistant Professor and Staff Surgeon, Department of Surgery, University of Minnesota and Minneapolis VAMC, Minneapolis, MN

Other Experience and Professional Memberships

2022- Surgical Biology Club II Board Director
2020-2023 Society for Surgery of Alimentary Tract Research Committee Chair
2018- American Surgical Association
2012- American College of Surgeons Cancer Care Delivery Research Committee
2011-2012 Member, Association for Academic Surgeons Outcomes Research Committee
2011- Member, Society of University Surgeons
2011- American College of Surgeons Clinical Research Program
2009-2011 Special Population committee of the Am College of Surgeons Oncology Group (ACOSOG)
2008- Member, The Society of Surgical Oncology
2007- Member, The Association for Academic Surgery
2005- Member, American Society of Clinical Oncology

Honors and Awards

2018 The John S. Dillon Medical Student Teaching Award
2015 The Inaugural Gulf Cooperation Council Merit Award for Achievement and Distinction
2014 Stephen R.T. Evans Teach Award
2006 M. D. Anderson Cancer Center, Dept. of Surgical Oncology Advanced Care Practitioner Award in Patient Care
2006 American Society of Clinical Oncology Foundation Merit Award
2005 American Society of Clinical Oncology Foundation Merit Award
2004 University of California at San Diego Department of Surgery Teaching Award
2004 University of California at San Diego Chairman of Surgery Award
2001 University of California at San Diego Chairman of Surgery Award
2000 San Diego Society for General Surgeons Award
1997 Desmond Lyons Memorial Prize in Surgery, Beaumont Hospital, Ireland
1997 Amir of Kuwait and Crown Prince Merit Award of Kuwait

C. Contributions to Science

1. The Affordable Care Act and surgical cancer care disparities: health care disparities are widely established in the US including surgical cancer care. While they represent a growing population of the US, these disparities have negatively impacted racial/ethnic minorities, the under- and uninsured the most of US populations. Extensive prior research publications from our team and others have documented these surgical care equity problems. Our team and others have shown that Medicaid beneficiaries do not have equal access to high quality care including access to high volume hospitals for complex cancer surgery. The ACA has passed to provide our patients at least safe, affordable, and accountable care. One of the main components of the ACA is to close gaps in US health care disparities. In this regard, I have co-authored several papers exploring issues related to Medicaid expansion and complex surgical care, surgical readmissions overall and across Medicaid and safety net hospitals. I have also highlighted possible unintended consequences of the ACA on surgical cancer care and those at risk of these negative consequences.

    a. Erin C. Hall, Chaoyi Zheng, Russell Langan, Lynt B. Johnson, Nawar Shara, **Waddah B. Al-Refaie**. Medicaid Beneficiaries Undergoing Complex Surgery At Quality Care Centers: Insights Into The Affordable Care Act. *American Journal of Surgery*. Accepted for publication.

b. AH Haider, VK Scott, KA Rehman, CG Velopulos, JM Bentley, EE Cornwell III and W Al-Refaie. Racial disparities in surgical care and outcomes in the United States: a comprehensive review of patient, provider and systemic factors. Journal of American College of Surgeons. 2013 Mar;216(3):482-492. PMID:23318117
c. Chaoyi Zheng, Elizabeth B. Habermann, Nawar M. Shara, Russell C. Langan, Young Hong, Lynt B. Johnson, **Waddah B. Al-Refaie**. Fragmentation of Care after Surgical Discharge: Non-Index Readmission after Major Cancer Surgery. *Journal of The American College of Surgeons*. Accepted for publication.
d. Hong Y, Zheng C, Hechenbleikner E, Johnson LB, Shara N, Al-Refaie WB. Vulnerable Hospitals and Cancer Surgery Readmissions: Insights into the Unintended Consequences of the Patient Protection and Affordable Care Act. J Am Coll Surg. 2016 Jul;223(1):142-51.

2. Variation in surgical cancer care: A large fraction of my publications has focused on quantifying variation in surgical oncology care among older adults and vulnerable populations. These publications have also focused on identifying reasons behind these variations. Older adults (65 yrs +) represent a growing cohort of the US population as well the surgical workload across many hospitals. These vulnerable persons and other, including multi-morbid, the under-or uninsured, are also at risk for worse operative and cancer-related outcomes. I served as the primary author or senior author providing clinical and methodological expertise in all of these studies.

   a. **Al-Refaie W**, Parsons HM, Henderson WG, Jensen EH, Tuttle TM, Rothenberger DA, Vickers SM, Virnig BA. Major Cancer Surgery in the Elderly: Results from the American College of Surgeons National Surgical Quality Improvement Program. *Annals of Surgery*: Feb;251(2):311-8, 2010. PMID: 19838107
   b. **Al-Refaie WB,** Habermann E, Jensen EH, Vickers SM, Tuttle TM, Virnig BA. “Extremity Soft Tissue Sarcoma Care in the elderly: Insights into the generalizability of NCI trials”. *Annals of Surgical Oncology*: 17 (7):1732-38, 2010. PMID: 20354801
   c. **Al-Refaie WB**, Weinberg A, Nelson H. Are older adults adequately represented in surgical oncology trials? Bull Am Coll Surg. 2013 May;98(5):52-3. PMID: 23841323

3. Cancer Trials vs. The Real World: Cancer clinical trials remain the gold standard to guide and inform clinical practice and oncologic decision making. However, the applicability of these trials to a real-world perspective of day-day persons with solid cancers and their surgeons remains an open question. This limitation is specifically relevant when most of these trials are conducted on persons who are young, healthy, insured and who receive their care at high volume or academic centers. As such, I have designed several observation studies exploring such trends among surgical patients with solid organ cancers who enrolled in cancer clinical trials. These studies point toward specific gaps in applicability of cancer trials in our current routine practice and future interventional investigations.

   a. **Al-Refaie W**, Pisters PWT, Rothenberger DA. Surgical oncology trials and surgeons in the real world!. Annals of Surgical Oncology. 2010 July; 17 (7):1727-28. PMID: 20393802.
   b. **Al-Refaie WB**, Vickers SM, Zhong W, Parsons HM, Rothenberger D, Habermann EB. Cancer trials versus the real world in the United States. Annals of Surgery. 2011 Sept; 254(3): 438-42. PMID: 21775882.
   c. **Al-Refaie W**, Vickers SM. Are cancer clinical trials useful and valid for the general surgeon and surgical oncologist? Advances in Surgery. Volume 46. 2012;46:269-81. PMIID: 22873045

Complete List of Published Work in My Bibliography:

https://pubmed.ncbi.nlm.nih.gov/?term=al-refaie+w&sort=date

BIOGRAPHICAL SKETCH

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

NAME: Walters, Ryan William

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

POSITION TITLE: Associate Professor, Vice Chair for Clinical Research, Director of Statistics and Analytics

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Minnesota State University, Mankato	BS	05/2005	Psychology
Missouri State University	MS	08/2007	Experimental Psychology
University of Nebraska-Lincoln	PhD	07/2015	Quantitative Psychology

A. Personal Statement

I am Associate Professor, Vice Chair for Clinical Research, and Director of the Statistics and Analytics team within the Department of Clinical Research and Public Health at Creighton University. I am a quantitative methodologist with expertise in the design and analysis of repeated-measures and longitudinal data with a research program specific to methodological best practices for quantifying and explaining individual differences in the amount and variability of repeated-measures data. Methodologically, I have extensive experience in the design of retrospective cohort studies using large administrative data (e.g., National Inpatient Sample, Nationwide Readmissions Database, State Inpatient Databases, National Cancer Database, etc.). Statistically, my expertise includes the estimation of generalized linear mixed models, multilevel models, mixed-effects location-scale models, structural equation models including latent growth models, and multivariate models for dyadic data. I have expertise to estimate these models in both frequentist and Bayesian frameworks.

My primary responsibilities at Creighton University are to serve as primary research mentor to faculty and learners across the School of Medicine as well as to serve as instructor of record for the evidence-based medicine curriculum for medical students and research curriculum for faculty, fellows, and residents. My role as research mentor is a responsibility to which I dedicate considerable effort; I have shared consistent success in developing my mentee's skills to position themselves to climb the next step of their careers. Individual differences abound, my mentorship is tailored to the mentee with specific focus on question and hypothesis development, study design, outcome measurement, statistical analysis, and reporting for presentation and publication. Further, my approachable, yet rigorous, style has offered me numerous opportunities to deliver local, national, and international methodological and statistical colloquia and workshops.

I am also course director for the evidence-based medicine curriculum for medical students as well as the research curriculum for residents and fellows; I am instructor of record for a 3-credit biostatistics course for basic science master's and doctoral students.

B. Positions, Scientific Appointments, and Honors

2021-Present	Associate Professor with tenure, Department of Clinical Research (primary), Department of Medicine (secondary), Creighton University
2021-Present	Vice Chair for Clinical Research, Creighton University
2015-2021	Assistant Professor, Department of Medicine, Creighton University

2009-2015 Senior Research Analyst & Instructor, Department of Medicine, Creighton University
2007-2009 Research Analyst, School of Pharmacy and Health Professions, Creighton University

C. Contributions to Science

1. I am a quantitative methodologist focused on methodological and statistical best practices for modeling longitudinal data using the multilevel model or mixed-effects location-scale model. In my experience, there is a substantial disconnect between mathematical statisticians who develop new statistical methods and empirical scientists who could use these methods in practice. My research program is dedicated to bridging the gap between these two types of individuals, disseminating information that is statistically rigorous while remaining accessible. Specifically, as an applied statistician, I use state-of-the-art statistical models to attain accurate and informative analyses of empirical data; as a methodologist, I conduct simulation studies designed to inform the empirical scientist about how the statistical models perform in circumstances often encountered in their research settings. Below are peer-reviewed publications specific to my research program.
    a. Hoffman, L, & Walters, R. W. (2022). Catching up on multilevel modeling. *Annual Review of Psychology, 73*, 659-689. https://doi.org/10.1146/annurev-psych-020821-103525. PMID: 34982593.
    b. Lester, H. F., Cullen-Lester, K. L., Walters, R. W. (2021). From Nuisance to Novel Research Questions: Using Multilevel Models to Predict Heterogeneity of Variance. Organizational Research Methods, 24(2), 342-388. https://doi.org/10.1177/1094428119887434.
    c. Walters, R. W., Hoffman, L., & Templin, J. (2018). The power to detect and predict individual differences in intra-individual variability using the mixed-effects location-scale model. Multivariate Behavioral Research, 53(3), 360-374. https://doi.org/10.1080/00273171.2018.1449628. PMID: 29565691.
    d. Cushing, C. C., Walters, R. W., & Hoffman, L. (2014). Aggregated N-of-1 randomized controlled trials: Modern data analytics applied to a clinically valid method of intervention effectiveness. *Journal of Pediatric Psychology, 39*, 138-150. https://doi.org/10.1093/jpepsy/jst083. PMID: 24284134.

2. Below are select publications for which I have provided primary research mentorship for projects using large administrative datasets with a focus on health disparities.
    a. Petersen, J., Abusnina, W., Beesabathina, S., Desu, S. S., Walters, R. W., & Alla, V. M. (In Press). Racial disparities in outcomes of delivery and cardiac complications among pregnant women with congenital heart disease. Journal of Racial and Ethnic Health Disparities. https://doi.org/10.1007/s40615-024-01950-0. PMID: 38416292.
    b. Latif, A., Tran, A. M., Ahsan, M. J., Niu, F., Walters, R. W., & Kim, M. H. (2023). Relationship of health-related social needs and hospital readmissions in patients following a hospitalization for atrial fibrillation. American Heart Journal Plus: Cardiology Research and Practice, 36, 100340. https://doi.org/10.1016/j.ahjo.2023.100340. PMID: 38510101.
    c. Ismayl, M., Abbasi, M. A., Al-Abcha, A., El-Am, E., Walters, R. W., Goldsweig, A. M., et al. (2023). Racial and ethnic disparities in the utilization and outcomes of transcatheter mitral valve replacement: Analysis from the National Inpatient Sample database. Journal of the American Heart Association, 12(7), e028999. http://doi.org/10.1161/JAHA.122.028999. PMID: 36974752.
    d. Pajjuru, V. S., Thandra, A., Guddeti, R. R., Walters, R. W., Jhand, A., Andukuri, V. G., et al. (2022). Sex differences in mortality and 90-day readmission rates after transcatheter aortic valve replacement (TAVR): A nationwide analysis from the United States. European Heart Journal: Quality of Care and Clinical Outcomes, 8(2), 135-142. https://doi.org/10.1093/ehjqcco/qcab012. PMID: 33585884.

3. Additional contributions have required me to provide study design expertise, develop statistical analysis plans, analyze and interpret the data, and write up the Method and Results sections for publication. These expectations have netted me 99 peer-reviewed publications, 6 book chapters, and hundreds of national conference presentations. Below is a selected sample of recent peer-reviewed publications for which I served as the primary research mentor and estimated generalized linear mixed effects models.

a. Schuster, H., Walters, R. W., Mathy, J., Ramaswamy, S., & Alsakaf, I. (In Press). Correlation between ECT quality measures and likelihood to transition from acute to continuation and maintenance ECT. Journal of ECT.
b. Guck, T. P., Walters, R. W., Abdul-Greene, C., Doll, J., Greene, M. A., & McGaha, A. L. (2024). Sustainable and replicable clinical and charge outcomes in an interprofessional education and collaborative practice nexus. Journal of Interprofessional Care, 38(1), 70-77. https://doi.org/10.1080/13561820.2021.1932776. PMID: 34139943.
c. Cote, J. J., Cote-Arsenault, D., Handelzalts, J. E., Badura-Brack, A. S., Kalata, M., Walters, R. W., et al. (2023). Effects of 3D printed models and 3D printed pictures on maternal and paternal-fetal attachment, anxiety, and depression. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 52*(3), 223-234. https://doi.org/10.1016/j.jogn.2023.02.002. PMID: 36940782.
d. Jagan, N., Walters, R. W., Maldonado, F., Pilli, S., & DePew, Z. S. (2022). Uncovering objective improvements in physical activity using digital actigraphy after therapeutic thoracentesis: A Pilot study. Annals of the American Thoracic Society, 19(8), 1438-1440. https://doi.org/10.1513/AnnalsATS.202202-165RL. PMID: 35587360.

BIOGRAPHICAL SKETCH

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

NAME: Erin A Gillaspie

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

POSITION TITLE: Assistant Professor of Thoracic Surgery

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Florida, Gainesville FL	BS	05/2004	Microbiology and Cell Science
University of Miami Miller School of Medicine, Miami FL	MD	05/2008	Medicine
Spartanburg Regional Medical Center, Spartanburg SC		06/2009	Intern in General Surgery
Bassett Medical Center (Columbia University), Cooperstown NY		06/2013	Residency in General Surgery
Mayo Clinic, Rochester MN		06/2016	Fellowship in Cardiothoracic Surgery
Vanderbilt University	MPH	05/2018	Masters of Public Health

A. Personal Statement

I am a thoracic surgeon with a primary interest in the multidisciplinary treatment of lung cancer, lung cancer prevention and minimally invasive surgery. Throughout my training and early career, I have dedicated a significant portion of my time to research. In fact, during my first two years as an attending, I also pursued a Master of Public Health to further bolster my skills in biostatistics and clinical trial development. I have participated in all aspects of projects including design, budget, staffing, patient recruitment, data collection, analysis and have produced several peer-reviewed publications in addition to having the opportunity to present my research at national and international meetings. I have been privileged to participate in projects that will continue to help to define our surgical care of patients.

My current research focuses on the development of novel technology to optimize diagnosis and treatment of patients using minimally invasive methods.

B. Positions and Honors

Positions and Employment:

2016 - present Assistant Professor of Thoracic Surgery, Vanderbilt University Medical Center, Nashville TN
2012-2013 Instructor of Surgery, Mayo Clinic, Rochester MN
2013-2016 Fellow, Division of Thoracic Surgery, Mayo Clinic, Rochester MN

Other Experiences and Professional Memberships

2018- present Southern Thoracic Surgical
2018-present Society of Thoracic Surgery
2018-present Fellow of the American College of Surgeons
2017- present American College of Chest Physicians
2017- present ECOG-ACRIN Thoracic Surgery Committee

2017- present ECOG-ACRIN Thoracic Oncology Committee
2017-present IASLC Member
2017-present Nashville Surgical Society
2016- present Women in Thoracic Surgery Member
2014- 2016 Thoracic Surgery Resident's Association Executive Officer
2013- 2016 Thoracic Surgery Resident's Association
2015- present International Society of Heart and Lung Transplantation
2014- present European Society of Thoracic Surgery
2013- present American College of Surgeons Associate Fellow
2013- 2016 Women in Thoracic Surgery Candidate Member
2013- 2016 Society for Thoracic Surgeons Candidate Member

Honors:

2018 Association for Academic Surgery Young Investigator Award
2017 Women in Thoracic Surgery- Intuitive Surgical Robotic Fellowship Award
2016 STS- Advocacy Legislative Fly-in Scholarship Winner
2016 Priestley Mayo Clinic Surgical Honor Society
2015 AATS Graham Foundation Intuitive Surgical Robotics Fellowship
2015-2016 O.T. Claggett Travelling Fellowship Award
2015 Jeopardy Thoracic Surgery Competition Finalist
2014 Mayo Clinic Bronze Quality Fellow
2014 Master's Cup Champion (Thoracic Surgery International Knowledge Competition)
2014 Top Gun Suturing Competition Finalist
2012-2013 E Donnall Thomas Excellence in Research Award
2008-2009 Charles B Hanna MD Surgical Intern of the Year Award

C. Contributions to Science

1. Trials involving the efficacy of a new devices, the economics and implementation into practice have been a focus of my research since medical school. The past two decades have ushered in a technological revolution with the incorporation of robotics, new imaging and navigational technologies. I have worked the last two years with the Vanderbilt Institute for Surgical Engineering to help improve upon currently available instruments, develop new instruments to fill unmet needs as well as new robotic platforms to further develop and expand the minimally invasive diagnostic and therapeutic fields.

a. Gafford J, Webster S, Dillon N, Blun E, Hendrick R, Maldonado F, **Gillaspie EA**, Rickman O, Herrell W, Webster R. A Concentric Tube Robot System for Rigid Bronchoscopy: A Feasibility Study on Central Airway Obstruction Removal. Annals of Biomedical Engineering.
b. **Gillaspie EA**. Device clinical trials: the importance of "repurposing" technology. Video-assist Thorac Surg, 2018; 3:12.
c. Schild AF, **Gillaspie E**. Interrupted Clips Give a Better Outcome. *The Journal of Vascular Access.* Jul-Sep 2005; 6(3): 147.
d. Shild AF, **Gillaspie EA**, Patel AR, Noicely K, Baltodano N. Use of Polyetherurethaneurea Grafts in HIV-positive Patients. *Vascular Access for Hemodialysis X*. Jul 2008; 113-117.

2. Oncology has long been a passion of mine with the goal of optimizing both quality and quantity of life for patients and trying to determine optimal treatment regimens. Obesity has reached epidemic proportion globally and has been linked to an increased incidence of cancer – in particular colon cancer. I received a foundational grant that allowed me to create a new animal model of diet induced obesity in which to study cancer. Once established, I studied growth patterns of colon cancer and treatment efficacy using standard chemotherapeutic regimens. In addition to establishing a more aggressive growth pattern, but we also observed a notable impairment in the effectiveness of 5-Fluorouracil when treating these tumors. These publications document the emerging challenge we face in improving outcomes for colon cancer. I served as primary investigator or co-investigator in all of these studies.

a. O'Neill AM**, Gillaspie EA,** Burrington CM, Lynch DT, Horsman MJ, Greene MW. High-fat Western diet-induced obesity contributes to increased tumor growth in mouse models of human colon cancer. Nutr Res. 2016, (Epub ahead of print). PMID 27866828.

b. Ammannagari N, **Gillaspie EA**, Burrington C, Horsman M, Greene M. Obesity impairs the efficacy of colon cancer treatment. *National American College of Physicians*. San Francisco, CA. April 2013. PMCID: PMC4259412
c. Ammannagari N, **Gillaspie EA** et al "Obesity impairs the efficacy of colon cancer treatment" Poster Presentation. New York Chapter American College of Physicians Meeting. Albany, New York. 2013. Winner of Stanley & Eleanor Wallace Memorial Award.
d. **Gillaspie EA**, Lynch D, Burrington C, Green M. Insulin Resistance and the Impact on Colon Cancer. *E. Donnall Thomas Poster Competition*. Cooperstown, NY. April, 2012. Winner of the E Donnall Thomas Award for Research Excellence.

3. The management of lung cancer, in particular later stages of disease, as of yet remains controversial. Trying to delineate which patients benefit most from surgical intervention and who should be relegated to chemotherapy/radiation is complex. In the last three years I have dedicated significant time to creating and defining treatment algorithms for Stage IIIA non-small cell lung cancer patients, to the use of technology to improve outcomes for complex resections of advanced stage tumors and to the development of minimally invasive techniques to reduce patient morbidity.

a. **Gillaspie EA**, Wigle DA. Management of Stage IIIA (N2) Non-Small Cell Lung Cancer. *Thorac Surg Clin*. Aug 2016; 26(3):271-85. PMID: 27427522
b. **Gillaspie EA.** Locally Advanced Lung Cancer. In LaPar DJ, Mery CM, Turek JW (Ed), *TSRA Review of Cardiothoracic Surgery, 2nd ed*. Pg 38-44. Thoracic Surgery Residents Association; Chicago, 2015.
c. **Gillaspie EA,** Blackmon SH. Minimally invasive approaches to chest wall and superior sulcus tumors-hybrid resections. In Want J, Ferguson MK (Ed), *Atlas of Minimally Invasive Surgery for Lung and Esophageal cancer*. Springer Netherlands, 2017.
d. **Gillaspie EA**, Matsumoto JS, Norris NE, Downey RJ, Shen KR, Allen MS, Blackmon SH. From 3D Printing to 5D Printing: Enhancing Thoracic Surgical Planning and Resection of Complex Tumors. *Ann Thorac Surg*. May 2016; 101(5): 1958-62. PMID

Please find a list of my published works available through PubMed at the following link:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Gillaspie+EA%5BAuthor%5D

BIOGRAPHICAL SKETCH

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

NAME: Singh, Awinder Preet

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

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 *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Government Medical College Amritsar, India	MBBS	02/2000	Medicine
Government Medical College Baroda, India	MS	06/2005	Residency, General Surgery
Thomas Jefferson University Hospital, Philadelphia, PA		06/2014	Residency, General Surgery
Thomas Jefferson University Hospital, Philadelphia, PA		06/2015	Fellowship, Minimally Invasive Gastrointestinal Hepatobiliary and Pancreatic Surgery
Providence Hospital and Medical Center, Southfield, MI		06/2016	Fellowship, Hepatopancreatobiliary Surgery

A. Personal Statement

I am an Associate Professor of Surgery, in the Department of Surgery at Creighton University Omaha, NE. My previous work was focused on clinical outcomes and disparities including Gender Disparity in Outcome of Post-Surgery Colorectal Cancer Patients and Breast Cancer Treatment Practices in Elderly Women in a Community Hospital. My recent work is on disparities and the outcome of pancreatic cancer among rural and urban populations. In addition, I have collaborated with my other colleagues, and produced peer-reviewed publications.

As a result of the above work, I have experience and understand the importance of frequent communication with research team members and getting timely outcomes within desired budget.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2023 – present Associate Professor, Department of Surgery, Creighton University
2016 – 2023 Assistant Professor, Department of Surgery, Creighton University

Other Experiences and Professional Membership -

2023 Member of the Society for Surgery of the Alimentary Tract
2023 Member of International Hepato-Pancreato-Biliary Association
2023 Fellow of American college of surgeons
2013 Member of American college of surgeons.

C. Contributions to Science

My early publications directly addressed the disparities in post cancer surgery outcomes among different genders. Also, other work reflects racial disparities in breast cancer patients. These publications document the urgent need to provide the same highest standard of care to every patient irrespective of their age group or gender. Some of my recent work is looking for similar kinds of disparities among patients living in rural areas of our country compared to urban populations.

1. Cullen E.Worsh BA,.TalarTatarian MD, Awinder Singh MD, Michael J.Pucci MD, Jordan Winter MD,.Charles J.Yeo MD, HarishLavu MD Total parenteral nutrition in patients following pancreaticoduodenectomy: lessons from 1184 Patients. Journal of Surgical Research.Volume 218, October 2017, Pages 156-161.
2. Stacey Martindale, Awinder Singh, Hua Wang, Ashley Steinberg, Alan Go, Peter Pappas, Haidi Zhang, and Amer Homsi. Racial disparities in survival and age-related outcome in post-surgery breast cancer patients in a New York City community hospital. Accepted 23 December 2013 in ISRN Oncology
3. HuaWang, Awinder P.Singh, SerenaSt.Luce,and Alan R. Go. Breast Cancer Treatment Practices in Elderly Women in a Community Hospital. International Journal of Breast Cancer.Volume 2011 (2011), Article ID 467906,7pages.

BIOGRAPHICAL SKETCH

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

NAME: Timperley, Jillian

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

POSITION TITLE: Research Assistant

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Nebraska Wesleyan University, Lincoln, NE	BS	05/2022	Biology

A. Personal Statement

My experiences as a research assistant with at the University of Nebraska Medical Center provided me with skills in conducting neuropsychological testing and working with an interdisciplinary team to explore cognitive functioning in older adults following allogeneic hematopoietic cell transplantation. Building upon this foundation, ss a research assistant with the Department of Surgery at Creighton University, I have been able to continue to advance healthcare knowledge in a collaborative environment on several current projects focused on surgical equity. Through constant communication our Creighton team produced a peer- reviewed publication on Social Determinants of Health and Surgery. My experiences as a research assistant have provided me with the skills to work with this team and support this research endeavor.

