NIH MEDICAL RESEARCH SCHOLARS PROGRAM RESEARCH COMPENDIUM

2022-2023

National Institutes of Health
Turning Discovery Into Health

About the NIH Medical Research Scholars Program

The NIH's mission is to strengthen our nation's research capacity, broaden our research base and inspire a passion for science in current and future generations of clinician-scientists. Recognizing that successful biomedical research depends on the talent and dedication of the scientific workforce, the NIH supports MRSP and other innovative training programs that foster scientific creativity and exploration. MRSP is a 10-12 month residential research immersion program in which scholars engage in mentored basic, clinical, or translational research projects that match their professional interests and career goals.

The MRSP is distinguished from other training programs by the scholars' unique access to the full continuum of NIH biomedical research—the bench, the bedside, and beyond—from crystallography to molecular biology, from computational biology to clinical trials and epidemiology. The MRSP scholars join laboratories and clinical research facilities that are among the most extensive and highly regarded in the world, with access to the NIH's 27 intramural Institutes and Centers, NIH seminars and tutorials, and teaching rounds at the NIH Clinical Center, America's Research Hospital. Scholars spend the majority of time in their research laboratories, under the mentorship of a fulltime NIH investigator whom they select, and also participate in a complementary curriculum of professional development and leadership opportunities.

The MRSP academic curriculum offers:

- A "Process of Discovery" seminar series on basic, translational and clinical research topics that highlight the continuum of discovery, including issues in bioethics, science policy and emerging technologies. This provides scholars with opportunities to meet and interact with NIH leaders, including institute directors, scientific and clinical directors, as well as established principal investigators from the intramural institutes.
- Participation in mentored Journal Clubs

- MRSP Clinical Teaching Rounds focusing on the patient population participating in clinical protocols at the NIH Clinical Center
- "Great Teachers" colloquia with nationally renowned clinician-scientists who are invited to the NIH as part of the Clinical Center Grand Round series.
- Workshops in CV writing and interviewing

MRSP Funding:

- Support for the MRSP occurs through a public-private partnership, supported by the NIH and private donations procured by the Foundation for the National Institutes of Health (FNIH).
- The Shared Resources Subcommittee (SRS) of the NIH Board of Scientific Directors funds the MRSP as a signature program in the NIH's mission of training future clinician-scientists. The MRSP also works in partnership with the National Institute of General Medical Sciences (NIGMS) to fund students from NIGMS Institutional Development Award (IDeA) states and commonwealths, to promote scientific careers in those states.
- The FNIH was established by Congress in 1990 as a not-for-profit 501(c)(3) charitable organization. As an independent organization, it raises private funds and creates public private partnerships to support the mission of the NIH—making important discoveries that improve health and save lives. Generous support for the NIH Medical Research Scholars Program was received through the FNIH from the American Association for Dental Research, the Colgate-Palmolive Company, and other private donors.

Summary of Research Achievements

During the 2022-2023 MRSP year, the scholars celebrated many research accomplishments, as shown by the number of manuscripts they produced (Figure 1); the number of scholars who were first authors on these manuscripts (Figure 2); the number of scholars who presented their work at professional meetings (Figure 3); and the number of scholars who attended professional meetings, either virtually or in-person (Figure 4). Specifically, **24** scholars (**47%**) produced a total of **47** manuscripts for peer-reviewed publication, including **20** articles that were published or in press in peer-reviewed journals and **27** papers under review. MRSP scholars were first authors on **23 of 47 (49%)** of these published or submitted manuscripts. Forty-three scholars (84%) attended 85 professional meetings where they presented a total of 102 abstracts; 12 scholars (24%) received awards for outstanding research achievement. Two scholars were invited to spend a second year at NIH as an intramural research training awardee; one scholar was accepted into the NIH Oxford-Cambridge PhD Program and will pursue further research at NIH as a doctoral candidate.

Figure 1. Completion of 47 MRSP Manuscripts

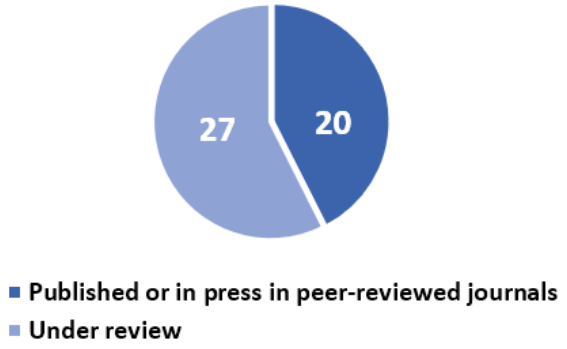


Figure 2. Authorship of MRSP Publications

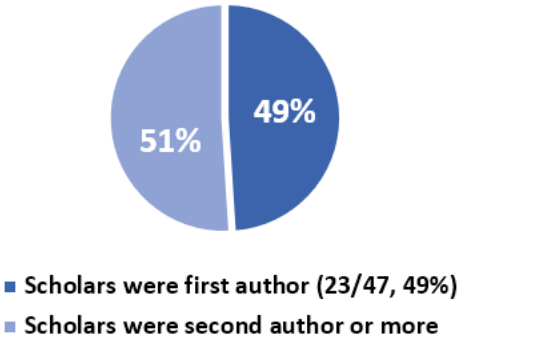


Figure 3. Presentations at Scholarly Meetings
n=102 total abstracts

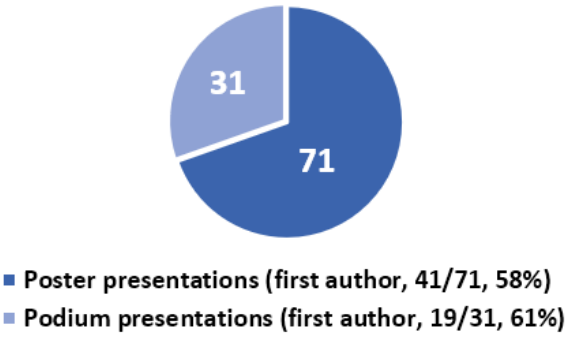
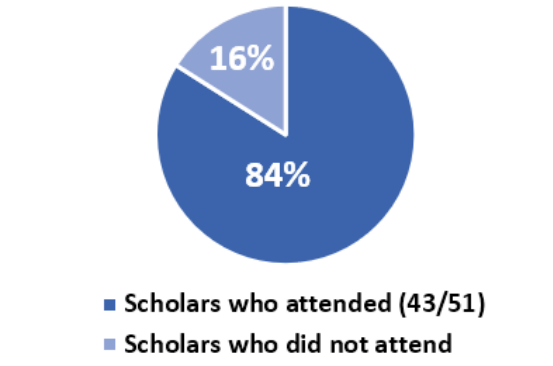


Figure 4. Attendance at Professional Meetings



Scholar Research Summary Directory

46. Eszter Toth, Medical College of Georgia at Augusta University
47. Rajiv Trehan, Rutgers Robert Wood Johnson Medical School
48. Alex Valenzuela, Charles R. Drew University of Medicine and Sciences
49. Sarita Walvekar, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
50. Philip Wang, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
51. Georgina Whelan, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Scholar	Sarfraz R. Akmal
School	Rutgers Robert Wood Johnson Medical School
Mentor	Jonathan M. Hernandez, M.D., Investigator, Surgical Oncology Program
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Leveraging a Novel *Ex vivo* Human Tumor System to Interrogate Promising Pre-clinical Immuno-oncology Agents

Research Summary

Recapitulating the complexities of human tumors *ex vivo* remains a major obstacle in the advancement of cancer care. Our lab has developed and validated the SMART (Sustained Microenvironment for Analysis of Resected Tissue) system as an *ex vivo* human tumor model for evaluation of novel immuno-oncology agents. We investigated the effects of tivozanib, a multi-kinase inhibitor, in cholangiocarcinoma and other cancers using this model. Tivozanib has been shown to reduce tumor growth via SLK inhibition in a patient-derived xenograft model. We hypothesized that tivozanib might also inhibit selective arms of the immune system through LCK inhibition.

To investigate the effects of tivozanib on the immune system, we evaluated the activity of T cells following treatment with tivozanib. T cells were plated and incubated with tivozanib 2 µM or with control diluent for 5 days. Following incubation, the T cells were stimulated with anti-CD3 antibody and evaluated for IFN-y production using flow cytometry.

IFN-y production in FoxP3+ T cells and CD4+ T cells was inhibited by tivozanib treatment as compared to untreated control cells ($p<0.005$ and $p<0.0005$, respectively). IFN-y production in CD8+ T cells was not inhibited ($p=0.33$). FoxP3+ cells are regulatory T cells, and their inhibition can lead to pro-inflammatory behavior by the immune system. We hypothesize that this selective inhibition may facilitate anti-tumor activity and also synergize with other immuno-oncology agents approved to treat cholangiocarcinoma, such as durvalumab, a PD-L1 inhibitor.

Future studies will evaluate the native T cell population within tumors using the SMART system. Tumor tissue from patients will be incubated and treated with tivozanib in the SMART system for up to 4 days. Dissociative methods will be used to evaluate T cell

activity within the tumor. Our model is perfectly configured to evaluate these effects and we anticipate that our *ex vivo* results may be translatable to *in vivo* patient responses.

Publications

- Gregory SN, Sarvestani AL, Ryan CE, Teke ME, **Akmal SR**, Hernandez JM, Gupta S. Oregovomab plus chemo in newly diagnosed patients with advanced epithelial ovarian cancer following optimal debulking surgery (FLORA-5/GOG-3035). *Ann Surg Oncol*. 2023 Mar;30(3):1299-1301. PMID: 36400892.

Abstracts

- Teke M, Saif A, Verbus E, Lux SC, Remmert K, Sarvestani AL, Gregory SN, Ryan CE, Pu T, Lin Y, **Akmal SR**, Lambdin JT, Luberice K, Sinha S, Blakely AM, Davis J, Hernandez JM. Leveraging a novel ex-vivo human tumor system to interrogate peritoneal surface malignancies in children. American Pediatric Surgical Association Annual Meeting, Orlando, FL; May 10-13, 2023. [Podium presentation]
- Pu T, Remmert K, Massey A, Gregory SN, **Akmal SR**, Garmendia-Cedillos M, McCormick M, Do K, Davis JL, Blakely AM, Cartagena-Rivera A, Hernandez JM. Hyperthermic intraperitoneal chemotherapy restores physiologic tumor stiffness and stimulates lymphocyte proliferation & recruitment. Society of Surgical Oncology, Boston, MA; Mar. 22-25, 2023. [Poster presentation]
- Ryan CE, Gregory SN, Teke M, Sarvestani AL, Remmert K, Lambdin J, Smith EC, Sinha S, **Akmal SR**, Lux SC, Pu T, Luberice K, Kleiner D, Hernandez JM. Peribiliary gland injury by floxuridine may be an early mechanistic insult en route to sclerosis. The Americas Hepato-Pancreato-Biliary Association Annual Meeting, Miami Beach, FL; Mar. 9-12, 2023. [Podium presentation]
- Lambdin J, Luna AJ, Lux SC, Luberice K, Ryan CE, Stepp H, Rossi A, Sarvestani AL, Gregory SN, **Akmal SR**, Davis JL, Yaffe M, Kleiner DE, Blakely AM, Hernandez JM. Creation of a perfusion machine for prolonged ex-vivo animation of resected tumor-bearing organs. The Americas Hepato-Pancreato-Biliary Association Annual Meeting, Miami Beach, FL; Mar. 9-12, 2023. [Podium presentation]
- Pu T, Ghabra S, Luberice K, Sarvestani AL, **Akmal SR**, Davis JL, Blakely AM, Hernandez JM. Surgery for gastroenteropancreatic neuroendocrine tumors with synchronous liver metastasis. The Americas Hepato-Pancreato-Biliary Association Annual Meeting, Miami Beach, FL; Mar. 9-12, 2023. [Podium presentation]
- Gregory SN, Ryan CE, Teke ME, Savestani Al, Ke X, **Akmal SR**, Lux SC, Lambdin JT, Pu T, Luberice K, Remmert KC, Smith EC, Sinha S, Rainey A, Satterwhite A, Hannah C, Davis JL, Hernandez JM, Blakely AM. In-vivo tumor model to understand the effect of HIPEC in peritoneal malignancies. Society for Surgical Oncology Advanced Cancer Therapies, San Diego, CA; Feb. 18-20, 2023. [Podium presentation]

Professional Meetings

- The Americas Hepato-Pancreato-Biliary Association Annual Meeting, Miami Beach, FL; Mar. 9-12, 2023.

Scholar	Martin A. Arhin
School	University of North Carolina School of Medicine
Mentor	Prashant Chittiboina, M.D., Investigator, Neurosurgery Unit for Pituitary and Inheritable Diseases, Surgical Neurology Branch
NIH Institute	National Institute of Neurological Disorders and Stroke (NINDS)
Project Title	Monocyte-derived Pro-tumor Macrophages Drive Vestibular Schwannoma Growth
Research Summary	Vestibular schwannomas (VS) are tumors that originate from the Schwann cells of cranial nerve VIII. These tumors typically develop within the internal acoustic canal and may extend into the cerebellopontine angle. Symptoms such as sensorineural hearing loss, disequilibrium, and those arising from brainstem compression occur primarily due to the volume of VS displacing neural structures. Despite best treatments (surgery and/or radiation), morbidities such as permanent hearing loss or imbalance may occur. Given limited therapeutic options, there is a need for novel therapies. Emerging evidence shows that the tumor microenvironment (non-Schwann cell milieu) is important in VS growth. Tumor-associated macrophages polarized to a pro-tumorigenic M2 phenotype may drive sporadic and neurofibromatosis type 2 (NF2)-related VS. However, the origin and role of macrophages within VS are not well understood. We created parallel single-cell (n=2 sporadic; n=2 NF2) and single nucleus (n=4 sporadic; n=5 NF2) transcriptomic maps. We used marker-based (scSorter) and cluster-based classifications to identify macrophages and their transcriptomic profiles. We then validated the canonical cell classes with multiplexed immunohistochemistry (n=6 NF2). We found that cell fractions corresponded best with single-nucleus RNA datasets (macrophages were 14-73% of all cells). Next, we sought to determine macrophage phenotypes and whether macrophages were tissue-resident or monocyte-derived. We found a predominance of M2 phenotype macrophages that expressed CD163 (51%) and CD204 (46%) in NF2 and sporadic schwannomas. The M1 marker CD86 was expressed in 32% of macrophages. A small population was microglial-derived (11%) while the majority were monocyte-derived. Approximately 51% and 23% of M2 macrophages expressed the monocyte markers HLA-DRA and CSF1R, respectively, and 6% expressed the microglial marker P2RY12.

	These findings indicate that targeting macrophage influx and expansion may hinder tumor expansion and offer promising avenues for managing this disorder.
Abstracts	• **Arhin M**, Mandal D, Gandhi J, Moore E, Asuzu D, Chittiboina P. Transcriptomic characterization of locally derived microglial-like macrophages that drive tumor volume in vestibular schwannomas. American Association of Neurological Surgeons Annual Meeting, Los Angeles, CA; Apr. 20-24, 2023. [Poster presentation] • Moore E, Chen K, Kyeyoon P, Asuzu D, Ramavenkat N, **Arhin M**, Maric D, Mandal D, Chittiboina P. Corticotroph fate weighted cell differentiation paradigm for human induced pluripotent stem cells. American Association of Neurological Surgeons Annual Meeting, Los Angeles, CA; Apr. 20-24, 2023. [Podium presentation]
Professional Meetings	• American Association of Neurological Surgeons (AANS) Annual Meeting, Los Angeles, CA; Apr. 20-24, 2023.

Scholar	Bhargav Arimilli
School	University of Texas Southwestern Medical School
Mentors	W. Marston Linehan, M.D., Chief, Urologic Oncology Branch Daniel R. Crooks, Ph.D., Staff Scientist, Urologic Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Metabolic Pathways of Oncometabolite Accumulation in Fumarate Hydratase-Deficient Renal Tumor Cell Lines
Research Summary	Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is a rare, autosomal dominant disease characterized by a germline mutation in fumarate hydratase (FH), the tricarboxylic acid cycle (TCA) enzyme that mediates the conversion of fumarate to malate. Increased fumarate levels inhibit HIF prolyl hydroxylase, leading to stabilization of HIF1α and increased transcription of downstream targets, such as VEGF and GLUT1. FH-deficient cells also undergo a metabolic shift to aerobic glycolysis. Patients with HLRCC present with benign cutaneous leiomyomas and uterine fibroids, and are also at risk for developing an aggressive form of type II papillary renal cancer. To assess how fumarate is metabolized in HLRCC cells, we conducted a series of isotope tracer experiments in which cells were cultured with ^{13}C-glutamine and then analyzed via NMR and mass spectrometry. We found that in two HLRCC cell lines, UOK271 and UOK348, fumarate was not fully labelled by ^{13}C-glutamine despite the presence of fully labelled succinate, fumarate's immediate precursor in the TCA cycle. These data suggested a problem with succinate dehydrogenase (SDH), which converts succinate to fumarate. Further analysis demonstrated very high succinate levels relative to fumarate levels in these two cell lines, which was unexpected given that HLRCC is classically considered a "high-fumarate" disease. Our data suggest sources of cellular fumarate that are distinct from SDH. One alternative pathway is purine biosynthesis, in which fumarate is produced during the conversion of PRPP into AMP. To determine whether purine biosynthesis was implicated in fumarate production in these two cell lines, we performed another ^{13}C-glutamine tracer experiment and detected ^{13}C-glutamine incorporation into the purine intermediate

	adenylosuccinate, demonstrating that this pathway contributes to the intracellular fumarate pool in these two HLRCC cell lines. We demonstrate that HLRCC tumor cells may become reliant on purine biosynthesis, in lieu of functional FH, for fumarate production. This raises the possibility that purine antimetabolites such as 6-mercaptopurine or azathioprine could have therapeutic potential.
Publications	• Brown K, Jenkins LMM, Crooks DR, Surman DR, Mazur SJ, Xu Y, **Arimilli B**, Yang Y, Lane AN, Fan TWM, Schrump DS, Linehan WM, Ripley RT, Appella E. Targeting mutant p53-R248W reactivates WT p53 function and alters the onco-metabolic profile. *Front Oncol*. 2023;12:1094210. PMID: 36713582.
Abstract Publications	• **Arimilli B**, Crooks D, Yang Ye, Yang Y, Vocke C, Ricketts C, Ball M, Fan T, Lane A, Linehan WM. The impact of loss and mutation of mitochondrial DNA on oncometabolite accumulation in patient-derived fumarate hydratase-deficient renal tumor cell lines. J Urol. 2023; 209(Supplement 4):e495.
Professional Meetings	• American Urological Association Annual Meeting, Chicago, IL; Apr. 28-May 1, 2023.

Scholar	Thilaka Arunachalam
School	Albany Medical College
Mentors	Emily Y. Chew, M.D., Senior Investigator, Division of Clinical Trials and Applications, Clinical Trials Branch Tiarnan D. L. Keenan, M.D., Ph.D., Staff Clinician, Division of Clinical Trials and Applications, Clinical Trials Branch
NIH Institute	National Eye Institute (NEI)
Project Title	Geographic Atrophy: A Prospective Longitudinal Analysis of Macular Sensitivity using Microperimetry
Research Summary	In geographic atrophy (GA) secondary to age-related macular degeneration (AMD), optimal approaches to evaluating macular sensitivity over time are unclear. The purpose of this study was to characterize longitudinal changes in macular sensitivity using microperimetry during the 9-month natural history period of a prospective study of GA. Patients with GA in ≥ 1 eye were enrolled prospectively at the National Eye Institute (NCT02564978). Participants underwent mesopic microperimetry at baseline, 3 months, and 9 months using a Nidek MP-1 microperimeter. The T-shaped test grid extended 15° temporally, 12° superiorly, and 12° inferiorly from the fovea and included 40 testing loci evenly spaced 1° apart. The following parameters were assessed at each time point: (i) number of scotomatous loci (S), mean sensitivity in decibels (dB) (ii) of all loci, (iii) of all non-scotomatous loci (NS), and (iv) of only para-scotomatous loci averaged separately within the temporal (TPS), superior (SPS), and inferior (IPS) axes. Rate of change was analyzed by linear regression. The study population comprised 30 eyes of 30 patients (mean age 74.1 years). Mean follow-up for microperimetry was 8.2 months. Mean sensitivity ± SE (dB/month) decreased significantly for TPS loci (-0.33 ± 0.11; p=0.002), SPS loci (-0.28 ± 0.09; p=0.004) and IPS loci (-0.40 ± 0.10; p=0.001) during the follow-up period. There were no significant changes in the number of S loci (-0.02 ± 0.23 loci/month; p=0.94), mean sensitivity of all loci (-0.09 ± 0.10; p=0.37), and mean sensitivity of NS loci (-0.12 ± 0.10; p=0.21) over time.

Meaningful changes in macular sensitivity following GA progression can be detected by concentrating testing within para-scotomatous loci, corresponding to GA transitional zones. In contrast, time-dependent changes may not be evident when averaging many loci across scotomatous and non-scotomatous areas. Therefore, para-scotomatous sensitivity may be a useful functional endpoint for measuring GA progression.

Abstracts

- **Arunachalam T**, Jeffrey B, Cukras C, Chew E, Wong W, Keenan T. Geographic atrophy: a prospective longitudinal analysis of macular sensitivity using microperimetry. Association for Research in Vision and Ophthalmology Annual Meeting, New Orleans, LA; Apr. 23-27, 2023. [Poster presentation]
- Le J, Vitale S, **Arunachalam T**, Bradley C, Goldstein J, Chew E. Recalibration of the National Eye Institute Visual Functional Questionnaire and associations with age-related macular degeneration in the Age-Related Eye Disease Study. Association for Research in Vision and Ophthalmology Annual Meeting, New Orleans, LA; Apr. 23-27, 2023. [Poster presentation]
- Vitale S, Le J, Goldstein J, Bradley C, **Arunachalam T**, Chew E. Rasch-analysis calibration of the National Eye Institute-Visual Function Questionnaire (NEI VFQ-25) items in AREDS participants. Association for Research in Vision and Ophthalmology Annual Meeting, New Orleans, LA; Apr. 23-27, 2023. [Poster presentation]
- Keenan T, Bailey C, Bellur S, **Arunachalam T**, Kangale-Whitney C, Abraham M, Orndahl C, Menezes S, Jeffrey B, Wiley H, Thavikulwat A, Cukras C, Chew E, Wong W. A phase II trial evaluating oral minocycline in the treatment of geographic atrophy in age-related macular degeneration. Association for Research in Vision and Ophthalmology Annual Meeting, New Orleans, LA; Apr. 23-27, 2023. [Podium presentation]

Professional Meetings

- Association in Research in Vision and Ophthalmology (ARVO) Annual Meeting, New Orleans, LA; Apr. 23-27, 20

Awards

- Knights Templar Eye Foundation Travel Grant Award, Association for Research in Vision and Ophthalmology, 2023.