B. Positions, Scientific Appointments, and Honors

2023-Present Research Assistant, Department of Surgery, Creighton University School of Medicine, Omaha, NE

2022-2024 Research Assistant, Department of Internal Medicine, University of Nebraska Medicine, Omaha, NE

C. Contributions to Science

1. My first publication reported on case of a patient with a critical gastrointestinal manifestation of SARS-CoV-2 infection amidst the COVID-19 pandemic. This case study shed light on a rare yet critical manifestation of the virus and highlighted the importance of monitoring for atypical presentations of the disease.
    a. Samlowski MD, Erica, Emily Brown MD, **Jillian Timperley**, Sue Sasse MD “COVID-19 Patient Presenting with Total Pancreatic Necrosis, Colopancreatic Fistula.” American College of Surgeons, 1 Aug. 2022, https://www.facs.org/for-medical-professionals/news-publications/ journals/case-reviews/issues/v3n6/brown-covid/.
2. This letter to the American Journal of Surgery explored incoming national policies on Health-Related Social Need Screening and their potential utilization for surgical patient populations to address long standing health disparities. The letter highlights several gaps in knowledge and future research priorities on this topic.
    a. **Timperley, Jillian**, Doll, Joy OTD, Tade, Yanick, Al- Refaie, Waddah MD “Health related social needs and social determinants of health: Navigating the convoluted path of health equity in surgery.” American Journal of Surgery https://www.americanjournalofsurgery.com/article/S0002-9610(24)00032-1/ abstract

Statement of Project's Relevance to Cancer or Smoking Diseases

Nearly 15 million rural persons live in Nebraska and its surrounding states in the Midwest.[1] These persons represent nearly 30% and 25% of the Midwest and entire US rural populations, respectively, and face significant cancer mortality relative to urban residents.[2] Rural America has suffered disproportionately higher rates of disparities in access to and outcomes after quality cancer surgery. Also, nearly 40% of rural people on Medicaid, who are uninsured, who live in poverty, or who are ethno-racial minorities suffer even poorer cancer surgery access and outcomes, in part, due to their complex medical and social determinants of health. These issues are further complicated in states with high rural hospital closure rates likely leading to profoundly detrimental effects on cancer surgery access.

The ACA's Medicaid Expansion has contributed to a significant reduction in the number of uninsured Americans in states that opted to expand.[5,30] Expanding Medicaid eligibility to low-income Americans (people at or below 138% of the federal poverty level) drastically reduced the number of uninsured Americans from 44 million in 2013 to under 28 million in 2016.[5] However, concerns remain whether this increased insurance coverage has improved access to quality surgical cancer care in rural America, particularly among vulnerable populations that include the uninsured, the poor, or ethno-racial minorities. As such, this study will explore the effect of the Medicaid expansion on cancer surgery access and outcomes among surgical patients living in rural counties with focus on disparities among those vulnerable patient populations. This unique study, representing one of the largest multi-rural state evaluations of the Medicaid Expansion, will drive understanding of how this historic health policy impacted cancer treatment in rural America.

Reasons behind selecting lung, pancreatic, and colorectal cancer surgery are highly relevant to Nebraska and the Midwest. First, these three cancers are the most common and leading causes of rural cancer mortality.[7-9] Second, the combination of smoking, under- or no-insurance, and lack of transportation are key reasons behind advanced rates of lung cancer in Nebraska and rural America. Third, lack of adequate insurance, short provider supply, health literacy, and transportation challenges are also key reasons for advanced colorectal stage at diagnosis. Fourth, these cancers are best treated at centers of excellence, which depend on adequate provider supply and transportation means.

In addition to Nebraska, we propose to use data from 9 rural Midwest states chosen due to similarity to Nebraska, a recently expanded state. It is particularly important that the study team include Midwest states that expanded early given that Medicaid expansion in Nebraska is relatively new (2020 Q4). Insight into outcomes for states that expanded early will likely provide comparable outcomes in Nebraska and inform the team's subsequent grant application to address disparities specifically in Nebraska.

Results from this study represent one of the largest Midwest state evaluations that have policy, healthcare delivery, and research implications for the State of Nebraska. Findings from this study will be leveraged to solicit a successor NIMHD R01 grant in 2026.

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

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
FY24/25	1	0

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

CELLULAR SIGNALING AND MOLECULAR TRAFFICKING IN CANCER
Dr. Laura Hansen

Project Title: Checkpoint Signaling and
Cell Survival in Normal and Tumorigenic Skin Keratinocytes
Principal Investigator: Dr. Laura Hansen

I. Progress Report Summary

A. Specific Aims

As described in last year's progress report, Flower (FWE) expression was increased in differentiated regions (keratin pearls) of human cutaneous squamous cell carcinoma (cSCC) subcutaneous xenografts using cell lines overexpressing isoforms hFWE3 or hFWE4. Since then, we have extended this finding to endogenously expressed FWE in human cSCC samples classified as poorly or well-differentiated by a dermatopathologist. In addition, we utilized CRISPR technology to ablate FWE expression in our cSCC cell line to evaluate the effect of no FWE expression on xenograft tumor differentiation. Results described below have led us to further refine the direction of the project to assess the role of FWE isoforms in late-stage keratinocyte differentiation in normal human epidermis and its expression in cSCC. We show that FWE is abundant in the stratum granulosum (SG), where it is essential for maturation of the epidermal barrier, and also show that expression and subcellular localization is dysregulated in two genetic disease of cornification.

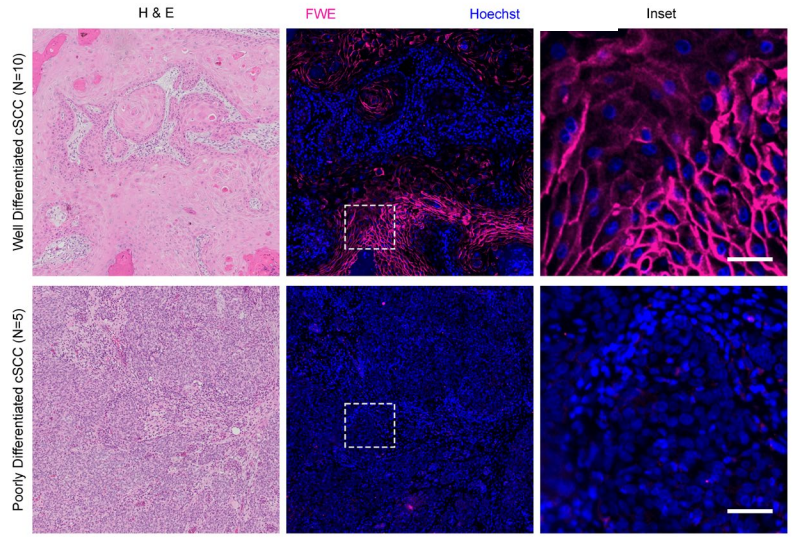

Fig 1. Flower is abundant in terminally differentiated layers of well-differentiated cSCCs. *FWE immunofluorescence was performed on biopsies of well (N=10) and poorly differentiated (N=5) cSCCs. Well and poorly differentiated keratinocyte regions were identified by a dermatopathologist. Shown are representative images of well-differentiated and poorly differentiated foci.*

B. Studies and Results

Endogenous Flower protein levels are increased in differentiated regions of human cSCC tumors. Acquisition of an FWE-specific antibody from a collaborator enabled us to extend our analyses of the role of FWE in tumor development to human cSCC samples. Well-differentiated tumors express high levels of FWE, but poorly differentiated tumors express little to no FWE protein (Fig 1).

Loss of Flower protein leads to decreased

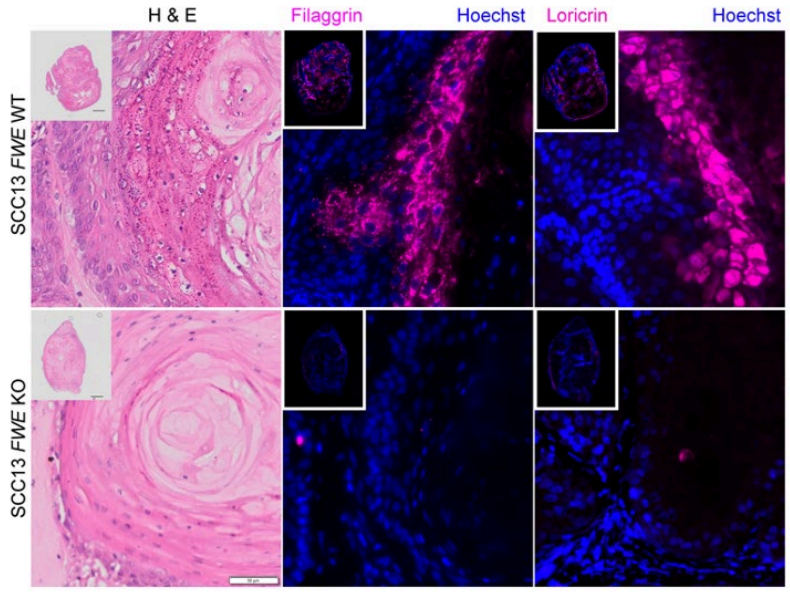

Fig 2. *hFWE* KO impairs terminal differentiation in cSCC xenografts. *hFWE* KO and WT SCC-13 cells were grafted subcutaneously onto NCG immunocompromised mice (N=12) and tumors were allowed to develop for 1 month before collection. Shown are representative routine histology and immunofluorescent staining for late terminal differentiation markers Filaggrin and Loricrin.

markers of differentiation in cSCC xenograft tumors. The results with human cSCC samples led us back to the xenograft model to evaluate the differentiation potential of cSCC cells expressing endogenous FWE compared to cSCC cells lacking FWE expression (CRISPR KO). FWE KO xenografts displayed decreased keratohyalin granules, Filaggrin and Loricrin expression (all markers of keratinocyte differentiation) relative to xenografts expressing endogenous FWE (Fig. 2).

Flower is upregulated in terminally differentiating epidermal keratinocytes and localizes to apically polarized vesicles

In human epidermis, confocal microscopy revealed that FWE was largely restricted to keratinocytes in the upper stratum spinosum (SS) and throughout the three layers of the stratum granulosum (SG), termed SG3, SG2, and SG1 from inner to outer, respectively. Cells in the uppermost SG1 layer frequently displayed the most intense, apically polarized signal for FWE (Fig 1A). Double immunostaining for the desmosomal cadherin desmoglein-1 (DSG-1) and FWE demonstrated that, in upper SS and lower SG3 layers, FWE puncta accumulated just underneath the apical plasma membrane and appeared to fuse with this membrane in SG2 and SG1 cells (Fig 3). Airyscan2 super-resolution microscopy further revealed FWE-positive vesicles of 140-200nm in diameter aligning between DSG-1 positive puncta (marking desmosomes between each cell layer) along the apical membrane of SG1, and to a lesser extent SG2 cells (Fig 3B). To validate the specificity of the FWE antibody used in these immunofluorescence and subsequent immunoblotting assays, *FWE* knockout (KO) N/TERT2-G keratinocytes were generated using Cas9:sgRNA RNP delivery to target exon 1, which is shared among all *FWE* transcript variants, and clonal knockout cell lines were established and characterized (data not shown). Immunoblotting in differentiated monolayers and immunofluorescence in epidermal organoid culture revealed specificity for a single <25kDa product and apically polarized signal in the SG, respectively (data not shown). These observations suggested that FWE labels a subset of apically secreted vesicles in SG keratinocytes of the upper epidermis where the extracellular lipid-rich matrix that fortifies the cutaneous barrier is actively assembled.

To determine whether this differentiation-associated expression pattern could be recapitulated *in vitro*, normal human epidermal keratinocytes (NHEKs) and TERT-immortalized NHEKs (N/TERT2-G) were differentiated for either one, three, or five days in high calcium (1.4mM) medium. Immunoblotting revealed a progressive increase in total FWE protein during differentiation, along with the differentiation markers keratin 10 (K10) and loricrin (LOR) (Fig 3C). As additional validation of the FWE antibody, a CRISPR-Cas9 strategy was used to knock-

in a 3xFLAG tag immediately upstream of the stop codon in the shared exon 6b of *hFWE3* and *hFWE4* in N/TERT2-G cells and isolated a heterozygous knock-in (KI) clone (data not shown). KI N/TERT-2G were subjected to a Ca^{2+} induced differentiation time course and immunoblotting revealed a progressive increase in 3xFLAG-tagged FWE levels during differentiation that mirrored endogenous expression (Fig 3D).

Importantly, immunoblotting for epitope-tagged protein in the KI cells yielded only a single specific band that runs at the expected molecular weight for hFWE4-3xFLAG (Fig 3D). The second most abundant isoform, hFWE3, is ~5kDa smaller than the canonical hFWE4, but was not observed. Detection of only the canonical FWE isoform (hFWE4) is consistent with our recent analysis of the GTEX database, which suggests *hFWE4* transcripts account for ~80% of all *hFWE* transcripts, and is in line with protein-level data in mice where only the hFWE4 orthologue (mFwe2) is detected. Lastly, to assess whether *FWE* expression changes are evident at the transcript level during keratinocyte differentiation, we reanalyzed two publicly available bulk RNA-sequencing (RNA-seq) datasets from NHEK differentiation time courses, which also revealed a progressive increase in *hFWE* expression during differentiation (Fig 3E).

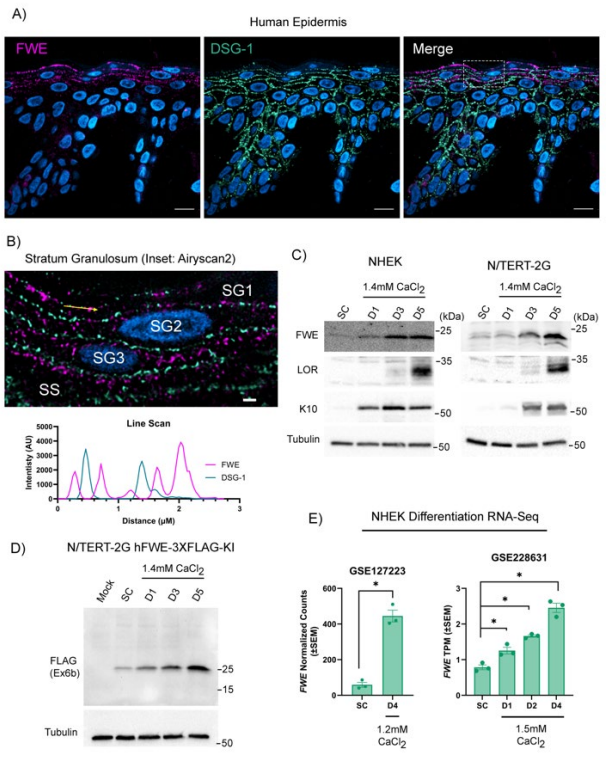

Fig 3. Flower is upregulated in terminally differentiating epidermal keratinocytes and localizes to apically polarized vesicles. (A) Confocal microscopy of desmoglein-1 (DSG-1) and Flower (FWE) IF in human epidermis. B) Airyscan super-resolution of stratum granulosum inset. (C) Immunoblotting for FWE and differentiation marker expression in primary NHEK and immortalized N/TERT-2G keratinocytes during a 5-day differentiation timecourse. (D) FLAG immunoblotting of differentiated monolayer N/TERT-2G cells harboring a 3X FLAG knocked into the endogenous *hFWE* locus. (E) *FWE* transcript expression in differentiated primary keratinocytes from publicly available RNA-seq datasets.

Collectively, these data identify FWE as a novel marker of late-stage terminal differentiation localized to the upper epidermal layers and suggest that FWE is trafficked to the apical surface of SG keratinocytes at the onset of cornification.

Flower-deficient epidermal organoids exhibit impaired barrier function

Given the distribution of FWE in the differentiated layers of human epidermis and its elevated expression at both transcript and protein levels during keratinocyte differentiation *in vitro*, we hypothesized that FWE plays an active role in the epidermal differentiation program.

As late terminal differentiation and cornification are not well replicated in two-dimensional monolayer cultures, we assessed the effect of *FWE* deficiency on the maturation of epidermal organoid cultures grown at the air-liquid interface. This organotypic model of human epidermis undergoes cornification over the course of 10-14 days to produce histologically normal *in-vitro* epidermis and is amenable to subsequent barrier function testing. During organoid maturation, FWE accumulation in SG keratinocytes peaked between 4-6 days of air-liquid interface culture and declined abruptly after 10 days (Fig 4A), consistent with FWE accumulation preceding complete acquisition of epidermal barrier function, which occurs at

day 10 as previously measured using electrical impedance spectroscopy (EIS) in our recent work. *FWE* KO organoids showed a significant reduction in electrical impedance within the range of frequencies that correlates with differentiation of viable keratinocytes (EIS^{diff}) (Fig 4B-C), providing additional evidence that FWE functionally contributes to epidermal barrier development. Histological analysis of tissue morphology revealed a small reduction in the number of layers containing keratohyalin granules (KHGs), suggesting that the barrier deficit may result from impaired SG maturation (Fig 4D). Collectively, these data indicated that *FWE* expression functionally contributes to the development of the epidermal barrier.

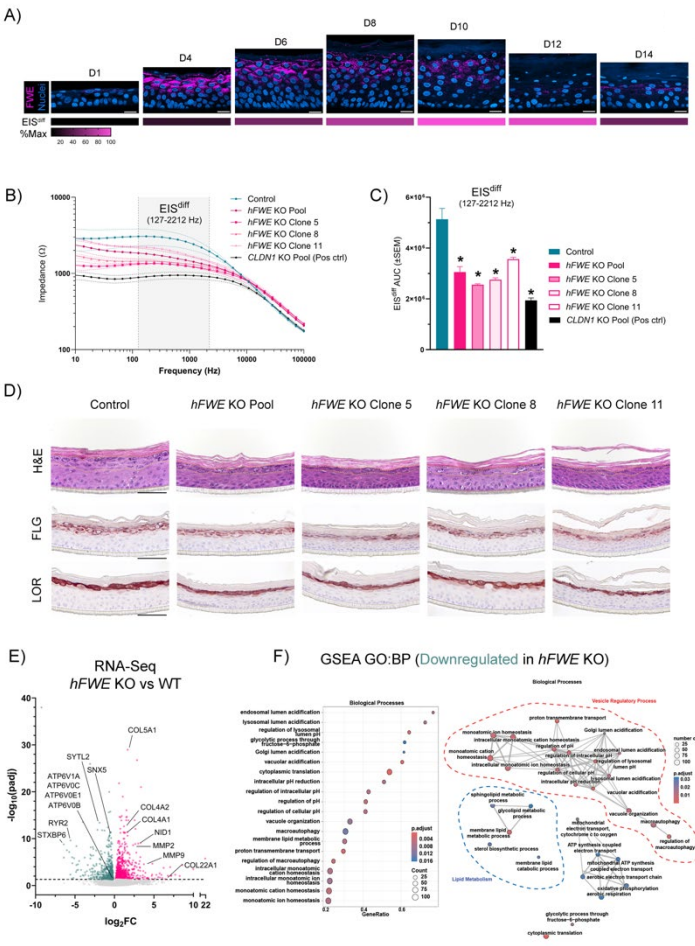

Fig. 4. Flower-deficient epidermal organoids exhibit impaired barrier function. (A) FWE IF (pink) on N/TERT-2G epidermal organoids grown at air-liquid interface (ALI). Heatmap beneath images reports percent maximal barrier function at each timepoint. (B,C) Electrical impedance spectroscopy (EIS) spectra of WT and *hFWE* KO organoids grown at ALI for 10d. Representative of two independent experiments, each N=3. (D) Representative routine histology and IHC for differentiation markers loricrin and filaggrin in epidermal organoids. (E) Volcano plot showing differentially expressed genes from bulk RNA-seq of WT (N=3) and *hFWE* KO (four independent cell lines, each N=3) epidermal organoids. (F) Gene set enrichment analysis of DEGs from RNA-seq of *hFWE* KO organoids, showing significant enrichment for downregulated genes involved in lysosome acidification/function, lipid metabolism, and calcium handling.

In line with the observation that KO organoids contained fewer KHG-containing layers, immunohistochemistry for epidermal differentiation markers revealed modestly reduced signal for both filaggrin (FLG), a constituent of KHGs, and LOR, the major component of the cornified cell envelope that forms in the SG (Fig 4D). Similarly, when differentiated as confluent monolayers under high Ca^{2+} conditions for four days, *hFWE* KO keratinocytes exhibited variable reductions in LOR, while minimal effect was observed in K10 levels, which is expressed in the lower epidermal layers prior to FWE induction (data not shown). Reduced LOR expression did not appear to result from a proliferation defect in *FWE* KO cells. In monolayer culture, there was a modest increase in the S-phase fraction of some KO clones (data not shown), while in organoid cultures, there was no apparent change in the fraction of proliferating Ki67+ cells between conditions (data not shown). These findings are consistent with the previous report of a small but significant increase in the S-phase fraction of breast cancer cells upon KO of *hFWE.*

To identify other factors contributing to the significant barrier deficit, transcriptomic profiling (bulk RNA-seq) performed on WT and KO organoids showed significant alterations in the expression of 733 genes (438 UP, 295 DOWN; p-adj <0.05) (Fig 4E). Consistent with the morphological

analysis showing minor changes in the uppermost epidermal layers of KO cultures, major differences in markers of various stages of keratinocyte differentiation were not observed. Across the 57 genes comprising the epidermal differentiation complex (31), only 7 (*IVL, LCE3C-E, LCE5A, S100A11, S100A16, TCHH*, and *CRNN)* showed significant reduction in expression in *FWE* KO organoids (data not shown). However, we were intrigued by gene set enrichment analysis (GSEA) showing that downregulated genes were enriched for biological functions related to vesicle function, including organelle acidification, lysosome maturation, autophagy, and cation homeostasis, along with those related to lipid metabolism, all of which are known drivers of the latest stages of epidermal differentiation (Fig 4F). *FWE* orthologues in mouse and fly have previously been described to function as putative calcium-permeable channels that regulate the trafficking of lysosome-related organelles (LROs) in other cell types (e.g., synaptic vesicles and lytic granules). Therefore, we reasoned that FWE may be exerting calcium-dependent control over biogenesis and trafficking of epidermal lamellar bodies (LBs), LROs potentially derived from the trans Golgi network that are generated and secreted in the SG to drive cutaneous barrier formation.

FWE localizes to epidermal LBs and facilitates their apically directed secretion.
Based on the apically polarized subcellular distribution of FWE in SG cells, we hypothesized that FWE may physically associate with epidermal LBs, which are secreted apically in cornifying keratinocytes. Airyscan2 super-resolution microscopy of double immunofluorescence for FWE and the LB cargo molecule corneodesmosin (CDSN) in normal human skin revealed clear colocalization between FWE and CDSN along the apical membrane of SG1 in vesicles exhibiting a diameter of roughly 140-200nm (Fig 5A-C), a size consistent with that described for LBs in previous electron and confocal microscopy studies. In SG2 cells, colocalization was also evident in both the cytosol and along the apical membrane (Fig 5A). Based on these imaging data, we hypothesized that FWE may directly control LB biogenesis and/or apical trafficking for secretion during cornification. Supporting this hypothesis, CDSN immunohistochemistry on WT organoids revealed strong expression and apical polarization in SG keratinocytes along with SC signal, reflecting its localization to corneodesmosomes (Fig 5B). In contrast, *FEW*-deficient organoids displayed diminished CDSN signal that had lost polarized distribution in the SG and was absent from SC layers (Fig 5B).

We further hypothesized that this impaired LB trafficking in KO organoids could cause retention of the desmosomal cadherin DSG-1 in the SC due to failed proteolysis by enzymes that are normally secreted from LBs onto the SC surface. In WT samples, consistent with normal cornification, CDSN was retained in the upper SC layers, while DSG1 labeled only the lower SC layers (Fig 5C). In *FWE* KO organoids, this pattern was reversed, with DSG1 often being retained in the most apical SC layers, while CDSN was present predominantly in the lower SC layers (Fig 5C). Collectively, these data reveal that in SG keratinocytes, FWE localizes to LBs and is essential for their trafficking and apical secretion to allow proper delivery of CDSN and processing of DSG-1 during cornification.

FWE-positive LBs are mislocalized in cornification disorders driven by impaired cytosolic Ca^{2+} handling

Proper execution of end-stage terminal differentiation and cornification in the epidermis is largely governed by calcium-dependent signal transduction. The critical role of calcium homeostasis in the epidermis is well-illustrated by disorders of cornification, such as Darier disease (DD), which is caused by germline mutation in the ER calcium reuptake pump, SERCA2 (encoded by *ATP2A2*). Impaired cytosolic calcium handling in DD keratinocytes prevents normal desmosome trafficking, leading to loss of keratinocyte cohesion (acantholysis) in suprabasal layers and accelerates differentiation to produce prematurely cornified cells

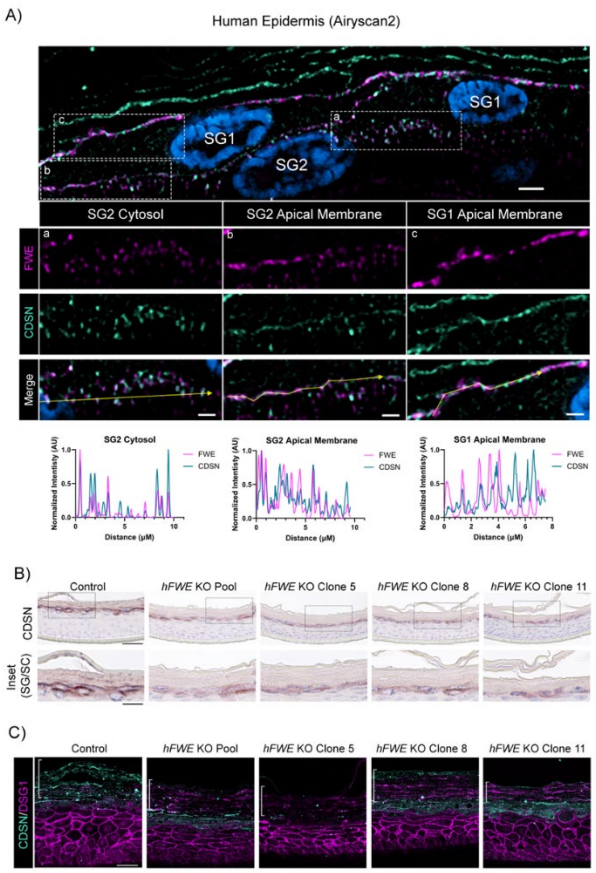

Fig 5. Flower localizes to epidermal lamellar bodies (LB) and facilitates their apically directed secretion. (A) Representative Airyscan2 super-resolution imaging of double IF for FWE and the LB cargo molecule corneodesmosin (CDSN) in stratum granulosum of human epidermis. Line scans present normalized fluorescence intensity values. (B) CDSN IHC on WT and *hFWE* KO epidermal organoids, representative of N=3. Inset images show stratum granulosum and corneum. (C) Double DSG-1 and CDSN IF on WT and *hFWE* KO organoids, representative of N=3. Apical brackets in white denote the width of the stratum corneum.

known as 'corps ronds' and 'grains' that are typically found in the upper epidermal layers. Recent work has also demonstrated that Grover's disease (GD), which shares identical histological features with DD, can be driven by somatic mutations in *ATP2A2*. Given data presented here that describe FWE as a new regulator of calcium-dependent polarized LB trafficking during cornification, we hypothesized that expression or subcellular localization of FWE might be dysregulated in these diseases.

Supporting this hypothesis, reanalysis of bulk RNA-seq data from recent work characterizing transcriptomic alterations in skin biopsies from patients with DD and GD revealed a significant increase in *FWE* expression in both lesion types relative to normal skin (Fig 6A). There was a non-significant trend toward increased expression in a related acantholytic disorder called Hailey-Hailey disease, which typically exhibits much less dyskeratosis on pathology. FWE immunofluorescence performed on clinical samples from Grover's and Darier patients (N=3), as well as normal human skin (N=5), demonstrated that, relative to normal skin, the apically polarized FWE signal was expanded downward into additional spinous layers in acantholytic regions of the epidermis (Fig 6B). Interestingly, the most striking increase in FWE signal intensity was observed in dyskeratotic foci characterized by numerous 'corps ronds' and grains on H&E-stained tissue sections (Fig 6B, arrows). Cells in these regions displayed a marked loss of the polarized distribution of FWE, which often encased the entire plasma membrane (Fig 6B, arrows) or exhibited aberrant basal or lateral polarity (Fig 6B, arrowheads). Double immunofluorescence for CDSN and FWE in DD disease suggested that these alterations in FWE signal likely reflect an accumulation and loss of polarity of FWE-associated LBs as there was markedly increased intensity of both proteins and significant colocalization in lesional areas of epidermis (Fig 6C). Airyscan2 super-resolution imaging of dyskeratotic foci in DD samples showed particularly striking colocalization of FWE and CDSN in 'corps ronds' with both proteins lacking apparent subcellular polarity, suggesting a failure of polarized delivery of LBs to the cell

surface (Fig 6D). Collectively, these findings suggest that dysregulation of FWE expression and localization may result in the loss of polarized delivery of LB contents in DD and GD to drive the premature cornification associated with these diseases.

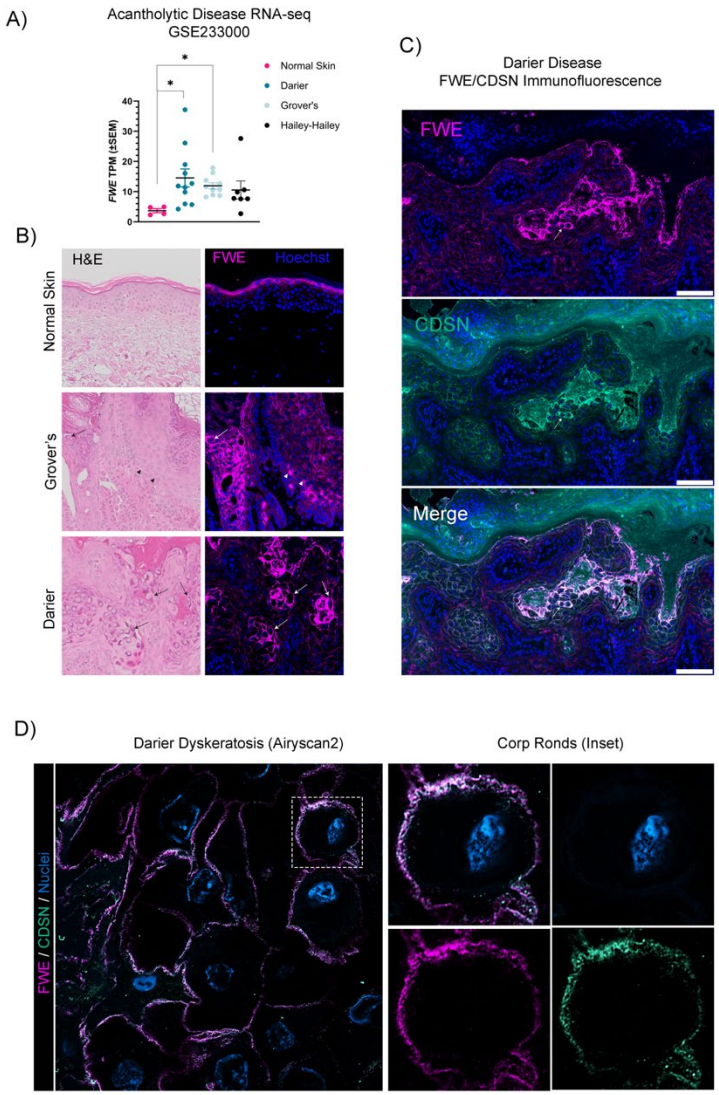

Fig 6. Dysregulation of FWE-associated LBs characterizes epidermal pathologies driven by impaired cytosolic calcium handling (A) *hFWE* expression retrieved from publicly availably bulk RNA-seq of normal skin, Darier or Grover's disease (N=4, 11, 10, respectively) samples. (B) Representative FWE IF from N=3 normal skin or disease samples showing accumulation and loss of polarization of FWE expression in dyskeratotic foci of Darier and Grover's. (C) Double IF for FWE (pink) and CDSN (green) in Darier samples, representative of N=3, showing significant colocalization of FWE and CDSN (white) in regions of dyskeratosis. (D) Representative Airyscan2 super-resolution confocal of FWE/CDSN double IF in a dyskeratotic focus of a Darier disease biopsy. Inset images show a pathognomonic 'corp rond' containing abundant FWE/CDSN+ lamellar bodies surrounding the entire cell periphery.

C. Significance

Our results provide new insight into the final stage of epidermal barrier formation, revealing that hFWE4, a small integral membrane protein with no prior documented function in normal skin, localizes to LBs and elevates cytosolic Ca^{2+} to facilitate delivery of cargo to the cell surface during cutaneous barrier formation.

Additionally, we show that instead of the endocytic role described for its orthologues in presynaptic neurons and cytotoxic T-lymphocytes (CTLs), in the SG of epidermis, hFWE4 controls apically polarized exocytosis of epidermal LBs. This discrepancy in endo- versus exo-cytic function across cell types is perhaps not surprising when considering the differences in the biology of the associated tissue and the cargo contained in the LRO. Unlike CTLs and presynaptic neurons, which must survive upon exocytosis of lytic granules or synaptic vesicles, SG1 keratinocytes undergo functional cell death via corneoptosis shortly after extrusion of LB contents, making it unlikely that the limiting membrane of LBs are retrieved via endocytosis after fusion with the apical cell surface.

Additionally, for LBs exocytosed in still viable SG2 keratinocytes, the lipid rich content of the vesicle and cross-linking of proteins under the membrane to form the rigid cornified envelope may slow exocytic release, preventing endocytic retrieval.

Our data demonstrate a function of the canonical hFWE isoform in epidermal morphogenesis and also reveal dysregulation in diseases of cornifcation that have a well understood genetic basis. We further hypothesize that given its importance in cornification, somatic mutation in hFWE itself may lead to ichthyosis. We also have extensive data not reported here showing that hFWE expression in epidermal cancers like cutaneous squamous cell carcinoma (cSCC) drives terminal differentiation, and that level of hFWE expression is predictive of tumor differentiation status. Results generated from our LB595 work will focus on further revealing Flower mechanisms and function in both diseases of cornification and skin cancer in the coming year.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

Rudd, et al. (Under review) Flower directs apically polarized trafficking of lamellar bodies to establish the epidermal barrier. *Nat Comm,* 2024

III. List of extramural grants submitted from 7/1/2023–6/30/2024

Agency: NIH R01
PI: Laura Hansen
Submitted: December 2023
Title: Flower Regulates Late-Stage Epidermal Differentiation and Barrier Function
Amount: $3,117,352
Status: Not awarded

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

Agency: Nebraska Dept. of HHS Stem Cell Research Grant Application
PI: Laura Hansen
Awarded: August 2023
Title: Flower Lineage Tracing in CRAINBOW Mice Stem Cells
Amount: $109,125
Status: Active as of 7/1/2023

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(July 1, 2023 – June 30, 2024)

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

Project Title: 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 focused a large portion of our effort on Aim 2, which 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 periods, 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 continued to focus 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 skinfold 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. In this reporting period, we did further analysis of our bulk-RNA-seq data to further define the effects of NMN on upregulation of the epithelial to mesenchymal transition, angiogenesis, and KRAS signaling. In addition, we have performed whole exome sequencing to assess mutation burden in tumors isolated from water- and NMN-treated tumors. First, we found that the mutation spectrum in UV-induced cSCC from SKH-1 mice has a similar mutation gene spectrum to human cSCCs. We also found that NMN supplementation was associated with a higher mutation load in UV-treated mice, with nearly twice the number of mutations observed. In addition, while tumors isolated from NMN-treated mice had no difference in phosphorylated gamma H2Ax, they did have a significant increase in Ki67 staining, suggesting that mutation burden increase could be due to increased proliferation and outgrowth of tumor cells versus any differential response to the DNA damaging effects of UV light. Supporting this notion, NAD+ precursors, including NMN and Nicotinamide, promote proliferation and survival of cSCC cell lines.

C. Significance

NAD$^+$ boosters have gained notoriety lately due to their potential to delay aging at the molecular and cellular level. Due to this, compounds such as NMN and Nicotinamide Riboside

(NR), which are cell permeable molecules that are components of the NAD^+ salvage pathway and are available over-the-counter, have been heavily marketed to the public. Our studies suggest that long-term utilization of these compounds to boost NAD^+ may in fact have adverse effects in certain circumstances, such as UV-induced skin tumorigenesis.

These studies will provide the data for an initial publication, as well as preliminary data for a grant submission focused on understanding the consequences of long-term supplementation of NAD precursors on skin biology and disease.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

None to date. We are drafting a manuscript on the effects of NMN in UV-induced skin cancer for submission this fall.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

National Institute of Diabetes and Digestive and Kidney Diseases/NIH
4/01/2025 - 3/31/2030
Project Number: Not available yet
PI: Brian J. North
Title: BubR1 as a Novel Regulator of Intestinal Homeostasis: Implications in Disease and Aging
Major Goals: The goals of these studies are to elucidate the cellular, molecular, and tissue consequences of BubR1 loss on intestinal epithelial tissue homeostasis, disease states such as ulcerative colitis, and during intestinal aging.

National Heart, Lung, and Blood Institute/NIH
4/01/2025 - 3/31/2030
Project Number: R01 HL178792-01
PI: Brian J. North
Title: The Role of BubR1 in Cardiac Development
Major Goals: The goals of these studies are to elucidate the molecular and cellular basis for why heart specific BubR1 knockout leads to embryonic lethality with failure of cardiac maturation and features similar to congenital heart defects.

Nebraska Health and Human Services
07/01/2024 - 06/30/2025
Project Number: NHHS LB606 Stem Cell Research Program
PI: Brian J. North
Title: Intestinal Stem Cell Maintenance by BubR1
Major Goals: The goal of this research proposal is to utilize single-cell RNA-sequencing and cellular characterization to define the role for BubR1 in maintenance of intestinal stem cells.

American Heart Association
7/01/2024 - 6/30/2027
Project Number: N/A
PI: Brian J. North
Title: Regulation of Heart Development by the Mitotic Checkpoint Factor BubR1.
Major Goals: The goal of these studies is to define the role of BubR1 in regulating proper cardiac maturation during embryonic development.

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

Nebraska Health and Human Services 08/01/2023 - 06/30/2024
Project Number: Creighton University LB595 Development Program.
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.
Effort: 0.6 Calendar Months
Direct Costs: $65,000 ($65,000 Total)

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

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

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

I. Progress Report Summary

A. Specific Aims

The original specific aims are as follows:

1. Establish the cellular localization of RAG1 degradation and identify factors required for this process.
2. Determine whether 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 previous progress reports, progress on Aim I was hindered by untimely departures of students and staff for other employment or educational opportunities. Currently, I have a PharmD-MS student who was initially 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 a truncated form of RAG1 lacking the amino-terminal third of the protein (which does not associate with $CRL4^{VPRBP(DCAF1)}$ E3 ubiquitin ligase complex and is stabilized against degradation). The approach relied on transfecting HEK293TK cells with GFP-tagged forms of the two RAG1 proteins, and monitoring drug-dependent changes in GFP expression and/or localization using confocal microscopy. He made considerable progress developing and optimizing the microscopy portion of the project, and worked on assessing how inhibitors of cullin and ubiquitin transfer activity alter RAG1 localization.

During the course of those experiments, he observed that when HEK293TK cells were treated with bortezomib and subjected to live cell imaging, the cells underwent significant morphological changes that were not apparent in non-imaged cells, or imaged, but untreated, cells. This led us to consider the possibility that exposure to the violet light used to image the cells synergized with bortezomib to induce the changes in morphology. Further testing using flow cytometry provided some experimental support for this hypothesis, and led us to submit an internal grant to follow up this finding (Kicks for a Cure), which was funded in January 2024.

Meanwhile, because we have shown that VprBP/DCAF1 regulates full-length RAG1 degradation, and that the amino-terminal portion of RAG1 also controls nucleolar localization, we asked whether co-expressing VprBP/DCAF1 with full-length RAG1 alters its localization. These studies, though preliminary, suggest VprBP/DCAF1 mediates relocalization of full-length RAG1. VprBP/DCAF1 truncation mutants fail to mediate substantive relocalization. His progress has been hampered by his clinical rotations, but he will have a research rotation this summer in which we hope to significantly move this study forward.

As noted in the previous progress report, we have also been 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. We obtained data showing loss of RACK1 in B cells significantly increases levels of Bim, but not HIF1α, in B cells. In addition, given RACK1's reported role in regulating various signaling nodes, we used flow cytometry to investigate the activation status of several signaling pathways in response to cell treatment with pervanadate. We found that loss of RACK1 led to significant alterations in Src-dependent signaling, leading to hyperactivation of MAPK and suppression of NF-κB signaling. Together with RNAseq datasets, we used the data to submit a new R01 application involving LB595 investigators Drs. Yusi Fu and Brian North in 2023. While the grant was under review, we pursued follow-up experiments during the current cycle to investigate potential alterations in Bim degradation, but these efforts have been complicated by difficulties detecting Bim or phospho-Bim at the protein level after treating cells in culture. Meanwhile, recognizing that reviewers may ask for additional experiments related to effects of RACK1 loss on cell cycle, proliferation, and apoptosis, we completed experiments to address these potential concerns.

Also mentioned in the previous progress report was our initiation of a second new subaim, partly related to the new subaim above, to acquire and begin breeding RAG1 knock-in mice that harbor deletions or mutations in the amino-terminal region of RAG1 that regulates 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-terminal region of RAG1 leads to Bim accumulation and/or an inversion of the ratio of Igκ^+:Igλ^+ 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 successfully treated these animals and completed phenotypic analysis of B cell development and evaluated patterns of V(D)J rearrangement in mice lacking the amino-terminal 215 residues of RAG1. We did not find loss of this region increased Bim levels by flow cytometry. Intriguingly, however, these mice showed a selective defect in mediating a rearrangement involving cryptic recombination signals that result in deletion of the immunoglobulin kappa constant region exon, which occurs to inactivate the locus. Together with recent published evidence that VprBP stabilizes the histone methyltransferase and polycomb repressor component Ezh2 (Ghate *et al.* Nature Communications **2023**; 14:2140), and preliminary data from our group suggesting murine B cells lacking VprBP also show lower Ezh2 levels, we submitted a new R21 to explore the possibility that full-length RAG1, through its association with VprBP, and in turn, VprBP's regulation of Ezh2, may function to control epigenetic modification of chromatin and transcription within the immunoglobulin kappa locus. This R21 was favorably reviewed and funded in May 2024 and also involved Dr. Yusi Fu as a co-investigator at 2.5% FTE.

<u>For specific aim 2</u>, as noted in an earlier 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 VprBP controls intracellular RAG1 localization and, through VprBP's regulation of Ezh2, potentially directs chromatin modifications within the immunoglobulin loci in B cells to regulate V(D)J recombination. Regarding the role of RACK1, while our evidence argues against its direct role as a regulator of RAG1 turnover, RACK1 nevertheless is required for B cell development. 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. Specifically, our data now point to a potential novel role of RACK1 in negatively regulating MAPK signaling. Data and reagents obtained with LB595 support were used to support submission of two scored NIH proposals (one now funded) that included LB595 co-investigators, and two other smaller state and local grants (one funded).

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

None, but a completed manuscript is being reviewed by co-authors who are no longer at Creighton.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

Agency: NIH/NIAID 1R21AI185362-01
Role: PI (co-investigator, Dr. Yusi Fu 2.5% FTE)
Title: Role of a RAG1-DCAF1(VprBP)-Ezh2 Axis in B Cell Development
Dates: 07/01/2024 to 06/30/2026
Amount: $404,250

Agency: Kicks for a Cure
Role: PI (co-Investigator Laura Hansen)
Title: Ubiquitin-Proteasome Inhibition to Sensitize Skin Cancer to Ultraviolet Radiation
Dates: 01/01/2024 to 12/31/2024
Amount: $40,000/yr

Agency: State of Nebraska LB506 Cancer and Smoking Disease Research Program
Role: PI
Title: Role of RACK1 in SAPK signaling and cell fate determination in B cells
Dates: 07/01/2024 to 06/30/2025
Amount: $65,000/yr

Agency: NIH/NIGMS 3P20GM139762-04S1 (Supplement)
Role: Co-investigator (Steyger, PI)
Title: Bigfoot Cell Sorter
Dates: 02/01/2024 to 01/31/2025
Amount: $250,000/yr

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

Agency: NIH/NIAID 1R21AI185362-01
Role: PI
Title: Role of a RAG1-DCAF1(VprBP)-Ezh2 Axis in B Cell Development
Dates: 06/05/2024 to 04/30/2026
Amount: $382,000

Agency: NIH/NIGMS 3P20GM139762-04S1 (Equipment Supplement)
Role: Co-investigator (Steyger, PI)
Title: Bigfoot Cell Sorter
Dates: 02/01/2024 to 01/31/2025
Amount: $250,000/yr

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

CELLULAR SIGNALING AND MOLECULAR TRAFFICKING IN CANCER
Laura Hansen, PhD, Director

Project Title: 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 US. 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 the role of Rac Guanine nucleotide exchange factors in tumor-associated macrophage activation. The preliminary data we obtained laid the foundation for an upcoming submission of an R21 to NIH.

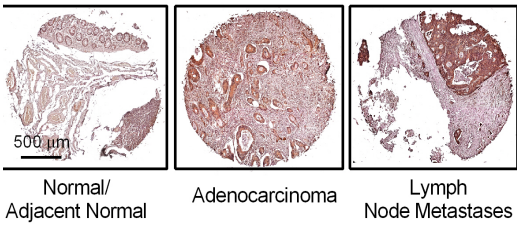


Fig.1. Upregulation of Drp1 protein expression in human CRC and metastases to lymph node. Representative immunostaining of Drp1 protein in human CRC tissue microarray using a mouse anti-Drp1 antibody.

1) Increased Drp1 protein levels in human invasive CRC and metastases to lymph nodes: To examine the clinical relevance of our data, we performed immunohistochemical (IHC) analysis of Drp1 protein expression in commercial microarrays of 266 human CRC specimens and adjacent normal tissues (US Biomax Inc., BC05115, BC05112, CO702b, CO1002b). We used a mouse anti-Drp1 antibody (BD Biosciences) with non-immune mouse IgG as the negative control. **Fig.1** shows that Drp1 immunostaining was very weak in normal tissue, increased in carcinoma, but much more intense in lymph node metastases. To semi-quantify these differences, expression levels of Drp1 protein in all microarray cases were graded from 1–4 based on overall staining intensity. As shown in **Table 1**, average Drp1 staining intensities in carcinoma were increased as compared with normal or adjacent normal tissues (2.34±0.05 vs 1.12±0.08, P<0.001).

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

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

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

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

2) Drp1-deficient CRC cells have impaired migratory abilities *in vitro.* Metastatic HCT-116 cells highly express Drp1, providing a model to address the role of Drp1 in CRC metastasis. We introduced plasmids expressing Drp1-targeted shRNA (OriGene) or its control shRNA with a Zeocin-selection marker into HCT-116 cells. Drp1-silenced stable cell lines were established through Zeocin selection and confirmed by Western blot assays. The selected Drp1-silenced clones 5 and 9 showed a reduction of 80-90% in Drp1 expression levels (**Fig. 2A**). Transwell migration abilities of these cell lines were compared and showed that that silencing Drp1 decreases migratory abilities of CRC cells *in vitro* by 70%.

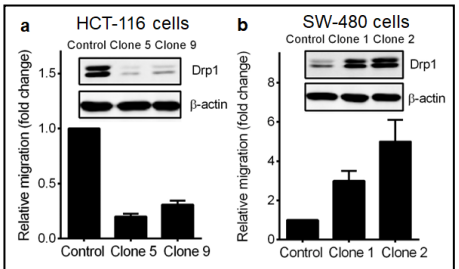


Fig.2. Drp1 positively regulates CRC cell migration ability. (a) Knockdown of endogenous Drp1 inhibits migration abilities of CRC HCT-116 cells. Inset: Western blot analysis of Drp1 expression in cells stably expressing scramble (Control) or Drp1 shRNA (Clones 5 and 9). **(b)** Overexpression of recombinant Drp1 promotes migration of CRC SW480 cells. Inset: Western blot analysis of Drp1 expression in cells stably transfected with control vector or Drp1 (Clones 1 and 2).

3) Overexpression of Drp1 promoted CRC cell migration *in vitro.* SW480 cells express little Drp1, and thus were used to examine effects of exogenous Drp1 on CRC metastasis. We generated three SW480 cells stably expressing Drp1. Cells were selected with G418, and positive clones were identified by western blot assay. Drp1 was three- and four-fold increased in selected clones (Clones 1 and 2) (**Fig.2B**). Analysis of transwell migration abilities of these cell lines demonstrated that expression of Drp1 increases migratory abilities of CRC cells *in vitro.*

Since lymph node and liver are the most common metastasis sites for CRC, we are currently performing experimental lymph node and liver metastasis assays in nude mice (BALB/C nu/nu) to determine if silencing Drp1 significantly reduces metastasis of HCT-116 cells, whereas overexpression of Drp1 increased metastasis of SW480 cells *in vivo*.

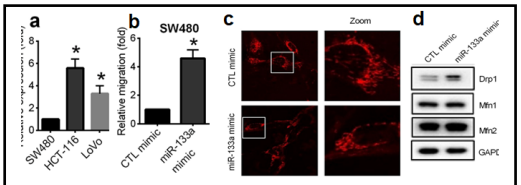


Fig.3. Upregulated MiR-133a increased Drp1 and promoted mitochondrial fission and migration of CRC cells. **(a)** Relative miR-133a levels. **(b)** Transwell migration. **(c)** Mitochondrial staining. **(d)** Western blot.

4) Upregulated miR-133a increased Drp1 and promoted mitochondrial fission and migration 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 metastatic CRC HCT-116 and LoVo cells as compared to non-metastatic SW480 cells (**Fig.3a**). Transfection of miR-133a mimic enhanced the migratory potential of SW480 cells (**3b**). Importantly, mitochondria are more fragmented in cells transfected with miR-133a mimic compared to CTL mimic (**3c**). Western blot assay showed that miR-133a increased Drp1 expression without effects on mitochondrial fusion proteins Mfn1 and Mfn2 (**3d**).