Scholar	Santiago Avila
School	University of Chicago Pritzker School of Medicine
Mentor	P. Sheila Rajagopal, M.D., Physician-Scientist Early Investigator, Cancer Data Science Laboratory
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Identifying Associations Between Reported Germline Somatic Interactions and Clinical Treatment Response in Breast Cancer
Research Summary	Clinical implementation of genomic testing is increasingly shaping prediction and prognostication for patients with breast cancer. While germline (inherited) and somatic (tumor-specific) variant data have traditionally been siloed in the clinic, recent therapeutic agent approvals in breast cancer, such as PARP inhibitors in 2018, rely on both data types being collected clinically in patients with breast cancer. Despite these advances, patients who are eligible for targeted therapies do not experience consistent disease response. Few studies examine possible interactions between germline and somatic variants, with no data on effects associated with treatment response. We hypothesize that interactions between germline and somatic variants may mediate response. This study aims to identify associations between germline-somatic variant interactions and clinical treatment response in breast cancer. In the context of this study, germline data and somatic data will focus on genes covered in clinical panel testing, and expand to whole-exome or whole-genome where possible. Variant types will include single nucleotide variants/small-scale insertions/deletions. To identify these interactions, we are obtaining clinical trial datasets with germline and somatic sequencing and clinical response to treatment in breast cancer. Sequencing data for 830 patients with general treatment response and 252 patients with detailed clinical response data, respectively, were obtained from eight clinical trials or biobanks. Variant-calling pipelines using updated benchmarked methods will be used to uniformly call and annotate variants. Individual-level patient data will be aggregated as possible through treatments and outcomes to improve statistical power. We will use statistical association testing via logistic regression to identify and define interactions between germline and somatic variants.

	This project will result in an assessment of germline and somatic data as associated with treatment response, providing contextual understanding for current biomarkers and insight into possible mechanisms for treatment resistance.
Professional Meetings	• American Association for Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 14-19, 2023.

Scholar	Taylor Badger
School	Southern Illinois University School of Medicine
Mentor	Veronica Gomez-Lobo, M.D., Director, Pediatric and Adolescent Gynecology
NIH Institute	Eunice Kennedy Shriver National Institute for Child Health and Human Development (NICHD)
Project Title	Combinatory Effects of Unilateral Oophorectomy plus Cyclophosphamide Treatment on Ovarian Reserve and Fertility in Mice
Research Summary	Ovarian tissue cryopreservation via unilateral oophorectomy (ooph) prior to gonadotoxic treatment is the gold standard for fertility preservation in pre-pubertal girls. The deleterious effects of cyclophosphamide (Cy) on ovarian follicles have been well studied, but it is not known how unilateral oophorectomy in combination with Cy may influence the ovary. The aim of this study was to develop a mouse model to investigate whether oophorectomy worsens the effects of Cy on ovarian reserve and fertility. 4–5-week-old C57BL/6 mice underwent unilateral oophorectomy or sham surgery and were treated with 75 mg/kg Cy or 0.9% saline via single intraperitoneal injection 10-11 days after surgery. Four groups were compared: 1) sham+saline, 2) sham+Cy, 3) ooph+saline, 4) ooph+Cy. Mice were sacrificed at 10 weeks old, approximately 30 days after injection, and ovary(ies) and blood were collected for histological analysis and anti-Mullerian hormone (AMH) levels, respectively. Ovarian follicles were counted, classified, and averaged in five tissue sections per mouse. We performed a TUNEL assay to evaluate the status of apoptosis in each group. AMH levels and follicles were analyzed for 15 mice. AMH levels were lowest in ooph+Cy as compared to sham+saline. Follicular count showed a greater reduction in primordial follicles (PMF) in the sham+Cy group compared to sham+saline. There were no differences in PMFs in both ooph groups compared to sham+saline. TUNEL staining revealed an increase in apoptosis in growing follicles in Cy-treated groups vs. saline-treated groups. The present data suggest that Cy may initially induce follicular growth, which may be followed by accelerated follicular loss due to apoptosis. Here we have demonstrated the feasibility of a mouse model to evaluate the effect of oophorectomy in addition to Cy as

	a risk for primary ovarian insufficiency (POI). The overall effect of oophorectomy on POI will be evaluated with mating trials, currently in progress.
Abstracts	• Kastury R, **Badger TC**, Maher J, Gomez-Lobo V. Measurement of ovarian reserve from ovarian tissue cryopreservation samples in classic galactosemia. North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting; Mar. 24-26, 2023. *J Pediatr Adolesc Gynecol.* 2023 Apr;36(2):227-228. doi: https://doi.org/10.1016/j.jpag.2023.01.082 [Podium presentation] • **Badger TC**, Dowlut-McElroy T, Lightbourne M, Maher JY, Gomez-Lobo V. Assessment of BMI and other cardiometabolic parameters in Turner syndrome. North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting; Mar. 24-26, 2023. *J Pediatr Adolesc Gynecol.* 2023 Apr;36(2):184-185. doi: https://doi.org/10.1016/j.jpag.2023.01.114 [Poster presentation] • Mascoe C, Mumford K, **Badger TC**, Lou H, Maher JY, Arlova A, Brown GT, Gomez-Lobo V. Ovarian tissue histopathology and correlation to serum markers in Turner syndrome. North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting; Mar. 24-26, 2023. *J Pediatr Adolesc Gynecol.* 2023 Apr;36(2):220. doi: https://doi.org/10.1016/j.jpag.2023.01.253 [Poster presentation] • **Badger TC**, Kavarthapu R, Balasubramanian R, Grinberg A, Mumford K, de la Luz Sierra M, Lou H, Levine J, Pfeifer K, Kim SY, Maher JY, Gomez-Lobo V. Combinatory effects of unilateral oophorectomy plus cyclophosphamide treatment on ovarian reserve and fertility in a mouse model. American Society for Reproductive Medicine Scientific Congress; Oct. 14-18, 2023. [Submitted] • **Badger TC**, Kastury R, Kavarthapu R, Balasubramanian R, Mumford K, de la Luz Sierra M, Lou H, Maher JY, Gomez-Lobo V. Investigating the role of Pi3k/Akt mediated ovarian follicle depletion in classic galactosemia. American Society for Reproductive Medicine Scientific Congress; Oct. 14-18, 2023. [Submitted]
Professional Meetings	• North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting, Nashville, TN; Mar. 24-26, 2023.

Scholar	Emma M. Byrne
School	Drexel University College of Medicine
Mentor	Mark R. Gilbert, M.D., Chief, Neuro-Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Differentiating Pseudo-progression from Progression in Immunotherapy-treated Primary Brain Tumor Patients: A Diagnostic Dilemma
Research Summary	Immune checkpoint inhibitors are under investigation as possible therapy for primary brain tumor (PBT) patients. While on treatment, patients are monitored for progression using imaging surveillance, with MRI, and patient-reported outcome (PRO) measures. However, pseudo-progression (PsP), which is seen on MRI as an increase in contrast enhancement without true tumor growth, is a diagnostic challenge when evaluating patients. Current dogma states that PsP is less symptomatic than true progression (TP), but this has not been investigated in PBT patients treated with immune therapy. Thus, this study investigated the symptomology of PBTs on immune therapy, the symptom burden of TP vs. PsP patients, and the MRI characteristics associated with TP and PsP. Six clinical trials conducted at the NIH NOB using immune therapy for PBT pts were queried. Patient demographics, prior treatments, trial data, and PROs while on trial, including MDASI and PROMIS assessment vehicles, were collected. Pre-operative MRI sequences, including T1-post contrast, T2 FLAIR, Perfusion, and DWI of the patients who had re-operation for TP vs. PsP were assessed using criteria established with a neuro-radiologist. MRI data were collected in a manner blinded to PsP or TP. One hundred sixteen patients were treated on immune therapy trials at the NCI NOB. Fifty-one underwent a re-operation to differentiate TP vs. PsP. Five patients were sampled more than once, resulting in 45 samples of TP and 13 samples of PsP. Trial and symptom data were matched and mixed-model linear regression will be used to understand the symptomology of PBT patients treated on immune therapy. Logistic regression will be used to analyze the symptoms of patients who underwent re-operation to determine TP vs PsP. Finally, inferential statistics will be used to associate MRI characteristics seen in TP and PsP.

Publications

- **Byrne E**, Pascoe M, King A, Cooper D, Armstrong TS, Gilbert MR. The challenges and limitations of clinical trials in the adolescent and young adult (AYA) CNS cancer population: a systematic review. [In preparation]

Scholar	Manuel A. Cintron
School	University of Illinois College of Medicine, Peoria
Mentor	Tiffany Powell-Wiley, M.D., Stadtman Investigator, Social Determinants of Obesity and Cardiovascular Risk Laboratory
NIH Institute	National Heart Lung and Blood Institute (NHLBI)
Project Title	Chronic Stress-induced Inflammation and Epigenetic Dysregulation: A Potential Pathway for Increased Cardiovascular Disease Risk Through Trimethylamine N-oxide: Data from the Washington, DC Cardiovascular Health and Needs Assessment
Research Summary	Chronic stress is known to be a risk factor for cardiovascular disease (CVD), but the underlying mechanisms remain unclear. Research suggests that chronic stress increases neural and amygdalar activity (AmygA) and may be associated with elevated levels of trimethylamine n-oxide (TMAO), a metabolite that has been linked to CVD risk. We investigated whether TMAO could serve as a mediator of DNA epigenetic regulation advancing CVD development and progression. 60 African American adults (93% female, mean age 61 ± 11 years) at risk for CVD living in the Washington DC area participated in a cross-sectional, community-focused study. Participants had a ^{18}FDG-PET/CT to assess chronic stress-related AmygA. DNA methylation was assessed on buffy coat samples, and serum and plasma samples were used for cytokine and TMAO measurement using ELISA-based techniques. Multivariable regression analyses adjusted for atherosclerotic cardiovascular disease (ASCVD) 10-year risk score and body mass index (BMI)-identified associations between AmygA and TMAO. IL-1β, TNF-α, IL-6, IL-8, and IFN-γ were evaluated as mediators of indirect associations between AmygA and TMAO. Multivariable regression modeling revealed significant associations between AmygA and TMAO (β=0.32, p=0.02), AmygA and IL-1β (β=0.36, p=0.003), AmygA and TNF-α (β=0.31, p=0.024), IL-1β and TMAO (β=0.34, p=0.007), TNF-α and TMAO (β=0.43, p=0.0010), TMAO and NF-kB2 cg26810474 methylation site (β=0.31, p=0.017), and NF-kB, in the fully adjusted model. IL-1β and TNF-α mediated the AmygA and TMAO relationship at effect rates of 43.51% and 38.06%, respectively.

	The association between AmygA as a measure of chronic stress and TMAO, a CVD risk biomarker, is mediated by IL-1β and TNF-α. Furthermore, TMAO has been found to promote NF-kB gene expression, which is associated with vascular inflammation, suggesting the potential for chronic stress-related inflammation in the brain-gut axis, potentially influencing CVD in underserved communities. These hypothesis-generating results should be studied further by including large, diverse populations.
Publications	• Baumer Y, Pita M, Baez A, Ortiz-Whittingham L, **Cintron M**, Rose R, Gray V, Baah, Powell-Wiley T. By what molecular mechanisms do social determinants impact cardiometabolic risk? *Clin Sci (Lond).* 2023 Mar 31;137(6):469-494. PMID: 36960908.
Abstracts	• **Cintron M**, Ortiz-Whittham L, Baumer Y, Farmer N, Collins B, Wallen G, Powell-Wiley T. Interleukin-1 β mediation of chronic stress-related neural activity and trimethylamine n-oxide – data from the Washington DC cardiovascular health and needs assessment. American Heart Association Epidemiology and Prevention-Lifestyle and Cardiometabolic Health Scientific Sessions, Boston, MA; Feb. 28-Mar. 3, 2023. [Poster presentation] • Baumer Y, Ortiz-Whittham L, **Cintron M,** Tarfa H, Dave A, Saurab A, Collins B, Mitchell V, Powell-Wiley T. Corticosterone-mediated associations between neighborhood environment perceptions and lysosomal acid lipase levels-data from the Washington D.C. cardiovascular health and needs assessment. American Heart Association Arteriosclerosis, Thrombosis, and Vascular Biology Vascular Discovery: From Genes to Medicine 2023 Scientific Sessions, Boston, MA; May 10-13, 2023. [Poster presentation]
Professional Meetings	• American Heart Association Epidemiology and Prevention - Lifestyle and Cardiometabolic Health Scientific Sessions, Boston, MA; Feb. 28-Mar. 3, 2023.
Awards	• American Heart Association Council on Epidemiology and Prevention's Underrepresented Racial and Ethnic Groups Travel Grant, 2023.

Scholar	Leah H. Cobb
School	Medical University of South Carolina
Mentors	Catherine Cukras, M.D., Ph.D., Lasker Clinical Research Scholar, Unit on Clinical Investigation of Retinal Disease Laryssa Huryn, M.D., Medical Officer, Ophthalmic Clinical Genetics Section
NIH Institute	National Eye Institute (NEI)
Project Title	Relationship of Patient-Reported Outcomes with Visual Function in Retinitis Pigmentosa
Research Summary	Patients with retinitis pigmentosa (RP) suffer peripheral vision loss while retaining good central visual acuity (VA) until late in the disease. As therapies are developed and tested in clinical trials for RP, outcome measure selection becomes critical to evaluate the impact of these treatments on the patient. Patient-reported outcomes (PROs) are often considered. Understanding the correlation of PROs to measures of visual function (VF) would help justify the choice of PRO. In this study, we administered three different PROs to RP patients and healthy volunteers (HV) and analyzed their association with objective visual measures. In this single-center observational cross-sectional study, RP (n=26) and HV (n=15) subjects completed the National Eye Institute Visual Function Questionnaire (NEI-VFQ) and Vision Quality of Life (VisQoL) survey. At follow-up, the Independent Mobility Questionnaire (IMQ) was administered. At every visit VA, mean deviation (MD) measures of VF, and ophthalmic examination were performed. RP patients were divided into severity groups of mild, moderate, and severe based on MD values of ≥-15, -15 to -20, and ≤-20 dB, respectively. All analyses were performed utilizing Graphpad® with significance set at $p<0.05$. HV and RP scores were significantly different for almost every question on all 3 PROs. Several questions on the NEI-VFQ and IMQ displayed stepwise changes in score with increasing severity of RP. Only two PRO questions correlated with VA, both from the NEI-VFQ: Q7 (finding something on a crowded shelf, $R^2=0.2$, $p=0.022$) and Q8 (reading street signs, $R^2=0.22$, $p=0.015$). The strongest associations of PRO scores and VF were for IMQ questions on mobility in public places (Q6,8 $R^2=0.30$-0.39), bumping into things (Q25,26

	R^2=0.28-0.30) and NEI-VFQ questions on driving status (Q15, R^2=0.40), and finding something on a crowded shelf (Q7, R^2=0.34). Our results demonstrate the potential of PROs to reflect meaningful functional differences across RP severities, which supports their utility as outcome measures.
Publications	• Xiao C, **Cobb L**, Jeffrey B, Ferreira C, Das S, Gahl W, Huryn L. Peripheral retinopathy in patients with *ELOVL4*-related spinocerebellar ataxia. *Gene*. [Under review]
Abstracts	• **Cobb L**, Huryn L, Zein W, Brooks B, Vitale S, Chew E, Cukras, C. Association of patient-reported outcomes with objective visual function measures in retinitis pigmentosa. Association for Research in Vision and Ophthalmology (ARVO) Annual Conference, New Orleans, LA; Apr. 23-27, 2023. [Poster presentation] • Dave A, **Cobb L**, Huryn L, Zein W, Brooks B, Cukras C. Correlations of optical coherence tomography measurements to visual function in patients with retinitis pigmentosa. Association for Research in Vision and Ophthalmology (ARVO) Annual Conference, New Orleans, LA; Apr. 23-27, 2023. [Poster presentation]
Professional Meetings	• Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, New Orleans, LA; Apr. 23–27, 2023.

Scholar	Rod Carlo Columbres
School	William Carey University College of Osteopathic Medicine
Mentors	Andrea B. Apolo, M.D., Chief, Bladder Cancer Section, Genitourinary Malignancies Branch A. Rouf Banday, Ph.D., Stadtman Investigator, Genitourinary Malignancies Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Exploring the Therapeutic Potential of SF3B1 Inhibitors in Bladder Cancer
Research Summary	RNA splicing dysregulation is considered to be a hallmark of cancer, occurring in multiple solid and hematological malignancies. Splicing factor 3 subunit B1 (SF3B1), a key component of the U2 small nuclear ribonucleoprotein (snRNP) complex that regulates RNA splicing, is frequently altered in a variety of cancer types, including non-muscle-invasive and muscle-invasive bladder cancer, where the E902K mutation is common. In this study, we investigated the therapeutic potential of the SF3B1 inhibitor, pladienolide B, in bladder cancer using *in vitro* models. We used a recombinant plasmid containing the SF3B1^{E902K} mutation and established a UMUC3^{E902K} mutant urothelial cell line. Both UMUC3^{WT} and UMUC3^{E902K} cells were treated with pladienolide B alone or in combination with cisplatin to evaluate differences in cell viability and cell apoptosis. We determined that the IC50 for pladienolide B is 5nM for UMUC3 cells, which is observed at 72 hours post-treatment. Pladienolide B halted the cell cycle and caused cell death by apoptosis, as assessed by annexin V staining and propidium iodide exclusion, respectively, for both UMUC3^{WT} and UMUC3^{E902K} cell lines. However, UMUC3^{E902K} cells were significantly more sensitive to pladienolide B compared to UMUC3^{WT} cells ($p \leq 0.05$). We also observed a synergistic effect after co-treatment with low-dose pladienolide B (2 nM) and cisplatin (3 µM) for both UMUC3^{WT} and UMUC3^{E902K} cell lines, with UMUC3^{E902K} cells showing greater sensitivity to combination treatment. These results provide insights into the potential use of splicing inhibitors, alone or in combination with chemotherapy, for treating bladder cancer, specifically for tumors that bear driver mutations in splicing factors. Currently, we are evaluating the mechanism by which SF3B1 mutations make cells sensitive to pladienolide B *in vitro* and *in vivo,* with the goal of identifying specific mRNA splicing vulnerabilities that can be targeted by anti-sense or splice-switching oligonucleotides to achieve anti-tumor outcomes.
Publications	• **Columbres RC**, Apolo AB, Banday AR. Regulation of alternative splicing in bladder cancer: clinical and diagnostic implications. [In preparation]

Abstracts

- **Columbres RC**, Simon NI, Chandran E, Lei K, Verdini NP, Lin J, Vega A, Niglio SA, Cordes LM, Ley L, Wang T, Mortazavi A, Pal SK, Knopp MV, Wright C, Jung A, Steinberg SM, Gonzalez EM, Lidenberg L, Apolo AB. FDG PET/CT and NaF PET/CT imaging quantification of osseous metastatic lesions in patients with metastatic genitourinary cancer and their association with survival outcomes. American Society of Clinical Oncology Annual Meeting, Chicago, IL; June 2-6, 2023. J Clin Oncol 41, 2023 (suppl 16; abstr e16573).
- Simon NI, **Columbres RC**, Chandran E, Niglio SA, Cordes LM, Ley L, Wang T, Mortazavi A, Pal SK, Munian-Govindan R, Perk TG, Gonzales EM, Lidenberg L, Apolo AB. Association of 18F-FDG PET characteristics and survival outcomes using whole body tumor analysis in patients with metastatic genitourinary malignancies. American Society of Clinical Oncology Annual Meeting, Chicago, IL; June 2-6, 2023. J Clin Oncol 41, 2023 (suppl 16; abstr 4600). [Poster Presentation]
- **Columbres RC**, Chakraborty A, Apolo AB, Banday AR. Characterizing the role of spliceosome factor 3 isoform B1 (SF3B1) alterations in bladder cancer. Association of American Physicians, American Society for Clinical Investigation, American Physician Scientists Association Joint Meeting, Chicago, IL; Apr. 21-23, 2023. [Poster presentation]
- **Columbres RC**, Chakraborty A, Apolo AB, Banday AR. Exploring therapeutic potential of SF3B1 inhibitors in bladder cancer. National Cancer Institute RNA Biology Symposium, Bethesda, MD; Apr. 27-28, 2023. [Poster presentation]

Professional Meetings

- American Society of Clinical Oncology (ASCO) Genitourinary Cancers Symposium, San Francisco, CA; Jan. 25-27, 2023.
- American Urological Association / Johns Hopkins Greenberg Bladder Cancer Initiative Bladder Cancer Research Symposium, Linthicum, MD; Mar. 3-4, 2023.
- Association of American Physicians (AAP), American Society for Clinical Investigation (ASCI), American Physician Scientists Association (APSA) Joint Meeting, Chicago, IL; Apr. 21-23, 2023.
- National Cancer Institute (NCI) RNA Biology Symposium, Bethesda, MD; Apr. 27-28, 2023.
- American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago, IL; June 2-6, 2023.

Scholar	Briana N. Cortez
School	University of Texas Rio Grande Valley School of Medicine
Mentor	Nitin Roper, M.D., Lasker Clinical Research Scholar, Developmental Therapeutics Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Advancing Precision Medicine in Neuroendocrine Tumors using Patient-Derived Organoids
Research Summary	Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms that most commonly develop in the gastrointestinal tract, pancreas and lungs. Everolimus, an mTOR inhibitor, is one of very few approved therapies for NETs. Drug development has been challenging due to limited pre-clinical models, with only three single cell lines available in the field (BON-1, QGP-1, and NT-3). The NCI Natural History Study of Children and Adults with Neuroendocrine Neoplasms (NCT05237934) aims to address this need by developing preclinical models. Hundreds of compounds were screened for anti-tumor effect in the BON-1 cell line by the National Center for Advancing Translational Science, and TAK243, a ubiquitin inhibitor, was shown to have the greatest anti-tumor effect. We tested whether TAK243 would show synergy with everolimus in patient-derived organoids (PDOs), and against BON-1 and QGP-1. PDOs were developed from liver metastasis of a primary small bowel tumor and lung primary NET tissue from patients NET27 and NET28, respectively. Tissue enzymatic digestion was performed and cells were plated in Matrigel to allow for organoid growth. After 24 hours, everolimus and TAK243 were added to wells individually and in combination. After 72 hours, the Cell Titer Glo assay was performed to determine percent viability. Overall, TAK243 showed potent anti-tumor activity in BON-1 (EC50=20.2 nM), QGP-1 (EC50=15.6 nM), NET27 (EC50=681 nM), and NET28 (EC50=10.5nM). Everolimus did not show strong anti-tumor effects in BON-1 (EC50=7873 nM), QGP-1 (EC50=11729 nM), NET27 (EC50=14481 nM), or NET28 (EC50=2587 nM), which was expected due to its known cytostatic behavior in NETs. Synergistic effects were seen with the two-drug combination against BON-1, QGP-1, NET27, and NET28.

We have developed PDOs which can be used as a tool for *in vitro* drug testing. Combination of TAK243 and everolimus showed synergy in NET cell lines and PDOs. Future work will assess *in vivo* effects and dose-toxicity relationships in rodents.