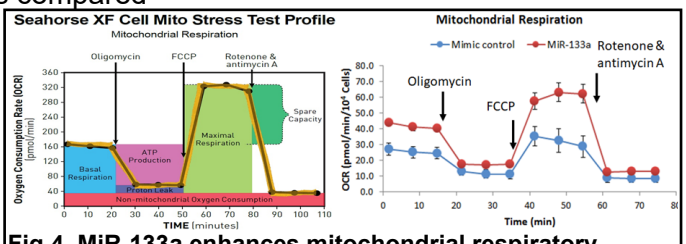


Fig.4. MiR-133a enhances mitochondrial respiratory.

5) Upregulated miR-133a enhanced 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.4**), suggesting a significant elevation of the mitochondrial respiration upon miR-133a mimic treatment.

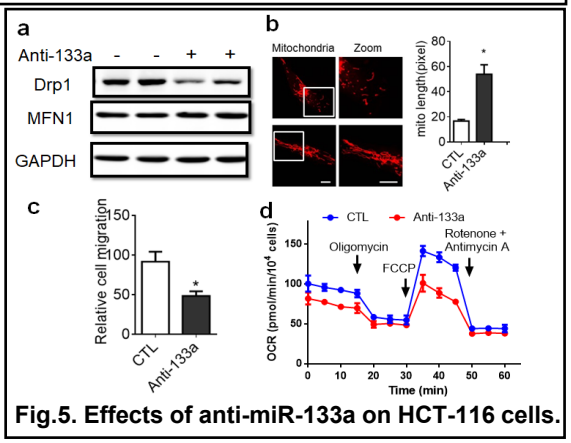


Fig.5. Effects of anti-miR-133a on HCT-116 cells.

6) Anti-miR-133a (a miR-133a hairpin inhibitor) reduced Drp1 expression and on CRC cells. Anti-miR-133a (50 nM, Thermo Scientific) or its negative control was introduced into HCT-116 cells for 72 h. Inhibition of endogenous miR-133a by the anti-miR-133a reduced Drp1 but not Mfn1 expression (**5a**), promoted mitochondrial elongation (**5b**), decreased cell migratory ability, (**5c**) and reduced the mitochondrial respiration of HCT-116 cells (**5d**).

7) Parkin is a target of miR-133a in CRC cells. We searched for the miR-133a direct targets that negatively regulate Drp1 expression. Among the putative targets of miR-133a predicted by online algorithms (Targetscan, mirSVR), Parkin fits our hypothesis because the 3-UTR of *Parkin* contains a binding site for miR-133a (**Fig.6**). It also induces Drp1 degradation, and loss of Parkin leads to mitochondria fragmentation. The mutation of the *Parkin* gene is a cause of familial Parkinson's disease. Parkin also functions as a tumor suppressor and its mutations were found in many types of cancers, but its roles in cancer metastasis are unknown. We found that HCT-29 and SW-480 cells express higher levels of Parkin as compared to HCT-116 and LoVo cells (**Fig.6a**). Expression of miR-133a downregulates Parkin expression with increased Drp1 protein in SW480 cells (**Fig.6b**). 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 (**Fig.6c**). SW480 cells were co-transfected with miR-133a and luciferase reporter plasmids. As shown in **Fig. 6d**, miR-133a represses wild-type Parkin-3′UTR reporter activity without inhibition of the mutant Parkin-3′UTR reporter activity, suggesting a direct regulation of miR-133a in the 3′UTR of *Parkin* mRNA.

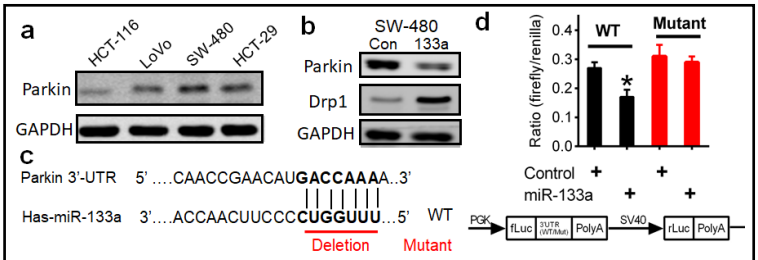


Fig.6. Parkin is a target of miR-133a. (a) Decreased Parkin expression in metastatic CRC cells. **(b)** miR-133a reduced Parkin expression with increased Drp1 expression. **(c)** The putative miR-133a binding site in the 3′-UTR of the *Parkin* and its deletion mutant at the seeding region. **(d)** Firefly 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.

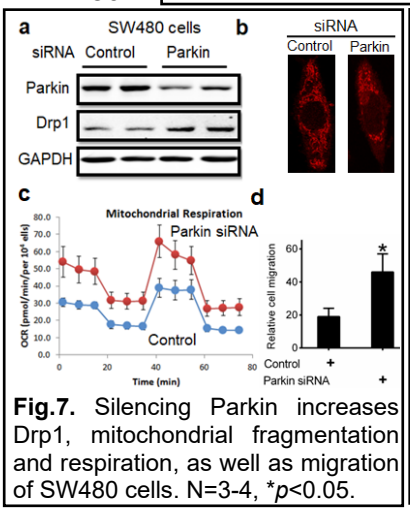


Fig.7. Silencing Parkin increases Drp1, mitochondrial fragmentation and respiration, as well as migration of SW480 cells. N=3-4, **p*<0.05.

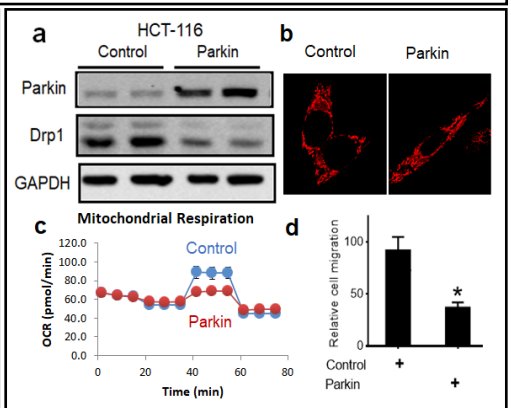


Fig.8. Parkin regulates Drp1, mitochondrial fission, respiration, and HCT-116 cell migration. N=3-5, **p*<0.05.

8) Effects of silencing endogenous Parkin on SW480 cells. SW480 cells highly express Parkin. Silencing endogenous Parkin increases Drp1 expression (**Fig.7a**), mitochondrial fragmentation, (**7b**) and respiration (**7c**), as well as cell migration (**7d**).

9) Effects of overexpression of Parkin on HCT-116 cells. HCT-116 cells express lower levels of endogenous Parkin. Expression of recombinant Park1 decreases Drp1 expression (**Fig.8a**), promotes mitochondrial elongation (**8b**), and reduces mitochondrial respiration (**8c**) and HCT-116 cell migration (**8d**).

EXPANSION OF THE PROJECT

Rac Guanine nucleotide exchange factors and tumor-associated macrophage activation

The tumor microenvironment (TME) is the pathological extracellular environment that harbors various immune cells and components that can turn intrinsic immune components against healthy cells and promote tumorigenesis. Cancer-associated gene mutations can generate antigens to activate cancer-cell-reactive CD8+ cytotoxic T lymphocytes (CTLs). However, CTL activities are frequently suppressed in the tumor, in part due to T cell exhaustion induced by cells in TME. Although inhibitory receptor programmed cell death protein 1 (PD-1) and its ligand PD-L1 have been successfully targeted for cancer therapy, T cell exhaustion is unlikely to be reversed by anti-PD-1/PD-L1 therapies. Macrophages, one of the major immune components, are known to contribute to the proliferation of tumor cells in TME. Macrophages polarize into two major subtypes, M1 and M2, in response to environmental stimuli. M1 is a pro-inflammatory and anti-tumor subtype, whereas M2 is an anti-inflammatory and pro-tumor subtype. A specific subtype of the M2 macrophage subtype known as tumor-associated macrophages (TAM), also referred to as "M2d type macrophages," is more prominent in promoting tumorigenesis and progression than all other subtypes of macrophages. Arginase-1 (Arg-1) and vascular endothelial growth factor (VEGF) are the markers of M2d macrophages. A recent study suggests that TAM promotes T cell exhaustion in cancer. The various cytokines secreted by TAMs not only cause the TME immunosuppression but also promote tumor cell proliferation, drug resistance, and angiogenesis (3,4). Thus,

understanding the molecular mechanisms underlying TAM activation will provide new molecular targets for developing new immunotherapies against various cancers.

The Rho GTPases family (Rho, Rac, and Cdc42) controls many aspects of cell behavior through the regulation of multiple signal transduction pathways. The members of the Rac sub-class (Rac1 and Rac2) are known to be regulators of macrophage functions. Previous studies suggest that Rac2 is required for M2 macrophage activation and the deletion of Rac2 skews macrophages towards classical M1 activation and mice are defective in tumor growth. Our preliminary studies showed that inhibition of Rac2 but not Rac1 blocked Lewis lung carcinoma LL-2 cell-derived conditioned medium (LLC)-induced TAM activation. Rac proteins switch between an inactive (GDP-bound) and an active (GTP-bound) conformational state. Guanine nucleotide exchange factors (GEFs) stimulate the exchange of GDP for GTP to generate the activated form, which then regulates downstream effector proteins. A previous proteomic and transcriptomic analysis has identified Arhgef6 and P-Rex1 as the most abundant RacGEFs in mouse bone marrow-derived macrophages (BMDM) and the macrophage cell line RAW264.7 (5). Interestingly, treatment with LLC rapidly downregulated Arhgef6 and P-Rex1 expression and silence of Arhgef6 or P-Rex1 significantly enhanced LLC-induced TAM activation. Thus, **we hypothesize** that loss of Arhgef6 and P-Rex1 contributes to TAM activation.

<u>1) Treatment with LLC promoted TAM-like polarization of primary mouse BMDMs and RAW267.4 cells.</u> Many research works have investigated crosstalk between tumor cells and macrophages. We found that LLC treatment promoted M2d-like polarization of macrophages, indicated by elevated Arg-1 and VEGF (**Fig.1**). These results suggest that LL-2 cancer cells may secrete some factors to induce macrophages in TME to polarize to TAM, which would facilitate immune evasion of tumor cells.

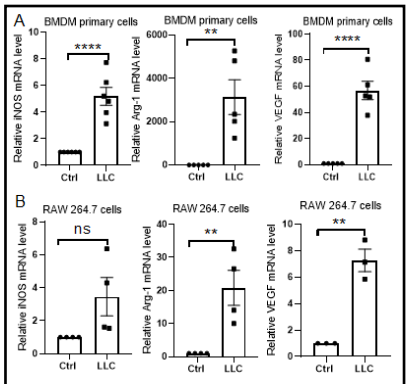


Fig.1. Treatment with LLC induced TAM-like polarization of mouse BMDM and RAW267.4 cells. Cells were stimulated with LLC/2% FBS for 6 h (BMDM, **A**) or 24 h (RAW264.7, **B**). M1 (iNOS) and M2 (Arg-1 and VEGF) markers were analyzed by qRT-PCR (n =4-5). ns, not significant; **p<0.01, ****p<0.0001.

<u>2) Inhibition of Rac2 but Rac1 blocked LLC-induced TAM-like polarization.</u> Mouse BMDM and RAW264.7 cells highly express Rac1 and Rac2. However, inhibition of Rac2 but not Rac1 attenuated LLC-induced TAM polarization (**Fig.2**).

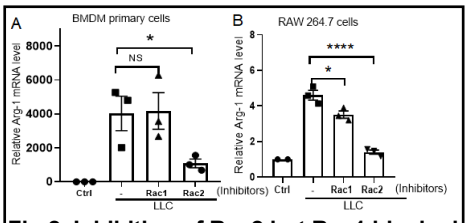
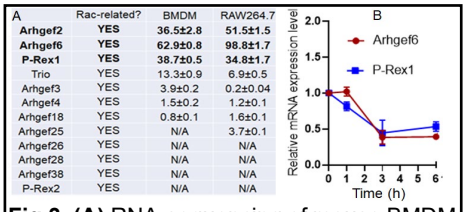


Fig.2. Inhibition of Rac2 but Rac1 blocked LLC-induced TAM-like polarization. Cells were pre-treated with 5μM Rac1 inhibitor (NSC27366) or Rac2 inhibitor (EHT-1864) for 1 h prior to LLC stimulation (BMDM), **A;** RAW264.7, **B**). N=3, *p<0.05, ****p<0.0001.



Rac-related?	BMDM	RAW264.7
Arhgef2	**YES**	**36.5±2.8**	**51.5±1.5**
Arhgef6	**YES**	**62.9±0.8**	**98.8±1.7**
P-Rex1	**YES**	**38.7±0.5**	**34.8±1.7**
Trio	YES	13.3±0.9	6.9±0.5
Arhgef3	YES	3.9±0.2	0.2±0.04
Arhgef4	YES	1.5±0.2	1.2±0.1
Arhgef18	YES	0.8±0.1	1.6±0.1
Arhgef25	YES	N/A	3.7±0.1
Arhgef26	YES	N/A	N/A
Arhgef28	YES	N/A	N/A
Arhgef38	YES	N/A	N/A
P-Rex2	YES	N/A	N/A

Fig.3. (A) RNA-sequencing of mouse BMDM and RAW267.4 cells (cited from Wang et al. Nat. Commun. 2022; 13:7389). **(B)** LLC induced a rapid downregulation of Arhgef6 and P-Rex1 mRNA in BMDM cells. N=3.

<u>3) Treatment with LLC induced a rapid downregulation of Arhgef6 and P-Rex1 mRNA levels in BMDM cells.</u> A previous proteomic and transcriptomic analysis identified Arhgef6 and P-Rex1 as the most abundant RacGEFs in BMDM and RAW264.7 cells (**Fig.3A**) (5). Arhgef6 has been reported to exert crucial biological functions in immune response and the migration of mature T- and B-cells in blood. P-REX1 is the only RacGEF synergistically activated by G protein-coupled receptors and receptor tyrosine kinases, thus integrating signals from several pathways and playing important physiological roles. We and other research groups previously reported that P-Rex1 was overexpressed in cancer and aberrantly upregulated P-Rex1 promotes cancer growth and metastasis (6,7). However, the relationship between Arhgef6, P-Rex1, and TAM and how these RacGEFs pertain to tumorigenesis in TME has not yet been explored. We found that treatment with LLC induced a rapid downregulation of Arhgef6 and P-Rex1 mRNA expression in BMDM cells (**Fig.3B**). Interestingly, the immune landscape analysis in TME revealed that Arhgef6

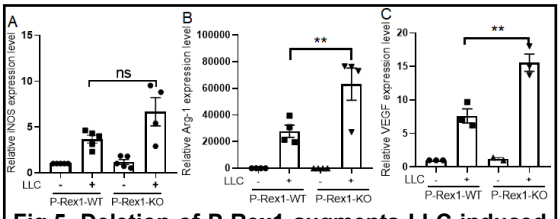


Fig.5. Deletion of P-Rex1 augments LLC-induced TAM-like polarization. WT and P-Rex1 KO mouse BMDM were cultured with and without LLC for 6 h and mRNA levels of M1 (iNOS) **(A)**, M2 (Arg-1) **(B)** and M2d (VEGF) markers **(C)** were measured by qRT-PCR. N= 3-5, ns, not significant, **p<0.01.

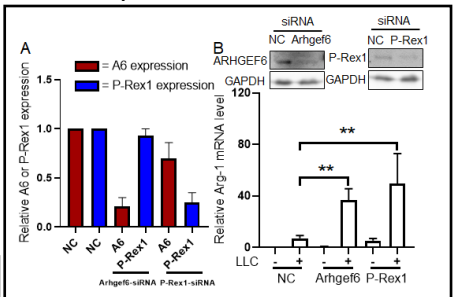


Fig.4. siRNA knockdown of Arhgef6 or P-Rex1 enhanced LLC-induced Arg-1 upregulation in RAW264.7 cells. Cells were dual-transfected with 50 nM siRNAs for Arhgef6, P-Rex1 or negative control (NC) siRNA for 48 h and then stimulated without or with LLC for 24 h. **(A)** Arhgef6 and P-Rex1 mRNA levels assessed by qRT-PCR. **(B)** The Arg-1 expression levels were assessed by qRT-PCR. **Inset**: western blot analysis of Arhgef6 and P-Rex1 protein. N=3, **p<0.01.

expression was positively associated with immune cell infiltration and immune checkpoints but was remarkably downregulated in lung adenocarcinoma cells and tumor tissues (7).

4) siRNA knockdown of Arhgef6 or P-Rex1 significantly enhanced LLC-induced Arg-1 upregulation of RAW264.7 cells. To determine the functional roles of Arhgef6 and P-Rex1 in TAM, Arhgef6 or P-Rex1 in RAW264.7 cells was silenced by their specific siRNAs (**Fig.4A**). Knockdown of Arhgef6 or P-Rex1 by 70-80% enhanced LLC-induced Arg-1 upregulation by five-fold in RAW264.7 cells (**Fig.4B**).

5) Deletion of P-Rex1 augmented LLC-induced TAM-like polarization of BMDM. Primary BMDM cells were isolated from WT and P-Rex1 knockout mice. Deletion of P-Rex1 in BMDM augments LLC-induced TAM-like polarization, as indicated by increased Arg-1 and VEGF (**Fig.5**).

C. Significance

Colorectal cancer (CRC) is the third most common cancer worldwide and the secnd 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, which 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 a biomarker and therapeutic target. For example, miR-133a expression correlates with metastasis and poor prognosis of CRC patients. Our recent data suggest that miR-133a orchestrates EMT, which endows epithelial cancer cells with enhanced motility and invasiveness. Therefore, **we hypothesize** that miR-133a-dependent upregulation of Drp1 promotes mitochondrial fission, which in turn promotes CRC metastasis. Our studies will address the following two 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, tumor-associated macrophages (TAM) not only cause the TME immunosuppressive but also promote tumorigenesis and progression. In our preliminary studies, we identified two RacGEFs, Arhgef6 and P-Rex1 that highly express in macrophages as major negative regulators of TAM activation. Interestingly, treatment with tumor-derived conditioned medium induced a rapid downregulation of both Arhgef6 and P-Rex1.

We will first determine the mechanisms underlying Arhgef6 and P-Rex1 suppression of TAM activation (**Aim 1**). We will then elucidate the mechanism of tumor-derived conditioned medium induced Arhgef6 and P-Rex1 repression (**Aim 2**). Finally, we will investigate the pathologic importance of macrophage Arhgef6 and P-Rex1 repression in cancer development and progression (**Aim 3**). Completion of these studies could unravel Arhgef6 and P-Rex1 repression as a novel mechanism underlying TAM activation, thus providing potential clinical impact by guiding development of novel therapeutics enhancing macrophage Arhgef6 and P-Rex1 expression and activity for prevention and treatment of cancer. The data generated will also form the basis for a future R21 application focusing on establishing the best potential targets in the Arhgef6 and P-Rex1 regulation and action pathways to enhance Arhgef6 and P-Rex1 effects for therapeutic benefit, which may prevent cancer progression.

References:

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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. DeNardo DG, Ruffell B. (2019) Macrophages as regulators of tumor immunity and immunotherapy. *Nat Rev Immunol.* 19:369-82.
4. Moeini P, Niedźwiedzka-Rystwej P. (2021) Tumor-Associated Macrophages: Combination of Therapies, the Approach to Improve Cancer Treatment. *Int J Mol Sci.* 22:7239.
5. Qie J, Liu Y, Wang Y, Zhang F, Qin Z, Tian S, Liu M, Li K, Shi W, Song L, Sun M, Tong Y, Hu P, Gong T, Wang X, Huang Y, Lin B, Zheng X, Zhou R, Lv J, Du C, Wang Y, Qin J, Yang W, He F, Ding C. (2022) Integrated proteomic and transcriptomic landscape of macrophages in mouse tissues. *Nat Commun.* 13:7389.

6. Qin J, Xie Y, Wang B, Hoshino M, Wolff DW, Zhao J, Scofield MA, Dowd FJ, Lin MF, Tu Y. 92009) Upregulation of PIP3-dependent Rac exchanger 1 (P-Rex1) promotes prostate cancer metastasis. *Oncogene*. 28:1853-63.
7. Srijakotre N, Liu HJ, Nobis M, Man J, Yip HYK, Papa A, Abud HE, Anderson KI, Welch HCE, Tiganis T, Timpson P, McLean CA, Ooms LM, Mitchell CA. (2020) PtdIns(3,4,5)P_3-dependent Rac exchanger 1 (P-Rex1) promotes mammary tumor initiation and metastasis. *Proc Natl Acad Sci U S A.* 117:28056-67.

II. List of related publications (7/1/2023– 6/30/2024)

None

III. List of extramural grants submitted from 7/1/2023 – 6/30/2024

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: 12/2024 - 11/2028
Role: Co-Investigator; Juan Carlos Cardet (PI)
Total funds requested: $2,031,637; **Under review.**

IV. List of extramural grants awarded from 7/1/2023 – 6/30/2024

National Institutes of Health – NHLBI and NIGMS
R01 HL164593
Title: A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-Resistant (Refractory) Asthma
$1,653,185 (8/20/2023 - 5/31/2027)
Role: Tu (PI)

Creighton University Cancer & Smoking Disease Research Program
FY20/21 Progress Report
(July 1, 2023 – June 30, 2024)

CELLULAR SIGNALING AND MOLECULAR TRAFFICKING IN CANCER
Laura A. Hansen

Project Title: 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, in the past years we have synthesized several analogs of the C-terminal fragment of chlorotoxin (CTX), which is a 36-amino acid peptide, and has high-binding selectivity for glioblastoma cells. These included β-turn stabilizing motifs. We tested these peptides for their biological activity.

2. We have completed the analysis all of the molecular dynamics (MD) simulations trajectories, including the MM-PBSA calculations for obtaining binding energies. The analysis revealed that CTX and its analogs frequently bound to regions on MMP-2 other than the catalytic site. All docking methods showed large negative ΔE_b, indicating favorable interactions between Ctx and its analogs with MMP-2. These data indicate that if CTX inhibits MMP-2 enzymatic activity, it should be through allosteric sites.

Aim 2.

1. To determine experimentally the interaction between CTX and our synthetic peptide analogs with the active form of recombinant MMP-2, last year we developed a differential scanning fluorometry (DSF) assay technique. We have repeated the experiments several times and it appears that the interaction with MMP-2 is rather weak. We have completed the MMP-2 enzyme inhibition assays and found no significant inhibitory activities for the peptide analogs.

2. We completed U-87 glioblastoma cells migration inhibitory testing by using the scratch assay. Quantitative analyses showed that neither CTX nor P75 and P76 analogs have inhibited cell migration significantly. The latest and shortest analog (P78), however, significantly inhibited cell migration.

3. Using cell invasion assay, CTX, P75 and its analogs inhibited significantly U-87 glioblastoma cell invasion. In preliminary experiments, P78 also inhibited cell invasion.

C. Significance

In blind molecular docking studies, we have found that CTX and its C-terminal analogs are not

binding to the catalytic site of MMP-2, but rather to neighboring sites, indicating possible allosteric mechanism of action of the peptides. With several time repetition of the MMP-2 enzyme inhibition assays, we found no significant inhibitory activities for the peptide analogs. These data are agreement with recent publication by Farkas, S. et al. *J.Biol. Chem.* **2023**, *299* (9), 104998. Using DSF, we found weak binding between CTX, peptides, and MMP-2. Peptide analogs have cell migration and invasion inhibitory activity.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

Rudd, J.C.; Maity, S.; Grunkemeyer, J.A.; Snyder, J.C.; **Lovas, S.** and Hansen, L.A. Membrane Structure and Internalization Dynamics of Human Flower Isoforms. *J. Biol. Chem.* **2023**, *299(8),* 104945. doi: 10.1016/j.jbc.2023.104945. PMID: 37348560 PMCID: PMC10366549

Kamayirese, S.; Maity, S.; Dieckman, L.M.; Hansen, L.A.; **Lovas, S.** Optimizing Phosphopeptide Structures That Target 14-3-3ε in Cutaneous Squamous Cell Carcinoma. *ACS Omega* **2024**, *9*, 2719−2729, doi:10.1021/acsomega.3c07740, PMID: 38250398, PMCID:PMC10795040.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

NIH, PI: S. Dravid
Dates: 07/01/24 – 11/30/2029
Project title: Striatal Trans-Synaptic Signaling Mechanism in Parkinsonism
Role: Co-Investigator

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

None

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

Lynch Cancer Research Center
Dr. Laura Hansen, Basic Sciences Director LCCRC

I. Progress Report Summary

A. Specific Aims

The overall goal of this funding is to further the growth of the Lynch Cancer Research Center (LCRC) 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

Dr. Laura Hansen successfully completed the Hedwig van Ameringen Executive Leadership in Academic Medicine fellowship program in April 2024. During this fellowship, Dr. Hansen developed a roadmap for the growth of the LCRC in the form of a detailed strategic plan. As detailed in this strategic plan, our vision is to establish an international reputation for excellence in cancer research, cancer research training, and cancer care by building on the legacy of Dr. Henry Lynch. This initiative will lead to groundbreaking interdisciplinary research, enhanced opportunities for learners to participate in research, and better health for our communities.

Over the past year, a 15-year strategic plan to guide the expansion of the Lynch Cancer Research Center was established. In developing this plan, multiple strategies were utilized to gather information, build a coalition of stakeholders, synthesize information, and socialize the resulting strategic plan within leadership. Information was accumulated through searches of online and Medline sources, interviews with directors of NCI-designated cancer centers, and a SWOT (strengths, weaknesses, opportunities, threats) analysis using a survey of CUSOM/CHI alliance faculty and leaders. Coalition building was initiated through stakeholder meetings with CUSOM basic science faculty, leaders of key clinical departments, health system leaders, and university relations representatives.