Abstracts

- **Cortez BN**, Varghese DG, Naimian A, Mallorson R, Kelley S, Magee T, Roper N, Del Rivero, J. Severe hypertriglyceridemia and acute pancreatitis in a patient with adrenocortical carcinoma (ACC) taking mitotane. *Endocrine (ENDO) Society Conference,* Chicago, IL; June 15-18, 2023. [Poster presentation]
- **Cortez BN**, Varghese DG, Naimian A, Mallorson R, Kelley S, Magee T, Roper N, Del Rivero, J. A rare disease masked by a common syndrome: small bowel neuroendocrine tumor case report. American College of Physicians (ACP) Internal Medicine Meeting, San Diego, CA; Apr. 27-29 2023. [Poster presentation]
- Mallorson R*, **Cortez BN***, Kumar S, Arakawa Y, Varghese D, Kaplan R, Reilly K, Widemann B, Thomas C, Pommier Y, Roper N, Del Rivero J. Natural history study and tissue procurement of neuroendocrine neoplasms. Gastrointestinal Cancers Symposium, American Society of Clinical Oncology (ASCO), San Francisco, CA; Jan. 18-20, 2023. [Poster presentation]
- **Cortez BN**, Kumar S, Arkcawa Y, Varghese D, Kaplan R, Reilly K, Widemann B, Thomas C, Pommier Y, Roper N, Del Rivero J. Patient-derived organoids and their potential for precision medicine in neuroendocrine tumors. Neuroendocrine Tumor Research Foundation Symposium, Boston, MA; Nov. 16-18, 2022. [Poster presentation]

Professional Meetings

- North American Neuroendocrine Tumor Society (NANETS) Symposium, Washington DC; Oct. 27-29, 2022.
- Neuroendocrine Tumor Research Foundation (NETRF) Symposium, Boston, MA; Nov. 16-18, 2022.
- Endocrine Society Future Leaders Advancing Research in Endocrinology (FLARE) Workshop, Los Angeles, CA; Mar. 16-18, 2023.
- American College of Physicians (ACP) Internal Medicine Meeting, San Diego, CA; Apr. 27-29, 2023.

Scholar	Julia M. Denniss
School	Duke University School of Medicine
Mentor	Avindra Nath, M.D., Clinical Director, NINDS; Chief, Section of Infections of the Nervous System
NIH Institute	National Institute of Neurological Disorders and Stroke (NINDS)
Project Title	Extracellular Vesicle-derived HIV RNA in CSF and Serum Reservoirs and Its Role in Mediating Neurocognitive Dysfunction
Research Summary	Having previously demonstrated the presence of HIV-1 RNA in extracellular vesicles (EVs) isolated from patient CSF and serum in a cohort of HIV-infected individuals with well-controlled disease on combined antiretroviral therapy (cART), we used seven Digital Droplet PCR (ddPCR) assays to evaluate the transcriptional status of the reservoir at various sequence regions, including (i) Readthrough (affected by transcriptional interference), (ii) TAR (required for transcriptional initiation), (iii) R-U5/pre-Gag (long LTR, suggests elongation proximal to the 5' end), (iv) Pol (indicating elongation past the region of the initial Gag viral protein), (v) Nef (distal protein), (vi) U3-polyA (indicating complete transcription of the HIV protein), and (vii) Tat-Rev (the multiply-spliced complete protein, indicating productive infection). We found that levels of the RNA transcripts differed significantly depending on the reservoir from which they were derived. The transcripts were found at higher levels in the CSF, suggesting poorer viral control in the CNS reservoir. Additionally, the percent of patients with detectable levels of viral transcripts (defined as a concentration > 0) differed by reservoir. We did not find a correlation between matched serum and CSF RNA concentrations. These findings provide support for our hypothesis of a separate CNS reservoir with a unique transcriptional profile. Our results indicate significant correlations between Long LTR and global deficit score, attention/working memory, information processing, and verbal fluency, as well as a summative T-score of the seven categories (all of which decrease with worsening deficit). The Long LTR transcript serves as a scaffold which recruits suppressive transcription remodelers, contributing to viral latency via epigenetic silencing. Our findings suggest that there is basal viral transcription associated with neurological complications of long-

	term disease. This novel mechanism, which contributes to neurological dysfunction, could be targeted to improve quality of life and achieve a functional cure for HIV-1 infection.
Publications	• **Denniss J**, DeMarino C, Smith B, Snow J, Henderson L, Pandya D, Nath A. Extracellular vesicle-derived HIV RNA in CSF and serum reservoirs and its role in mediating neurocognitive dysfunction. *Nat Microbiol* [Under review]
Abstracts	• **Denniss J**, DeMarino C, Smith B, Snow J, Henderson L, Pandya D, Nath A. Extracellular vesicle-derived HIV RNA in CSF and serum reservoirs and its role in mediating neurocognitive dysfunction. Extracellular RNA Communication Consortium (ERCC) Annual Meeting, Bethesda, MD; May 1-2, 2023. [Poster presentation] • **Denniss J**, DeMarino C. HIV RNA in CSF extracellular vesicles: An update. NIH Intramural NeuroHIV Research Workshop, Bethesda, MD; Feb. 10, 2023. [Podium presentation]
Professional Meetings	• Viral Infections and Inflammation Workshop 2022, Rockville, MD; Sept. 8-9, 2022. • NIH Intramural NeuroHIV Research Workshop, Bethesda, MD; Feb. 10, 2023. • Extracellular RNA Communication Consortium (ERCC) Annual Meeting, Bethesda, MD; May 1-2, 2023.

Scholar	Barrett Dryden
School	Washington University School of Medicine in Saint Louis
Mentor	Diane Damiano, Ph.D., Senior Investigator, Rehabilitation Medicine Department
NIH Institute	Clinical Center (CC)
Project Title	The Role of Dopamine for Implicit Learning in Cerebral Palsy

Research Summary

Cerebral palsy (CP) is the most common child-onset motor disability and is caused by a multitude of environmental and genetic factors. Individual responses to treatments can vary widely among patients. It is crucial to understand how individual characteristics drive the spectrum of response to therapy. Dopamine transmission and synaptic plasticity contribute to motor learning, through multiple brain pathways. The role of dopamine and the genes that influence the level of brain dopamine have rarely been studied in CP.

Motor and implicit procedural learning were evaluated by a randomized, controlled prospective crossover study in 28 subjects, ages 6.5-25 years, with the Serial Reaction Time Task and Weather Prediction Task. These tasks were administered with and without rewards to modulate reward (dopamine-related) pathways. A random, improbable sequence was used to examine visuomotor plasticity and a repeated, probable sequence was used to examine implicit sequence learning. The effects of polymorphisms of five genes related to dopamine transmission were aggregated into a single gene score and compared against measures of performance on the tasks. The second hypothesis examined how CP patients (n=13, ages 6.5-25 years) would differ from a control group (n=12, ages 8.1-25 years) on the tasks since damage to dopamine pathways due to brain injury can also affect learning. No correlation was found between gene score and visuomotor learning ($p = 0.8$, $p = 0.9$) or sequence learning ($p = 0.7$, $p = 0.4$). There was an association between diagnosis of CP and sequence learning when rewards are given ($p = 0.03$) and similarly between diagnosis of CP and response to rewards for visuomotor learning ($p = .04$) and implicit sequence learning ($p = .02$).

An association between genetic variation in the dopamine system and learning ability in subjects with CP was not observed. The differential response to rewards between those with a diagnosis of CP and healthy individuals suggests that reward-based training can compensate for damage to dopamine pathways in CP and thereby improve motor and procedural learning.

Scholar	Riley S. Ferguson
School	University of Cincinnati College of Medicine
Mentor	Wei Li, Ph.D., Chief, Retinal Neurophysiology Section
NIH Institute	National Eye Institute (NEI)
Project Title	Microglia in Spaceflight-Associated Neuro-ocular Syndrome: Adaptations to Chronic Simulated Microgravity
Research Summary	Spaceflight-Associated Neuro-ocular Syndrome (SANS) has been identified as one of the greatest barriers to expedition-length human spaceflight and is of translational value to other pressure-related pathologies including glaucoma and idiopathic intracranial hypertension. We endeavored to better understand the ocular pathophysiology, especially the role of microglia, in the chronically (>60 days) microgravity-exposed eye as a model of SANS. Hindlimb-unloading (HLU) was used to simulate chronic microgravity exposure. Using two different mouse strains, Piezo1(Pz1)-flox and Pz1-/-, four cohorts of 12 mice each were exposed to four experimental conditions: Normal Gravity Pz1-flox, HLU Pz1-flox, Normal Gravity Pz1 -/-, and HLU Pz1 -/-. All mice underwent tonometry, optical coherence tomography (OCT), and electroretinogram recording every 14 days over a 60-day exposure. Retinal tissue was analyzed for the phenotypes of microglia, Pz1 activation, and retinal ganglion cell quantification at the conclusion of the exposure. Intraocular pressure (IOP) was differently regulated between Normal Gravity cohorts and their respective HLU comparisons for each strain [p = 0.0018]. OCT imaging demonstrated significant increase in optic nerve head width (ONHW) in HLU animals of both strains [p=0.0001]. Interestingly, PZ1 -/- optic nerve head width was less increased than Pz1-flox. In both strains, HLU demonstrated a significant loss of retinal ganglion cells in the central regions compared to normal gravity counterparts [p = 0.008]. Chronic HLU effectively reproduced the hypothesized effects of SANS on the mouse eye in both Pz1-flox and Pz1 -/- strains. Pz1 -/- demonstrated faster downregulation of IOP and less optic nerve head swelling, indicating Pz1 in microglia may contribute to

	SANS pathophysiology and offering a potential therapeutic target.
Publications	• **Ferguson RS,** Pantalos G. Central venous pressure in microgravity: A review. • *Aerosp Med Hum Perform.* [Under review]
Abstracts	• **Ferguson RS**; Pantalos G. Cardiovascular pressure responses to microgravity: a review. Aerospace Medical Association Annual Scientific Meeting, New Orleans, LA; May 2023. [Podium presentation] • McNerlin C; **Ferguson RS**. Mental health in aviation: a review of accident reports. Aerospace Medical Association Annual Scientific Meeting. New Orleans, LA. May 2023. [Podium Presentation]. • **Ferguson RS**, Nadal-Nicolas F, Li W. Characterizing the rod A-II amacrine bipolar cell pathway in cone-dominated thirteen-line ground squirrels. Association for Research in Vision and Ophthalmology (ARVO), New Orleans, LA; Apr. 2023. [Poster presentation] • **Ferguson RS**, Nadal-Nicolás F, Li W. Thirteen-lined ground squirrels: A novel animal model for Spaceflight-Associated Neuro-ocular Syndrome. International Human in Space Conference, Sydney, Australia; Oct. 2022. [Virtual podium presentation]. • Girgla N, **Ferguson RS,** Girgla T, Bagian J. Creation of an online Aerospace Medicine course for medical students. International Conference of Aerospace Medicine, Paris, France; Sep. 2022. [Poster presentation].
Professional Meetings	• Aviation Mental Health Symposium, Grand Forks, ND; Nov. 16, 2022. • Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, New Orleans; Apr. 21-25. 2023. • Aerospace Medical Association Annual Scientific Meeting (AsMA), New Orleans, LA; May 21- 26, 2023.
Awards	• Space Surgery Future Researcher Award, Space Surgery Association & Aerospace Medical Association, 2023. • Exceptionally Good Review Award, Investigative Ophthalmology & Visual Science, 2022.

Scholar	Caitlin Elise Foster
School	University of Connecticut School of Medicine
Mentor	Laura Kerosuo, Ph.D., Stadtman Investigator, Neural Crest Development and Disease Unit
NIH Institute	National Institute of Dental and Craniofacial Research (NIDCR)
Project Title	The Central Role of Centrioles: How a Loss-of-function Mutation in *CenpJ* Leads to Craniofacial Dysgenesis
Research Summary	Mutations in the gene centromeric protein J (CENPJ) have been associated with human birth defects including primary microcephaly (reduced brain size) and Seckel syndrome, a primordial dwarfism disorder characterized by the presence of microcephaly and craniofacial defects including reduced jaw size (micrognathia) and expansion of the facial midline (hypertelorism). CENPJ is an important structural protein critical for centriole duplication and maintenance. Centrioles perform two distinct cellular functions: (i) they form centrosomes, which function as microtubule organizing centers of the cell, and (ii) they form basal bodies that template formation of the cilium, a microtubule-based specialized signaling organelle. While the role of CENPJ in brain development has been extensively studied, the molecular basis driving the development of craniofacial defects is less clear. Since global deletion results in midgestation lethality, we created a *CenpJ* conditional knockout mouse (cKO) model using a Sox9-cre driver, which deletes the gene in multiple tissues of the craniofacial complex. We find that the Sox9-cre; *CenpJ* cKO mice present with hypertelorism, hypoplastic maxilla and mandible, and retrognathia, recapitulating Seckel patient phenotypes and rendering these mice an excellent model to investigate the molecular mechanism driving craniofacial dysmorphology. Developmental analysis revealed that midfacial widening emerges as early as mouse embryonic stage E11.5 ($p<0.01$). Preliminary results also show that deletion of *CenpJ* leads to progressive centriole loss ($p<0.01$) and increased cell death ($p<0.01$) in stages E10.5, E11.5 and E12.5, likely due to upregulation of p53 activation. Consequent knockout of p53 rescued the craniofacial defects in the Sox9-*cre*; *CenpJ* cKO mice, demonstrating the central role of p53 activation in driving this phenotype. Ongoing investigations aim to elucidate the spatiotemporal role of centrioles in craniofacial development to gain insights into the etiology of Seckel syndrome.

Abstracts	• **Foster CE**, Abrams SR, Xie C, Reiter J, Kerosuo L. Central role of centrioles: Loss-of-function mutation leads to craniofacial dysgenesis. American Association for Dental, Oral and Craniofacial Research/Canadian Association for Dental Research (AADOCR/CADR) Annual Meeting & Exhibition, Portland, OR; Mar. 15-18, 2023. [Podium presentation] • **Foster CE**, Abrams SR, Xie C, Reiter J, Kerosuo L. Tissue specific requirements for centrioles in craniofacial morphogenesis. Society for Developmental Biology 12th Structural Birth Defects Meeting, Rockville, MD; Oct. 18-20, 2022. [Poster presentation]
Professional Meetings	• Society for Developmental Biology 12th Structural Birth Defects Meeting, Rockville, MD; Oct. 18-20, 2022. • American Association for Dental, Oral and Craniofacial Research/Canadian Association for Dental Research (AADOCR/CADR) Annual Meeting & Exhibition, Portland, OR; Mar. 15-18, 2023. • Society for Developmental Biology Mid-Atlantic Regional Meeting, Princeton, NJ; Apr. 20-22, 2023.
Awards	• First Place Award, Fellows Poster Competition, National Institute of Dental and Craniofacial Research (NIDCR) Annual Fellows Scientific Training, 2023. • Second Place Award, Poster Competition in Graduate Student Category, Society for Developmental Biology Mid-Atlantic Regional Meeting, 2023.

Scholar	Thomas R. Gossard
School	Creighton University School of Medicine
Mentor	Michael E. Ward, M.D., Ph. D., Investigator, Neurogenetics Branch
NIH Institute	National Institute of Neurological Disorders and Stroke (NINDS), Center for Alzheimer's and Related Dementias (CARD)
Project Title	Evaluation of Neurodegeneration via the iPSC Neurodegenerative Disease Initiative
Research Summary	The iPSC neurodegenerative disease initiative (iNDI) is the largest ever iPSC genome engineering project, designed to model more than 134 variants spanning 74 genes in Alzheimer's disease (AD) and related dementias. As part of this project, we are using CRISPR inhibition (CRISPRi) to evaluate the effects of gene knockdown on various common mutations in neurodegenerative disease. CRISPRi uses CRISPR/Cas9 machinery to knock down genes by targeting a catalytically dead Cas9 protein combined with a repressor protein to specific regions of the genome. Greater than 800 cell lines are being analyzed, so that each step of the process must be optimized. First, we evaluated several Cas9 variants and two Cas12f variants containing different nuclear localization sequences. Using flow cytometry, we discovered that each Cas9 variant achieved 89-95% efficiency in gene knockdown, while the Cas12f variant did not achieve any knockdown. Second, iPSC-derived neurons most closely resemble embryonic neurons, which makes it difficult to generalize our findings to neurodegeneration, which commonly occurs in the elderly. We modeled several methods to induce aging in our iPSC-derived neurons, based on proposed mechanisms of neuronal aging. In our initial studies, we used pharmacological agents and employed biological mechanisms that induce DNA breaks, hindering cell cycle checkpoints, impairing transcription-coupled repair, and knocking down genes necessary for methylation maintenance. The 'age' of these iPSC-derived neurons will be measured by their methylation signature, which can be translated to a DNA 'age' by a well-characterized method. Analysis of our initial findings are currently in progress. The first phase of the project is approaching completion, and our preliminary data has led to proposals for new mechanisms of neurodegeneration.
Professional Meetings	• 23rd Annual Packard Center ALS Research Symposium, Baltimore, MD; Feb. 27-28, 2023.

Scholar	Alexis L. Green
School	University of Maryland School of Medicine
Mentor	Anna Nápoles Ph.D., Scientific Director, Division of Intramural Research Paula Strassle Ph.D., Staff Scientist, Division of Intramural Research
NIH Institute	National Institute on Minority Health and Health Disparities (NIMHD)
Project Title	Association between Patient-Provider Racial-Ethnic and Gender Concordance and Adherence to Preventive Services Guidelines Across Race-Ethnicity and Gender
Research Summary	Patient-provider racial-ethnic and gender concordance have been shown to improve healthcare utilization and reduce disparities, but their impact on preventive services utilization remains unclear. This study aimed to assess if patient-provider racial-ethnic or gender concordance was associated with increased preventive services utilization. This study utilized data from the 2018 and 2020 Medical Expenditure Panel Survey. Responses were weighted to be nationally representative. Respondents were excluded if they were <18 years old or reported not having a usual healthcare provider. Associations between racial-ethnic and gender concordance with preventive services were estimated using Poisson regression, adjusting for patient and provider demographics and patient health characteristics. Analyses were restricted to the guideline-relevant population (e.g., only women 50-74 were included when assessing breast cancer screening). The majority of White (82.1%), Hispanic (74.0%), and Asian (52.7%) adults had race-ethnicity concordant providers; prevalence was much lower among Black (23.8%), American Indian/Alaska Native (11.8%), and Pacific Islander (1.6%) adults. Gender concordance was higher among men (69.7%) than women (51.1%). Black/African American participants with racial-ethnic concordance were significantly more likely to have received influenza (PR=2.02, 95% CI=1.08-3.79), zoster (PR=1.48, 95% CI=1.20-1.83) and pneumococcal (PR=1.78, 95% CI=1.31-2.42) vaccinations, as well as colon cancer screening (PR=1.26, 95% CI=1.05, 1.52), compared to those without concordance. Racial-ethnic concordance was also associated with increased likelihood of receiving the pneumococcal vaccine among Hispanic participants (PR=2.06, 95% CI=1.48-2.85) and the zoster vaccine among Asian participants (PR=1.28, 95% CI=1.04-1.58). Among women,

	gender concordance was associated increased utilization of all vaccinations, cancer screenings, and cardiovascular screenings studied. Lack of provider diversity continues to be a healthcare issue that, if improved, could help decrease racial-ethnic and gender disparities in preventive services utilization. Healthcare provider education should address the potential impact of racial-ethnic and gender concordance on preventive services utilization and strategies for engendering trust, especially in discordant medical encounters.
Publications	• Strassle PD, **Green AL**, Colbert CA, Stewart AL, Nápoles AM. COVID-19 vaccination willingness and uptake among rural Black/African American, Latino, and White adults. *J Rural Health*. 2023;10.1111/jrh.12751. PMID: 36863851. • **Green AL**, Stewart AL, Nápoles AM, Strassle PD. COVID-19 vaccination willingness and uptake among low-income Black/African American, Latino, and White adults. *Prev Med Rep*. [Under review] • Wilkerson MJ, **Green AL**, Forde AT, Ponce SA, Stewart AL, Nápoles AM, Strassle PD. COVID-related discrimination and inability to get needed health care, COIVD-19 tests, and COVID-19 vaccines among a nationally-representative, diverse sample of U.S. adults. *BMC Public Health*. [Under review]
Abstracts	• **Green AL**, Nápoles AM, Mendez I, Strassle PD. Association between COVID-19 vaccine information sources and vaccine uptake among a diverse sample of U.S. adults. Academy Health Annual Research Meeting, Seattle, WA; June 24-27, 2023. [Poster presentation] • **Green AL**, Nápoles AM, Strassle PD. Impact of patient-provider racial-ethnic and gender concordance on use of preventive services in the U.S. Academy Health Annual Research Meeting, Seattle, WA; June 24-27, 2023. [Poster presentation]
Professional Meetings	• Academy Health Annual Research Meeting, Seattle, WA; June 24-27, 2023.

Scholar	Dennis A. Gross
School	University of Central Florida College of Medicine
Mentors	Michael A. Solomon, M.D., Head, Cardiology Section, Critical Care Medicine Department Jason Elinoff, M.D., Head, Pulmonary Vascular Biology Section, Critical Care Medicine Department, CC Sean Agbor-Enoh, M.D., Ph.D., Lasker Clinical Research Scholar, Applied Precision Omics, NHLBI
Institutes	NIH Clinical Center (NIH-CC); National Heart, Lung, and Blood Institute (NHLBI)
Project Title	Evaluating Cell-Free Mitochondrial DNA as a Diagnostic Tool in Pulmonary Arterial Hypertension
Research Summary	Pulmonary arterial hypertension (PAH) is a rare disease, characterized by elevated PA pressures and high mortality. Cell free nuclear DNA (cf-nDNA) fragments released into circulation by injured cells have been shown to be a biologically plausible non-invasive biomarker for PAH detection. In addition to cf-nDNA, cell-free mitochondrial DNA (cf-mtDNA) is also released. Cf-mtDNA could provide greater diagnostic sensitivity, given higher circulating abundance. Our aims were twofold: (1) to assess cf-mtDNA's utility as a PAH biomarker; and (2) to understand relative cf-mtDNA contribution by known cell/tissue types injured in PAH. Biomarker utility of cf-mtDNA was assessed using plasma collected from 96 PAH patients and 16 controls. Quantification was performed using ddPCR and a mtDNA-specific primer set. Cf-mtDNA concentrations distinguished PAH patients from controls by 1.36 $\log_{10}$ cf-mtDNA copies/mL ($p=<0.0001$). The PAH group had a higher cf-mtDNA/nDNA ratio compared to controls (393.7 vs 63.7 copies/mL, $p=0.002$). In addition, no correlation was observed between concentrations of cf-mtDNA and cf-nDNA. To further understand the lack of correlation between cf-mtDNA and cf-cDNA, we assessed whether four of seven previously described sources of cfnDNA in PAH (neutrophils, natural killer cells, monocytes, and vascular endothelial cells) demonstrate varying mtDNA content, measured as mtDNA copy number. MtDNA copy numbers of these cells were determined in healthy controls (n=5) using ddPCR with dual FAM/HEX probes and nDNA/mtDNA primer sets. Vascular endothelial cells had the highest mtDNA

	copy number at 1082.1 copies/cell, followed by monocytes (254.1 copies/cell), natural killer cells (159.5 copies/cell), and neutrophils (84.8 copies/cell). Cf-mtDNA is a promising novel PAH biomarker. However, cf-mtDNA concentrations do not correlate with cf-nDNA, likely due to considerable differences in mtDNA copy number between cell types. This work provides insight into the potential of cf-mfDNA as a novel PAH biomarker and the need to determine cellular mtDNA copy number variance in interpreting findings.
Publications	• Roman BS, Mastoor Y, Zhang Y, **Gross D**, Springer D, Glancy B, Murphy T. Loss of mitochondrial Ca^{2+} uptake protein 3 (MICU3) impairs skeletal muscle calcium handling and exercise capacity. *J Physiol*. [Under review]
Abstracts	• **Gross D**, Murphy E, Roman B. A knock-in mutation in F-ATP synthase decreases sensitivity of the mitochondrial permeability transition pore in mice. American Heart Association Scientific Session, Chicago, IL; Nov. 5-7, 2022. [Poster presentation]
Professional Meetings	• American Heart Association Scientific Session, Chicago, IL; Nov. 5-7, 2022. • 17th Annual Cardiometabolic Health Congress, Boston, MA; Oct. 19-22, 2022.

Scholar	John Han
School	University of California, Irvine School of Medicine
Mentor	Ji Luo, Ph.D., Senior Investigator, Laboratory of Cancer Biology and Genetics
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Investigating the Selectivity Mechanism of a Novel Non-Covalent KRAS Inhibitor

Research Summary

The recent discovery of small molecule inhibitors against mutant KRAS protein was a breakthrough in targeted therapy. MRTX1133 was the first non-covalent inhibitor against the KRASG12D mutant that demonstrated specificity and potency in pre-clinical tumor models.

Using isogenic cell lines expressing a single RAS allele, we found that in addition to KRASG12D, MRTX1133 shows significant activity against wildtype KRAS and several other KRAS mutants. In contrast, MRTX1133 exhibits no activity against both G12D and wildtype forms of HRAS and NRAS proteins. Of the amino acid residues that directly interact with MRTX1133 in co-crystal structure with KRASG12D, all are conserved among KRAS, HRAS and NRAS proteins except histidine 95 (H95). In HRAS and NRAS, the corresponding residues are glutamine (Q95) and leucine (L95), respectively. Thus, we hypothesized that the non-conserved residue H95 on KRAS provides a critical selectivity handle for MRTX1133.

To test this hypothesis, we generated reciprocal mutations in G12D mutant KRAS, HRAS and NRAS where H95 in KRAS was mutated to Q95 and L95 as in HRAS and NRAS, respectively, and Q95 in HRAS and L95 in NRAS was mutated to H95 as in KRAS. We stably expressed the cDNA of these mutants in isogenic RASless mouse embryonic fibroblasts (MEFs) so we could directly compare their responses to MRTX1133. Expression of KRASG12D/H95Q, KRASG12D/H95L, HRASG12D and NRASG12D rendered cells completely resistant to MRTX1133. In contrast, cells expressing the KRASG12D, HRASG12D/Q95H, and the NRASG12D/L95H remained fully sensitive to MRTX1133. These sensitivity profiles were corroborated by the impact of MRTX1133 on the level of active RAS and phosphorylated MAP kinases in these cells.

	Our results indicate that the non-conserved H95 residue on KRAS presents an essential selectivity handle that drives the selectivity of MRTX1133 towards KRAS over HRAS and NRAS. This knowledge could aid the development of future KRAS-selective inhibitors.
Publications	• Keats MA*, **Han JJW***, Lee YH, Lee CS, Luo J. A non-conserved histidine residue on KRAS drives paralog selectivity of the $KRAS^{G12D}$ inhibitor MRTX1133. *Cancer Res*. 2023 Sep 1;83(17):2816-2823. PMID: 37339170
Abstracts	• **Han J**, Keats M, Lee YH, Lee CS, Luo J. A non-conserved histidine residue on KRAS drives paralog selectivity of the $KRAS^{G12D}$ inhibitor MRTX1133.. 2023 Annual NCI Center for Cancer Research Fellow and Young Investigators Conference. National Cancer Institute, Shady Grove Campus, Rockville, MD; May 4-5, 2023 [Poster presentation]
Professional Meetings	• 2022 Fourth RAS Initiative Symposium. NCI Center for Cancer Research, Frederick Campus, Frederick, MD; Oct. 17-19,2022
Awards	• Travel Award for Outstanding Poster Presentation, 2023 Annual NCI Center for Cancer Research Fellows and Young Investigators Conference

Scholar	Pranay S. Hegde
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor	Christopher S. Hourigan, M.D., Ph.D., Chief, Laboratory of Myeloid Malignancies
NIH Institute	National Heart, Lung, and Blood Institute (NHLBI)
Project Title	Utility of *FLT3* Tyrosine Kinase Domain-Mutated Measurable Residual Disease for Prognostication in Acute Myeloid Leukemia
Research Summary	Acute myeloid leukemia (AML) is a heterogeneous group of blood cancers affecting over 20,000 Americans annually. Despite advanced treatments such as chemotherapy and allogeneic hematopoietic cell transplantation (alloHCT), 75% of patients die within three years of diagnosis. *FLT3* gene variants are common in AML, with tyrosine kinase domain (TKD) mutations present in 7-10% of patients. Measurable residual disease (MRD) refers to lingering leukemic cells in the blood despite clinical remission. It is unclear if detection of *FLT3*-TKD MRD during clinical remission after initial chemotherapy treatment predicts worse outcomes post-alloHCT. We conducted error-corrected next-generation sequencing on remission blood samples from 342 patients positive for *FLT3*-TKD variants at diagnosis. We examined overall survival (OS) as a primary endpoint, along with relapse-free survival (RFS) and cumulative incidence of relapse (CIR) as secondary endpoints with Kaplan-Meier and Fine and Gray regression. Non-relapse mortality (NRM) was treated as a competing risk. 34 (9.9%) patients were found to be *FLT3*-TKD MRD positive (+) in remission prior to alloHCT. *FLT3*-TKD MRD+ patients had significantly increased rates of relapse (HR 3.11, 95% CI 1.82 – 5.31; 3-year CIR 50.2% vs. 19.7%, $p < 0.001$) and lower overall survival at 3 years (HR 2.12, 95% CI 1.3 – 3.44; 3-year OS 41.7% vs. 66.9%, $p = 0.002$) compared to *FLT3*-TKD MRD negative (-) patients. The presence of detectable *FLT3*-TKD mutations in pre-transplant peripheral blood samples from patients in remission prior to first alloHCT was associated with increased relapse and decreased OS at variant allele fractions (VAF) greater than or equal to 0.01%. More studies incorporating co-mutations in a large, diverse patient cohort are needed to further characterize this potentially high-risk group with unmet need.
Awards	• NHLBI Outstanding Fellows' Pitch Award, 2023.

Scholar	Gerard D. Hoeltzel
School	Sidney Kimmel Medical College at Thomas Jefferson University
Mentor	Mitchell Ho, Ph.D., Deputy Chief, Laboratory of Molecular Biology; Director, NCI/CCR Antibody Engineering Program
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Biparatopic CAR-T Cells Targeting GPC1 for the Treatment of Pancreatic Ductal Adenocarcinoma
Research Summary	Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal solid epithelial cancers, with a 5-year mortality of 90% following diagnosis. Engineering CAR-T cells for the treatment of solid tumors has proved challenging, especially in 'immune cold' tumors such as PDAC. To improve CAR-T cell efficacy in PDAC, we developed biparatopic CARs targeting glypican-1 (GPC1), an oncofetal antigen highly expressed in malignant but not normal pancreatic tissue. Biparatopic CARs enable a single receptor, comprised of two distinct antigen-binding peptides (HM2 scFv and D4 camelid V_HH nanobody) to target two distinct epitopes on the GPC1 molecule. A biparatopic approach may enhance CAR-T binding, mitigate risk of antigen escape, and improve anti-tumor efficacy. Biparatopic CAR-T cells were effectively synthesized with transduction efficiencies ranging from 30 to 45%. Biparatopic CAR-T cells in the HM2 followed by D4 orientation (HM2-D4) exhibited increased binding to GPC1 antigen at low antigen concentrations (0.16 ug/mL to 0.64 ug/mL) compared to those in the reverse orientation (D4-HM2). HM2-D4 biparatopic CAR-T cells, but not D4-HM2, demonstrated significantly increased *in vitro* killing efficacy against GPC1(+) PDAC cell lines BXPC3, T3M4, and PANC1 compared to D4 or HM2 monospecific CAR-T cells. Neither monospecific nor biparatopic CAR-T cells led to tumor regression in BXPC3 subcutaneous xenograft models, likely due to an excessive tumor burden of > 200 mm^3 at time of treatment. HM2-D4 biparatopic CAR-T cells also showed greater cytotoxicity against T3M4 GPC1 *knockout* cells compared to their monospecific counterparts, raising concerns for heightened risk of off-target off-tumor toxicity using this CAR format. This work demonstrates the potential of scFv and V_HH combinatorial, biparatopic CAR-T cell therapies for the treatment of GPC1(+) PDAC. Biparatopic CARs offer a potential new

	engineering strategy for the development of potent CAR-T cells targeting multiple distinct epitopes. Additional *in vivo* and mechanistic studies are required.
Publications	• Li N, Quan A, Li A, Pan J, Ren H, **Hoeltzel GD**, de Val N, Ashworth D, Ni W, Zhou J, Mackay S, Hewitt SM, Cachau R, Ho M. The IgG4 hinge with CD28 transmembrane domain improves V_HH-based CAR T cells targeting a membrane-distal epitope of GPC1 in pancreatic cancer. *Nat Commun* **14**, 1986 (2023). PMID: 37031249.

Scholar	Jordan J. Juarez
School	Lewis Katz School of Medicine at Temple University
Mentor	Eliseo J. Pérez-Stable, M.D., Director, National Institute on Minority Health and Health Disparities (NIMHD)
NIH Institute	National Heart, Lung, and Blood Institute (NHLBI)
Project Title	Differential Exposure to Discrimination Stress as a Mediator between Racial Identity and Health among Hispanic/Latino Individuals
Research Summary	Current racial and ethnic paradigms do not adequately capture the identities of Hispanic/Latino individuals. This creates methodological and conceptual limitations that obscure health patterns. The present study aimed to identify disparities in health for Hispanic/Latino individuals who are differentially racialized, and to test discrimination stress as a mechanism linking racial identity to health. Data came from Visit 1 (2008-2011), Visit 2 (2014-2017), and the Sociocultural Ancillary Study (2010-2012) of the Hispanic Community Health Study/Study of Latinos, a multi-center cohort study of Hispanic/Latino adults. Participants (*n*=4081) self-identified as: White (*n*=1689), Black (*n*=135), American Indian/Alaska Native (AI/AN) (*n*=130), Multiracial (*n*=680), and Racially Unidentified (*n*=1447). We used multinomial logistic regression to examine demographic predictors of racial identification and structural equation modeling to test discrimination stress as a mediator in the relationship between racial identity (main predictor) and changes in depression and anxiety symptoms and diabetes and hypertension incidence (outcomes) over six years. Demographic factors related to socioeconomic status and heritage were most strongly associated with racial identification. Unequal exposure to discrimination contributed to greater increases in depression and anxiety symptoms for Black-identified (β =0.13, C.I.=0.01, 0.31; β=0.02, C.I.=0.00, 0.05, respectively), AI/AN-identified (β =0.39, C.I.=0.17, 0.79; β=0.05, C.I.=0.02, 0.10), multiracial-identified (β=0.07, C.I.=0.00, 0.17; β=0.01, C.I.=0.00, 0.02), and racially unidentified individuals (β=0.08, C.I.=0.02, 0.16; β=0.01, C.I.=0.00, 0.02) compared to White-identified individuals. Discrimination also contributed to higher incidence of hypertension for multiracial-identified individuals (β=0.03, C.I.=0.00, 0.09) and hypertension and diabetes for AI/AN-identified (β=0.14, C.I.=0.02, 0.34; β=0.15,

C.I.=0.04, 0.36) and racially unidentified persons (β=0.03, C.I.=0.00, 0.09; β=0.03, C.I.=0.00, 0.07).

Findings suggest discrimination stress is an underlying mechanism linking racial identity to health for Black, AI/AN, multiracial, and racially unidentified Hispanic/Latino individuals. Research should continue to advance conceptualizations of race among Hispanic/Latino individuals to further understand health patterns at the intersection of racial and ethnic identity.

Publications

- **Juarez JJ**, Khalid MU, Ulloa B, Romero CM, Maruthi R, Shah D, Chang E, Shafi I, Lakhter V, Zhao H, Rodriquez EJ, Pérez-Stable EJ, Bashir R. Racial and ethnic disparities in inferior vena cava filter placement for deep vein thrombosis in the United States. *J Vasc Surg: Venous Lymphat Disord.* 2023 Sep 12:S2213-333X(23)00335-9. PMID: 37708935.
- Zajdel RA, Rodriquez EJ, **Juarez JJ**, Gallo LC, Perreira KM, Daviglus M, Pirzada A, Suglia S, Chambers EC, Pérez-Stable EJ. Differential stress exposure as a mediator between racial identity and health among Hispanic/Latino individuals: results from the HCHS/SOL Sociocultural Ancillary Study. *SSM Popul Health.* [Under review]
- Pérez-Stable EJ, Beard Morgan L, **Juarez JJ**, Rodriquez EJ. Black race matters in the Latino population. *Am J Public Health*. [Under review]

Abstracts

- **Juarez JJ**, Zajdel RA, Rodriquez EJ, Pérez-Stable EJ. Differential stress exposure as a mediator between racial identity and health among Hispanic/Latino individuals. Society of General Internal Medicine Annual Meeting, Aurora, CO; May 10-13, 2023. [Poster presentation]
- **Juarez JJ**, Khalid MU, Ulloa B, Romero CM, Maruthi R, Chang E, Shafi I, Lakhter V, Zhao H, Pérez-Stable EJ, Bashir R. Racial and ethnic disparities in inferior vena cava filter placement for deep vein thrombosis in the United States. American College of Cardiology Annual Meeting, New Orleans, LA; Mar. 4-6, 2023. [Poster presentation]

Professional Meetings

- Society of General Internal Medicine (SGIM) Mid-Atlantic Regional Virtual Meeting; Oct. 20-21, 2022.
- American College of Cardiology (ACC) Annual Meeting, New Orleans, LA; Mar. 4-6, 2023.
- Society of General Internal Medicine (SGIM) Annual Meeting, Aurora, CO; May 10-13, 2023.

Awards

- Josiah Macy Jr. Foundation Scholarship (National Medical Fellowship), 2022.
- AL DÍA News Emerging Leader Scholarship, 2023.

Scholar	Conor S. Kelly
School	Georgetown University School of Medicine
Mentor	Stefan Cordes, M.D., Ph.D., Assistant Clinical Investigator, Translational Stem Cell Biology Branch
NIH Institute	National Heart, Lung, and Blood Institute (NHLBI)
Project Title	High-Throughput Screening of Chimeric Antigen Receptor (CAR) T Cells Using Native and Non-Native Costimulatory Domains
Research Summary	Chimeric antigen receptor (CAR) T-cells have shown great promise in the treatment of hematologic malignancies. Their therapeutic utility is limited by lack of CAR T cell durability and by toxicities, which are known to correlate with T cell subset distribution. It is well known that without costimulatory domains, CAR T cells are dysfunctional. Different costimulatory domains result in skewing of T-cell subsets. A limited number of costimulatory domains have been used clinically and there has been relatively little research into the effect of non-native costimulatory domains. We performed high throughput *in vitro* screening of 1,243 costimulatory domain candidates from receptors found in the innate and adaptive immune systems. To design the pool, we queried the Uniprot database for the primary sequences of the intracellular portions of transmembrane immune signaling molecules. Most of the elements in this pool are not naturally expressed in T cells and include receptors involved in activation, regulation, development, and cytokine signaling of innate and adaptive immune cells. The motifs in our pool include those previously studied, such as ITAMs, and many not yet studied such as TIR and SPRY/B30.2. We inserted codon-optimized versions of candidate costimulatory domains into an anti-CD20 CAR with a truncated EGFR marker. This CAR plasmid pool was lentivirally transduced into T cells from several healthy human donors and co-cultured with CD20(+) Raji cells and CD20(-) K562 cells. The CAR T cells were then FACS sorted by memory vs effector and exhausted vs non-exhausted phenotype. We counted the number of costimulatory candidates in each group via DNA sequencing. In the memory (low exhaustion) group, we found CAR T-cells expressing CD74 as their costimulatory domain were enriched and those expressing CD33 were depleted (figure). We performed multinomial logistic regression to identify important costimulatory domain

	features and are using this data to build predictive and generative machine learning models.
Abstracts	• **Kelly C,** Zhu W, Sellers S, Dagur P, Dunbar C, Cordes S. High-throughput screen of CAR T cells using native and non-native costimulatory domains. American Society of Gene and Cellular Therapy 26th Annual Meeting, Los Angeles, CA; May 16-20, 2023. [Podium presentation]
Professional Meetings	• American Society of Hematology (ASH) Summit on Immunotherapies, Washington, DC; Mar. 2-3, 2023. • American Society of Gene and Cellular Therapy (ASGCT) 26th Annual Meeting, Los Angeles, CA; May 16-20, 2023.

Scholar	Suzie Kim
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor	Brian Brooks, M.D., Ph.D., Chief, Ophthalmic Genetics and Visual Function Branch
NIH Institute	National Eye Institute (NEI)
Project Title	Genetic Characterization of FAT1 and the Hippo Signaling Pathway in Human Retinal Pigment Epithelium
Research Summary	Coloboma is a congenital disorder that results in childhood blindness and is caused by failure of optic fissure closure (OFC), the initial steps of which are mediated by the presumptive retinal pigment epithelium (RPE). The atypical cadherin FAT1 was previously demonstrated to have a conserved role in mediating OFC. Preliminary data suggest FAT1 may do this via the Hippo signaling pathway, which induces a phosphorylation cascade that ultimately prevents nuclear translocation of transcriptional activator YAP/TAZ. Aberrant YAP/TAZ is independently correlated with coloboma; however, it has not been characterized in human RPE. We studied the interactions between FAT1 and core proteins of the Hippo pathway with three patient lines of induced pluripotent stem cell-derived RPE (i-RPE). With a fully-confluent RPE monolayer, we developed a novel *in vitro* model of OFC via chemical cellular junction disruption and reformation to study the transcriptome of these interactions via bulk RNA sequencing. Protein assays demonstrated the expression of FAT1 as well as both phosphorylated and non-phosphorylated Hippo proteins in the RPE. In sub-confluent RPE cultures in areas of low cellular density, YAP/TAZ localized strongly in RPE cell nuclei (45.6% of nuclei), while it was strongly excluded from the nucleus and localized predominantly to the membranes in confluent RPE (13.1% of nuclei, $p<0.001$). This suggests that actively proliferating RPE demonstrates increased nuclear localization of transcriptional activator YAP/TAZ, which is then sequestered to the membranes with increased cell packing. This was concurrent with the localization of FAT1 at the leading edges of proliferating sub-confluent RPE.

	Taken together, our findings suggest an important role for FAT1 in modulating YAP/TAZ during the proliferation of RPE and formation of cell-cell junctions. These results evidence the potential significance of FAT1-mediated Hippo signaling in RPE-mediated optic fissure closure and the pathogenesis of coloboma.
Publications	• George A, Lee J, Liu J, **Kim S,** Brooks BP. Zebrafish model of RERE syndrome recapitulates key ophthalmic defects that are rescued by small molecule inhibitor of shh signaling. *Dev Dyn.* 2022 Dec; 252:495-509. PMID: 36576487. • **Kim S,** Shih G, Brooks BP. Emery and Rimoin's Principles and Practice of Medical Genetics, 5th Edition. Eds: Rimoin DL, Connor JM, Pyeritz RE, Korf BR. Cambridge: Academic Press; c2023. *Optic Atrophy*. [In press]
Abstracts	• **Kim S**., Leigh A., George A., Pfister T., Bharti K., Brooks B.P. Characterization of FAT1 and Hippo signaling pathway proteins in human retinal pigment epithelium. Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, New Orleans, LA; Apr. 22-27, 2023. [Podium presentation]
Professional Meetings	• American Association for Pediatric Ophthalmology and Strabismus (AAPOS) Annual Meeting, New York, NY; Mar. 29 - Apr. 2, 2023. • Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, New Orleans, LA; Apr. 22-27, 2023.
Awards	• Medical Student and Resident Trainee Travel Award, American Association for Pediatric Ophthalmology & Strabismus, 2023.

Scholar	Rebecca Kuan
School	Western University of Health Sciences - College of Osteopathic Medicine of the Pacific
Mentor	Peter Grayson, M.D., Chief, Vasculitis Translational Research Program
NIH Institute	National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)
Project Title	T Cell Subset Analysis in Patients with Large Vessel Vasculitis

Research Summary

Takayasu's arteritis (TAK) is a life-threatening form of large-vessel vasculitis. Prior studies of disease-associated immunology are limited, but suggest roles for T cell subsets. We aimed to characterize T helper (Th) and cytotoxic T cell (Tc) subsets based on cytokine production and transcription factor (TF) expression utilizing a longitudinal observational cohort that allowed for within-patient comparison of disease activity.

For this study, a 30-color spectral flow cytometry panel was designed. Each antibody was titrated to determine its optimal concentration and then further adjusted until the staining pattern was comparable between multicolor and single-color staining. Nine patients with TAK (ages 14 to 38) contributed 16 active and 19 remission visits. Cells were incubated in stimulated (PMA, ionomycin, and Brefeldin A) and unstimulated (media alone) conditions. Treg, Th1, Th2, Th17, Tc1, Tc2, and Tc17 subset abundance were defined in two ways as the number of cells per 1000 CD4 or CD8 T cells that 1) expressed a lineage-defining TF or 2) produced a lineage-defining cytokine. Wilcoxon rank sum test was used to compare cell populations in association with disease activity.

Results from TF-defined unstimulated subsets did not correlate with that from cytokine-defined stimulated subsets (r -0.12 to 0.67). The TF-defined subsets were more strongly associated with disease activity. Considering TFs, most patients (at least 6 out of 9) had lower Treg, Th1, Th17, and Tc1 abundance and higher Tc2 and Tc17 abundance in active disease. Considering cytokines, most patients had lower Th2 and higher Tc1 and Tc2 abundance in active disease. Statistically significant differences were not observed.

Our findings suggest that the method of defining T cell subsets can substantially influence results. Strong differences in abundance of subsets were not observed in association with TAK disease activity. This may reflect disease heterogeneity or limitations of studying peripheral blood rather than affected arterial tissue.

Professional Meetings	• American College of Rheumatology (ACR) Convergence, Philadelphia, PA; Nov. 10–14, 2022.