Information compiled from these activities was consolidated in the development of a 15-year strategic plan that includes five major goals and multiple tactics for each goal during each of the three 5-year phases. The goals of the strategic plan are to 1) increase funded cancer research, primarily through the targeted recruitment of new faculty, 2) establish necessary infrastructure for the center, 3) foster interdisciplinary research and build a campus-wide coalition, 4) expand cancer care and clinical trials, and 5) obtain philanthropic support. The strategic plan was presented to the dean and other leaders in a PowerPoint overview and in a detailed spreadsheet, and further revised to incorporate their feedback. The project was also presented to medical school deans from across the country and was well received.

Additionally, guidelines for the recruitment and mentoring and benchmarks for continued support of research-intensive faculty in clinical departments, primarily clinician investigators,

were developed in collaboration with clinical chairs.

The philanthropy initiative was prioritized on Creighton's Annual Giving Day, providing visibility to the project while raising funds in support of the center. The strategic plan will serve as the roadmap for both coalition-building and prioritizing efforts for expansion of the cancer center. Ultimately, this initiative is expected to advance research, augment research opportunities for Creighton learners, and improve the health of our communities.

The search committee to begin recruiting a new department chair for the Department of Biomedical Sciences should be announced this summer. This chair is targeted to be a cancer researcher to help drive this project. The leadership committee, including selected clinical department chairs, will set a priority list of the research-intensive faculty to be recruited. Along with the dean, we will also establish the funding model to support these positions, in conjunction with our clinical partners.

The Lynch Comprehensive Cancer Research Center's name was officially changed with the university to Lynch Cancer Research Center (LCRC). Dr. Laura Hansen is the current director for the center. Dr. Lesley Conrad left Creighton University; however, collaborative projects with clinician investigators have continued to increase. Dr. Waddah Al-Refaie, MD, successfully recruited a chief of thoracic surgery, Dr. Erin Gillaspie, MD. Both of these clinicians are active collaborators with Dr. Jun Xia, PhD. Dr. Jun Xia also has built successful collaboration partnership with Dr. Kalyana Nandipati, MD, revitalizing his research program.

Salary support was provided in the past year to Dr. Yusi Fu and Dr. Jim Grunkemeyer, who were recruited and hired by the previous LCRC 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 co-author 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, co-authoring two publications and submitting four grant applications to a mix of internal, foundation, and federal research opportunities.

Jim Grunkemeyer's Research Progress

For details, see Dr. Hansen's Progress Report. Additional information on Dr. Grunkemeyer's work is available on request.

Yusi Fu's Research Progress

We performed whole genome sequencing to identify the differences and similarities between Type 1 and Type 2 endometrial cancer (EC), and to find potential biomarkers for Type 2 EC (endometrial serous carcinoma, ESC). This study included three ESC samples (samples 1, 3, and 4) and one Type 1 EC sample (sample 2). The DNA concentration of each sample was measured using a Qubit Analyzer. For DNA sequencing, 100 ng of DNA were used from samples 2, 3, and 4, while 50 ng were used from sample 1 due to variation in DNA concentration. The DNA sequencing library for each sample was prepared using the NEBNEXT Ultra II kit. The volume of each sample was adjusted to 26 microliters with nuclease-free water, then 7 microliters of NEBNext Ultra II FS Reaction Buffer and 2 microliters of NEBNext Ultra II FS Enzyme Mix were added, bringing the final volume to 35 microliters. Each sample was incubated at 37°C for 30 minutes and then at 65°C for 30 minutes. Adaptor ligation involved adding 30 microliters of NEBNext Ultra II Ligation Master Mix, 1 microliter of NEBNext Ligation

Enhancer, and 2.5 microliters of NEBNext Adaptor for Illumina to reach a final volume of 68.5 microliters. Samples were incubated at 20°C for 15 minutes without a heated lid, then with 3 microliters of USER Enzyme at 37°C for 15 minutes with a heated lid. For size selection, 57 microliters of SPRI beads were added to each sample, incubated at room temperature for 5 minutes, placed on a magnetic stand, and the supernatant removed. Samples were washed twice with 200 microliters of fresh 80% ethanol, incubated on a magnetic stand for 30 seconds each time, and air-dried for 5 minutes. The DNA was eluted by adding 17 microliters of 0.1X TE buffer, mixing well, incubating for 5 minutes, and transferring the supernatant to a new tube. PCR enrichment involved combining 15 microliters of DNA fragments, 25 microliters of NEBNext Ultra II Q5 Master Mix, and 10 microliters of Index-Primer Mix to a total volume of 50 microliters. PCR amplification consisted of initial denaturation at 98°C for 30 seconds, denaturation at 98°C for 10 seconds, annealing/extension at 65°C for 75 seconds, 4 repeated cycles, and a final extension at 65°C for 5 minutes. The PCR cleanup step involved adding 45 microliters of SPRI beads to each PCR sample, mixing well, incubating at room temperature for 5 minutes, placing on a magnetic stand for 5 minutes to remove the supernatant, and washing twice with 200 microliters of fresh 80% ethanol. The DNA was eluted by air-drying the beads for 5 minutes, adding 33 microliters of 0.1X TE buffer, mixing well, incubating for 5 minutes, and transferring the supernatant to a new tube.

Each sample was assessed for quality using the Tapestation analyzer for fragment size and concentration, and qPCR to determine the molar concentration of PCR product. Sequencing was done using the Illumina NextSeq 2000 Sequencer. Tapestation analyzer results showed fragment sizes of 208 bp for Sample 1, 207 bp for Sample 2, 261 bp for Sample 3, and 218 bp for Sample 4. MultiQC reports indicated duplication rates, GC content, and adapter sequences for each sample. For example, Sample 1 had 4.9% duplications, 40.6% GC content, and 37.3% adapter sequences.

Sequencing read depth was computed within 50kbp genomic bins, and the R package DNAcopy was used to identify whole genome copy number profiles. Type 1 EC (sample 2) displayed normal copy numbers, while Type 2 EC samples (1, 3, and 4) showed various gene locations with copy number variations.

Key findings included:

1) *ERBB2* on chromosome 17 showed copy number variations of 7 (Sample 1), 1.9 (Samples 2 and 4), and 1.2 (Sample 3).
2) *STARD10* on chromosome 11 showed variations of 3.8 (Sample 1), 3.1 (Sample 3), and 2.0 (Sample 4).
3) *TNFSF10* on chromosome 3 showed variations of 12.0 (Sample 1), 2.2 (Sample 3), and 2.1 (Sample 4).
4) *TACC3* on chromosome 4 showed variations of 11.8 (Sample 1), 2.0 (Sample 3), and 1.9 (Sample 4).
5) *SMOC2* on chromosome 6 showed variations of 4.6 (Sample 1), 1.7 (Sample 3), and 2.04 (Sample 4).

Sample 1 generally showed copy number gains, Sample 3 showed more losses, and Sample 4 rarely deviated from Sample 2.

The results suggest that ESC exhibits higher genome instability and copy number changes compared to Type 1 EC, with significant alterations in cancer-related genes. These findings could guide personalized treatment for ESC patients, identifying unique mutational signatures and potential drug targets to improve prognosis.

II. List of refereed publications germane to this project from 7/1/2023–6/30/2024

Gao, S. M. et al. Aging atlas reveals cell-type-specific effects of pro-longevity strategies. Nat. Aging 1–16 (2024) doi:10.1038/s43587-024-00631-1.

Plowman, J. N. et al. Targeted sequencing for hereditary breast and ovarian cancer in BRCA1/2-negative families reveals complex genetic architecture and phenocopies. Hum. Genet. Genom. Adv. 5, 100306 (2024).

III. List of extramural grants submitted from 7/1/2023–6/30/2024

NIAID - NIH
Title: Role of a RAG1-DCAF1(VprBP)-Ezh2 Axis in B cell Development
PI: Patrick Swanson; Co-I: Yusi Fu
Submitted, awarded

Mary Kay Ash Foundation
Title: Genomic Characterization of Endometrial Serous Carcinoma with Ultra-Sensitive and Accurate Duplex DNA Sequencing
PI: Yusi Fu
Submitted, not funded

NIH
Title: Epigenetic Regulation of the Neuroendocrine Axis in Autism with Global Delays
PI: Holly Stessman; Co-I: Yusi Fu
Submitted, pending

NIH
Title: KMT5B Contribution to Genomic Imprinting Maintenance
PI: Holly Stessman; Co-I: Yusi Fu
Submitted, pending

Kicks for a Cure
Title: Lineage Tracking of Copy Numbers: From Barrett's Esophagus to Esophageal Adenocarcinoma
PI: Jun Xia; Co-I: Yusi Fu
Submitted, awarded

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

NIGMS-NIH (COBRE Pilot Grant)
Title: WGS to Identify Candidate Genetic Variants Associated with Susceptibility to AIHL
PI: Peter Steyger; Pilot Project Lead: Yusi Fu

NIAID - NIH
Title: Role of a RAG1-DCAF1(VprBP)-Ezh2 Axis in B cell Development
PI: Patrick Swanson; Co-I: Yusi Fu

Kicks for a Cure
Title: Lineage Tracking of Copy Numbers: From Barrett's Esophagus to Esophageal Adenocarcinoma
PI: Jun Xia; Co-I: Yusi Fu

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

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

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. All participants with a biospecimen in the biobank have now been manually audited for future use. Approximately 85% of participants are approved for future use, 14% are not approved for future use, and <1% have questions that require final IRB ruling, which is forthcoming. For all re-approved participants, a high-quality DNA sample has been identified, quantified, and stored for request. All blocks and slides in storage have been inventoried. As they are requested, data files associated with specimens are being redacted to a deidentified format and attached to the LIMS. An updated biobanking consent form allowing for ongoing identifiable contact is currently being sent out to 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 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. SA2-2 is considered complete. 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 in the final stages of a RedBerry security audit. Consenting and data collection will be performed going forward using this interface after IRB approval (expected to be complete in the next six months).

Specific Aim 3. Modernize laboratory techniques. To support other LB595-funded work on campus, we continue to add and offer new techniques/services to the Biorepository.

Current offerings:

- High-throughput DNA/RNA extraction, quantification, and storage
- 10X single cell genomics library preparation
- RNA-sequencing
- Whole genome sequencing
- Whole exome sequencing
- Targeted sequencing
- Basic cell culture training and services (adherent and suspension)
- ELISA
- DNA shearing (Covaris)
- Custom cloning
- Sanger sequencing preparation/variant validation

These techniques continue to allow us to partially offset the cost of employing core staff. For example, 25% of personnel salary/benefits were covered by a NIGMS CONDA supplement (PI: Stessman) in 2023-2024 for 10X single cell genomics sample preparations, 5% of the personnel salary/benefits were covered by an IDeA grant (PI: Cote) for ELISA assays resulting in a publication (PMID: 38723407), and several 10X single cell genomics sample preparations have also been billed for an Investigator at Boys Town National Research Hospital.

New Projects

Five new projects are also utilizing 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, identify families carrying variants of undetermined significance (VOUSs) for additional family and functional work-up, and identify families at high risk for novel and/or modifier mutations that would benefit from additional whole genome sequencing. Using these preliminary data, Dr. Stessman is developing an ARA R01 as a three-way multi-PI award with collaborators at MD Anderson Cancer Center (Dr. Chad Huff) and the University of Washington (Dr. Elizabeth Blue) to whole genome sequence a large portion of the Lynch Legacy Collection.

Second, a Kicks for a Cure award (PI: Yaping Tu; Co-I: Stessman) was awarded to perform targeted sequencing for *FBXW7* variants on all biobanked (former Lynch collection) samples with hopes of identifying samples carrying variation for additional functional work.

Third, a Haddix Interprofessional Award (PI: formerly Lesley Conrad – replaced with John Coté; Co-Is: Stessman and Yusi 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. Individuals in this study are also offered the option to biobank any remaining sample identifiably to support future research.

Fourth, a mid-cycle LB595 award was added to this program to support a whole genome sequencing pilot on five families carrying no known hereditary cancer risk variants from the Lynch Legacy Collection. Sequencing has been completed and data analysis is currently underway.

Finally, we are participating in a Li-Fraumeni syndrome (LFS) group sequencing effort led by City of Hope PI: Dr. Stephen Gruber. Individuals and families carrying *TP53* variants are being collected by this group and whole genome sequenced. Genetic and phenotypic data are being

combined to better understand the LFS spectrum. Sequencing data will be provided back to the Biorepository and attached to these participants' records.

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 7/1/2023–6/30/2024

Plowman JN, Matoy EJ, Uppala LV, Draves SB, Watson CJ, Sefranek BA, Stacey ML, Anderson SP, Belshan MA, Blue EE, Huff CD, Fu Y, Stessman HAF. Targeted sequencing for hereditary breast and ovarian cancer in BRCA1/2-negative families reveals complex genetic architecture and phenocopies. HGG Adv. 2024 May 10;5(3):100306. doi: 10.1016/j.xhgg.2024.100306. Epub ahead of print. PMID: 38734904.

Matoy EJ, Plowman JN, Watson CJ, Belshan MA, Blue EE, Huff CD, Stessman HAF. In vitro data suggest a role for PMS2 Kozak sequence mutations in Lynch syndrome risk. HGG Adv. 2024 Apr 22;5(3):100298. doi: 10.1016/j.xhgg.2024.100298. Epub ahead of print. PMID: 38654521; PMCID: PMC11087717.

III. List of extramural grants submitted from 7/1/2023–6/30/2024

COBRE Pilot Project (PI: Stessman)
Impact of KMT5B Expression on Choroid Plexus Development and Macrocephaly

National Institutes of Health, 1 R21 HD114018-01A1 NIH/NICHD (R21; PI/PD: Stessman)
KMT5B contribution to genomic imprinting maintenance

National Institutes of Health, 1 R01 MH133600-01A1 NIH/NIMH (R01; PI/PD: Stessman)
Epigenetic regulation of the neuroendocrine axis in autism with global delays

National Institutes of Health, COBRE Research Project Leader (PI/RPL: Stessman)
Epigenetic Regulation Linking Autism to the Neuroendocrine Growth Axis

IV. List of extramural grants awarded from 7/1/2023–6/30/2024

COBRE Pilot Project (PI: Stessman)
TREM1 as a Novel Therapeutic Target for Global Ischemia

Other 3 grants pending review/council.

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

Investigator	Submitted FY 23/24	# Submitted
Juliane Strauss-Soukup	$0	0
Holly Feser Stessman	$2,667,380	3
Laura Hansen	$3,117,352	1
Brian North	$6,872,847	5
Patrick Swanson	$509,250	3
Yaping Tu	$95,792	2
Sandor Lovas	$2,084,590	3
Janee Gelineau-van Waes	$100,000	2
Jun Xia	$40,000	1
Gajanan Shelkar	$0	0
TOTAL SUBMISSIONS	$15,487,211	20

Investigator	Awarded FY 23/24	# Awarded
Juliane Strauss-Soukup	$1,145,112	4
Holly Feser Stessman	$416,396	3
Laura Hansen	$540,060	4
Brian North	$348,275	3
Patrick Swanson	$376,733	3
Yaping Tu	$505,694	2
Sandor Lovas	$0	0
Janee Gelineau-van Waes	$25,000	1
Jun Xia	$436,221	3
Gajanan Shelkar	$25,089	1
TOTAL AWARDS	$3,818,580	24

LB595 Investigator Awards FY23/24

Principal Investigator	Sponsor Name	Sponsor Name	Project Title	Awarded Project Period Start Date	Awarded Project Period End Date	Directs	Indirects	Total
Holly Feser Stessman	National Institutes of Health/University of NE Medical Center	University of Nebraska Medical Center/UNMC	Impact of KMT5B Expression on Choroid Plexus Development and Macrocephaly	14-Dec-2023	31-Jan-2025	$50,000.00	$23,500.00	$73,499.99
	National Institutes of Health/University of NE Medical Center	University of Nebraska Medical Center/UNMC	COBRE Administrative Supplement: TREM1 as a Novel Therapeutic Target for Global Ischemia	01-Aug-2023	31-Jan-2025	$182,242.00	$85,654.00	$267,896.00
	State of Nebraska - LB692	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
						Total: $307,242.00	Total: $109,154.00	Total: $416,395.99
Janee Gelineau-van Waes	Dr. George F. Haddix President's Faculty Research Grant	Dr. George F. Haddix President's Faculty Research Grant	Probing an Unexpected Pharmacological Target of Dolutegravir	01-Apr-2024	31-Mar-2025	$25,000.00	$0.00	$25,000.00
						Total: $25,000.00	Total: $0.00	Total: $25,000.00
Laura Hansen	State of Nebraska - LB692	State of Nebraska - LB692	Research Salary Support	01-Jul-2023	30-Jun-2024	$45,392.07	$0.00	$45,392.07
	National Institutes of Health/NIH	National Institutes of Health/NIH	Targeting Aberrant Anti-Apoptotic Signaling for Prevention of Skin Cancer	01-Aug-2020	30-Apr-2025	$217,313.00	$98,877.00	$316,190.00
	State of Nebraska - LB692	State of Nebraska - LB692	Research Salary Support	01-Jul-2023	30-Jun-2024	$69,353.18	$0.00	$69,353.18
	State of Nebraska Stem Cell Research Project - LB606	State of Nebraska Stem Cell Research Project - LB606	Flower Lineage Tracing in CRAINBOW Mice Stem Cells	01-Jul-2023	30-Jun-2024	$109,125.00	$0.00	$109,125.00
						Total: $441,183.25	Total: $98,877.00	Total: $540,060.25
Brian North	Health Sciences Strategic Investment Fund/HSSIF	Health Sciences Strategic Investment Fund/HSSIF	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
	Dr. George F. Haddix President's Faculty Research Grant	Dr. George F. Haddix President's Faculty Research Grant	Regulation of Intestinal Homeostasis by BubR1	01-Apr-2024	31-Mar-2025	$25,000.00	$0.00	$25,000.00
	National Institutes of Health/NIH	National Institutes of Health/NIH	Regulatory Mechanisms Governing BubR1 Protein Stability During Stress and Aging	01-Jun-2022	31-Mar-2025	$205,000.00	$93,275.00	$298,275.00
						Total: $255,000.00	Total: $93,275.00	Total: $348,275.00
Gajanan Shelkar	Health Sciences Strategic Investment Fund/HSSIF	Health Sciences Strategic Investment Fund/HSSIF	Role of GluN2C-Containing NMDA Receptor in Cocaine Addiction	01-Jul-2022	30-Jun-2024	$25,089.00	$0.00	$25,089.00
						Total: $25,089.00	Total: $0.00	Total: $25,089.00
Juliane Strauss-Soukup	State of Nebraska - LB692	State of Nebraska - LB692	Research Personnel Salary Support	10-Mar-2021	30-Jun-2024	$69,220.00	$0.00	$69,220.00
	National Institutes of Health/University of NE Medical Center	University of Nebraska Medical Center/UNMC	INBRE: Detection and Characterization of Compounds that Target the glmS Riboswitch and Act as Antibiotics	01-May-2020	30-Apr-2025	$80,998.36	$34,581.00	$150,160.36
	National Institutes of Health/University of NE Medical Center	University of Nebraska Medical Center/UNMC	Nebraska Research Network in Functional Genomics INBRE - CU Administration	01-May-2020	30-Apr-2025	$333,150.00	$151,583.00	$484,733.00
	National Institutes of Health/NIH	National Institutes of Health/NIH	Examination of Ornithine Decarboxylase Antizyme RNA Structure and Function from Various Organisms for the Development of Antibiological Agents	01-Aug-2023	31-Jul-2026	$299,999.06	$140,999.94	$440,999.00
						Total: $783,367.42	Total: $327,163.94	Total: $1,145,112.36
Patrick Swanson	National Institutes of Health/NIH	National Institutes of Health/NIH	Drug Discovery and Delivery Core	05-Mar-2021	31-Jan-2026	$95,040.00	$43,243.20	$138,283.20
	Kicks for a Cure, Inc.	Kicks for a Cure, Inc.	Ubiquitin-Proteasome Inhibition to Sensitize Skin Cancer to Ultraviolet Radiation	02-Jan-2024	31-Dec-2024	$40,000.00	$0.00	$40,000.00
	National Institutes of Health/NIH	National Institutes of Health/NIH	Role of a RAG1-DCAF1(VprBP)-Ezh2 Axis in B cell Development	05-Jun-2024	30-Apr-2026	$135,000.00	$63,450.00	$198,450.00
						Total: $270,040.00	Total: $106,693.20	Total: $376,733.20

Principal Investigator	Sponsor Name	Sponsor Name	Project Title	Awarded Project Period Start Date	Awarded Project Period End Date	Directs	Indirects	Total
Yaping Tu	National Institutes of Health/NIH	National Institutes of Health/NIH	A Novel Approach to Target Neutrophilic Airway Inflammation and Airway Hyperresponsiveness in Therapy-Resistant (Refractory) Asthma	20-Aug-2023	31-May-2025	$356,821.00	$123,873.38	$480,694.00
	Health Sciences Strategic Investment Fund/HSSIF	Health Sciences Strategic Investment Fund/HSSIF	Molecular Mechanisms and New Targets of Refractory Asthma	01-Jul-2023	30-Jun-2024	$25,000.00	$0.00	$25,000.00
						Total: $381,821.00	Total: $123,873.38	Total: $505,694.00
Jun Xia	Kicks for a Cure, Inc.	Kicks for a Cure, Inc.	Lineage Tracking of Copy Numbers: From Barrett's Esophagus to Esophageal Adenocarcinoma	02-Jan-2024	31-Dec-2024	$40,000.00	$0.00	$40,000.00
	State of Nebraska - LB692	State of Nebraska - LB692	Start-Up: Mechanisms of Genome Instability Induced by Environmental and Endogenous Sources	01-Aug-2022	30-Jun-2025	$150,000.00	$0.00	$150,000.00
	National Institutes of Health/NIH	National Institutes of Health/NIH	The Role of Aquaporin 3 in Arsenic-Induced DNA Damage and Mutagenesis	15-Aug-2022	31-Jul-2025	$180,169.00	$66,052.00	$246,221.00
						Total: $370,169.00	Total: $66,052.00	Total: $436,221.00
								Total: $3,818,580.80

LB595 Investigator Submissions FY23/24

Principal Investigator	Sponsor Name	Project Title	Requested Project Period Start Date	Requested Project Period End Date	Directs	Indirects	Total
Holly Feser Stessman	National Institutes of Health/NIH	Epigenetic Regulation of the Neuroendocrine Axis in Autism with Global Delays	01-Dec-2024	30-Nov-2029	$1,489,543.96	$700,085.66	$2,189,629.62
	National Institutes of Health/NIH	KMT5B Contribution to Genomic Imprinting Maintenance	01-Jul-2024	30-Jun-2026	$275,000.00	$129,250.00	$404,250.00
	National Institutes of Health/NIH	Impact of KMT5B Expression on Choroid Plexus Development and Macrocephaly	01-Feb-2024	31-Jan-2025	$50,000.00	$23,500.00	$73,499.99
					Total: $1,814,543.96	Total: $852,835.66	Total: $2,667,379.61
Janee Gelineau-van Waes	Dr. George F. Haddix President's Faculty Research Grant	Probing an Unexpected Pharmacological Target of Dolutegravir	01-Apr-2024	31-Mar-2025	$25,000.00	$0.00	$25,000.00
	State of Nebraska - LB692	Establishing a Central Role for the Calcium-Sensing Receptor (CaSR) in Mediating Adverse Off-target Effects of Dolutegravir	01-Jul-2024	30-Jun-2025	$75,000.00	$0.00	$75,000.00
					Total: $100,000.00	Total: $0.00	Total: $100,000.00
Laura Hansen	National Institutes of Health/NIH	Flower Regulates Late-Stage Epidermal Differentiation and Barrier Function	01-Jul-2024	30-Jun-2029	$2,176,839.67	$940,512.04	$3,117,351.71
					Total: $2,176,839.67	Total: $940,512.04	Total: $3,117,351.71
Sandor Lovas	Defense Threat Reduction Agency	Novel Antibiotics that Selectively Inhibit the Essential Protein-Protein Interfaces of the SSB Interactome with ESKAPE-E-Bacteria	01-Jul-2024	30-Jun-2029	$765,931.53	$359,987.82	$1,125,919.35
	National Institutes of Health/NIH	Striatal Trans-Synaptic Signaling Mechanism in Parkinsonism	01-Jul-2024	30-Jun-2029	$21,603.33	$10,153.57	$31,756.90
	Defense Threat Reduction Agency	Novel Antibiotics that Inhibit the Essential Protein-Protein Interfaces of the SSB Interactome in Tier 1 WMD Bacteria	01-Jul-2024	30-Jun-2029	$630,553.40	$296,360.10	$926,913.50
					Total: $1,418,088.26	Total: $666,501.49	Total: $2,084,589.75
Brian North	National Institutes of Health/NIH	BubR1 as a Novel Regulator of Intestinal Homeostasis: Implications in Disease and Aging	01-Apr-2025	31-Mar-2030	$3,091,744.18	$599,129.77	$3,690,873.95
	Nebraska Department of Health and Human Services/DHHS	Intestinal Stem Cell Maintenance by BubR1	01-Jul-2024	30-Jun-2025	$75,000.00	$0.00	$75,000.00
	National Institutes of Health/NIH	The Role of BubR1 in Cardiac Development	01-Apr-2025	31-Mar-2030	$1,892,499.07	$889,474.56	$2,781,973.63
	American Heart Association	Regulation of Heart Development by the Mitotic Checkpoint Factor BubR1	01-Jul-2024	30-Jun-2027	$272,727.30	$27,272.70	$300,000.00
	Dr. George F. Haddix President's Faculty Research Grant	Regulation of Intestinal Homeostasis by BubR1	01-Apr-2024	31-Mar-2025	$25,000.00	$0.00	$25,000.00
					Total: $5,356,970.55	Total: $1,515,877.03	Total: $6,872,847.58

Principal Investigator	Sponsor Name	Project Title	Requested Project Period Start Date	Requested Project Period End Date	Directs	Indirects	Total
Patrick Swanson	National Institutes of Health/NIH	Role of a RAG1-DCAF1(VprBP)-Ezh2 Axis in B cell Development	01-Jul-2024	30-Jun-2026	$275,000.00	$129,250.00	$404,250.00
	Kicks for a Cure, Inc.	Ubiquitin-Proteasome Inhibition to Sensitize Skin Cancer to Ultraviolet Radiation	02-Jan-2024	31-Dec-2024	$40,000.00	$0.00	$40,000.00
	State of Nebraska - LB506	Role of RACK1 in SAPK Signaling and Cell Fate Determination in B Cells	01-Jul-2024	30-Jun-2025	$65,000.00	$0.00	$65,000.00
					Total: $380,000.00	Total: $129,250.00	Total: $509,250.00
Yaping Tu	Health Sciences Strategic Investment Fund/HSSIF	Dysregulation of RGS2 and Cigarette Smoke-related Glucocorticoid Insensitivity of Airway Smooth Muscle Cells in Refractory Asthma	01-Jul-2024	30-Jun-2026	$50,000.00	$0.00	$50,000.00
	National Institutes of Health/NIH	Impact of Genetic Variation in the RGS Pathway on Airway Smooth Muscle Mechanophenotypes and Asthma Severity Outcomes	01-Dec-2024	30-Nov-2028	$31,151.00	$14,641.00	$45,792.00
					Total: $81,151.00	Total: $14,641.00	Total: $95,792.00
Jun Xia	Kicks for a Cure, Inc.	Lineage Tracking of Copy Numbers: From Barrett's Esophagus to Esophageal Adenocarcinoma	02-Jan-2024	31-Dec-2024	$40,000.00	$0.00	$40,000.00
					Total: $40,000.00	Total: $0.00	Total: $40,000.00

Creighton University Cancer & Smoking Disease Research Program
FY23/24 Progress Report
(July 1, 2023 – June 30, 2024)

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

Cellular Signaling and Molecular Trafficking in Cancer Program

Publications for Hansen:

1. Rudd, et al. (Under review) Flower directs apically polarized trafficking of lamellar bodies to establish the epidermal barrier. *Nat Comm*, 2024.