Scholar	Christina T. LaGamma
School	Penn State College of Medicine
Mentor	Hugo Tejeda, Ph.D., Stadtman Investigator; Chief, Unit on Neuromodulation and Synaptic Integration
NIH Institute	National Institute of Mental Health (NIMH)
Project Title	Prefrontal Cortex Opioid Regulation of Adaptive Conflict Resolution in a Murine Model
Research Summary	Survival depends on competing drives between foraging for food and avoiding threats. Conflict resolution entails integration of these approach/avoidance behaviors. The ventromedial prefrontal cortex (vmPFC) is necessary for fear suppression. Dynorphin (Dyn) is a neuropeptide enriched in the vmPFC. However, the role of vmPFC Dyn in conflict resolution remains unknown. We assessed the necessity of vmPFC Dyn for conflict resolution by knocking down local Dyn expression via PDyn shRNA viral infusion in mice exposed to a conflict task. Mice learned that a light cue predicted reward availability and that an auditory cue predicted onset of a foot shock, which they could avoid by accessing a nearby platform. Conflict occurred by co-presenting light and tone cues. Before conflict, Dyn knockdown did not affect foraging, suggesting that Dyn is not necessary for reward learning. Control mice learned to resolve conflict by prioritizing reward and delaying shock avoidance. In contrast, Dyn knockdown mice remained on the platform at the expense of reward, suggesting that vmPFC Dyn is necessary for adaptive conflict resolution. Using fiber photometry and a Cre-dependent activity marker in Dyn-Cre mice, we observed that vmPFC Dyn cell activity increased with foraging only under conflict, suggesting that activity of vmPFC Dyn-neurons encodes conflict presence. To test the generalizability of our results, we developed a novel conflict paradigm where mice are placed into an arena for an hour with sucrose reward centrally located. Each approach to the reward triggered a stimulus that simulated an aerial predator, which could be avoided by hiding in a nearby shelter. vmPFC Dyn knockdown mice remained in the shelter longer and triggered fewer looming stimuli, consistent with excessive avoidance patterns observed in our earlier experiments. These results underscore the

	critical role of vmPFC control in approach/avoidance behaviors. Our study advances the understanding of neural mechanisms underlying adaptive conflict resolution.
Publications	• Enriquez-Traba J, Yarur-Castillo H, Flores RJ, Weil T, Roy S, Usdin T, **LaGamma CT**, Arenivar M, Wang H, Tsai V, Mortiz A, Sibley DR, Moratalla R, Freyberg ZZ, Tejeda HA. Dissociable control of motivation and reinforcement by distinct ventral striatal dopamine receptors. *bioRxiv* [Preprint]. 2023 Jun 28:2023.06.27.546539. PMID: 37425766
Abstracts	• **LaGamma CT**, Bravo-Rivera H, Limoges A, Tejeda HA. Prefrontal cortex opioid regulation of adaptive conflict resolution. American Association of Neurological Surgeons Annual Meeting, Los Angeles, CA; Apr. 21–24, 2023. [Podium presentation] • Enriquez-Traba J, Yarur-Castillo H, Flores-Garcia R, Roy S, Usdin T, **LaGamma CT**, Tejeda HA. Dissociable control of motivation and goal-directed behavior by distinct ventral striatal dopamine receptors. Winter Conference on Brain Research Annual Meeting, Snowbird, UT; Jan. 20–25, 2023. [Poster presentation] • **LaGamma CT**, Traba JE, Tejeda HA. Dopamine D1 receptors in the nucleus accumbens are necessary for reward-based learning in mice. Howard University College of Medicine Annual Neuroscience Research Symposium, Washington DC; Sep. 17, 2022. [Podium presentation]
Professional Meetings	• Howard University College of Medicine 1st Annual Neuroscience Research Symposium, Washington DC; Sep. 17, 2022. • Winter Conference on Brain Research 55th Annual Meeting, Snowbird, UT; Jan. 20–25, 2023. • American Association of Neurological Surgeons 91st Annual Meeting, Los Angeles, CA; Apr. 21–24, 2023.

Scholar	Riley M. Larkin
School	University of Miami Miller School of Medicine
Mentor	Nyall R. London Jr., M.D., Ph.D., Principal Investigator, Sinonasal and Skull Base Tumors, Surgical Oncology Program
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Characterization of the Olfactory Neuroblastoma Tumor Microenvironment Reveals Novel Immunotherapeutic Approaches to Treatment
Research Summary	Olfactory neuroblastoma (ONB), also known as esthesioneuroblastoma, is a rare malignancy of the nasal cavity and anterior skull base. The standard of care is a multimodal approach centered on surgical resection coupled with adjuvant radiation and consideration for chemotherapy in locally advanced or metastatic disease. There are no approved targeted therapies for ONB, and current treatments can lead to significant long-term morbidity. Through multispectral immunofluorescence (mxIF) staining, thorough characterization of the tumor microenvironment (TME) can guide future rational translational immunotherapeutic approaches. Validated panels to analyze the T cell (CD4, CD8, FOXP3, Ki-67, PD-1, and synaptophysin), myeloid derived suppressor cell (MDSC) (CD11b, CD14, CD15, CD68, HLA-DR, and synaptophysin), and natural killer (NK) cell (CD16, NCAM1, CD3, granzyme B, and synaptophysin) compartments were instituted on a tissue microarray (TMA) containing 47 clinically annotated ONB samples. Synaptophysin positivity was used to segment tumor from stroma. A retrospective chart review was then performed collecting clinical variables of interest such as tumor stage and pathological grade. HALO (Indica Labs) was used to objectively quantify and annotate immune cell densities and spatial relationships. Immune cells were largely restricted to the stroma and excluded from the tumor parenchyma; this included cytotoxic T cells, helper T cells (except their PD-1+ subset), regulatory T cells, macrophages, MDSC-polymorphonuclear leukocytes, and MDSC-monocytes. There was no difference in NK cell density in tumor parenchyma vs stroma; however, NK cells were sparse in the ONB TME. Therefore, a subsequent mxIF panel testing for major histocompatibility complex class I was implemented. RNAScope – an *in-situ* hybridization technique – was used to determine levels of IL-8, CXCL9, and CXCL10. Comparison between low and high Hyams grade tumors, Kadish A/B and Kadish C/D stage

	tumors, and presence of dural infiltration yielded novel targets for immunotherapeutic approaches.
Publications	• **Larkin R**, Hermsen MA, London NR Jr. Translocations and gene fusions in sinonasal malignancies. *Curr Oncol Rep*. 2023 Apr;25(4):269-278. PMID: 36753024.
Abstracts	• **Larkin R**, Lopez D, Robbins Y, Lassoued W, Gallia G, Allen C, London N. Multispectral immunofluorescence analysis of the olfactory neuroblastoma tumor immune microenvironment reveals polymorphonuclear leukocyte and macrophage stroma localization and tumor parenchyma exclusion. American Association for Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 14-19, 2023. [Poster presentation] • **Larkin R**, Lopez D, Robbins Y, Gallia G, Allen C, London N. Multispectral immunofluorescence analysis of the esthesioneuroblastoma tumor immune microenvironment reveals T cell stroma localization and tumor parenchyma exclusion. North American Skull Base Society 32nd Annual Meeting, Tampa, FL; Feb. 17-19, 2023. [Podium presentation]
Professional Meetings	• North American Skull Base Society 32nd Annual Meeting, Tampa, FL; Feb. 17-19, 2023. • American Association for Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 14-19, 2023

Scholar	Yue Lin
School	Boston University Chobanian & Avedisian School of Medicine
Mentors	Baris Turkbey, M.D., Director, Artificial Intelligence Resource; Director, Magnetic Resonance Imaging Section, Molecular Imaging Branch Peter L. Choyke, M.D., Chief, Molecular Imaging Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Evaluation of a Cascaded Deep Learning-Based Algorithm for Prostate Lesion Detection on Biparametric MRI
Research Summary	The performance evaluation of artificial intelligence (AI) models developed for detection of intraprostatic lesions requires large datasets and extensive testing. The purpose of this study was to evaluate a deep learning algorithm for intraprostatic lesion detection and segmentation and to compare its performance with radiologist and biopsy results. This retrospective study included consecutive participants who were imaged with mpMRI and subsequently received MRI/ultrasound fusion-guided targeted and systematic biopsy from April 2019 to September 2022. All lesions were prospectively evaluated using PI-RADSv2.1. A previously developed AI algorithm was evaluated. Algorithm performance was compared to a radiologist at both lesion- and participant-level using sensitivity, positive predictive value (PPV), and Dice similarity coefficient (DSC). Algorithm performance was also examined using histopathological outcomes, and the Wald test was used to compare the performance of the algorithm and the radiologist for cancer detection. A total of 658 participants (median age, 67 years; interquartile range, 61-71 years) with 1029 MRI-visible lesions were evaluated. On participant-level analysis using the radiologist as ground truth, detection sensitivity with the AI was 93% (519/559; 95% confidence interval [CI]: 90, 95) and PPV was 88% (519/594; 95% CI: 85, 90). Mean number of false positive per participant was 0.61 (range 0–3). DSC for lesion segmentation was 0.34. On histopathologic analysis, the algorithm identified 96% (282/294; 95% CI: 94, 98) of all participants with clinically significant prostate cancer versus 98% (287/294; 95% CI: 96, 99) identified by the radiologist (p = 0.23). The

algorithm detected 84% (103/122), 96% (152/159), 96% (47/49), 95% (38/40), and 98% (45/46) of participants with International Society of Urological Pathology grade groups of 1, 2, 3, 4, and 5 lesions, respectively.

This fully automated cascaded deep learning algorithm can detect lesions suspicious for cancer on biparametric MRI with a reasonable performance, comparable to a well-trained radiologist. Moreover, it was able to reliably predict lesions that were clinically significant on histopathology.

Publications

- **Lin Y**, Yilmaz EC, Belue MJ, Turkbey B. Prostate MRI and image quality: It is time to take stock. *Eur J Radiol*. 2023 Apr;161:110757. PMID: 36870241.
- **Lin Y**, Yilmaz EC, Belue MJ, Harmon SA, Phelps TE, Merriman KM, Hazen L, Garcia C, Yang D, Xu Z, Lay NS, Toubaji A, Merino MJ, Xu D, Gurram S, Wood BJ, Choyke PL, Pinto PA, Turkbey B. Evaluation of a cascaded deep learning-based algorithm for prostate lesion detection on biparametric MRI. *Radiology* [Under review]
- **Lin Y**, Johnson L, Fennessy F, Turkbey B. Prostate cancer local staging with magnetic resonance imaging. *Radiol Clin N AM* [Under review]
- **Lin Y**, Belue MJ, Yilmaz EC, Harmon SA, Phelps TE, Merriman KM, Hazen L, Garcia C, Lay NS, Toubaji A, Merino MJ, Gurram S, Wood BJ, Choyke PL, Pinto PA, Turkbey B. Deep learning-based T2W MR image quality assessment and its impact on prostate cancer detection rates. *J Urol* [Under review]
- Yilmaz EC, Harmon SA, Belue MJ, Merriman KM, Phelps TE, **Lin Y**, Garcia C, Hazen L, Patel K, Merino MJ, Wood BJ, Choyke PL, Pinto PA, Citrin DE, Turkbey B. Evaluation of a deep learning-based algorithm for post-radiotherapy prostate cancer local recurrence detection using biparametric MRI. *Eur J Radiol* 2023 Sep 13; 168:111095. PMID: 37717420
- Belue MJ*, Blake Z*, Yilmaz EC, **Lin Y**, Harmon SA, Nemirovsky DR, Enders JJ, Kenigsberg AP, Mendhiratta NM, Rothberg M, Toubaji A, Merino M, Gurram S, Wood BJ, Choyke PL, Turkbey B, Pinto PA. Multiparametric MRI-based radiomics features of prostatic adenocarcinoma with cribriform architecture. *Prostate* 023 Aug 25. PMID: 37622756 **Equal contribution*
- Belue MJ, Law YM, Marko J, Turkbey E, Malayeri A, Yilmaz EC, **Lin Y**, Johnson L, Merriman KM, Lay NS, Choyke PL, Harmon SA, Turkbey B. Deep learning-based interpretable AI for prostate T2W MRI quality evaluation. *Radiology* [Under review]

Abstracts

- **Lin Y**, Belue MJ, Yilmaz EC, Harmon SA, Choyke PL, Turkbey B. Deep learning-based MR image quality assessment and its impact on prostate cancer detection rates. American Roentgen Ray Society Annual Meeting, Honolulu, HI; Apr. 16-20, 2023. [Podium presentation]
- Blake Z, Belue MJ, Nemirovsky DR, Harmon SA, Enders JJ, Kenigsberg AP, Mendhiratta NM, Yilmaz EC, **Lin Y**, Rothberg M, Toubaji A, Merino M, Gurram S, Wood BJ, Choyke PL, Turkbey B, Pinto PA. Multiparametric MRI-based radiomics features of prostatic adenocarcinoma with cribriform architecture. American Urological Association Annual Meeting, Chicago, IL; Apr. 28-May 1, 2023. [Podium presentation]
- **Lin Y**, Belue MJ, Yilmaz EC, Merriman KM, Phelps TE, Lay NS, Merino MJ, Wood BJ, Choyke PL, Harmon SA, Pinto PA, Turkbey B. Impact of image quality on detection of extraprostatic extension on MRI: Evaluation with a deep learning-based AI algorithm. Radiological Society of North America Annual Meeting, Chicago IL; Nov. 26-30, 2023. [Submitted]

- **Lin Y**, Yilmaz EC, Belue MJ, Harmon SA, Phelps TE, Merriman KM, Hazen L, Garcia C, Yang D, Xu Z, Lay NS, Toubaji A, Merino MJ, Xu D, Gurram S, Wood BJ, Choyke PL, Pinto PA, Turkbey B. Evaluation of a cascaded deep learning-based algorithm for prostate lesion detection on biparametric MRI. Radiological Society of North America Annual Meeting, Chicago IL; Nov. 26-30, 2023. [Submitted]
- Belue MJ, Blake Z, Yilmaz EC, **Lin Y**, Harmon SA, Nemirovsky DR, Enders JJ, Kenigsberg AP, Mendhiratta NM, Rothberg M, Toubaji A, Merino M, Gurram S, Wood BJ, Choyke PL, Pinto PA, Turkbey B. Is prostatic adenocarcinoma with cribriform architecture more difficult to detect on prostate MRI? Radiological Society of North America Annual Meeting, Chicago IL; Nov. 26-30, 2023. [Submitted]
- Belue MJ, Law YM, Marko J, Turkbey E, Malayeri A, Yilmaz EC, **Lin Y**, Johnson L, Merriman KM, Lay NS, Choyke PL, Harmon SA, Turkbey B. Deep learning-based interpretable AI for prostate T2W MRI quality evaluation. Radiological Society of North America Annual Meeting, Chicago IL; Nov. 26-30, 2023. [Submitted]
- Yilmaz EC, **Lin Y**, Belue MJ, Harmon SA, Phelps TE, Merriman KM, Johnson L, Lay NS, Toubaji A, Merino MJ, Wood BJ, Gurram S, Choyke PL, Pinto PA, Turkbey B. Correlating radical prostatectomy pathology with MRI-guided targeted biopsy: Prospective comparison between PI-RADS v2.0 vs. v2.1. Radiological Society of North America Annual Meeting, Chicago IL; Nov. 26-30, 2023. [Submitted]

Professional Meetings

- American College of Radiology Annual Meeting, Washington, D.C.; May 6-10, 2023.
- American Roentgen Ray Society Annual Meeting, Honolulu, HI; Apr. 16-20, 2023.
- American Society for Radiation Oncology Annual Meeting, San Antonio, TX; Oct. 23-26, 2022.

Scholar	Keagan G. Lipak
School	Ohio University Heritage College of Osteopathic Medicine
Mentor	Nirali Shah, M.D., Lasker Clinical Research Scholar; Head, Hematologic Malignancies Section, Pediatric Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Impact of Fludarabine Exposure on the Depth and Duration of Lymphopenia and CAR T-Cell Outcomes
Research Summary	Chimeric antigen receptor (CAR) T-cells are highly effective therapy for patients with relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL). Recent data identified an association of fludarabine exposure with reduced relapse rates, longer leukemia-free survival, and more prolonged B-cell aplasia following treatment with tisagenlecleucel, a CD19-directed CAR T-cell. However, the relationship of fludarabine exposure to lymphopenia and the mechanism by which dosing impacts outcomes following CAR T-cells remains unknown. We conducted a retrospective review of children and young adults with B-ALL who received fludarabine prior to infusion of CAR T-cells. The primary study objective was to investigate the impact of fludarabine on the depth and duration of lymphopenia. Secondary objectives included examining the impact of fludarabine exposure on: 1) overall clinical response, and 2) CAR T-cell expansion. Fludarabine exposure (AUC per dose) for each patient was predicted using a validated population PK model. In 137 participants, the median cumulative AUC was 8.3 mg*hr/mL (range, 3.00-13.83 mg*hr /mL). When stratified by median fludarabine exposure, there was no difference in depth or duration of lymphopenia or CAR T-cell expansion collectively or per trial. Median fludarabine exposure also did not differ between responders and non-responders. However, depth and duration of lymphopenia did differ in the CD22 CAR T-cell trial. Specifically, in those achieving complete remission (CR), the duration of lymphopenia was longer than in non-responders (7 versus 4 days, p=0.030). Similarly, CAR T-cell expansion was higher if lymphopenia duration was greater than 5 days (p=0.019). Although lymphodepletion impacts CAR T-cell response, we did not find an association between CR rate, depth of lymphopenia, or duration of lymphopenia with varying

	fludarabine exposures. This raises additional questions regarding the impact of lymphodepleting therapy on CAR T-cell outcomes, particularly since more prolonged lymphopenia was associated with high CR rates and greater peak expansion in at least one of our studies (CD22 CAR T-cells).
Publications	• **Lipak KG**, Peer CJ, Binder N, Yuan CM, Wang HW, Wang Y, Yates B, Shalabi H, Mackall CL, Fry TJ, Inglefield J, Figg WD, Gertz EM, Shah NN. Impact of fludarabine exposure on the depth and duration of lymphopenia and CAR T-cell outcomes. [In preparation]
Abstracts	• **Lipak KG**, Yates B, Binder N, Shalabi H, Peer CJ, Figg WD, Gertz EM, Shah NN. Impact of fludarabine exposure on the depth and duration of lymphopenia and CAR T-cell outcomes. American Society of Clinical Oncology, Chicago, IL; June 2-6, 2023. [Poster presentation]
Professional Meetings	• INSPIRED Symposium: Insights in Pediatric CAR T-cell Immunotherapy: Recent Advances and Future Directions, Bethesda, MD; Mar. 1, 2023. • American Society of Hematology Summit on Immunotherapies for Hematologic Diseases, Washington, DC; Mar. 2-3, 2023. • 4th NCI Symposium on Cancer Health Disparities, Bethesda, MD; Apr. 4-5, 2023. • American Society of Clinical Oncology, Chicago, IL; June 2-6, 2023. • 7th Annual Children's Cancer Foundation Research Symposium, Greenbelt, MD; June 14, 2023.

Scholar	Sarah B. Lynn
School	University of Minnesota School of Dentistry
Mentor	Janice S. Lee, D.D.S., M.D., Clinical Director, NIDCR; Chief, Craniofacial Anomalies and Regeneration Section
NIH Institute	National Institute for Dental and Craniofacial Research (NIDCR)
Project Title	The Condyle Conundrum: Investigating Temporomandibular Joint Disorders in Loeys-Dietz Syndrome Through a Murine Model
Research Summary	Temporomandibular joint disorders (TMDs) affect 4.8% to 42.7% of the United States population, with women affected twice as frequently as men. Loeys-Dietz Syndrome (LDS) is a rare connective tissue disorder caused by autosomal-dominant mutations along the transforming-growth-factor-beta (TGF-β) pathway, and characterized mainly by aortic aneurysm, mandibular and maxillary hypoplasia, and enamel anomalies. We previously determined that patients with LDS have a high prevalence of TMD, especially those with LDS Type-I (*TGFBR1* mutation) and Type-II (*TGFBR2* mutation), which contributes to a lower oral health quality of life. The etiology for TMD in LDS has not been studied. Our objective was to investigate the pathogenesis of TMD in LDS. A retrospective radiologic analysis of the skeletal morphology of mandibular condyles using cone-beam computed tomography (CBCT) in 25 patients with LDS and age/gender/skeletal class matched healthy controls was performed. We also studied an LDS Type-II mouse model (*Tgfbr2*$^{G357W/+}$) that was previously shown to recapitulate the cardiovascular phenotype of LDS. The condylar morphology of *Tgfbr2*$^{G357W/+}$ mice and their wild-type littermates was analyzed using micro–CT imaging and histology, at ages 2, 6, 12, and 24 weeks. Premature or accelerated signs of condylar degeneration, with no predilection for sex, were observed in patients with LDS. Degenerative findings included osteophytes, pseudocysts, and osseous erosion. Bone remodeling (sclerosis, flattening) was also observed. *Tgfbr2*$^{G357W/+}$ mice exhibited degeneration, remodeling, and increased bone volume with decreased bone mineral density, when compared to wild-type mice. Condyle histology in the *Tgfbr2*$^{G357W/+}$ mice exhibited degenerative subchondral changes that were not present in wild-type mice.

	The mandibular condyles of patients with LDS demonstrate accelerated degeneration. An LDS mouse model recapitulates this phenotype. These condylar manifestations may explain the high prevalence of TMD in LDS patients, and demonstrate the critical role of TGF-β signaling in TMJ development and function.
Abstracts	• **Lynn SB**, Jani P, Almpani K, Keyvanfar C, Devine K, Duverger O, Lee JS. Mandibular condyle abnormalities associated with Loeys-Dietz Syndrome, a TGF-β-opathy. American Association for Dental and Craniofacial Research, 52nd Annual Meeting, New Orleans, LA; Mar. 15-18, 2023. *J Dent Res.* 102(Special Issue A): 0050. [Podium presentation] • **Lynn SB**, Jani P, Almpani K, Keyvanfar C, Devine K, Duverger O, Ahmad, M Lee JS. Unraveling rare disease pathogenesis using interplay between clinical data and an animal model. Colgate Clinical Research Innovation Day, Piscataway, NJ; May 24, 2023. [Podium presentation]
Professional Meetings	• American Association for Dental and Craniofacial Research 52nd Annual Meeting, Portland, OR; Mar. 15-18, 2023 • American Association of Oral and Maxillofacial Surgeons 104th Annual Meeting, New Orleans, LA; Sept. 12-17, 2022

Scholar	Jose Alberto Maldonado
School	University of Texas Medical Branch John Sealy School of Medicine
Mentor	Christine Alewine, M.D., Ph.D., Lasker Clinical Research Scholar, Laboratory of Molecular Biology
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Genomic Landscape of Pancreatic Adenocarcinoma in Different Races and Ethnicities
Research Summary	Non-Hispanic Black (NHB) persons have a higher incidence of pancreatic ductal adenocarcinoma (PDAC) and a shorter overall survival than non-Hispanic White (NHW) persons with PDAC, while Asian and Hispanic persons generally have better outcomes than NHW patients. The goal of our study was to determine whether somatic mutational frequency differs among these racial and ethnic groups and whether such differences could contribute to disparities in outcomes. PDAC mutational data was downloaded from AACR Project GENIE (Genomics Evidence Neoplasia Information Exchange) v13.0. We compared mutation frequencies between NHW and minority groups with respect to the most common PDAC mutations. To maximize power, we included any mutation with a frequency ≥5.0% where the database included at least ≥150 patients tested for that gene mutation. Specific point mutations in KRAS were also extracted with stratification for racial and ethnic group. Finally, homologous recombination repair (HRR) gene mutation frequency was also analyzed between groups. Our PDAC cohort included 5,292 individuals (NHW, n=4296, 80.7%; NHB, n=264, 5.0%; Hispanic, n=460, 8.7%; Asian, n=299, 5.7%). As compared to the NHW group, NHB patients had higher rates of TP53 (78.7% vs 69.0%, p= 0.0006, FDR (false discovery rate) = 0.0055) and PTPRT (5.3% vs 1.8%, p= 0.0095, FDR= 0.043) mutations. KRAS G12D mutation was more prevalent in Hispanic patients (40.9% Hispanic vs 36.1% NHW, p= 0.0443) while KRAS G12V was highest in Asian patients (36.1% Asian vs 27.9% NHW, p= 0.0031). All other targetable genes, including HRR genes and other KRAS point mutations, had similar mutation frequencies among different patient races and ethnicities.

In summary, somatic mutation profile is largely similar among PDAC patients across race and ethnicity. Additional factors likely contribute to differences in clinical outcomes among these groups. It is important for future intervention initiatives to address the socio-cultural differences that are likely contributing to unequal survival outcomes among PDAC patients.

Publications

- Skorupan N, Ghabra S, **Maldonado JA**, Zhang Y, Alewine C. Two rare cancers of the exocrine pancreas: to treat or not to treat like ductal adenocarcinoma? *J Cancer Metastasis Treat*. 2023; 9:5. PMID: 37538977
- Monge C, **Maldonado JA**, McGlynn K, Greten TF. Hispanic individuals are underrepresented in phase III clinical trials for advanced liver cancer in the United States. *J Hepatocell Carcinoma*. 2023 Jul 27;10:1223-1235. eCollection 2023
- **Maldonado JA**, Tai CH, Luke B, Alewine C. Genomic landscape of pancreatic adenocarcinoma in different races and ethnicities. *JCO Precis Oncol*. [Under review]

Abstracts

- **Maldonado JA**, Tai CH, Alewine C. Genomic characterization of somatic mutations by race and ethnicity in pancreatic cancer defined through AACR project GENIE. American Society of Clinical Oncology Annual Meeting; June 5, 2023. [Poster presentation]
- **Maldonado JA**, Tai CH, Alewine C. Somatic mutational landscape in pancreatic ductal adenocarcinoma by race and ethnicity. Medical Student & Resident Abstract Forum at the American Society of Clinical Oncology Annual Meeting; June 3, 2023. [Poster presentation].
- **Maldonado JA**, Graubard BI, McGylnn KA, Greten TF, Monge C. Racial and ethnic disparities in U.S. clinical trials in liver cancer in the last nineteen years. American Association of Cancer Research Annual Meeting; Apr. 17, 2023. [Poster presentation].
- **Maldonado JA**, McGylnn KA, Greten TF, Monge C. Disparities in multinational Phase III liver cancer trials by regions of the world. American Association of Cancer Research Annual Meeting; Apr. 17, 2023. [Poster presentation].
- **Maldonado JA**, Greten TF, Monge C. Cost-effectiveness of gemcitabine plus cisplatin with and without durvalumab in patients with advanced cholangiocarcinoma. American Society of Clinical Oncology Gastrointestinal Symposium; Jan. 20, 2023. [Poster presentation].

Professional Meetings

- American Society of Clinical Oncology (ASCO) Gastrointestinal Cancers Symposium, San Francisco, CA; Jan. 19-21, 2023.
- American Association of Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 14-19, 2023.
- American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago, IL; June 2-6, 2023.

Awards

- Conquer Cancer Annual Meeting Research Award, American Society of Clinical Oncology, 2023
- Conquer Cancer William T. Leslie, MD, Endowed Merit Award, American Society of Clinical Oncology, 2023
- Minority Scholar in Cancer Research, American Association of Cancer Research, 2023

Scholar	Danielle McAuliffe
School	Temple Lewis Katz School of Medicine
Mentor	Kareem Zaghloul, M.D., Ph.D., Senior Investigator, Functional Neurosurgery Section Julio Chapeton, Ph.D., Staff Scientist, Functional Neurosurgery Section
NIH Institute	National Institute of Neurological Disorders and Stroke (NINDS)
Project Title	Investigating Sequences of Interictal Epileptiform Discharges (IEDs) and Underlying Connections
Research Summary	Epilepsy is one of the most common neurological disorders, affecting more than 50 million people worldwide and more than 3 million in the US. Hypersynchrony is a hallmark of epilepsy, wherein groups of neurons can be seen to fire in unison. When hypersynchrony occurs in artifact-free baseline data, it is defined as an interictal epileptiform discharge (IED). IEDs are thought to mimic seizure onset. Because of this parallel, understanding the patterns and constraints of propagation of IEDs could provide insight into the patterns and constraints of propagation of seizures. We hypothesized that sequences of IEDs could be identified and could proxy epileptic networks, and that underlying functional connectivity could provide pathways that IEDs can utilize to spread. Twenty participants underwent surgical placement of electrodes for intracranial monitoring. We identified IEDs and developed algorithms to capture sequences. We defined statistically over-represented sequences (ORS) by comparisons against distributions of jittered data. We compared functional connectivity measures between ORS, non-ORS, and shuffled data. We also investigated relationships between ORS score and connectivity strength, and between ORS co-spike timings and connectivity timings (tau). Connectivity was calculated using Granger causality and tau was established using a time delay cross correlation. Seizure spread was considered by high amplitude distance and generalizability (GTCs). ORS had higher connectivity than non-ORS (p=0.02) and than distance-matched pseudo sequences (p=0.004). Additionally, ORS connectivity was correlated with ORS score (rho=0.17 p=0.0003) and co-spike time delays were correlated with connectivity tau (rho=0.21 p=0.002). Further, we could relate these ORS to seizure spread. We found that distance between ORS communities was higher in patients with GTCs and correlated with

	distance of high amplitude spread during a seizure. Taken together, these data demonstrate that underlying connectivity can contribute to the spread of IEDs and that IEDs can be used to proxy epileptic networks for seizure propagation.
Abstracts	• **McAuliffe D,** Chapeton J, Zaghloul K. Stereotyped sequences of interictal epileptiform discharges can utilize underlying functional connectivity to propagate. American Association of Neurological Surgeons Annual Meeting, Los Angeles, CA; Apr. 20-25, 2023. [Poster presentation] • **McAuliffe D,** Chapeton J, Zaghloul K. Interictal epileptiform discharge sequences repeated during seizures. Annual Neuroscience Research Symposium, American Association of Neurological Surgeons, Sep. 17, 2022. [Podium presentation]
Professional Meetings	• Annual Neuroscience Research Symposium, American Association of Neurological Surgeons; Sep. 17, 2022. • American Association of Neurological Surgeons Annual Meeting, Los Angeles, CA; Apr. 20–25, 2023.

Scholar	Kelsey C. Mumford
School	Dell Medical School at The University of Texas at Austin
Mentor	Benjamin Berkman, J.D., Head, Section on the Ethics of Genetics and Emerging Technologies, Department of Bioethics, NIH-CC Veronica Gomez-Lobo, M.D., Director, Pediatric and Adolescent Gynecology, NICHD
NIH Institute	NIH Clinical Center (NIH-CC)
Project Title	Couples' Decision-Making About Expanded Non-Invasive Prenatal Testing
Research summary	This project assessed the preferences of and decision-making processes between pregnant women and their partners on non-invasive prenatal whole genome sequencing. A survey was administered to 247 pregnant couples. The pregnant women and their partners were independently asked to envision being offered non-invasive prenatal testing for conditions that varied by severity, treatability, age of onset, and reliability of the test and discuss their preferences for seeking testing. Additional questions explored how they would make decisions with their partner, how much guidance they would seek from their obstetrician and others, and how the ability to terminate a pregnancy in their state would impact their decision to seek testing. Respondents were most likely to seek testing for earlier-onset conditions of higher severity that are readily treatable ($p=0.0310$, $p<0.0001$, and $p=0.0032$). 44.9% of pregnant women and 44.5% of partners reported that the inability to terminate a pregnancy in their state would make them more likely to seek testing, and this response was highly predictive of the pregnant person ($p=0.0009$) and partner ($p=0.0006$) seeking testing in general. Couples agreed on testing decisions for almost 90% of vignettes and highly agreed on seeking guidance from a doctor or genetic counsellor if they had difficulty coming to a decision about testing with their partner ($k=0.442$) and on using the genetic information to inform decisions on whether to terminate the pregnancy as being important ($k=0.426$). Our data suggests that it might be prudent for providers to elucidate patient views on abortion when discussing which tests to pursue, and that relevant professional societies should develop recommendations and resources to guide patients through this decision-making process. It also challenges the current paradigm of non-directiveness in genetic

counselling and suggests that couples desire firmer guidance in the face of so many testing options.

Publications

- **Mumford K**, Roesner N, Berkman B. The potential role of nudging in expanded non-invasive prenatal testing. *Am J Bioethics*. 2023;23(3):61-63. PMID: 36919549.
- **Mumford K**. Ethically navigating the evolution of gender-affirmation surgery. *AMA J Ethics*. 2023 Jun 1;25(6):E383-385. PMID 37285290.
- **Mumford K**. Capacity assessment during labor and the role of opt-out consent. *J Med Ethics*. 2023 Sep;49(9):620-621. PMID: 37419670
- **Mumford K**, Hendriks S, Gomez-Lobo V. Should ovarian tissue cryopreservation in pediatric patients with Turner syndrome be limited to the research setting? *J Ped Adol Gynecol*. [Under review]

Abstracts

- **Mumford K**, Maher J, Hendriks S, Gomez-Lobo V. The ethics of ovarian tissue cryopreservation as innovative therapy in girls with Turner syndrome. North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting, Nashville, TN; Mar. 24-26, 2023. *J Ped Adol Gynecol*. 2023; 36(2):215. [Poster presentation]
- Mascoe C, **Mumford K**, Badger T, Lou H, Maher J, Arlova A, Brown GT, Gomez-Lobo V. Ovarian tissue histopathology and correlation to serum markers in Turner syndrome. North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting, Nashville, TN; Mar. 24-26, 2023. *J Ped Adol Gynecol*. 2023; 36(2):220. [Poster presentation]
- Badger T, Maher JY, Kavarthapu R, Kim S, Grinberg A, Balasubramanian R, Lou H, De La Luz Sierra M, **Mumford K**, Pfeifer K, Levine J, Gomez-Lobo V. Combinatory effects of unilateral oophorectomy plus cyclophosphamide treatment on ovarian reserve and fertility in a mouse model. American Society for Reproductive Medicine (ASRM) Scientific Congress, New Orleans, LA; Oct. 14-18, 2023. [Poster presentation]
- Badger T, Maher JY, Kastury R, Kavarthapu R, Balasubramanian R, **Mumford K**, De La Luz Sierra M, Lou H, Gomez-Lobo V. Investigating the role of PI3K/AKT mediated ovarian follicle depletion in classic galactosemia. American Society for Reproductive Medicine (ASRM) Scientific Congress, New Orleans, LA; Oct. 14-18, 2023. [Podium presentation]
- **Mumford K**, Hendriks S, Miner S, Huelsnitz C, Wakim P, Berkman B. Couples' decision-making about expanded prenatal cell-free DNA screening. St. André International Center for Ethics and Integrity Bioethics Colloquium, Rochefort-du-Gard, France; Oct. 25-28, 2023. [Oral presentation]

Professional Meetings

- North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting, Nashville, TN; Mar. 24-26, 2023.
- American College of Obstetricians and Gynecologists (ACOG) Annual Clinical & Scientific Meeting, Baltimore, MD; May 19-21, 2023.
- American Medical Association (AMA) Annual Meeting of the AMA House of Delegates, Chicago, IL; June 9-14, 2023.

Awards

- Scholarship Recipient, Medical Review Auschwitz Scholarship Program, 2022.
- P.E.O. Scholar Award, Philanthropic Education Organization (P.E.O.) International, 2023.
- Best Poster Award (Mascoe et al.), North American Society for Pediatric and Adolescent Gynecology (NASPAG) 37th Annual Clinical & Research Meeting, 2023.
- Invited Speaker, St. André International Center for Ethics and Integrity Bioethics Colloquium, 2023.

Scholar	Fiona Obiezu
School	David Geffen School of Medicine at the University of California, Los Angeles, Charles R. Drew University of Medicine and Sciences
Mentor	Ashkan Malayeri, M.D., Chief, Body Imaging Section, Radiology and Imaging Sciences Department
NIH Institute	NIH Clinical Center (CC)
Project Title	Detection of Anatomical Structures in Laparoscopic Nephrectomy Videos using Convolutional Neural Networks (YOLOV7)
Research Summary	Laparoscopy is the preferred method used in urological surgeries and therefore generates large amounts of video data that can be extremely useful in training artificial intelligence (AI) algorithms for object detection. Due to the complex nature of laparoscopic procedures, AI can serve as a tool for anatomical landmark recognition, instrument detection, and error identification for training and educational purposes. The use of You Only Look Once, version 7 (YOLOv7), a real-time object detection algorithm, is a powerful tool for detecting anatomical structures and surgical instruments to provide information that aids decision-making during surgery. In this study, we examined the accuracy and speed of YOLOv7 for detecting anatomical structures using surgical nephrectomy videos. The dataset consisted of 112,532 frames from 45 laparoscopic nephrectomy surgery videos. Videos were randomly divided into 10 benchmarks of training (80%) and test set (20%). A model based on an open-source neural network, YOLOv7, was designed to recognize objects. The model detected the liver, spleen, renal artery, renal vein, and ureter. The anatomical structures of interest were annotated, and a bounding box was drawn around the structure using Label Studio. An expert urologist checked the bounding boxes. The learning rate was set as 0.01, and data were augmented to change the angle of the images each epoch. F1 score, precision, recall, and mean average precision (mAP) were reported. Spleen, liver, and ureter detection yielded mean average positive predictive values (mAP) of 0.93, 0.93, 0.65, precision of 0.80, 0.86, 0.78, and sensitivity of 0.93, 0.85, 0.52, respectively. The total detection speed was 9.5 ms.

	Our model based on YOLOv7 was developed and successfully validated for detecting anatomical structures in video recordings of laparoscopic nephrectomies. With acceptable accuracy and speed, the proposed model may aid in surgical training and real-time decision-making during surgery.
Publications	• **Obiezu F**, Yazdian P, Mendhiratta N, Ball MW, Malayeri AA. Detection of anatomical structures in laparoscopic nephrectomy videos using convolutional neural networks (YOLOV7). [In preparation]

Scholar	Charles C. Osamor III
School	McGovern Medical School at the University of Texas Health Science Center at Houston, Texas
Mentors	Alison Boyce, M.D., Lasker Clinical Research Scholar, Metabolic Bone Disorders Unit, NIDCR Babak Saboury, M.D., Chief, Clinical Data Science Officer, Department of Radiology and Imaging Sciences, NIH Clinical Center
NIH Institute	National Institute of Dental and Craniofacial Research (NIDCR)
Project Title	Lesion Activity in Craniofacial Fibrous Dysplasia: Natural History and Response to Denosumab
Research Summary	Fibrous dysplasia (FD) is a genetically mosaic disorder resulting in poorly mineralized fibro-osseous lesions. FD is heterogeneous; lesion appearance, activity, and sequelae vary by involved body region. FD activity can be monitored using ^{18F}NaF-PET/CT scans, a combined functional and anatomical technique. Recently denosumab, an anti-RANKL antibody, has shown promise in decreasing FD activity; however, research is needed to define use of ^{18}F-NaF PET/CT to assess treatment response. ^{18}F-NaF PET/CT scans were evaluated from 2 cohorts: a natural history protocol (n=27) and a phase 2 trial of denosumab (n=8). Lesions were segmented in two fashions: CT-defined contours determined by radiographic dysmorphic features, and PET-defined contours determined by intra-lesion areas of tracer avidity. PET-based estimates of lesion activity were automatically generated: SUV_{mean}, Volume, Total Activity ($TA=SUV_{mean} \times$ Volume). Generalized equations evaluated associations between lesion activity and clinical parameters. CT-defined contours encompassed the entire dysplastic region for each lesion, while PET-defined contours showed variable uptake within dysplastic regions. For CT-defined lesions, SUV_{mean} was used to determine age and regional effects on FD activity. SUV_{mean} and age were negatively correlated for craniofacial ($p=0.03$) and appendicular ($p=0.04$), but not axial lesions. CT-defined craniofacial lesions had higher SUV_{mean} than appendicular (10.8 vs 7.4, $p<0.001$), but not axial lesions. Denosumab showed a strong effect on PET-defined contours with overall reduced FD activity, but did not impact CT-defined contours. Therefore, PET-defined lesions were used to evaluate regional

denosumab response, showing a larger decline in TA for craniofacial and axial lesions compared with appendicular lesions (678.3 vs 115.7, p=0.005).

Interpretation of ^{18}F-NaF PET/CT in FD must be guided by clinical endpoints. CT-defined contours encompass the entire lesion, making SUV_{mean} an appropriate metric to evaluate individual lesion activity. When assessing treatment response, TA using PET-defined contours is a more useful approach. These results demonstrate that craniofacial FD lesions have greater activity and response to denosumab.

Publications

- Sheppard A, Paravastu S, Wojnowski N, **Osamor CC 3rd**, Farhadi F, Collins M, Saboury B. Emerging role of ^{18}F-NaF PET/computed tomographic imaging in osteoporosis: A potential upgrade to the osteoporosis toolbox. *PET Clin.* 2023 Jan;18(1):1-20. PMID: 36442958.

Abstracts

- **Osamor CC 3rd,** Pan K, Paravastu S, Saboury B, Boyce A. Lesion activity in craniofacial fibrous dysplasia: Natural history and response to denosumab. American Cleft Palate Craniofacial Association 80th Annual Meeting, Raleigh, NC; May 2-6, 2023. [Poster presentation]
- Michel Z, Szymczuk V, Taylor J, Kram V, **Osamor CC 3rd**, Gun Z, Roszko K, Boyce A. Characterizing mutation burden in fibrous dysplasia. Pediatric Endocrine Society Annual Meeting, San Diego, CA; May 5-8, 2023 [Podium presentation]
- Gun Z, Szymczuk V, Taylor J, **Osamor CC 3rd**, Boyce A. Serum phosphorus levels as a driver of skeletal morbidity in patients with fibrous dysplasia. International Meeting of Pediatric Endocrinology, Buenos Aires, Argentina; Mar. 4-7, 2023 [Podium presentation]

Professional Meetings

- American Society of Bone and Mineral Research Annual Meeting, Austin, TX; Sep. 9-12, 2022.
- Student National Medical Education (SNMA) Annual Conference, Hartford, CT; Apr. 5-9, 2023.
- American Society for Aesthetics Eastern Meeting, Philadelphia, PA; Apr. 21-23, 2023.
- American Cleft Palate Craniofacial Association 80th Annual Meeting, Raleigh, NC; May 2-6, 2023

Scholar	Rebecca O. Oyetoro
School	University of Florida College of Medicine
Mentor	Véronique Roger, M.D., Chief, Epidemiology and Community Health Branch
NIH Institute	National Heart, Lung, and Blood Institute (NHLBI)
Project Title	Circulating Ketone Bodies and Mortality in Heart Failure: A Community Cohort Study
Research Summary	The relationship between ketone bodies (KB) and mortality in heart failure (HF) is not defined. The aim of this study was to identify potential determinants of KB and examine the association between KB and all-cause mortality in a population-based HF cohort. 1,389 patients from Southeastern Minnesota were enrolled in a HF community cohort between 2003 and 2012, using the EHR linkage system of the Rochester Epidemiology Project. Plasma KB levels were measured using nuclear magnetic resonance spectroscopy. Linear multivariable regression was used to determine associations between clinical characteristics and KB levels. A conditional inference tree method (ctree R Package) was used to determine optimal KB group cut points. Cox proportional hazard models were used to estimate the association between KB group and mortality, after adjustment for Meta-Analysis Global Group in Chronic HF (MAGGIC) risk score. In the analytic cohort (n= 1382), the median (IQR) KB was 180 (134, 308) µM. Most patients with HF had KB levels in line with healthy adults, 100-600 µM. Advanced HF (NYHA class III-IV) and elevated NT-proBNP ($p < 0.0001$) were positively associated with KB. The median follow-up of the study was 13.9 years, and 5-year mortality rate was 51.8% (95% CI: 49.1-54.4%). The risk of death was increased when KB were elevated (HR 1.07; 95% CI: 1.01-1.14) per 1 SD Log-transformed KB, independently of the clinical characteristics included in a validated clinical risk score. In secondary analyses, patients were divided into two groups with low KB (≤ 471.5 µM; n= 1172) and high KB (> 471.5µM; n= 210). Patients in the high KB group were at an increased risk of mortality, independent of a validated clinical risk score (HR 1.26; 95% CI, 1.08-1.47). In conclusion, in a HF community cohort, the distribution of KB in most

	patients were in line with healthy adults. Increasing KB levels were associated with advanced HF and mortality.
Publications	• **Oyetoro R**, Conners K, Joo J, Turecamo SE, Sampson M, Wolska A, Remaley AT, Otvos JD, Connelly MA, Larson NB, Bielinski SJ, Hashemian M, Shearer JJ, Roger VL. Circulating ketone bodies and mortality in heart failure: A community cohort study. *J Am Coll Cardiol* [Under review]
Abstracts	• **Oyetoro R**, Conners K, Joo J, Turecamo SE, Sampson M, Wolska A, Remaley AT, Connelly MA, Otvos JD, Larson NB, Bielinski SJ, Shearer JJ, Roger VL. Circulating ketone bodies and mortality in heart failure: A community cohort study. American Heart Association's Epidemiology and Prevention/Lifestyle and Cardiometabolic Health 2023 Scientific Sessions. Boston, MA; Feb. 28- Mar. 3, 2023. Circ 2022;147(Suppl 1). [Poster presentation]
Professional Meetings	• American Heart Association's Epidemiology and Prevention/Lifestyle and Cardiometabolic Health 2023 Scientific Sessions. Boston, MA; Feb. 28- Mar. 3, 2023.

Scholar	Zeynep Ozgur
School	Northeast Ohio Medical University
Mentor	Wade W. Chien, M.D., Principal Investigator, Inner Ear Gene Therapy Program
NIH Institute	National Institute on Deafness and Other Communication Disorders (NIDCD)
Project Title	Application of CRISPR Genome Editing to a Mouse Model of Autosomal Dominant Hereditary Hearing Loss, DFNA20/26
Research Summary	Sensorineural hearing loss is a common disorder which affects the world's population. Previous inner ear gene therapy studies have shown promising results in improving auditory function in various mouse models of sensorineural hearing loss. While many studies focus on non-syndromic autosomal recessive hereditary hearing loss (referred to as DFNB), non-syndromic autosomal dominant hereditary hearing loss (DFNA) may be a better candidate for inner ear gene therapy translation due to the later onset and slower progression of hearing loss, which offers a wider therapeutic window for treatment. In this study, we applied CRISPR genome editing to a mouse model of human DFNA20/26. DFNA20/26 is caused by mutations in the *ACTG1* gene, which encodes gamma actin, an isoform of actin that is abundant in the inner ear. The *Actg1*$^{P264L/P264L}$ mutant mouse has an orthologous human P264L knock-in mutation and develops progressive hearing loss starting at about 1 month of age due to outer and inner hair cell stereocilia bundle abnormalities. In this study, guide RNAs (gRNAs) were designed and validated to target the P264L mutation *in vitro*. The gRNA with the best sensitivity and specificity for targeting the P264L mutation was delivered via adeno-associated virus (AAV) along with Cas9 nuclease into neonatal *Actg1*$^{P264L/P264L}$ inner ears via the posterior semicircular canal approach. We found that *Actg1*$^{P264L/P264L}$ mutant mice treated with CRISPR gene therapy showed improved outer hair cell stereocilia morphology compared to untreated mutant mice on scanning electron microscopy (SEM) imaging. In addition, treated *Actg1*$^{P264L/P264L}$ mice (n = 11) showed improved auditory brainstem response (ABR) thresholds at about 1 month of age compared to untreated mutant mice (n = 13) at 8 kHz (29.09 vs 46.92 dB, p = 0.0048) and 16 kHz (56.82 vs 70.77 dB, p = 0.0348). Our results demonstrate that CRISPR genome editing was able to improve the stereocilia morphology and auditory function in *Actg1*$^{P264L/P264L}$ mice.