Publications for North:

None in this cycle.

Publications for Swanson:

None in this cycle.

Publications for Tu:

None in this cycle.

Publications for Lovas:

1. Rudd, J.C.; Maity, S.; Grunkemeyer, J.A.; Snyder, J.C.; Lovas, S. and Hansen, L.A. Membrane Structure and Internalization Dynamics of Human Flower Isoforms. J. Biol. Chem. 2023, 299(8), 104945. doi: 10.1016/j.jbc.2023.104945. PMID: 37348560 PMCID: PMC10366549. https://www.jbc.org/article/S0021-9258(23)01973-7/fulltext
2. Kamayirese, S.; Maity, S.; Dieckman, L.M.; Hansen, L.A.; Lovas, S. Optimizing Phosphopeptide Structures That Target 14-3-3ε in Cutaneous Squamous Cell Carcinoma. ACS Omega 2024, 9, 2719−2729, doi:10.1021/acsomega.3c07740, PMID: 38250398, PMCID:PMC10795040. https://pubs.acs.org/doi/10.1021/acsomega.3c07740

Biorepository Infrastructure

Publications for Stessman Project:

1. Plowman JN, Matoy EJ, Uppala LV, Draves SB, Watson CJ, Sefranek BA, Stacey ML, Anderson SP, Belshan MA, Blue EE, Huff CD, Fu Y, Stessman HAF. Targeted sequencing for hereditary breast and ovarian cancer in BRCA1/2-negative families reveals complex genetic architecture and phenocopies. HGG Adv. 2024 May 10;5(3):100306. doi: 10.1016/j.xhgg.2024.100306. Epub ahead of print. PMID: 38734904. https://www.cell.com/hgg-advances/fulltext/S2666-2477(24)00045-9

2. Matoy EJ, Plowman JN, Watson CJ, Belshan MA, Blue EE, Huff CD, Stessman HAF. In vitro data suggest a role for PMS2 Kozak sequence mutations in Lynch syndrome risk. HGG Adv. 2024 Apr 22;5(3):100298. doi: 10.1016/j.xhgg.2024.100298. Epub ahead of print. PMID: 38654521; PMCID: PMC11087717. https://www.cell.com/hgg-advances/pdf/S2666-2477(24)00037-X.pdf

Development Program

Publications for Gelineau-van Waes project:

None for this cycle.

Publications for North project:

None for this cycle.

Publications for Stessman project:

1. Plowman JN, Matoy EJ, Uppala LV, Draves SB, Watson CJ, Sefranek BA, Stacey ML, Anderson SP, Belshan MA, Blue EE, Huff CD, Fu Y, Stessman HAF. Targeted sequencing for hereditary breast and ovarian cancer in BRCA1/2-negative families reveals complex genetic architecture and phenocopies. *HGG Adv*. 2024 May 10;5(3):100306. doi: 10.1016/j.xhgg.2024.100306. Epub ahead of print. PMID: 38734904. https://www.cell.com/hgg-advances/fulltext/S2666-2477(24)00045-9

Publications for Xia project:

1. Fu Y[#], Agrawal S, Snyder D, Yin S, Zhong N, Grunkemeyer J, Hansen L, Waddah A, Nandipati K[#], Xia J[#]. Transcriptomic and gene fusion changes during the Progression from Barrett's Esophagus to Esophageal Adenocarcinoma. Under review at *Biomarker Research*, [#]Corresponding
2. Li Y, Xiao X, Li J, Han Y, Cheng C, Fernandes GF, Slewitzke SE, Rosenberg SM, Zhu M, Byun J, Bossé Y. Lung cancer in ever-and never-smokers: Findings from multi-population GWAS studies. *Cancer epidemiology, biomarkers & prevention*. 2024 Mar 1;33(3):389-99. https://aacrjournals.org/cebp/article/33/3/389/734667/Lung-Cancer-in-Ever-and-Never-Smokers-Findings

3. Long E, Yin J, Shin JH, Li Y, Kane A, Patel H, Luong T, Xia J, Han Y, Byun J, Zhang T. Context-aware single-cell multiome approach identified cell-type specific lung cancer susceptibility genes. bioRxiv (minor revision at *Nature Communications*). 2023 Sep 26. https://www.biorxiv.org/content/10.1101/2023.09.25.559336v1

Creighton University
Cancer & Smoking Disease Research Program
Report of Expenditures
July 1, 2023 - June 30, 2024

	Approved Budget	Total Expenses	Remaining Budget
Personnel	$708,238.00	$710,827.48	-$2,589.48
Consultant	26,000.00	39,674.88	-13,674.88
Equipment	0.00	0.00	0.00
Supplies	350,217.00	321,305.60	28,911.40
Other Expenses	215,545.00	145,219.84	70,325.16
Total	$1,300,000.00	$1,217,027.80	$82,972.20

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 2023 – June 2024

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 Fred & Pamela Buffett Cancer Center is to understand, prevent, and treat cancer in Nebraska through leading-edge research, exceptional patient care, premier education and training, and meaningful outreach to underserved populations.

The BCC continues to make substantial progress in pursuing its 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.

OVERALL

Dr. Joann Sweasy officially began her tenure as the seventh Director of the Fred & Pamela Buffett Cancer Center and the Eppley Institute for Research in Cancer and Allied Diseases at the University of Nebraska Medical Center (UNMC) on November 1, 2023. Her appointment was the unanimous choice of the multidisciplinary search committee seated by the UNMC Chancellor after they conducted a national search for the next Buffett Cancer Center Director following the retirement of Dr. Ken Cowan. The arrival of Dr. Sweasy in Nebraska was lauded as a critical step in taking the BCC to the next level of excellence in cancer research, clinical care, training, and outreach, and moving it towards comprehensive status. In the relatively short time since she assumed the held at the Cancer Center, Dr. Sweasy has achieved several key accomplishments highlighted here:

- Refreshed BCC leadership structure and committees with buy-in from senior leadership to increase operational clarity and efficiency and to facilitate effective strategic planning and decision-making.
- Partnered with the CEO of Nebraska Medicine, UNMC's hospital partner, to launch the BCC Clinical Executive Committee to align and execute on joint priorities across the academic and clinical entities.
- Made initial crucial investments in the BCC Office of Community Outreach and Engagement; partnered with the Associate Director for COE to characterize the Cancer Center's catchment area and collaborated with the COE AD to identify and select priority cancers; launched a population health assessment; and awarded a community partnership grant.
- Incorporated input from the BCC senior leadership team to revise the Center's policy on cancer relevance for increased rigor.
- Undertook a comprehensive assessment of BCC clinical research and initiated improvements to clinical research organization in order to increase impact based on catchment area priorities; refined staffing levels; and enhanced policies, workflows, and implementation of procedures aimed at improving efficiency and decreasing time to activation of clinical trials.
- Launched a comprehensive strategic planning process, including *ad hoc* external reviews focused on COE/Cancer Prevention and Control/Workforce Development and Belonging; Clinical Research; and Shared Resources. This process was supported by a BCC Members Strategic Planning Retreat facilitated by nationally recognized subject matter experts that was held in June 2024.
- Launched a multi-year strategic recruitment scheme, with key hires to include an Associate Director for Population Sciences and a Deputy Director for Clinical Research. In support of strategic recruitment efforts, partnered with the UNMC College of Medicine and the UNMC Vice Chancellor for Research on two recent faculty recruits..
- Refreshed leadership of the Cancer Prevention and Control developing Research Program and revised aims, goals, and themes.
- Began the process of expanding BCC Administration to improve efficiency through adding personnel to the administrative team and the acquisition and onboarding of research software management tools Research Logix (for collection and organization of real-time BCC member metrics, including grants and publications.

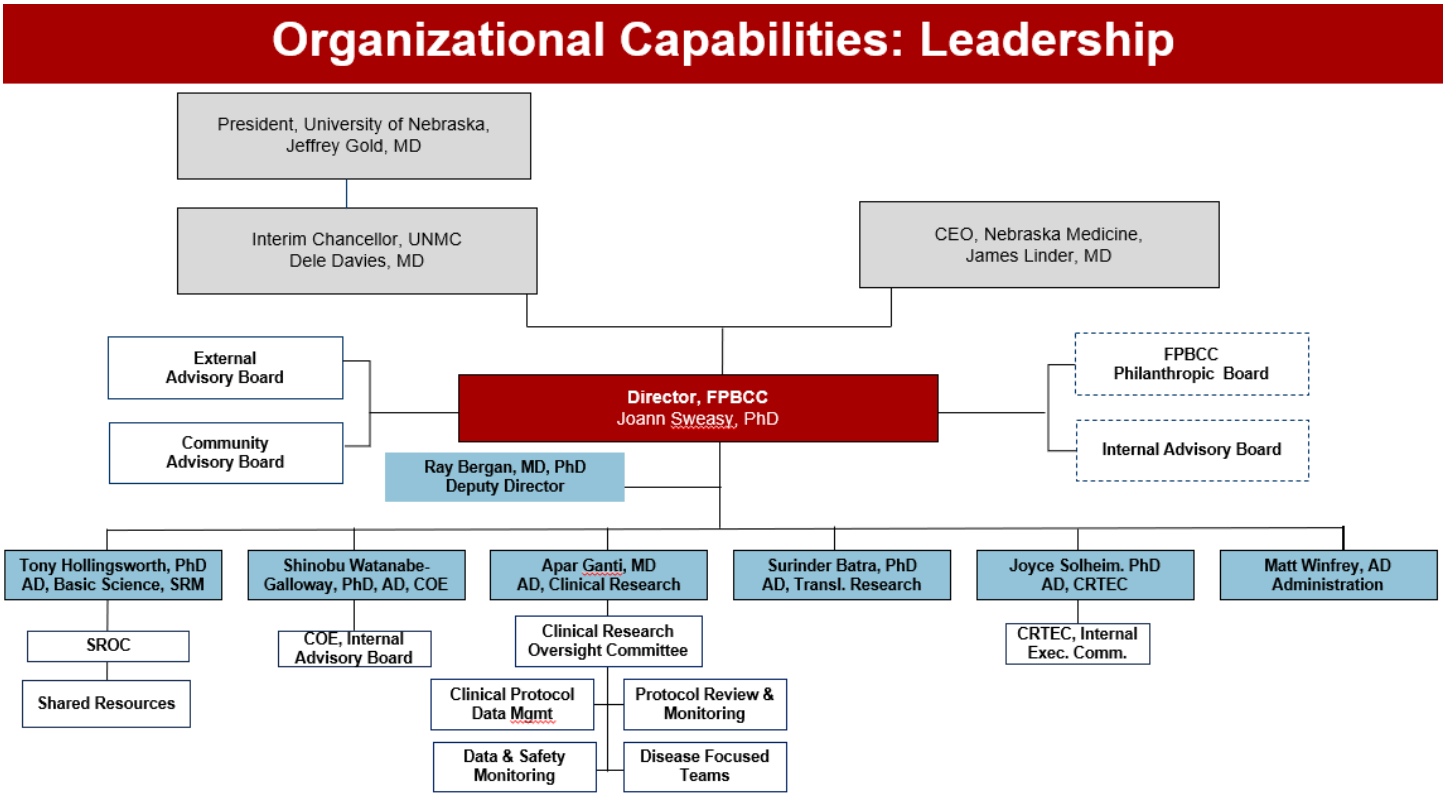
DIRECTOR'S OVERVIEW AND SIX ESSENTIAL CHARACTERISTICS

Physical Space

Physical Space: In this reporting period, the Buffett Cancer Center will build on its already exceptional strength in 400,000 square feet of physical space in Omaha, comprised of research laboratories; clinical trial office and administrative space with the opening of the Fred and Pamela Buffett Kearney Cancer Center, anticipated in late 2024. This partnership between Nebraska Medicine, the FPBCC, and the University of Nebraska at Kearney (UNK) campus will bring essential expanded cancer care, research, prevention, and education services to the central Nebraska region, in direct support of the BCC's developing strategic research theme focused on rural and frontier health, to include the related thematic areas of access to care, prevention and risk reduction, cancer and the environment, and population health. This will allow the BCC to increase its outreach across our catchment area, which is strongly characterized by its non-urban population, with 79 out of 93 counties in the state being categorized as rural or frontier. The $53-million, state-of-the-art FPBCC Kearney Cancer Center facility encompasses more than 53,000 square feet and will make the cutting-edge cancer research and care at the Buffett Cancer Center much more readily accessible to rural communities in the middle of the state. Importantly, this expansion of BCC facilities beyond the main UNMC campus in midtown Omaha; the Village Pointe Health Center clinic located in west Omaha; and the Bellevue Campus will provide further training and educational opportunities for both UNK and University of Nebraska Medical Center students and is also anticipated to bring auxiliary economic development benefits to the Kearny area. Renderings of the new facility are shown below.

Organizational Capabilities

Dr. Sweasy has initiated several important updates to the organizational structure of the Buffett Cancer Center. She has envisioned a dynamic and unified system of leadership committees, each with its own dedicated charge. This refreshed organization will not only allow the BCC to improve efficiency and effectiveness but will also make it more responsive to the needs of Center members and more agile as it works to achieve the various mandates that make up its mission, including those related to research, clinical care, training, and outreach. Below is an organizational chart depicting these updated leadership committees; the makeup and responsibilities of each are described in detail in the Accomplishments section of the Leadership, Planning and Evaluation component.



Transdisciplinary Collaboration and Coordination

The Buffett Cancer Center continues to build on its existing strengths in transdisciplinary collaboration and coordination, facilitated by a unique research space designed to promote collaboration and interaction among basic, clinical, and population science researchers. The BCC's emphasis on collaboration is evidenced by the success of recent publication and funding efforts. In the previous reporting period (April 2023-March 2024), the BCC had 245 cancer-relevant member publications, with 34% of those being intraprogrammatic, 27% interprogrammatic, 16% both intra- and interprogrammatic, 55% interinstitutional (representing key collaborations with other NCI-designated cancer centers), and 12% with an impact factor greater than 10. Another highlight of the current transdisciplinary collaboration strengths at the BCC are four ongoing multi-component grants.

A primary mechanism to facilitate collaboration across disciplines utilized by the BCC is the Cancer Center's successful pilot grant program. In 2023, the BCC received 33 pilot project applications, with 23 of those being multi-PI or multi-investigator applications. After an innovative peer review process, 12 projects were selected for funding, totaling more than $700,000 disbursed to PIs representing all the major cancer research-focused departments at UNMC (including Biochemistry and Molecular Biology, Eppley Institute, Genetics, Cell Biology and Anatomy, Internal Medicine - Division of Oncology and Hematology, Neurosurgery, Obstetrics and Gynecology, Pharmacy Practice and Science, Pharmaceutical Sciences, Surgery - Division of Surgical Oncology, as well as the University of Nebraska-Lincoln. 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 look to new internal funding mechanisms being developed by Dr. Sweasy and her leadership team designed to stimulate transdisciplinary collaborative success in a more targeted way, via a team science pilot funding programs that will bring basic and clinical researchers together to support innovative translational projects and enable them to secure external funding of multi-component large grants to expand these successful partnerships and enhance their research outcomes.

Further demonstration of the BCC's dedication to growing transdisciplinary collaboration can be found in recent investments in strategic recruitment and retention. Since Dr. Sweasy's arrival in November 2023, the Cancer Center has committed $1.02 million towards supporting the recruitment of two promising cancer researchers in the UNMC Department of Pathology, Microbiology and Immunology: Saber Tadros, MD, Assistant Professor, and Jennifer Bailey-Lundberg, PhD, Associate Professor. Dr. Tadros is a hematopathologist and researcher currently with the National Cancer Institute in Bethesda. He will begin at UNMC in August 2024. Dr. Bailey-Lundberg is a researcher focused on cancer immunology and immunoprevention, particularly in pancreatic cancer. She is scheduled to start at UNMC in July 2024. Drs. Tadros and Bailey-Lundberg were strategically recruited as a partnership effort, with the Buffett Cancer Center, the UNMC College of Medicine, and the UNMC Vice Chancellor for Research all participating in a reflection of the joint commitment to growing pancreatic cancer research capacity at the BCC in collaboration with the UNMC Pancreatic Cancer Center of Excellence.

Looking forward, the BCC will continue working to improve the translation of basic science discoveries successfully into clinical trials to ultimately improve cancer care and survivorship outcomes. To that end, the BCC hosted an *ad hoc* external clinical research review on July 12, with four nationally recognized clinical research experts from NCI-designated cancer centers to evaluate and advise the BCC on clinical trials infrastructure and operations. The BCC is currently implementing their recommendations. It is anticipated that their insight and guidance will assist us in improving upon our transdisciplinary success in subsequent funding periods.

Cancer Focus

Dr. Sweasy and her senior leadership team recently revised the policy on cancer relevance in recognition of the BCC's commitment to emphasizing rigor and cancer focus across the research portfolio. As of May 2024, the BCC's peer-reviewed research funding totaled more than $24.4 million, with $11.4 million of that being National Cancer Institute funding. This ratio remains nearly the same as it was in the previous renewal application in 2020. The BCC's newly instituted R01 Investment Program seeks to provide additional support for cancer-focused projects judged to have high impact by external reviewers and that barely missed the NCI funding payline to enable investigators to successfully resubmit their NCI research grant applications. The total peer-reviewed training funding at the BCC as of May 2024 was $2.5 million, with $1.1 million of that being NCI-sponsored. This represents a slight decrease in the percentage of NCI training funding as compared to the time of the previous renewal. The BCC Director is working with her leadership team, including the Associate Director for Training and Education, Dr. Joyce Solheim, to develop additional initiatives aimed at increasing NCI fellowship applications and other cancer-focused training grants.

Institutional Commitment

Institutional commitment from both the University of Nebraska Medical Center and its hospital partner, Nebraska Medicine, to the Buffett Cancer Center remains outstanding. Dr. Sweasy was recruited to serve as Director of the BCC and Eppley Institute for Research in Cancer with the support of a comprehensive start-up package that will allow her to meaningfully expand the BCC's research mission and footprint. She reports directly to the UNMC Chancellor (as well as current Provost and recent President-Elect of the University of Nebraska system), Dr. Jeffrey Gold, and to the Nebraska Medicine CEO, Dr. James Linder. These direct reporting relationships allow for more effective and efficient execution of the complex responsibilities of a director of a matrix cancer center. The structure of this elevated leadership position along with various direct authorities over state budgets for her units, over all BCC laboratory, other research, and office space (over 400,000 square feet), and over recruitment and promotion and tenure for Eppley Institute faculty are indicative of the strength of the ongoing institutional commitment to the success of Dr. Sweasy in particular and of the BCC in general.

Another demonstration of the ongoing strong institutional commitment to the BCC is the recent establishment of the Pancreatic Cancer Center of Excellence (PCCE). The PCCE is an equal institutional-state partnership that designated $30 million in funds to establish this special center of excellence aimed at building on existing

strengths in pancreatic cancer research at the BCC. The goal of the PCCE is to generate transformative discoveries in the early detection, diagnosis, prevention, and treatment of pancreas cancers. The BCC looks forward to working with the PCCE to build new and improved pancreas cancer projects and programs in order to ultimately improve patient outcomes for one of the most lethal of all cancers.

Center Director

The BCC was extremely fortunate to have recruited Dr. Joann Sweasy as Director of the Buffett Cancer Center and the Eppley Institute for Research in Cancer in November 2023. Dr. Sweasy assumed the directorship upon the retirement of Dr. Ken Cowan, who had served in that role since 1999. Dr. Sweasy is an internationally recognized expert in the genetics, cell biology, and biochemistry of DNA repair, who most recently served as director of the University of Arizona Cancer Center, an NCI-designated comprehensive cancer center. Dr. Sweasy has a remarkable track record of success in cancer research and leadership, and her innovative and dynamic vision for the BCC made her the clear priority candidate for the director search committee. Dr. Sweasy has already made important improvements to move the Center forward in her first six months as Director of the BCC and the EI, as previously outlined in the “Overall” section. We look forward to continuing to achieve swift and sustained progress on the Cancer Center’s developing strategic plan over the coming months with a vision aimed towards attaining comprehensive designation in the longer term.

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

A major focus for Dr. Sweasy in her initial months as Director of the Buffett Cancer Center has been to organize her leadership team in accordance with NCI best practices, to ensure they are appropriately equipped and positioned to achieve the goals as outlined in the developing strategic plan. BCC senior leaders are tasked with assisting the Director in implementing the Center's mission, including developing cancer research, clinical, training, and outreach initiatives. They are supported in this directive by the counsel and guidance of external advisors and incorporating feedback from related evaluation systems. The BCC senior leadership team continues to meet regularly within the context of various committees to discuss and shape the Cancer Center's strategic priorities. The BCC senior leadership team currently consists of the following individuals:

Fred & Pamela Buffett Cancer Center Senior Leadership Team	
Leader Name	**BCC Title**
Joann Sweasy, PhD	Director
Raymond Bergan, MD	Deputy Director
M.A. (Tony) Hollingsworth, PhD	Associate Director for Basic Research and Shared Resources
Surinder Batra, PhD	Associate Director for Translational Research
Apar Ganti, MD	Associate Director for Clinical Research
Shinobu Watanabe-Galloway, PhD	Associate Director for Community Outreach and Education
Joyce Solheim, PhD	Associate Director for Education and Training
Matthew Winfrey, MPP	Associate Director for Administration and External Affairs

There were three leadership changes over the previous reporting period. First, the previously appointed Associate Director for Workforce Development and Belonging, Dr. Quan Ly, stepped down from that role to focus on her clinical responsibilities in the UNMC Department of Surgery, Division of Surgical Oncology. Dr. Ly will remain involved in the BCC as a cancer clinician working on complex surgical oncology cases, particularly GI and pancreas surgeries. The search for an AD for Workforce Development and Belonging is currently underway. Second, the previously appointed Assistant Director for Shared Resources, Dr. Heather Jensen-Smith, stepped away from her role after accepting a faculty position within the UNMC Department of Genetics, Cell Biology, and Anatomy. Dr. Jensen-Smith remains the Director of the Advanced Microscopy Shared Resources and an active participant in the BCC Shared Resources Oversight Committee. Dr. Sweasy is currently reviewing and evaluating the best way to structure the BCC senior leadership team to ensure continuing active and involved oversight of Cancer Center shared resources. Third, Jixin Dong, PhD, was appointed to serve as Co-Leader of the Cancer Biology Program along with Dr. Hamid Band, filling a role that was opened after Dr. Pankaj Singh recently accepted a position as founding Chair of the Department of Oncology Science at the University of Oklahoma. Dr. Dong is a Professor in the UNMC Eppley Institute for Research in Cancer. His research is focused on the role of Hippo-YAP signaling as a therapeutic target in cancer, particularly in pancreatic cancer, as well as on the underlying mechanisms of paclitaxel chemo-resistance and protein kinases-mediated anti-tubulin chemosensitivity in pancreatic and ovarian cancer.