Publications

- Lau SC, Grati M, Isgrig K, Sinan M, Calabro KR, Zhu J, Ishibashi Y, **Ozgur Z**, Wafa T, Belyantseva IA, Fitzgerald T, Friedman TB, Boye SL, Boye SE, Chien WW. Dual-AAV vector mediated expression of *MYO7A* improves vestibular function in a mouse model of Usher Syndrome 1B. *Mol Ther Methods Clin Dev*. 2023 Aug 21;30:534-545. PMID: 37693946.

Abstracts

- **Ozgur Z**, Lau SC, Grati M, Isgrig K, Zhu J, Wang HJ, Ishibashi Y, Drummond M, Belyantseva IA, Friderici K, Friedman TB, Chien WW. Application of CRISPR genome editing to a mouse model of DFNA20/26. American Society of Gene and Cell Therapy 26th Annual Meeting, Virtual; May 16-20, 2023. [Poster presentation]
- Lau SC, Grati M, Isgrig K, Sinan M, Calabro KR, Zhu J, Ishibashi Y, **Ozgur Z**, Wafa T, Belyantseva IA, Fitzgerald T, Friedman TB, Boye SL, Boye SE, Chien WW. Dual-AAV-mediated gene replacement therapy improves the vestibular function in a mouse model of USH1B. Association for Research in Otolaryngology 46th Annual MidWinter Meeting, Orlando, FL; Feb. 11-15, 2023. [Poster presentation]
- Grati M, Faridi R, Inagaki S, Fenollar-Ferrer C, **Ozgur Z**, Boger E, Morell R, Friedman TB, Chien WW. Identification of a novel missense mutation in the LCCL domain of cochlin which causes DFNA9. Association for Research in Otolaryngology 46th Annual MidWinter Meeting, Orlando, FL; Feb. 11-15, 2023. [Poster presentation]

Professional Meetings

- American Society of Gene and Cell Therapy (ASGCT) 26th Annual Meeting, Los Angeles, CA; May 16-20, 2023.
- Association for Research in Otolaryngology (ARO) 46th Annual MidWinter Meeting, Orlando, FL; Feb. 11-15, 2023.
- Center for Comparative and Evolutionary Biology of Hearing (C-CEBH) and the Division of Intramural Research of the National Institute of Deafness and Other Communication Disorders (NIDCD) Joint Conference, Bethesda, MD; Oct. 12, 2022.

Scholar	Maeve M. Pascoe
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor	Terri S. Armstrong, Ph.D., Deputy Chief, Neuro-Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI-CCR)
Project Title	Sleep on the Brain: An Evaluation of Sleep and Related Patient Reported Outcomes in Primary Brain Tumor Patients

Research Summary

Patients with cancer experience a high burden of sleep disturbances, resulting from their tumor biology, stress, genetic factors, and cancer treatment, which subsequently may result in greater symptom burden, worse mood, and worse quality of life. Sleep disturbances have not been fully characterized in primary brain tumor (PBT) patients, and as such, this investigation aimed to characterize sleep disturbances in these patients using validated measures to identify risk factors associated with severity.

Patients wore smart-wearable devices for one month to monitor activity and sleep, and self-reported sleep data were collected at baseline and one month later using the PROMIS sleep-related impairment and sleep disturbance questionnaires and Morningness-Eveningness Questionnaire, measuring impact of chronotype, a sleep characteristic for which the eveningness type is associated with poorer health status in the general population. Patients (n=54) were predominantly young (56% age <50 yrs), male (56%), white non-Hispanic (85%), with diagnoses of high grade (65%) gliomas (15%), astrocytomas (31%), oligodendrogliomas (13%), and ependymomas (11%). Evaluation of subjective sleep data at baseline revealed that 17% of patients reported moderate-to-severe sleep impairment with 13% reporting moderate-to-severe sleep disturbance, but surprisingly, most patients had a morningness (39%) or intermediate chronotype (56%). Although the study was not designed to measure effects over time, the number of individuals with moderate-to-severe sleep disturbance significantly decreased (down to 4%) after one month (McNemar's test; p=0.031).

These results show that moderate-to-severe sleep impairment and disturbance occur in a subset of patients, and improvements seen in sleep disturbance warrant prospective analysis of sleep tracking as a potential intervention in this population. Finally, the morning-intermediate chronotype predominance in this sample is atypical compared to

	the general population, and evaluation of chronotype in a larger sample is warranted to identify if there is bias in this sample or if PBT patients have a different phenotypic distribution.
Publications	• **Pascoe M**, Byrne E, King A, Cooper D, Foldvary-Schaefer N, Mehra R, Lathia J, Gilbert MR, Armstrong TS. Sleep wake disorders associated with cranial radiation – a systematic review. [In preparation]

Scholar	Abdelrahman M. Rahmy
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentors	Javed Khan, M.D., Deputy Chief, Genetics Branch; Head, Oncogenomics Section John Glod, M.D., Ph.D., Associate Research Physician, Pediatric Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Exploring Mechanisms of Resistance to Tyrosine Kinase Inhibitors in RET-driven Medullary Thyroid Carcinoma Using Genome-Wide CRISPR Gene Knockout
Research Summary	Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor driven primarily by activating mutations in the *RET* proto-oncogene. It accounts for approximately 13% of thyroid cancer-related deaths. Total thyroidectomy is the standard treatment for MTC patients; however, its efficacy is limited due to the high prevalence of metastatic disease at diagnosis. Vandetanib and selpercatinib, both tyrosine kinase inhibitors (TKIs) that function by targeting RET signaling, have been approved for the treatment of advanced MTC. Despite their effectiveness, many patients eventually develop resistance to TKIs. In this study, we employed genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) gene knockout (KO) in MTC cell lines treated with selpercatinib or vehicle to investigate the genetic mechanisms of resistance to RET TKIs and identify potential synergistic or additive drug combinations. Our research identified key genes and pathways influencing the sensitivity or resistance of MTC cells to TKIs. Notably, RAS and mTOR signaling were found to play a significant role in resistance development. Knocking out negative regulators of these pathways (such as NF1 and TSC2) conferred growth advantages to cells treated with RET inhibitors. To validate the CRISPR screening, we selectively knocked down NF1 in MTC cells and found it led to a nearly twofold increase in IC50 for selpercatinib. These findings suggest that targeting these pathways in conjunction with RET inhibitors holds promise for combination therapies. *In vitro* testing revealed an additive effect and dose-dependent synergy between MEK inhibitors (downstream of the RAS pathway) and RET inhibitors. To progress towards clinical translation, *in vivo* mouse experiments will be conducted to validate the efficacy of this combined therapy. If successful, subsequent clinical trials will be initiated. In

conclusion, we have identified novel drug combinations to combat MTC resistance to RET TKIs and these results will inform future clinical trials aimed at improving patient outcomes.

Abstract

- Tian M, Wei JS, Cheuk A, Milewski D, Zhang Z, Kim YY, Liu C, Badr S, Kelly MC, Wu JT**,** **Rahmy A**, Chou H, Wen X, Khan J. FGFR4 and CD276 dual targeting CAR T cells demonstrate synergistic antitumor activity in childhood rhabdomyosarcoma. American Association of Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 14–19, 2023. [Poster presentation]

Scholar	Magdalena A. Rainey
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor	Clint T. Allen, M.D., Acting Chief, Surgical Oncology Program
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Effect of Combined TGF-β/PD-L1 Blockade on Systemic Antitumor Immunity in Head and Neck Cancer

Research Summary

Response rates to traditional immunotherapies are low among patients with head and neck squamous cell carcinoma and novel approaches such as the bifunctional fusion protein bintrafusp alfa (BA) that concurrently blocks PD-L1 signaling and neutralizes TGF-β are actively under investigation. Using single cell sequencing, our group determined that neoadjuvant BA expands exhausted tumor-specific T cells that recirculate into the peripheral blood, possibly through decreased expression of the tissue retention molecule CD103. We hypothesized that the addition of TGF-β neutralization to PD-L1 blockade will enhance systemic anti-tumor immunity compared to PD-L1 blockade alone.

Wild-type mice bearing established mouse oral cancer (MOC)-1 tumors were randomized into the following groups: 1) vehicle; 2) anti-PD-L1; 3) anti-TGF-β; 4) BA; and 5) CD8 depleting antibody alone or 6) in combination with BA. Three days after completing treatment, MOC-1 tumor cells were injected into the contralateral flank and engraftment and progression of secondary challenge tumors was analyzed.

Primary tumor progression was significantly inhibited by BA and to a lesser extent by anti-PD-L1 compared to control or anti-TGF-β. Engraftment and progression of secondary challenge tumors was significantly inhibited by BA and to a lesser extent by anti-PD-L1 compared to control or anti-TGF-β. The growth inhibitory effects of BA were abrogated by CD8 depletion in both the primary and challenge tumors. Flow cytometry analysis showed that BA increased the proportion of tumor-infiltrating $CD4^+$ and $CD8^+$ lymphocytes (TIL) in both the primary and challenge tumors and that CD103 expression was reduced in $CD4^+$ TIL.

Although TGF-β neutralization alone failed to induce anti-tumor immunity, the addition of TGF-β neutralization to PD-L1 blockade provided greater control of primary tumor growth and protection from engraftment of a challenge tumor compared to PD-L1 blockade

	alone. Preliminary studies indicate that enhanced systemic anti-tumor immunity may correlate with reduced tumor-infiltrating T cell expression of CD103.
Publications	• **Rainey MA**, Allen CT, Craveiro M. Egress of resident memory T cells from tissue with neoadjuvant immunotherapy: Implications for systemic anti-tumor immunity. *Oral Oncol* 2023 Sep 20;146:106570. PMID: 37738775. • **Rainey MA**, Clavijo PE, Allen CT. Heterogeneous characterization of neutrophilic cells in the tumor immune landscape. *Front Immunol* [Under review]
Abstracts	• **Rainey MA**, Craveiro M, Robbins Y, Sievers C, Allen CT. Combined TGF-β/PD-L1 blockade enhances systemic antitumor immunity in head and neck cancer. American Association for Cancer Research Annual Meeting, Orlando, FL; Apr. 15-19, 2023. [Poster presentation]
Professional Meetings	• American Association for Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 15-19, 2023.

Scholar	Anirudh Rao
School	Drexel University College of Medicine
Mentor	Andre Larochelle, M.D., Ph.D., Investigator, Regenerative Therapies for Inherited Blood Disorders, Hematology Branch
NIH Institute	National Heart Lung and Blood Institute (NHLBI)
Project Title	Dendrimer-Based Lipid Nanoparticles for Efficient, Low-Toxicity Gene Editing of Human Hematopoietic Stem and Progenitor Cells
Research Summary	Current gene therapy approaches to the treatment of hematopoietic diseases primarily rely upon *ex vivo* delivery of therapeutic transgenes in human hematopoietic stem and progenitor cells (HSPCs) with integrating retroviral vectors or, more recently, CRISPR/Cas9-based gene editing protocols. Systemic delivery of gene editing cargoes would circumvent the complexity of *ex vivo* processing and the toxicities of preparative regimens necessary for engraftment. Accordingly, we sought to develop a lipid nanoparticle (LNP)-based strategy for high-efficiency delivery of CRISPR/Cas9 components to HSPCs. In this study, we characterized LNP formulations based on the recently identified ionizable amino dendrimer lipid 4A3-SC8 for the ability to deliver various RNA cargos. As proof-of-concept, LNPs were first encapsulated with GFP-encoding mRNA and validated by physiochemical characterization. LNPs formulated with total lipid concentrations of 4-6 mM within the organic phase and nucleic acid concentrations of 50 ng/uL within the aqueous phase were determined to yield LNPs with optimal characteristics, including low particle diameter (130-170 nm), low polydispersity index (< 0.2), and high encapsulation efficiency (50-90%). To evaluate transfection efficiency, LNPs were applied to primary HSPCs *in vitro,* and GFP expression was quantified by flow cytometry. A dose-response experiment demonstrated high GFP expression (>80% GFP+ cells) at LNP dosages ≥200 ng per 250K cells, with minimal cytotoxicity. Moreover, colony forming unit assays of HSPCs transfected with these LNPs demonstrated preserved colony forming potential. In additional experiments, dendrimer-based LNPs were individually formulated with Cas9 mRNA and single guide RNA (sgRNA) targeting the human AAVS1 locus. Importantly, indel frequencies of up to 34% were achieved in HSPCs with a single application of Cas9/sgRNA

	encapsulated LNPs, providing an alternative to electroporation-mediated transfection approaches. Overall, dendrimer-based LNPs provide a promising, low-toxicity platform for human HSPC gene therapy. Future experiments will examine the ability of antibody-conjugated LNPs to target HSPCs *in vivo*.
Abstracts	• **Rao A,** Smith R, Vasalatiy O, Lane K, Swenson R, Larochelle A. Dendrimer-based lipid nanoparticles for efficient, low-toxicity gene editing of human hematopoietic stem and progenitor cells. American Society of Gene and Cell Therapy Annual Meeting, Los Angeles, CA; May 16-20, 2023. [Poster presentation]
Professional Meetings	• American Society of Gene and Cell Therapy (ASGCT) 26th Annual Meeting. Los Angeles, CA; May 16-20, 2023

Scholar	Aaron J. Sheppard
School	Louisiana State University Health - Shreveport
Mentors	Michael T. Collins, M.D., Chief, Skeletal Disorders and Mineral Homeostasis Section, NIDCR Babak S. Saboury, M.D., Chief, Clinical Data Science Officer, Department of Radiology and Imaging Sciences, NIH Clinical Center
NIH Institute	National Institute of Dental and Craniofacial Research (NIDCR)
Project Title	Development and Implementation of a Novel ^{18}F-NaF PET/CT-based Imaging Technique to Quantify Vascular Microcalcification in Hyperphosphatemic Familial Tumoral Calcinosis
Research Summary	Familial tumoral calcinosis (FTC) is a rare disorder of FGF23 deficiency, resulting in ectopic soft-tissue calcifications and severe peripheral vascular calcification. The spatial distribution and degree of vascular calcification in FTC have not been characterized. In this study, we investigated the pattern and quantity of vascular microcalcification in seven patients with FTC and four healthy controls using ^{18}F-NaF PET/CT. We measured maximum target-to-background ratios (TBRmax) at perpendicular slices throughout the vasculature from the aortic arch to popliteal arteries and plotted them on a standardized vascular atlas (SVA), which aligned each patient's vasculature to a common space. We computed mean TBRmax values (microcalcification score – mCS), as a summary measure of microcalcification within six vascular segments: the ascending aorta (AA), descending aorta (DA), abdominal aorta (AbA), iliac arteries (IAs), femoral arteries (FAs), and popliteal arteries (PAs). We compared the mCS at each vascular segment of the FTC group to healthy controls using a multiple comparison t-test and performed a repeated measures ANOVA to test if the mCS was more prominent within specific vascular segments among subjects for both groups. Our results showed that the mean mCS was greater in the distal arteries (IAs, FAs, and PAs) but not proximal arteries in patients with FTC compared to healthy controls. FTC patients had a significantly greater mCS in the AbA and FAs compared to other segments. In contrast, healthy controls only had an increase in mCS at the AbA compared to other segments. These findings suggest that microcalcification in FTC primarily affects the distal arteries. This method of fitting slice-wise TBRmax values to the SVA, which is readily applicable to other disease states, allowed for a spatially-aware quantification of ^{18}F-NaF PET and enabled the rapid assessment of vascular disease distribution, which may uncover patterns of vessel microcalcification that would otherwise go unnoticed.

Publications

- **Sheppard AJ**, Paravastu SS, Wojnowski NM, Osamor CC 3rd, Farhadi F, Collins MT, Saboury B. Emerging role of 18F-NaF PET/computed tomography imaging in osteoporosis: a potential upgrade to the osteoporosis toolbox. *PET Clin*. 2023 Jan;18(1):1-20. PMID: 36442958.
- **Sheppard AJ**, Theng E, Paravastu SS, Wojnowski NM, Farhadi F, Morris MA, Hartley IR, Gafni RI, Roszko KL, Collins MT, Saboury B. A spatial atlas for mapping the heterogeneity of vascular microcalcification using ^{18}F-NaF PET/CT: application in familial tumoral calcinosis. *Arterioscler Thromb Vasc Biol*. [Under review]

Abtracts

- **Sheppard AJ**, Theng E, Paravastu SS, Wojnowski N, Galisteo R, Farhadi F, Roszko KL, Collins MT, Saboury B. Novel spatially-aware method for quantification of vascular calcifications using ^{18}F-NaF PET/CT: detecting unique pattern of vascular microcalcification in a cohort of familial tumoral calcinosis. Society for Nuclear Medicine and Molecular Imaging Annual Meeting, Chicago, IL; June 24-27, 2023. [Poster presentation]
- **Sheppard AJ**, Theng E, Paravastu SS, Wojnowski N, Galisteo R, Farhadi F, Roszko KL, Collins MT, Saboury B. Utilizing ^{18}F-NaF PET/CT to inform pathologic and radiographic progression of an ultra-rare case of familial tumoral calcinosis plus a case for synergistic information. Society for Nuclear Medicine and Molecular Imaging Annual Meeting, Chicago, IL; June 24-27, 2023. [Podium presentation]
- **Sheppard AJ**, Paravastu SS, Farhadi F, Boykin W, Hartley IR, Roszko KL, Gafni RI, Saboury B, Collins MT. Utilizing ^{18}F-NaF PET/CT to inform pathologic progression of calcific lesions: exploration of voxel-level structural radiomics. Gordon Research Conference on Physiology, Biology, and Pathology of Phosphate. Galveston, TX; Feb. 12-17, 2023 [Poster presentation]
- **Sheppard AJ**, Paravastu SS, Wojnowski NM, Gafni RI, Roszko KL, Saboury BS, Collins MT. Image-based characterization of calcific lesions in hyperphosphatemic familiar tumoral calcinosis using sodium fluoride (^{18}F-NaF) PET/CT: spatial heterogeneity profiling through radiomics and nuclear medicine. Orthopedic Research Society Annual Meeting, Dallas, TX; Feb. 10-14, 2023. [Poster presentation]

Professional Meetings

- American Society for Bone and Mineral Research Annual Meeting, Austin, TX; Sep. 9-12, 2022
- Orthopedic Research Society Annual Meeting, Dallas, TX; Feb. 10-14, 2023
- Gordon Conference on the Physiology, Biology, and Pathology of Phosphate, Galveston, TX; Feb. 12-17, 2023
- Society of Nuclear Medicine and Molecular Imaging (SNMMI) 2023 Annual Meeting, Chicago, IL; June 24-27, 2023

Awards

- Young Investigator Award, American Society for Bone and Mineral Research, 2022
- Registration Award, Gordon Conference on the Physiology, Biology and Pathology of Phosphate, 2023

Scholar	Julie R. Solomon
School	Washington University School of Medicine in Saint Louis
Mentor	Mark W. Ball, M.D., Associate Research Physician, Urologic Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Characterization of the Pheochromocytoma-predominant Subgroup of von Hippel-Lindau Disease
Research Summary	von Hippel-Lindau disease is a hereditary tumor syndrome affecting multiple organs. A subset of von Hippel-Lindau patients present predominantly with pheochromocytomas. We developed a novel system to classify the pheochromocytoma-predominant subgroups of von Hippel-Lindau disease. von Hippel-Lindau patients who underwent adrenalectomy with pathology-proven pheochromocytoma at our institution were included. We defined pheochromocytoma-predominant von Hippel-Lindau *a priori* as patients with one or more of the following traits: early onset [age at first pheochromocytoma below the cohort's median age (28.2 years)], family history of pheochromocytomas, multiple pheochromocytomas, and paraganglioma(s). Patients with pheochromocytoma-predominant disease were compared to the remaining cohort to determine differences in genotype and phenotype. One hundred thirty-nine von Hippel-Lindau patients (56% male, 91% white) were examined. Preliminary analysis showed that three characteristics (early onset, family history, and multiple pheochromocytomas) correlated with one another. Having paraganglioma(s) did not correlate with the other factors, so it was excluded from the definition of pheochromocytoma-predominant disease. One hundred twelve (79%) of our patients met the final definition. Pheochromocytoma-predominant patients were less likely to have most additional von Hippel-Lindau tumor types, including renal cell carcinoma ($p<0.001$), whereas they were more likely to have missense mutations ($p<0.001$) than the remaining cohort. Overall, pheochromocytoma-predominant patients were most likely to have 0 ($p<0.001$) or 1 ($p=0.008$) extra-adrenal tumor types while non-pheochromocytoma-predominant patients were most likely to have 4 ($p=0.02$) or 5 ($p=0.02$).

Pheochromocytoma-predominant von Hippel-Lindau patients are phenotypically distinct from their non-pheochromocytoma-predominant counterparts and are significantly less likely to have more than one other von Hippel-Lindau manifestation, including renal carcinoma.

Publications

- **Solomon JR**, Ball MW. Adrenal Tumor (adenoma, adrenocortical carcinoma, pheochromocytoma, other hormonal). Elsamra S, Siddiqui M, eds. In: Urology Surgery Clerkship: A Guide for Senior Medical Students. Springer Nature; 2023. Contemporary Surgical Clerkships. [In press]
- **Solomon JR**, Lawson KA, Vocke C, Ricketts C, Linehan WM, Ball MW. Characterization of the pheochromocytoma-predominant subgroup of von Hippel-Lindau disease. [Under review]
- **Solomon JR**, Mendhiratta N, Linehan WM, Ball MW. Characterization of von Hippel-Lindau patients with metastatic pheochromocytoma. [Under review]
- Gomella PT, **Solomon JR**, Ahdoot M, Gurram S, Lebastchi AH, Levy E, Venkatesh K, Kassin MT, Chang R, Wood B, Linehan WM, Ball MW. Timing, incidence and management of delayed bleeding after partial nephrectomy in patients at risk of recurrent, bilateral, multifocal renal tumors. [Under Review]
- Owens-Walton J, **Solomon JR**, Chinonyerem O, Su D, Merino MJ, Metwalli AR, Linehan WM, Ball MW. Nonhereditary bilateral multifocal type I papillary renal cell carcinoma: clinical characteristics, surgical management, active surveillance and clinical outcomes. [Under review]
- Telfer SI, **Solomon JR**, Gurram S, Gomella PT, Li WL, Bratslavsky G, Metwalli A, Linehan WM, Ball MW. Surgical learning curve for robotic multiplex partial nephrectomy: the National Cancer Institute experience. [Under review]

Abstracts

- **Solomon JR**, Lawson KA, Vocke C, Ricketts C, Linehan WM, Ball MW. Characterization of the pheochromocytoma-predominant subgroup of von Hippel-Lindau disease. Annual Meeting of the American Urologic Association, Chicago, IL; Apr. 28 - May 2, 2023. [Podium presentation]
- Antony M, Kozel Z, Gopal N, Loebach L, **Solomon JR**, Metwalli A, Gurram S, Linehan WM, Ball MW. Cumulative impact of re-operative partial nephrectomy for the treatment of recurrent renal masses. Annual Meeting of the American Urologic Association, Chicago, IL; Apr. 28 – May 2, 2023. [Podium presentation]
- Antony M, Rompre-Brodeur A, Chaurasia A, Gopal N, Kozel Z, **Solomon JR**, Loebach L, Gurram S, Linehan WM, Ball MW. Survival benefit of renal transplantation following completion nephrectomy in von Hippel-Lindau disease. Annual Meeting of the American Urologic Association, Chicago, IL; Apr. 28 – May 2, 2023. [Poster presentation]
- Loebach L, Antony M, **Solomon JR**, Gurram S, Merino M, Linehan WM, Ball MW. Clinical, pathologic, and genetic predictors of nuclear grade in von Hippel-Lindau associated renal cell carcinoma. Annual Meeting of the Society of Urologic Oncology, San Diego, CA; Nov. 30 - Dec. 2, 2022. [Poster presentation]

Professional Meetings

- Annual Meeting of the American Urologic Association, Chicago, IL; Apr. 28 – May 2, 2023.