Planning and Evaluation

Several key changes to the Buffett Cancer Center planning and evaluation structure have been implemented by Dr. Sweasy since her arrival. Revisions and onboarding of new committees have been undertaken to promote highly effective, impactful, and efficient planning and evaluation, as suggested by the site visitors and the BCC EAB. Dr. Sweasy reports directly to the current Chancellor and Provost of UNMC, Dr. Jeffrey Gold, who is also the newly selected President-Elect of the University of Nebraska. She also reports directly to the CEO, Dr. James Linder, of UNMC's hospital partner, Nebraska Medicine. Dr. Sweasy solicits and incorporates external feedback via the BCC External Advisory Board and Community Advisory Board. She is also in the

process of instituting a BCC Philanthropic Board as well as reinvigorating an Internal Advisory Board for the BCC to provide additional advice on fundraising and institutional issues, respectively. Dr. Sweasy established two key groups to assist her in guiding planning and evaluation for the BCC: the Director's Council (DC) and the Research Executive Council (REC). Dr. Sweasy serves as Chair of both advisory committees, which meet bi-monthly and provide essential input on BCC strategic planning, investments, and other priority research initiatives. The DC is the primary internal governance committee, tasked with providing strategic advice, guidance, and recommendations to the Director on research initiatives and scientific priorities; it is primarily responsible for oversight of the strategic plan. The REC is charged with promoting the Center's research mission and fostering transdisciplinary collaboration among basic, population, and clinical scientists, to include coordination within and across Research Programs. These two key councils are supported by auxiliary committees focused on various aspects of the BCC's mission, including the following:

- Clinical Research Oversight Committee (CROC): Chaired by Dr. Apar Ganti, BCC Associate Director for Clinical Research; meets monthly. It oversees clinical research activities, including metrics, DFT performance, quality assurance outcomes, training and education initiatives, accrual monitoring, and accruals by gender, race, ethnicity, and lifespan.
- Community Outreach and Engagement Internal Advisory Board: Chaired by Dr. Shinobu Watanabe-Galloway, BCC Associate Director for COE; meets quarterly. It is tasked with advising Dr. Watanabe-Galloway on planning and evaluation activities within COE.
- CRTEC Internal Advisory Council: Chaired by Dr. Joyce Solheim, BCC Associate Director for Clinical Research; meets quarterly. Its directive is to advise Dr. Solheim on training and education coordination within the BCC.
- Shared Resources Oversight Committee: Chaired by Dr. Tony Hollingsworth, BCC Associate Director for Basic Research; meets quarterly. It aims to ensure that that shared resource services are meeting BCC membership needs and are fully aligned with the Center's strategic plan, in order to produce high-impact discoveries within the research programs.
- Clinical Executive Committee: Co-chaired by Drs. Sweasy and Steven Lisco, UNMC College of Medicine Senior Associate Dean for Clinical Affairs, and Nebraska Medicine Chief Academic Officer. This committee's goal is to foster a unified vision and goals for cancer care and patient-oriented research across the Buffett Cancer Center and Nebraska Medicine. Committee members include the Medical Director of the Oncology Service Line, the BCC AD for Clinical Research, the Division Chiefs of Oncology and Hematology and Surgical Oncology, the Chair of Radiation Oncology, the oncology service line administrator, as well as the BCC AD for COE.

Dr. Sweasy's major planning and evaluation focus is on developing a comprehensive strategic plan for the Buffett Cancer Center, with a refreshed mission, themes, and goals, in support of her vision to drive the BCC to comprehensive status. The strategic planning process began with an assessment of current state in November 2023. This ongoing phase is incorporating feedback from the Director's Council and Research Executive Committee meetings, stakeholder interviews, and Community Advisory Board meetings. It also includes multiple ad hoc reviews, including the first one focused on Community Outreach and Engagement, Cancer Prevention and Control, and Inclusive Excellence and Belonging. This review was held on April 19, 2024, and featured external reviewers Anita Kinney of the Rutgers Cancer Institute, Victoria Champion of the IU Simon Comprehensive Cancer Center, and Vanessa Sheppard of VCU Massey Cancer Center. Reviewers provided: 1) feedback and suggestions regarding impact and strategic expansion of COE, including thoughts on incorporating the Research Programs and clinical research; 2) a critique of the developing CPC Program, including thematic areas, catchment-informed research, strategic recruitment, and how to drive impact within the catchment community and beyond; and 3) advice on further developing the BCC's Plan to Enhance Diversity. Additional *ad hoc* reviews are planned to examine clinical research and shared resources, as described in more detail in Future Plans.

The current planning phase also featured a BCC members' survey, the results of which were used to structure the discussion outline and agenda for the Cancer Center's Members Strategic Planning Retreat that was recently held on June 14, 2024. Over 80 BCC members attended this all-day event to share their opinions and provide insight into what the Cancer Center's strategic priorities and strategies should be. The BCC will be moving into the next planning phase centered on setting goals, strategies, and action plans over the next few

months. This phase will culminate in the full BCC External Advisory Board meeting on September 6. The meeting is set to include all current EAB members, listed as follows:

Fred & Pamela Buffett Cancer Center External Advisory Board			
Member Name	**Cancer Center**	**Title**	**Status**
Kerry Burnstein, PhD	University of Miami Sylvester Comprehensive Cancer Center	Associate Director for Education and Training	Continuing
Victoria Champion, PhD	Indiana University Melvin and Bren Simon Cancer Center	Associate Director of Community Outreach and Population Science	Continuing
Robert Gerlach, MPA	Dartmouth Cancer Center	Associate Director for Administration and Scientific Affairs	Continuing
I. David Goldman, MD	Albert Einstein Cancer Center	Professor Emeritus	Continuing
Ernest Hawk, MD, MPH	The University of Texas MD Anderson Cancer Center	Vice President Division of Cancer Prevention and Population Sciences	Continuing
James Mulé, IPhD	Moffitt Cancer Center.	Associate Center Director, Translational Science	Continuing
Douglas Yee, MD	University of Minnesota Masonic Cancer Center	Director	Continuing
Ed Chu, MD, MMS	Albert Einstein Cancer Center	Director	New
Wafik El-Deiry, MD, PhD	Legorreta Cancer Center at Brown University	Director	New
Roy Jensen, MD	University of Kansas Comprehensive Cancer Center	Director	New
Anita Kinney, PhD	Rutgers Cancer Institute of New Jersey	Associate Director for Cancer Health Equity and Engagement	New
Patricia LoRusso, DO	Yale Cancer Center	Associate Cancer Center Director, Experimental Therapeutics	New
Yolanda Sanchez, PhD	University of New Mexico Cancer Center	Director	New
Vanessa Sheppard, PhD	VCU Massey Cancer Center	Associate Director for Community Outreach and Engagement	New
Louis Weiner, MD	Georgetown Lombardi Cancer Center	Director	New

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. Recipients who utilized LB 595 developmental funds in FY 2023/2024 are listed below along with a summary of their individual research program.

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*.

Punta Dhawan, PhD: Dr. Dhawan is a Professor in the Department of Biochemistry and Molecular Biology. Dr. Dhawan's lab aims to identify novel biomarkers and Therapeutics in colorectal cancer progression and

metastasis. Dr. Dhawan research program aims to understand the mechanism of colon cancer progression and metastasis, and to develop novel inhibitors for tight junction proteins for therapeutic implications. In addition, her research program seeks to understand the mechanism of regulation of cell-cell and matrix interaction in colonic homeostasis, and it is trying to determine the role of cell cycle protein MASTL in GI cancer and its therapeutic implications using cell, organoid and mouse models. Recent collaborative publications to which Dr. Ghosal's research contributed can be found in: *Bioorganic & Medicinal Chemistry, Tissue Barriers,* and *Biomedicine & Pharmacotherapy*.

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").

Katherine Hyde, PhD: Dr. Hyde is an Associate Professor in the Department of Biochemistry and Molecular Biology. Her laboratory's goal is to understand the mechanisms that regulate leukemia development, with the aim of developing new and improved treatments for hematological malignancies. Dr. Hyde's lab focuses on a subtype of acute myeloid leukemia that is caused by an inversion of chromosome 16 (Inv(16)). This inversion generates a fusion gene between the transcription factor CBFB and the gene for smooth muscle myosin heavy chain, MYH11, to generate CBFB-MYH11, which encodes the transcription factor CBFβ-SMMHC. It is known that expression of the fusion protein is the initiating event in leukemogenesis, but the role of CBFβ-SMMHC during leukemia progression is not understood. Using unique mouse models, Dr. Hyde and her lab are addressing this fundamental question. By identifying CBFβ-SMMHC's required binding partners and its downstream target genes, the team hopes to identify new treatment strategies for patients with Inv(16) AML. Her lab also uses mouse models, cell lines, and primary patient samples to address features common to many subtypes of AML.). Recent collaborative publications to which Dr. Hyde's research contributed can be found in: *Leukemia, Transplant Cell Therapy, Journal of Geriatric Oncology, Blood,* and *Proceedings of the National Academy of Sciences*

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).

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.

Rebecca Oberley-Deegan, PhD: Dr. Oberley-Deegan is a Professor in the Department of Biochemistry and Molecular Biology, and the Vice Chair for Education for the Department. The focus of Dr. Oberley-Deegan's laboratory is to reduce side effects associated with cancer therapy. The main goal of her research program is to reduce radiation and chemotherapy induced toxicity to normal tissues while not protecting the tumor cells from being killed by these therapies. Dr. Oberley-Deegan's laboratory is involved in several clinical trials currently ongoing at UNMC. There are two Phase II trials ongoing to investigate the role of a catalytic antioxidant as a radioprotector for anal and rectal cancer patients. There is an observational trial open to correlate adipose health and adiponectin levels to radiation-induced toxicities. Dr. Oberley-Deegan has recently published in the journals: *ChemMedChem*, *Redox Biolpgy*, *Cell Molecular and Bioengineering* and *Free Radical Biology and Medicine*

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. Sweasy, makes final decisions regarding the allocation of Cancer Center resources (space, funds, personnel) to Shared Resources. Dr. Sweasy 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.

The BCC established a new Shared Resources Oversight Committee (SROC). The SROC is comprised of Research Programs Leaders and SR Directors and chaired by the Associate Director for Basic Research**.** The SROC is convened for collective strategic planning to optimize scientific impact of SRs. It is within this committee that strategic planning to support Research Program initiatives will be integrated with service expansion and coordination of services within the SR portfolio. The SROC will meet quarterly to ensure that the SR services are aligned with the Strategic Plan and Research Program needs to produce high impact research discoveries. The Committee also monitors user satisfaction to ensure members have the resources and services to achieve desired results.

The Cancer Center is organizing an *ad hoc* external review of its shared resources that will be held on October 11, 2024. Feedback and recommendation for the management and strategies for shared resource prioritization will be solicited from the expert external reviewers.

ADMINISTRATIVE CORE

Buffett Cancer Center Administration has had several major accomplishments over the current reporting period, highlighted as follows:

- Providing organizational support and expert guidance for the administrative operations for the Offices of Community Outreach and Engagement, Cancer Research Training and Education Coordination, and Worforce Development and Belonging. The Office of Workforce Development and Belonging recently hired a new program associate as well as a new program coordinator; BCC Administration oversaw the evaluation and selection of candidates for these roles.
- Onboarding Dr. Joann Sweasy as the new Director of the BCC for Research in Cancer, upon Dr. Cowan's retirement in November 2023. This included overseeing the logistics of transferring her laboratory and current and pending grants to UNMC.

- Supporting Dr. Sweasy in her initial months as Director as she worked to establish her leadership team and institute various internal and external advisory committees, including the Buffett Cancer Center Director's Council, the BCC Research Executive Committee, the BCC Clinical Oversight Committee, and the BCC Shared Resources Oversight Committee.
- Facilitating the planning and execution of external advisory board meetings, including *ad hoc* meetings to review the following National Cancer Institute Cancer Center Support Grant required components: Community Outreach and Engagement/Cancer Prevention and Control/Plan to Enhance Diversity (held in April 2024), to review the clinical research operations and the BCC Clinical Trials Office (to be held in July 2024), to review BCC shared resources (scheduled for October 2024), as well as the full BCC External Advisory Board meeting set for September 2024.
- Managing the execution of a reenergized strategic planning process for the Buffett Cancer Center, including organizing a facilitated BCC members retreat focused on strategic planning that is being held in June 2024.
- Coordinating the BCC Pilot Projects Program 2023 Request for Applications, consisting of the acceptance, review, and evaluation of 30+ pilot project applications, as well as the awarding, tracking, and return on investment reporting for 12 funded projects totaling more than $700,000 in direct costs.
- Communicating and collaborating with external partner organizations, including the Nebraska Cancer Coalition (NC2) with whom we are partnering on data collection and analysis activities; the American Cancer Society with whom we are working on a Research Night event planned for the autumn of 2024, and the Cattlemen's Ball of Nebraska with whom we continue to partner to raise funds for supporting cancer research and care activities within the state.

Future Plans

Buffett Cancer Center Administration plans to add new personnel to the administrative team in the coming months, including two new project coordinators to assist in grants administration coordination, both extramurally and intramurally, as well coordination of cancer center operations and support of shared resources management. New initiatives being developed by Dr. Sweasy and the Administrative team are internal funding opportunities focused on investment in promising research grant application projects and in translational team science to build our multi-component grant research portfolio and stimulate success in catchment-informed, paradigm-shifting programmatic research. Another major focus in the coming reporting period will be to standardize operating procedures across the administrative team to improve overall effectiveness, maximize efficiency, and facilitate cohesive goal setting and achievement and tracking of related outcomes. BCC Administration will concentrate primarily on successful execution and integration of the Cancer Center's new strategic plan, to be supported by qualitative external reviews along with quantitative internal metrics collection and evaluation. Assessment of metrics will be aided by new research data software Administration is currently in the process of implementing (Research Logix, from Adminformatics), as well as a new data analyst team member who will be starting at UNMC in June 2024. Streamlining budgeting processes and integrating them into standardized administrative workflows will be another key aim for the coming year.

BCC Clinical Trials Office: As previously stated an *ad hoc* external review of clinical research operations and the BCC Clinical Trials Office is scheduled to be held July 12, 2024),. The goals are to increase efficiency and decrease the time to activation for clinical trials. BCC Administration continues to play a significant role during this ongoing process.

Dr. Juan Santamaria, the Clinical Assistant Director of Community Outreach and Engagement has been appointed as the liaison between the Clinical Trials Office and the Office of Community Outreach and Engagement. This will help better selection of clinical trials relevant to the catchment area.

Working with the Medical Director of the CTO and the Associate Director for Clinical Research, the CTO has developed metrics for each disease focused team (DFT). This has enabled each individual team to keep track of their portfolio and identify under-accruing trials sooner than in the past.

Principal Investigator: **Sweasy, Joann B.**

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) continued 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 also served as a principal investigator of the integrated Cancer Repository for Cancer Research (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: *Biochemical Pharmacology, 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).

Working with the Medical Director of the CTO and the Associate Director for Clinical Research, the CTO has developed metrics for each disease focused team (DFT). This has enabled each individual team to keep track of their portfolio and identify under-accruing trials sooner than in the past.

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*.

MOLECULAR BIOLOGY

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

The Director of the Molecular Biology/High-Throughput Screening Facility, Dr. David Kelly, unexpectedly passed away during the previous reporting period. The FPBCC is currently assessing the organization and services of the High-Throughput Screening Facility and will seek guidance from external reviewers at the October 11, 2024, ad hoc review of BCC Shared Resources.

Dr. James Eudy oversees the Genomics Facility located in the Durham Research Center II. The Genomics core provided services to approximately 59 different laboratories during the reporting period of September 2023 through April 2024. The facility continues to provide access to next-generation DNA sequencing (NGS) services (Illumina NovaSeq6000, NextSeq 550 and MiSeq sequencers), single cell sequencing (10x Genomics platform), and targeted gene expression assays (Nanostring Encounter platform) to the University of Nebraska system researchers. Researchers are performing RNAseq, both bulk and single cell RNAseq, whole genome metagenomics as well as 16s ribosomal profiling. In the context of single cell experimentation, a multi-omic approach is utilized to perform single cell RNA and T-cell receptor profiling on the same single cells to profile immune diversity. A new development during the last reporting period is that the core is collaborating with the Imaging Core to perform spatial transcriptomics. The Genomics Core director meets one on one with researchers to help them plan and optimize their experiments. Below is a list of recent highlighted cancer-related publications that utilized the resource:

Ahmad, R., Kumar, B., Thapa, I., Talmon, G. A., Salomon, J., Ramer-Tait, A. E., Bastola, D. K., Dhawan, P., & Singh, A. B. (2023). Loss of claudin-3 expression increases colitis risk by promoting Gut Dysbiosis. Gut Microbes. 2023 Dec;15(2): PMCID: PMC10730149

Ahmad, R., Kumar, B., Thapa, I., Tamang, R. L., Yadav, S. K., Washington, M. K., Talmon, G. A., Yu, A. S., Bastola, D. K., Dhawan, P., & Singh, A. B. (2023). Claudin-2 protects against colitis-associated cancer by promoting colitis-associated mucosal healing. The Journal of Clinical Investigation, 133(23). PMCID: PMC10688979

Coulter, D. W., Chhonker, Y. S., Kumar, D., Kesherwani, V., Aldhafiri, W. N., McIntyre, E. M., Alexander, G., Ray, S., Joshi, S. S., Li, R., Murry, D. J., & Chaturvedi, N. K. (2024). Marinopyrrole derivative MP1 as a novel anti-cancer agent in group 3 MYC-amplified Medulloblastoma. Journal of Experimental and Clinical Cancer Research, 43(1). PMCID: PMC10782703

Jung, O., Baek, M.-J., Wooldrik, C., Johnson, K. R., Fisher, K. W., Lou, J., Ricks, T. J., Wen, T., Best, M. D., Cryns, V. L., Anderson, R. A., & Choi, S. (2024). Nuclear phosphoinositide signaling promotes YAP/TAZ-TEAD transcriptional activity in breast cancer. EMBO Journal. PMCID: PMC11066040

Mallard, H. J., Wan, S., Nidhi, P., Hanscom-Trofy, Y. D., Mohapatra, B., Woods, N. T., Lopez-Guerrero, J. A., Llombart-Bosch, A., Machado, I., Scotlandi, K., Kreiling, N. F., Perry, M. C., Mirza, S., Coulter, D. W., Band, V., Band, H., & Ghosal, G. (2023). USP1 Expression Driven by EWS::FLI1 Transcription Factor Stabilizes Survivin and Mitigates Replication Stress in Ewing Sarcoma. Molecular Cancer Research : MCR, 21(11), 1186–1204. PMCID: PMC10618738

PATHOLOGY

The major aims of the Pathology Shared Resource are: (1) To provide comprehensive tissue resources, histology, immunohistochemistry, and digital pathology services; (2) To maximize the effectiveness of the resource by training and mentoring users; and (3) To provide long-term sustainability of core services through modernization and innovation.

The Pathology Shared Resource (PSR) continues to be led by Drs Benjamin Swanson, MD, PhD. And Dr. Kirk Foster.

Demand for tissue sciences services and expertise continues to be high. For the period of 7/1/2023-6/1/2024, there were 1148 facility requests from 132 unique PI users, including 42 (30%) FPBCC members (program areas TMD 33%, CB 38%, GIC 26%, and non-aligned CPC 3%) and 90 (70%) non-FPBCC faculty and external users. Utilization of services (based on order submissions) by FPBCC members over the same period was 51% and 49% by non-FPBCC faculty and external users. Currently, the top three services include (1) paraffin sample preparation, sectioning and histochemical staining (2) immunohistochemical staining (3) whole slide brightfield digital imaging.

For the time period of June 2023 through May 2024, 29 solid tumor specimens (many including matched “normal” tissues) consisting of more than 44 g of tissue, have been frozen and stored in the Pathology Shared Resource. Over a similar time period, the paraffin tissue bank has collected over 49 paraffin blocks. The Rapid Autopsy program has collected over 2156 specimens consisting of more than 17778 g of tissue during this time period. Finally, the lymphoma study group has collected over 18 fresh tissue samples and 44 paraffin blocks during this time period.

In the past 3 years, this resource has distributed: 559 specimens (264g) fresh frozen samples; 233 (1088g) fresh surgical samples; 4166 FFPE slides, and 331 (202 mL) blood/biofluid samples.

Recent highlighted cancer-related publications that utilized the resource re listed below:

Atri P, Shah A, Natarajan G, Rachagani S, Rauth S, Ganguly J, Carmicheal J, Ghersi D, Cox J. Smith L, Jain M, Kumar S, Ponnusamy M, Seshacharyulu P, & Batra S. Connectivity mapping-based identification of pharmacological inhibitor targeting HDAC6 in aggressive pancreatic ductal adenocarcinoma. NPJ Precis. Onc. 8, 66 (2024). https://doi.org/10.1038/s41698-024-00562-5

Shi W, Mirza S, Kuss M, Liu B, Hartin A, Wan S, Kong Y, Mohapatra, B, Krishnan M, Band H, Band V, Duan B, Embedded Bioprinting of Breast Tumor Cells and Organoids Using Low-Concentration Collagen-Based Bioinks. Adv. Healthcare Mater. 2023, 12, 2300905. https://doi.org/10.1002/adhm.202300905
Drug delivery and targeting to chemoresistant pancreatic cancer. Kumar V, Mahato RI.Cancer Lett. 2024 Mar 31;585:216648. DOI: 10.1016/j.canlet.2024.216648. Epub 2024 Feb 2. PMID: 38311056

Drug and nucleic acid delivery and targeting to the brain. Chitkara D, Mahato RI.J Control Release. 2024 May;369:684-686. DOI: 10.1016/j.jconrel.2023.09.046. Epub 2024 Apr 12. PMID: 37778467

Cancer-Associated Fibroblast Induces Acinar-to-Ductal Cell Transdifferentiation and Pancreatic Cancer Initiation Via LAMA5/ITGA4 Axis. Parte S, Kaur AB, Nimmakayala RK, Ogunleye AO, Chirravuri R, Vengoji R, Leon F, Nallasamy P, Rauth S, Alsafwani ZW, Lele S, Cox JL, Bhat I, Singh S, Batra SK, Ponnusamy MP.Gastroenterology. 2024 May;166(5):842-858.e5. DOI: 10.1053/j.gastro.2023.12.018. Epub 2023 Dec 27. PMID: 38154529

Principal Investigator: **Sweasy, Joann B.**

Integrative analysis of clinicopathological features defines novel prognostic models for mantle cell lymphoma in the immunochemotherapy era: a report from The North American Mantle Cell Lymphoma Consortium. Vose, J. M., Fu, K., Wang, L., Mansoor, A., Stewart, D., Cheng, H., ... & North American Mantle Cell Lymphoma Consortium. Journal of Hematology & Oncology. 2023 Dec 16. DOI:10.1186/s13045-023-01520-7

SAM-Competitive EZH2-Inhibitors Induce Platinum Resistance by EZH2-Independent Induction of ABC-Transporters. Groß E, Hilger RA, Schümann FL, Bauer M, Bouska A, Rohde C, Willscher E, Lützkendorf J, Müller LP, Edemir B, Mueller T. Cancers. 2023 Jun 3;15(11):3043. DOI: 10.3390-15113043

Long-term outcome of peripheral T-cell lymphomas: Ten-year follow-up of the International Prospective T-cell Project. Civallero M, Schroers-Martin JG, Horwitz S, Manni M, Stepanishyna Y, Cabrera ME, Vose J, Spina M, Hitz F, Nagler A, Montoto S. British Journal of Haematology. 2024 Mar 26. DOI: 10.1111/bjh.19433

Final results of a phase II study of CHOEP plus lenalidomide as initial therapy for patients with stage II–IV peripheral T-cell lymphoma. Stuver R, Horwitz SM, Advani RH, Vose JM, Lee HJ, Mehta-Shah N, Zain JM, Haverkos B, Lechowicz MJ, Moskowitz AJ, Pham LQ. British journal of haematology. 2023 Aug;202(3):525-9. DOI: 10.1111/bjh.18885

Integrative Genomic and Transcriptomic Analysis Reveals Targetable Vulnerabilities in Angioimmunoblastic T-Cell Lymphoma. Bouska A, Zhang W, Sharma S, Holte H, Lone WG, Cappelli LV, Fiore D, Gong Q, Heavican-Foral T, Cannatella J, Amador C. Blood. 2023 Nov 28;142:2993. DOI: 10.1182/blood-2023-186530

Clonal Hematopoiesis and Therapy-Related Myeloid Neoplasms After Autologous Transplant for Hodgkin Lymphoma. Yan C, Richard MA, Gibson CJ, He J, Bosworth A, Crossman DK, Singh P, Hageman L, Kalra R, Armenian SH, Vose J. Journal of Clinical Oncology. 2024 Apr: JCO-23. DOI: 10.1200/JCO.23.02547

High-grade B-cell lymphoma, not otherwise specified: a multi-institutional retrospective study. Zayac AS, Landsburg DJ, Hughes ME, Bock AM, Nowakowski GS, Ayers EC, Girton M, Hu M, Beckman AK, Li S, Medeiros LJ. Blood advances. 2023 Nov 14;7(21):6381-94. DOI: 10.1182/bloodadvances.2023009731

BET inhibition reforms the immune microenvironment and alleviates T cell dysfunction in chronic lymphocytic leukemia. Smith AL, Skupa SA, Eiken AP, Reznicek TE, Schmitz E, Williams N, Moore DY, D'Angelo CR, Kallam A, Lunning MA, Bociek RG. JCI insight. 2024 May 22;9(10). DOI: 10.1172/jci.insight.177054

Outcomes of patients with blastoid and pleomorphic variant mantle cell lymphoma. Gerson JN, Handorf E, Villa D, Gerrie AS, Chapani P, Li S, Medeiros LJ, Wang M, Cohen JB, Churnetski M, Hill BT. Blood Advances. 2023 Dec 26;7(24):7393-401. DOI: 10.1182/bloodadvances.2023010757

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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.

Below is list of accomplishments for the Structural Biology Shared Resource.

- The PrEP, CSG, and X-Ray labs continued to support a diverse cross section of research activities from contract work to education of graduate students. 14 research labs used these facilities. Of these 14 research labs, 86% were on the UNMC campus, and 36% of the PIs are BCC members.

- The NMR lab instruments (400MHz, 500 MHz and 600 MHz) were used by members of 20 research labs on the UNMC campus. Of the 20 research labs, 50% of the PI's are BCC members and 5% of the PIs are BCC associate members. An automatic sample changer was installed on the 600 MHz instrument in January 2024 to augment the ease of use on that spectrometer.

- Major Structural Biology Facility users include:
  - Martin Conda-Sheridan: Peptide Amphiphiles
  - Amar Natarajan: Molecular target discovery and development, therapeutic design, crystallization of proteins of interest with SMIs bound, including IKKb and Cbl
  - Corey Hopkins: Small molecule inhibitors of Claudine-1
  - Ram Mahato: Chemoresistant pancreatic cancer
  - Paul Trippier: AKR1C3 in prostate cancer
  - Piero Bianco: Single-stranded DNA binding proteins and their protein partners
  - Gloria Borgstahl: Homologous recombination DNA repair proteins, small molecule inhibitor (SMI) drug complexes and metalloenzymes such as MnSOD
  - Lynne Dieckman: Purification optimization and crystallization of PCNA complexes
  - Tony Hollingsworth: Purification of Muc1-CT and Alix for structural biology
  - Sarah Holstein: GGDPS purification and crystallization with SMI
  - Bhavesh Kevadiya: SAXS for delivery mechanisms for nanomedicine
  - DJ Murry: SAXS for liposome lamellarity and drug encapsulation
  - Don Ronning: Drug development for novel anti-infective and anti-cancer compounds
  - Juliane Strauss-Soukup: Protocol optimization and crystallization of RNA pseudoknots
  - Dong Wang: SAXS on thermal sensitive hydrogels and drug complexes for nanomedicine
  - Tahir Tahirov: Crystallization and data collection on DNA polymerases

- Jingwei Xie: SAXS for delivery mechanisms for nanomedicine

The 3 NMR instruments (400MHz, 500 MHz and 600 MHz) in the NMR lab were used by members of 20 research labs on the UNMC campus. Of the 20 research labs, 50% of the PI's are BCC members and 5% of the PIs are BCC associate members. The 500 MHz NMR spectrometer was retired from service after 32 years at field in January of 2024, as the resonance frequency was now too low for use. An automatic sample changer was installed on the 600 MHz instrument in January 2024 to augment the ease of use on that spectrometer. Major NMR users include:

- Martin Conda-Sheridan: Peptide Amphiphiles
- Amar Natarajan: Molecular target discovery and development
- Corey Hopkins: Small molecule inhibitors of Claudine-1
- Ram Mahato: Chemoresistant pancreatic cancer
- Paul Trippier: AKR1C3 in prostate cancer

Below is a list of recent highlighted cancer-related publications that utilized the resource:

Azadmanesh, J., Slobodnik, K., Struble, L. R., Lutz, W.E., Coates L., Weiss, K. Myles, D. A. A., Kroll, T. and Borgstahl, G. E. O." Revealing the atomic and electronic mechanism of human manganese superoxide dismutase product inhibition", Nature Communications in press (2024).

Pham, A.C., Holstein, S. A., and Borgstahl, G. E. O. "Structural insight into geranylgeranyl diphosphate synthase (GGDPS) for cancer therapy" Molecular Cancer Therapeutics, 23 14-23 (2024).

Wallin S, Singh S, Borgstahl GEO (CB), Natarajan A (TMD). Design, synthesis, and evaluation of a mitoxantrone probe (MXP) for biological studies. Bioorg. Med. Chem. Lett.. 2023 OCT 01; 94:129465. DOI: 10.1016/j.bmcl.2023.129465. 2023 Sep 3. PubMed PMID: 37669721; PMCID: PMC10528225.

Mashinson V, Webster TM, Vadukoot AK, Tolentino KT, Simeon P, Fatima I, Dhawan P (GIC), Hopkins CR (TMD). Discovery, synthesis and biological evaluation of a series of N-(phenylcarbamothioyl)-2-napthamides as inhibitors of Claudin-1. Bioorg. Med. Chem.. 2023 SEP 07; 92:117416. DOI: 10.1016/j.bmc.2023.117416. 2023 Jul 26. PubMed PMID: 37541070; PMCID: PMC10530161.

Carmona, A. V., Jonnalagadda, S., Case, A. M., Maddeboina, K., Jonnalagadda, S. K., Dow, L. F., Duan, L., Penning, T. M. & Trippier, P. C. "Discovery of an Aldo-Keto reductase 1C3 (AKR1C3) degrader" Communications Chemistry 7, 1, 95. Dec 2024

Eiken AP, Smith AL, Skupa SA, Schmitz E, Rana S, Singh S, Kumar S, Mallareddy JR, de Cubas AA, Krishna A, Kalluchi A, Rowley MJ, D'Angelo CR, Lunning MA, Bociek RG, Vose JM, Natarajan A, El-Gamal D. Novel Spirocyclic Dimer, SpiD3, Targets Chronic Lymphocytic Leukemia Survival Pathways with Potent Preclinical

Effects. Cancer Res Commun. 2024 May 22;4(5):1328-1343. doi: 10.1158/2767-9764.CRC-24-0071. PMID: 38687198; PMCID: PMC11110724.

Carmona AV, Jonnalagadda S, Case AM, Maddeboina K, Jonnalagadda SK, Dow LF, Duan L, Penning TM, Trippier PC. Discovery of an Aldo-Keto reductase 1C3 (AKR1C3) degrader. Commun Chem. 2024 Apr 29;7(1):95. doi: 10.1038/s42004-024-01177-4. PMID: 38684887; PMCID: PMC11059152.

Murthy D, Attri KS, Shukla SK, Thakur R, Chaika NV, He C, Wang D, Jha K, Dasgupta A, King RJ, Mulder SE, Souchek J, Gebregiworgis T, Rai V, Patel R, Hu T, Rana S, Kollala SS, Pacheco C, Grandgenett PM, Yu F, Kumar V, Lazenby AJ, Black AR, Ulhannan S, Jain A, Edil BH, Klinkebiel DL, Powers R, Natarajan A, Hollingsworth MA, Mehla K, Ly Q, Chaudhary S, Hwang RF, Wellen KE, Singh PK. Cancer-associated fibroblast-derived acetate promotes pancreatic cancer development by altering polyamine metabolism via the

ACSS2-SP1-SAT1 axis. Nat Cell Biol. 2024 Apr;26(4):613-627. doi: 10.1038/s41556-024-01372-4. Epub 2024 Mar 1.

Kumar V, Mahato RI. Drug delivery and targeting to chemoresistant pancreatic cancer. Cancer Lett. 2024 Mar 31;585:216648. doi: 10.1016/j.canlet.2024.216648. Epub 2024 Feb 2. PMID: 38311056.

Pramanik N, Gupta A, Ghanwatkar Y, Mahato RI. Recent advances in drug delivery and targeting for the treatment of pancreatic cancer. J Control Release. 2024 Feb;366:231-260. doi: 10.1016/j.jconrel.2023.12.053. Epub 2024 Jan 4. PMID: 38171473; PMCID: PMC10922996.

Kumar V, Mahato RI. Natural killer cells for pancreatic cancer immunotherapy: Role of nanoparticles. Cancer Lett. 2023 Nov 28;579:216462. doi: 10.1016/j.canlet.2023.216462. Epub 2023 Nov 2. PMID: 37924937; PMCID: PMC10842153.

Mashinson V, Webster TM, Vadukoot AK, Tolentino KT, Simeon P, Fatima I, Dhawan P, Hopkins CR. Discovery, synthesis and biological evaluation of a series of N-(phenylcarbamothioyl)-2-napthamides as inhibitors of Claudin-1. Bioorg Med Chem. 2023 Sep 7;92:117416. doi: 10.1016/j.bmc.2023.117416. Epub 2023 Jul 26. PMID: 37541070; PMCID: PMC10530161.

SYNTHETIC AND MEDICINAL CHEMISTRY

The major goal of the synthetic chemistry resource (SCR) is to synthesize compounds that will be used for studies in vitro, in cells or in animals to gain a better understanding of biology and / or for treatment of cancer.

The SCR supports senior chemists with expertise in synthetic and medicinal chemistry and Provides

1. Provide access to small molecules that are not commercially available (including compounds found in patents) for probing novel biological targets
2. Provide fully characterized material of biologically active molecules identified as hits from HTS campaigns for follow up studies
3. Provide chemical tools (e.g., fluorescently labeled or biotinylated compounds) to develop assays and/or identify biological targets
4. Provide scale up services (mg to grams) for cellular and / or in vivo studies
5. Provide hit-to-lead and / or lead optimization service through systematic structure activity relationship (SAR) 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.

Accomplishments (Completed)

- Generated a fluorescently labeled pH-sensitive peptide that was used to demonstrate "acetyl-CoA metabolism in pancreatic cancer cells regulates histone acetylation levels and is functionally critical for the survival of pancreatic cancer cells under low pH." (PI, Dr. Singh). Nature Cell Biology Volume 26 April 2024 613–627.

- Developed a novel method to generate biologically active isatin-derived spirolactone systems. (SCR Technology Development).
  Molecules 2024, 29, 3612.

- Scaled up SpiDs for evaluation in *in vitro* and *in vivo* CLL models (PI, Dr. ElGamal).
  Cancer Res Commun 4(5) May 2024
  1328. Hemato 2024, 5, 321–339.

- Generated superoxide solution required for MnSOD studies (PI, Dr. Borgstahl).
  Nat Commun 2024 (Manuscript in revision)

- Generated PKR activator (PI, Dr. Dong).
  Funded grant R01CA273226

- Synthesized RHNO1 mimics (PI, Dr. Karpf).
  Funded grant R21CA273399.
  Funded grant DOD HT9425-23-2-0238

- Gram scale synthesis of the heterobifunc. ional compound EN1 (PI, Dr. Bergan).
  Funded grant R01CA276846
  Work was presented at the American Chemical Society National Meeting (Mar 2024)
  Contributed to the submission of VA-Merit and DoD grants (PI, Dr. Bergan) and aplanned MPI R01 (Oct 2024 submission).

PROGRAM PUBLICATIONS

Listed below in reverse chronological order are program-related peer-reviewed journal articles by BCC investigators for whom developmental funds were budgeted and/or expended during the previous reporting period:

1: Dong R, Abazarikia A, Luan Y, Yu SY, **Kim SY**. Molecular Mechanisms Determining Mammalian Oocyte Quality with the Treatment of Cancer Therapy. *Adv Anat Embryol Cell Biol.* 2024;238:97-119. doi: 10.1007/978-3-031-55163-5_5. PMID: 39030356.

2: Raza S, **Siddiqui JA**, Srivastava A, Chattopadhyay N, Sinha RA, Chakravarti B. Autophagy as a Therapeutic Target in Breast Tumors: The Cancer stem cell perspective. *Autophagy Rep.* 2024 Jun 24;3(1):27694127.2024.2358648. doi: 10.1080/27694127.2024.2358648. PMID: 39006309; PMCID: PMC7616179.

3: Mirzapoiazova T, Tseng L, Mambetsariev B, Li H, Lou CH, Pozhitkov A, Ramisetty SK, Nam S, Mambetsariev I, Armstrong B, Malhotra J, Arvanitis L, **Nasser MW**, Batra SK, Rosen ST, Wheeler DL, Singhal SS, Kulkarni P, Salgia R. Teriflunomide/leflunomide synergize with chemotherapeutics by decreasing mitochondrial fragmentation via DRP1 in SCLC. *iScience*. 2024 May 27;27(6):110132. doi: 10.1016/j.isci.2024.110132. PMID: 38993482; PMCID: PMC11237869.

4: Kaushal JB, Raut P, Muniyan S, **Siddiqui JA**, Alsafwani ZW, Seshacharyulu P, Nair SS, Tewari AK, Batra SK. Racial disparity in prostate cancer: an outlook in genetic and molecular landscape. *Cancer Metastasis Rev.* 2024 Jun 20. doi: 10.1007/s10555-024-10193-8. Epub ahead of print. PMID: 38902476.

5: Valiveti CK, Kumar B, Singh AD, Biradar SK, Ahmad R, **Singh AB**, Tummala H. Stable Dietary Ora-Curcumin Formulation Protects from Experimental Colitis and Colorectal Cancer. *Cells*. 2024 Jun 1;13(11):957. doi: 10.3390/cells13110957. PMID: 38891089; PMCID: PMC11172195.

6: Bhat AM, Mohapatra BC, Luan H, Mushtaq I, Chakraborty S, Kumar S, Wu W, Nolan B, Dutta S, Storck MD, **Schott M**, Meza JL, Lele SM, Lin MF, Cook LM, Corey E, Morrissey C, Coulter DW, Rowley MJ, Natarajan A, Datta K, Band V, Band H. GD2 and its biosynthetic enzyme GD3 synthase promote tumorigenesis in prostate cancer by regulating cancer stem cell behavior. *Sci Rep.* 2024 Jun 12;14(1):13523. doi: 10.1038/s41598-024-60052-3. PMID: 38866755; PMCID: PMC11169677.

7: McDowell JA, Kosmacek EA, **Baine MJ**, Adebisi O, Zheng C, Bierman MM, Myers MS, Chatterjee A, Liermann-Wooldrik KT, Lim A, **Dickinson KA**, **Oberley-Deegan RE**. Exogenous APN protects normal tissues from radiation-induced oxidative damage and fibrosis in mice and prostate cancer patients with higher levels of APN have less radiation-induced toxicities. *Redox Biol.* 2024 Jul;73:103219. doi: 10.1016/j.redox.2024.103219. Epub 2024 May 31. PMID: 38851001; PMCID: PMC11201354.

8: Chaudhary S, **Siddiqui JA**, Appadurai MI, Maurya SK, Murakonda SP, Blowers E, Swanson BJ, **Nasser MW**, Batra SK, Lakshmanan I, Ganti AK. Dissecting the MUC5AC/ANXA2 signaling axis: implications for brain metastasis in lung adenocarcinoma. *Exp Mol Med.* 2024 Jun;56(6):1450-1460. doi: 10.1038/s12276-024-01255-6. Epub 2024 Jun 3. PMID: 38825648; PMCID: PMC11263355.

9: Plewes MR, Talbott HA, **Schott MB**, Wood JR, Cupp AS, Davis JS. Unraveling the role of lipid droplets and perilipin 2 in bovine luteal cells. *FASEB J.* 2024 Jun 15;38(11):e23710. doi: 10.1096/fj.202400260RR. PMID: 38822676; PMCID: PMC11347014.

10: Zheng D, Grandgenett PM, Zhang Q, **Baine M**, Shi Y, Du Q, Liang X, Wong J, Iqbal S, Preuss K, Kamal A, Yu H, Du H, Hollingsworth MA, Zhang C. radioGWAS links radiome to genome to discover driver genes with

somatic mutations for heterogeneous tumor image phenotype in pancreatic cancer. *Sci Rep.* 2024 May 29;14(1):12316. doi: 10.1038/s41598-024-62741-5. PMID: 38811597; PMCID: PMC11137018.

11: Huynh LM, Swanson S, Cima S, Haddadin E, **Baine M**. Prostate-Specific Membrane Antigen Positron Emission Tomography/Computed Tomography-Derived Radiomic Models in Prostate Cancer Prognostication. *Cancers (Basel)*. 2024 May 16;16(10):1897. doi: 10.3390/cancers16101897. PMID: 38791977; PMCID: PMC11120365.

12: **Schott MB**, Rozeveld CN, Bhatt S, Crossman B, Krueger EW, Weller SG, Rasineni K, Casey CA, McNiven MA. Ethanol disrupts hepatocellular lipophagy by altering Rab5-centric LD-lysosome trafficking. *Hepatol Commun.* 2024 May 22;8(6):e0446. doi: 10.1097/HC9.0000000000000446. PMID: 38780316; PMCID: PMC11124685.

13: Pravoverov K, Fatima I, Barman S, Jühling F, Primeaux M, Baumert TF, **Singh AB**, **Dhawan P**. IL-22 regulates MASTL expression in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol.* 2024 Aug 1;327(2):G123-G139. doi: 10.1152/ajpgi.00260.2023. Epub 2024 May 21. PMID: 38771154.

14: Andrianu K, Works D, Christiansen A, Enke C, Chaiken L, **Baine M**. Exploring the Impact of Sodium-Glucose Cotransporter-2 Inhibitors on Genitourinary Toxicities in Prostate Cancer Patients Undergoing Radiation Therapy: A Case Study and Discussion. *Pract Radiat Oncol.* 2024 Sep-Oct;14(5):373-376. doi: 10.1016/j.prro.2024.04.006. Epub 2024 May 16. PMID: 38752974.

15: AlMarzooqi SK, Almarzooqi F, Sadida HQ, Jerobin J, Ahmed I, Abou-Samra AB, Fakhro KA, **Dhawan P**, Bhat AA, Al-Shabeeb Akil AS. Deciphering the complex interplay of obesity, epithelial barrier dysfunction, and tight junction remodeling: Unraveling potential therapeutic avenues. *Obes Rev.* 2024 Aug;25(8):e13766. doi: 10.1111/obr.13766. Epub 2024 May 14. PMID: 38745386.

16: Kaushal JB, Takkar S, Batra SK, **Siddiqui JA**. Diverse landscape of genetically engineered mouse models: Genomic and molecular insights into prostate cancer. *Cancer Lett.* 2024 Jul 1;593:216954. doi: 10.1016/j.canlet.2024.216954. Epub 2024 May 10. PMID: 38735382.

17: West KL, Kreiling N, Raney KD, **Ghosal G**, Leung JW. Autophosphorylation of the Tousled-like kinases TLK1 and TLK2 regulates recruitment to damaged chromatin via PCNA interaction. *bioRxiv* [Preprint]. 2024 Apr 26:2024.04.22.590659. doi: 10.1101/2024.04.22.590659. PMID: 38712247; PMCID: PMC11071368.

18: Bonebrake BT, Parr E, Huynh LM, Coutu B, Hansen N, Teply B, Enke C, Lagrange C, **Baine M**. Predictive Value of Multiparametric Magnetic Resonance Imaging in Risk Group Stratification of Prostate Adenocarcinoma. *Adv Radiat Oncol.* 2024 Mar 12;9(6):101493. doi: 10.1016/j.adro.2024.101493. PMID: 38711959; PMCID: PMC11070813.

19: Carmona AV, Jonnalagadda S, Case AM, Maddeboina K, Jonnalagadda SK, Dow LF, Duan L, Penning TM, **Trippier PC**. Discovery of an Aldo-Keto reductase 1C3 (AKR1C3) degrader. *Commun Chem.* 2024 Apr 29;7(1):95. doi: 10.1038/s42004-024-01177-4. PMID: 38684887; PMCID: PMC11059152.

20: Rahman MS, Hadi Esfahani S, Zhang Y, Queen A, Aljarrah M, Kandil H, Baez A, Abbruscato TJ, Karamyan VT, **Trippier PC**. Imidazole Bioisostere Activators of Endopeptidase Neurolysin with Enhanced Potency and Metabolic Stability. *ACS Med Chem Lett.* 2024 Mar 29;15(4):510-517. doi: 10.1021/acsmedchemlett.4c00009. PMID: 38628788; PMCID: PMC11017387.

21: Hendricks BA, Kupzyk K, Poppert Cordts KM, **Lally RM**. Oncology's Silent Caregivers: A Mixed-Methods Exploration of the Experiences, Outcomes, and Unmet Needs of Caregiving Youth of a Parent With Cancer. *Cancer Nurs.* 2024 Apr 10. doi: 10.1097/NCC.0000000000001342. Epub ahead of print. PMID: 38598768.

22: Cox KE, Liu S, Hoffman RM, Batra SK, **Dhawan P**, Bouvet M. The Expression of the Claudin Family of Proteins in Colorectal Cancer. *Biomolecules*. 2024 Feb 24;14(3):272. doi: 10.3390/biom14030272. PMID: 38540693; PMCID: PMC10967842.

23: Takkar S, Sharma G, Kaushal JB, Abdullah KM, Batra SK, **Siddiqui JA**. From orphan to oncogene: The role of GPR35 in cancer and immune modulation. *Cytokine Growth Factor Rev.* 2024 Jun;77:56-66. doi: 10.1016/j.cytogfr.2024.03.004. Epub 2024 Mar 19. PMID: 38514303.

24: Abdullah KM, Sharma G, Qais FA, Khan I, Takkar S, Kaushal JB, Kanchan RK, Sarwar T, Chakravarti B, **Siddiqui JA**. Hydroxychloroquine interaction with phosphoinositide 3-kinase modulates prostate cancer growth in bone microenvironment: In vitro and molecular dynamics based approach. *Int J Biol Macromol.* 2024 May;266(Pt 1):130912. doi: 10.1016/j.ijbiomac.2024.130912. Epub 2024 Mar 20. PMID: 38513896.

25: Abdullah KM, Kaushal JB, Takkar S, Sharma G, Alsafwani ZW, Pothuraju R, Batra SK, **Siddiqui JA**. Copper metabolism and cuproptosis in human malignancies: Unraveling the complex interplay for therapeutic insights. *Heliyon*. 2024 Mar 7;10(5):e27496. doi: 10.1016/j.heliyon.2024.e27496. PMID: 38486750; PMCID: PMC10938126.

26: **Lally RM**, Schmidt R, Kupzyk K, Wengel SP, Cordts KP, Mills AC, Richards SE. Implementing Longitudinal Wellbeing Interventions and Evaluation Among Midwestern Healthcare Workers During COVID-19. *West J Nurs Res.* 2024 Apr;46(4):296-306. doi: 10.1177/01939459241237663. Epub 2024 Mar 11. PMID: 38465618.

27: Motzer RJ, Jonasch E, Agarwal N, Alva A, Bagshaw H, **Baine M**, Beckermann K, Carlo MI, Choueiri TK, Costello BA, Derweesh IH, Desai A, Ged Y, George S, Gore JL, Gunn A, Haas N, Johnson M, Kapur P, King J, Kyriakopoulos C, Lam ET, Lara PN, Lau C, Lewis B, Madoff DC, Manley B, Michaelson MD, Mortazavi A, Ponsky L, Ramalingam S, Shuch B, Smith ZL, Sosman J, Sweis R, Zibelman M, Schonfeld R, Stein M, Gurski LA. NCCN Guidelines® Insights: Kidney Cancer, Version 2.2024. *J Natl Compr Canc Netw.* 2024 Feb;22(1):4-16. doi: 10.6004/jnccn.2024.0008. PMID: 38394781.

28: Abdullah KM, Sharma G, Takkar S, Kaushal JB, Pothuraju R, Chakravarti B, Batra SK, **Siddiqui JA**. α-lipoic acid modulates prostate cancer cell growth and bone cell differentiation. *Sci Rep.* 2024 Feb 22;14(1):4404. doi: 10.1038/s41598-024-54479-x. PMID: 38388663; PMCID: PMC10884017.

29: Lim A, **Dickinson K**, **Lally RM**. Health Care Professional Education on Cancer Screening of SGM Individuals: An Integrative Review. *J Cancer Educ.* 2024 Jun;39(3):220-233. doi: 10.1007/s13187-024-02399-9. Epub 2024 Jan 31. PMID: 38291172.

30: Fatima I, Ahmad R, Barman S, Gowrikumar S, Pravoverov K, Primeaux M, Fisher KW, **Singh AB**, **Dhawan P**. Albendazole inhibits colon cancer progression and therapy resistance by targeting ubiquitin ligase RNF20. *Br J Cancer*. 2024 Apr;130(6):1046-1058. doi: 10.1038/s41416-023-02570-x. Epub 2024 Jan 26. PMID: 38278978; PMCID: PMC10951408.

31: Barkeer S, Pothuraju R, Malakar P, Pimentel TC, **Siddiqui JA**, Nair SA. Gum acacia dietary fiber: significance in immunomodulation, inflammatory diseases, and cancer. *Phytother Res.* 2024 Mar;38(3):1509-1521. doi: 10.1002/ptr.8125. Epub 2024 Jan 25. PMID: 38272848.

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SUMMARY OF EXPENDITURES

The following summary table includes the approved Revised Budget and Actual Expenditures from July 1, 2023, to June 30, 2024.

Budget Category	Revised LB 595 Budget	LB 595 Expenditures
Salaries and Fringe Benefits	$1,033,230	$1,107,371
Equipment	$78,275	$68,654
Supplies	$45,000	$66,231
Other Expenses	$143,495	$57,744
Total	**$1,300,000**	**$1,300,000**