Scholar	Trevor M. Stantliff
School	University of Cincinnati College of Medicine
Mentor	Daniel S. Chertow, M.D., M.P.H., Head, Emerging Pathogens Section
NIH Institute	Clinical Center (CC); National Institute of Allergy and Infectious Disease (NIAID); National Heart, Lung and Blood Institute (NHLBI)
Project Title	Tissue Compartment-specific Differences in T-cell Responses in 44 Fatal COVID-19 Cases
Research Summary	T-cells play an essential role in recognizing and clearing viruses from infected tissues. While peripheral blood T-cell responses among patients with coronavirus disease 2019 (COVID-19) have been characterized, little is known about T-cell repertoire diversity and SARS-CoV-2-specific T-cell responses in tissues. To determine the association between demographic, clinical, and virological variables and T-cell responses in tissues, we sequenced T-cell receptor beta (TCRβ) chains in lung, lymph node, spleen, and blood in 44 fatal COVID-19 cases. Tissues were collected at autopsy, preserved in buffered ethanol, and paraffin embedded. Blood was collected perimortem, and peripheral blood mononuclear cells (PBMCs) were isolated within 24 hours and cryopreserved until use. DNA was isolated from embedded tissues, and PMBCs and TCRβ sequencing was performed using the Adaptive ImmunoSeq platform. SARS-CoV-2-specific T-cells were determined by exact matching of the TCRβ sequences with those reported in the ImmuneCODE and VDJdb databases of functionally-confirmed SARS-CoV-2 specific T-cells. Significantly lower T-cell clonality and higher numbers of unique T-cell rearrangements were observed in lymph nodes compared with lung and PBMCs in all patients, indicating highest T-cell diversity in lymph nodes. Pearson correlations between age and SARS-CoV-2 specific T-cell depth, after controlling for sex and days from illness onset to death, significantly varied across tissue compartments in magnitude and directionality. Specifically, we observed a strong positive correlation between SARS-CoV-2 specific T-cell depth and increased age in lymph nodes and a strong negative correlation in PBMCs. Higher depth of SARS-CoV-2 specific T-cells in lymph nodes of older patients but not in PBMCs suggests impaired trafficking of SARS-CoV-2 T cells from lymph node to blood among older patients with COVID-19, potentially impacting viral clearance.

Abstracts	• **Stantliff TM**, Platt A, Stein SR, Oguz C, Vannella KM, Ramelli SC, Hewitt SM, Chertow DS. Tissue-specific T-cell responses among 44 COVID-19 autopsy cases. National Heart, Lung, and Blood Research Symposium, NIH, Bethesda, MD; Mar. 31, 2023. [Poster presentation]
Professional Meetings	• National Heart, Lung, and Blood Research Symposium, NIH, Bethesda, MD; Mar. 31, 2023

Scholar	Joshua M. Stark
School	University of Tennessee Health Sciences Center College of Medicine
Mentor	Jack F. Shern, M.D., Lasker Clinical Research Scholar, Pediatric Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Genotype and Clinical Outcome of TP53-mutant Rhabdomyosarcoma in a 78-Patient Cohort.
Research Summary	Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescence. Despite decades of study, the standard of care remains a chemotherapy regimen developed in the 1970s, even as the long-term survival for high-risk groups has remained <30% for more than 40 years. Recent genomic landscape studies have identified a number of somatic alterations in RMS. Notably, a recent international effort demonstrated that somatic TP53 mutations negatively impact clinical outcome. While this work provides insight into the overall role of TP53 mutations in RMS, it is not clear to what extent specific TP53 mutations drive therapy response and account for poor outcomes in these patients. To address this gap, we aimed to further characterize the genomic landscape of TP53-mutant RMS. Somatic tumor samples were collected from two cohorts, the Children's Oncology Group and UK malignant mesenchymal tumor and RMS2005 trials. DNA from 78 patients was placed through a custom-capture sequencing assay which targeted 39 oncogenic drivers previously implicated in RMS. Our analysis revealed a unique profile of TP53-mutant RMS cases, with a higher prevalence in young patients. TP53 mutations were found to be enriched in head and neck RMS locations, while the occurrence of TP53 mutations was notably absent in paratesticular RMS. TP53-mutant cases exhibited a higher overall mutation burden, suggestive of genomic instability. Survival analysis demonstrated that specific TP53 mutations, such as an R248Q gain-of-function mutation and Exon 6 mutations, as well as the presence of multiple TP53 mutations in the same tumor, negatively impacted patient survival. Unsupervised clustering based on functional features of TP53 mutations highlighted that dominant negative TP53 variants were associated with poor clinical outcomes. Furthermore, analysis of mutation patterns suggested that co-occurrence of TP53-mutation with FGFR4 V550L or PAX-FOXO1 resulted in poor clinical outcomes.

	This research significantly advances our understanding of TP53-mutant RMS and provides a foundation for further modeling and study of these tumors.
Publications	• **Stark JM**, Shern JF. Implications of recent genomic findings on potential therapies for rhabdomyosarcoma – a review. [In preparation]
Professional Meetings	• Childhood Cancer Data Initiative Annual Symposium, Washington DC; Mar. 28-30, 2023.

Scholar	Eszter Toth
School	Medical College of Georgia at Augusta University
Mentor	Steven M. Holland, M.D., Director, Division of Intramural Research, NIAID; Chief, Immunopathogenesis Section
NIH Institute	National Institute of Allergy and Infectious Diseases (NIAID)
Project Title	Dried Blood Spot Analyses for the Diagnosis of Anti-cytokine Autoantibodies
Research Summary	High-titer neutralizing anti-cytokine autoantibodies (ACAA) have been shown to be involved in several acquired diseases, including pulmonary alveolar proteinosis, cryptococcal meningitis, disseminated/extrapulmonary *Nocardia* infections (anti-GM-CSF autoantibodies), and disseminated mycobacterial disease (anti-IFN-γ autoantibodies). Currently, patient blood samples are shipped via courier and require temperature-controlled conditions for transport. This method is expensive and requires patients to have access to medical personnel to draw the blood. However, the well-established technique of collecting blood on a paper card as a dried blood spot (DBS) for diagnostic testing offers a point-of-care alternative which can be performed with a simple finger prick**.** 15uL of whole blood from patients was blotted on filter paper and stored at 4C until use. The filter paper was hole punched and each punched spot was eluted with 150uL of a 0.05% Tween PBS solution at room temperature overnight. The eluate and paired plasma were screened for ACAAs using a particle-based approach, and the neutralizing activity of antibodies was determined by using flow cytometry to assess downstream effects of cytokine stimulation in healthy monocytes. We confirmed the presence of autoantibodies in the DBS eluate from 5 previously diagnosed patients with anti-GM-CSF autoantibodies, 10 patients with anti-IFN-γ autoantibodies, and 2 patients with anti-IL-12 autoantibodies. We also confirmed the presence of ACAAs using dried plasma eluate from 29 patients with known anti-GM-CSF autoantibodies and 29 patients with anti-IFN-γ autoantibodies. Functional studies showed antibodies recovered from DBS eluate from 3 different patients with anti-IFN-γ autoantibodies were able to block IFN-γ-induced STAT-1 phosphorylation in normal

	PBMC. Temperature studies showed that the ACAAs were detected at similar levels when blotted filter paper was stored at 4C and 40C for a week. The diagnosis of pathogenic ACAAs should be considered in the context of unusual or adult-onset infections, and screening for this diagnosis can be performed with DBS testing.
Publications	• Lee SJ, **Toth E**, Browne SK, Rosen LB, Holland SM. Detection of anti-cytokine autoantibodies and clinical applications. Niewold T, Hooks J, eds. In: Manual of Molecular and Clinical Laboratory Immunology, 9th Edition. ASM Press; 2024. [In press]
Abstracts	• **Toth E**, Rosen LB, Zerbe CS, Holland SM. Dried blood spot analyses for the diagnosis of anti-cytokine autoantibodies. Clinical Immunology Society Annual Meeting, St Louis, MO; May 18-21, 2023. [Poster presentation]
Professional Meetings	• Clinical Immunology Society (CIS) Annual Meeting, St Louis, MO; May 18-21, 2023.

Scholar	Rajiv S. Trehan
School	Rutgers Robert Wood Johnson Medical School
Mentor	Tim F. Greten, M.D., Deputy Chief, Thoracic and GI Malignancies Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	Exploring the CD-8 T Cell Landscape in the Setting of Hepatocellular Carcinoma
Research Summary	Liver cancer is the fourth most common cause of cancer mortality, posing a significant global health challenge, where hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancer. Tumor-associated antigen and potentially tumor-reactive CD8 T cells have been found both in the tumor core and peritumoral tissues of HCC. Despite the common dogma surrounding CD8 T cell function, including their role in exerting a cytotoxic function in HCC, the presence of these tumor-reactive CD8 T cells has not necessarily correlated with improved clinical survival and tumor regression, making them a promising clinical target in HCC patients. Using a metastatic colon cancer (CT26) and melanoma (B16) model in mice, this study explored the frequency, phenotype, and function of CD8 T cells in both tumoral and peritumoral tissues in the liver, subcutaneous tissue, and lung tissue. Additionally, we further confirmed our functional flow results through CD8 T cell antibody depletion to explore their function in HCC. Our study revealed an unusually higher frequency of tumor antigen-specific CD8 T cells in the liver compared to other organ sites. Additionally, hepatic CD8 T cells exhibited ($p<0.05$) a uniquely dysfunctional phenotype, as evidenced by their high expression of exhaustion markers, loss of cytotoxic status, and lack of effect on tumor progression when depleted. Furthermore, their frequency and phenotype could not be explained through proliferation or interaction with regulatory T cells. The uniquely dysfunctional state of tumor antigen-specific CD8 T cells in the liver may underscore the significant disparity in clinical outcomes for primary liver cancer and metastatic disease to the liver, contributing to a higher tumor burden and lack of response to current immunotherapies. This work prompts further studies on possible

	mechanisms for clinical application in both primary and metastatic liver cancer to identify new targets to restore the function of tumor-specific CD8 T cells in cancer patients.
Publications	• Ruf B, Bruhns M, Babaei S, Kedei N, Ma L, Revsine M, Benmebarek MR, Ma C, Heinrich B, Subramanyam V, Qi J, Wabitsch S, Green BL, Bauer KC, Myojin Y, Greten LT, McCallen JD, Huang P, **Trehan R**, Wang X, Nur A, Murphy Soika DQ, Pouzolles M, Evans CN, Chari R, Kleiner DE, Telford W, Dadkhah K, Ruchinskas A, Stovroff MK, Kang J, Oza K, Ruchirawat M, Kroemer A, Wang XW, Claassen M, Korangy F, Greten TF. Tumor-associated macrophages trigger MAIT cell dysfunction at the HCC invasive margin. *Cell*. 2023 Aug 17;186(17):3686-3705.e32. PMID: 37595566. • Ma C, McCallen J, McVey JC, **Trehan R**, Bauer K, Zhang Q, Ruf B, Wang S, Lai CW, Trinchieri G, Berzofsky JA, Korangy F, Greten TF. CSF-1R+ macrophages control the gut microbiome-enhanced liver invariant NKT function through IL-18. J *Immunol*. 2023 Oct 1;211(7):1099-1107. PMID: 37624046.
Abstracts	• Myojin Y, Ruf B, Benmebarek MR, Bauer K, **Trehan R**, Coffman K, Ma C, Monge CB, Xie C, Greten T. Immune cell dynamics of patients and mice with hepatocellular carcinoma treated with anti-PD-L1 plus anti-CTLA-4 combination therapy. American Association for Cancer Research Annual Meeting, Orlando, FL; Apr. 14-29, 2023. *Cancer Research*. 2023;83 (Supplement):5465. [Poster presentation] • Ruf B, Bruhns M, Babaei S, Kedei N, Ma L, Revsine M, Ma C, Heinrich B, Subramanyam V, Qi J, Wabitsch S, Green B, Bauer K, Myojin Y, Benmebarek MR, Greten L, McCallen J, Huang P, **Trehan R**, Wang X, Pouzolles M, Kleiner D, Telford W, Dadkhah K, Ruchinskas A, Stovroff M, Kang J, Oza K, Ruchirawat M, Kroemer A, Wang XW, Claassen M, Korangy F, Greten TF. CSF1R+PD-L1+ tumor-associated macrophages trigger MAIT cell dysfunction at the HCC invasive margin. 1st International iFIT (Image-Guided and Functionally Instructed Tumor Therapies) Conference, Zell am See, Austria; Mar. 21-23, 2023. https://ifit2023.com/ [Podium presentation]
Professional Meetings	• American Association for Cancer Research (AACR) Annual Meeting, Orlando, FL; Apr. 14-29, 2023.

Scholar	Alex Valenzuela
School	David Geffen School of Medicine, University of California Los Angeles Charles R. Drew University of Medicine and Science
Mentor	Zhengping Zhuang, M.D., Ph.D, Senior Investigator, Cancer Stem Cell Biology Program, Neuro-Oncology Branch
NIH Institute	National Cancer Institute, Center for Cancer Research (NCI/CCR)
Project Title	A Novel Dendritic Cell Vaccine Utilizing Mannan Anchored to Irradiated Tumor Cells in the Presence of Immune Adjuvants
Research Summary	Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor and is characterized by a median survival of 15 months. Current standard of care consists of maximal surgical resection, radiation, and chemotherapy with temozolomide; there is no FDA-approved immunotherapy for GBM. Many clinical trials utilizing immune checkpoint inhibitors have failed to reach their primary end point, emphasizing the need for novel therapies. Our lab previously developed a subcutaneous autologous tumor vaccine utilizing the following combined components: irradiated (IR) tumor cells with phagocytosis-stimulating ligands (Mannan-BAM), toll-like receptor (TLR) agonists, and immunostimulant anti-CD40 antibody (collectively abbreviated as MBTA). The aim of our study was to generate a dendritic cell (DC) vaccine utilizing this strategy. To assess if MBTA could cause anti-tumor-directed maturation of DCs *in-vitro*, we harvested bone-marrow DCs (BMDCs) from mice and matured them in the presence of PBS, tumor-lysate (TL) or MBTA . By flow cytometry, we found robust up-regulation of co-stimulatory molecules CD40, CD80 and CD86 and preferential upregulation of major histocompatibility class I (MHC I) molecules in the MBTA group while TL-matured DCs preferentially upregulated MHC II. Furthermore, ELISA analysis showed MBTA DCs significantly upregulated Type 1 cytokines (TNFa and IL-6) and limited regulatory cytokine (IL-10) secretion. To assess if MBTA DCs could protect against tumor metastasis, we utilized a melanoma (B16) prophylactic model and found significant decrease in lung metastasis in mice

vaccinated with MBTA DCs compared to TL and PBS. Immunohistochemistry of metastatic lung lesions demonstrated robust perivascular lymphocytic infiltrate in MBTA DC-vaccinated mice.

Collectively, our results demonstrate that MBTA is an effective strategy to mature and load tumor antigens onto DCs *ex-vivo,* and has efficacy as a DC vaccine to prevent metastasis in a mouse model.

Scholar	Sarita Walvekar
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor	Irene Cortese, M.D., Director, Experimental Immunotherapeutics Unit
NIH Institute	National Institute of Neurological Disorders and Stroke (NINDS)
Project Title	Radiological Characterization of Cortical Lesions in Progressive Multifocal Leukoencephalopathy
Research Summary	Progressive Multifocal Leukoencephalopathy (PML) is caused by reactivation of the JC virus (JCV) in the setting of immune compromise, producing confluent demyelinating lesions seen on magnetic resonance imaging (MRI). Histopathological studies suggest that cortical lesions (CLs) may be an underrecognized feature of PML. The aim of this project was to 1) Determine the prevalence and appearance of CLs in PML on MRI; and 2) assess the relationship between clinical presentation of PML and CLs. Individuals with PML enrolled in the National Institutes of Health Natural History Study of PML (NCT 01730131) underwent 3T brain MRI including T1 weighted MPRAGE ($1mm^3$). Underlying predisposing conditions included human immunodeficiency virus (HIV), hematological malignancy, primary immune deficiencies and immune-mediated diseases. CLs were identified and manually segmented on 3T MPRAGE images by 2 independent raters. 3T MPRAGE images from 10 healthy volunteers and 5 participants with HIV were also evaluated for CLs. Association between CL burden and clinical presentation was explored. 35/42 individuals with PML (83.3%) had CLs on 3T MPRAGE images, median 6 lesions, range 0-84. No CLs were observed in 10 healthy volunteers and 5 participants with HIV. PML CLs were predominantly small, round, intracortical lesions or leukocortical lesions, some of which extended longitudinally along the cortex-white matter junction. No apparent association was found between CL burden and patient age, underlying diagnosis, time since PML diagnosis or specific signs of cortical dysfunction (aphasia or seizures) at the first timepoint. Our results suggest intracortical and leukocortical lesions are a common finding in PML and are detectable on clinical MRI scans. Characterization of cortical pathology in PML is

	expected to provide insight into the pathophysiology of this opportunistic infection and generally into cortical involvement across demyelinating diseases with different etiologies.
Abstracts	• **Walvekar S**, Nistri R, Al-Louzi O, Fletcher A, Bhagavatheeshwaran G, Reich DS, Beck ES, Cortese I. Radiological characterization of cortical lesions in progressive multifocal leukoencephalopathy. Americas Committee for Treatment and Research in Multiple Sclerosis Annual Meeting, San Diego, CA; Feb. 23-25, 2023 [Podium presentation]. • **Walvekar S**, Nistri R, Al-Louzi O, Fletcher A, Bhagavatheeshwaran G, Reich DS, Beck ES, Cortese I. Radiological characterization of cortical lesions in progressive multifocal leukoencephalopathy. American Academy of Neurology Annual Meeting, Boston, MA; Apr. 22-27, 2023 [Podium presentation]
Professional Meetings	• Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) Annual Meeting, San Diego, CA; Feb. 23-25, 2023. • American Academy of Neurology (AAN) 75th Annual Meeting, Boston, MA; Apr. 22–27, 2023.
Awards	• Finalist, Best Young Investigator Poster, Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS), 2023 • Educational Grant and Speaker Travel Grant, Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS), 2023

Scholar	Philip R. Wang
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentor	Carlos Zarate, M.D., Chief, Experimental Therapeutics and Pathophysiology Branch; Chief, Section on the Neurobiology and Treatment of Mood Disorders
NIH Institute	National Institute of Mental Health (NIMH)
Project Title	Dextromethorphan-quinidine in Treatment-resistant Depression: an Open-label Pilot Study
Research Summary	Approximately one third of patients with major depressive disorder have treatment-resistant depression (TRD). Drugs that target the glutamatergic system, such as the NMDA receptor (NMDAR) antagonist ketamine, have been shown to lead to rapid and robust antidepressant effects, but are limited by accessibility and route of administration. In this open-label, in-hospital pilot study, subjects with TRD received treatment with 20 mg/10 mg oral dextromethorphan/quinidine (DM/Q), a non-competitive NMDAR antagonist, three times daily. Montgomery-Asberg Depression Rating Scale (MADRS) scores were obtained at baseline prior to DM/Q administration and regularly during hospitalization. MADRS scores of 0 to 6 indicate no depression; 7-19, mild depression; 20-34 moderate depression, and >35, severe depression. Improvement of 2 or more points on the MADRS is considered clinically relevant. In this study, full response was defined as a ≥ 50% reduction in baseline MADRS score, partial response as a 25–50% decrease in baseline MADRS score, and non-response as a < 25% reduction or increase in baseline MADRS score. Seventeen inpatients (40.8 ± 12.3 years; 9F/8M) were given open-label DM-Q for 5.1 ± 2.7 weeks. 54% of patients responded to DM/Q; 15% achieved full response and 38% experienced a partial response. The largest MADRS difference observed at any timepoint was -6.4 ± 8.4 points (-21.0% ± 29.9) and the MADRS difference observed at time of DM/Q discontinuation or hospital discharge was -4.8 ± 8.4 points (-15.9% ± 29.7). 24% of patients had a non-serious adverse event. In this open-label pilot study, DM/Q was well tolerated and had mixed efficacy. Further research with larger, placebo-controlled trials are needed to determine the real-world efficacy of DM/Q and to identify predictors of response to NMDAR antagonists.

Publications	• **Wang PR**, Yavi M**,** Lee H, Kotb Y, Shora L, Park LT, Zarate CA. An open-label study of adjunctive dextromethorphan/quinidine in treatment-resistant depression. *J Clin Psychopharmacol.* 2023 Sep-Oct 01;43(5):422-427. PMID: 37683231.
Abstracts	• **Wang PR**, Yavi M, Park L, Zarate C. An open-label study of adjunctive dextromethorphan/quinidine in treatment-resistant depression. Society of Biological Psychiatry Annual Meeting, San Diego, CA; Apr. 27-29, 2023. *Biol Psychiatry.* 2023;93(9):S177-178. [Poster presentation]
Professional Meetings	• Society of Biological Psychiatry Annual Meeting, San Diego CA; Apr. 27-29, 2023.

Scholar	Georgina V. Whelan
School	Cleveland Clinic Lerner College of Medicine of Case Western Reserve University
Mentors	Gretchen Gierach, Ph.D., Chief, Integrative Tumor Epidemiology Branch Jacqueline B. Vo, Ph.D., R.N., Assistant Clinical Investigator, Radiation Epidemiology Branch
NIH Institute	National Cancer Institute, Division of Cancer Epidemiology and Genetics (NCI/DCEG)
Project Title	Association of Surgery and Radiotherapy with Risk of Thoracic Soft Tissue Sarcoma Among Older Breast Cancer Survivors
Research Summary	Although breast cancer survivors are living longer, they have an increased risk of treatment-related second cancers, including rare but deadly thoracic soft tissue sarcomas (tSTS) such as angiosarcomas, compared with the general population. Limited studies have examined the relationship between surgery type and radiotherapy on tSTS risk. We undertook this large-scale study to analyze the joint effects of surgery type and radiotherapy with tSTS risk. The SEER-Medicare analytic cohort included 162,507 patients diagnosed with primary breast cancer between 2000-2017, aged 66-84 years, who survived 1 year, and were followed until 2018. We calculated the cumulative incidence of tSTS and angiosarcoma, accounting for competing risks. We then examined the joint effects of breast surgery and radiotherapy receipt (mastectomy + no/unknown radiotherapy, mastectomy + radiotherapy, breast conserving surgery [BCS] + no/unknown radiotherapy, and BCS + radiotherapy), with risk of developing any tSTS or angiosarcoma specifically. We calculated hazard ratios (HRs) from multivariable Cox proportional hazard models, with attained age as the time scale, and adjusted for year of diagnosis (2000-2004, 2005-2009, 2010-2017), chemotherapy (yes, no/unknown), and stage (in situ, localized, regional/distant, unknown). Mean age at breast cancer diagnosis was 73.7 (SD 5.2) years. At 5 years, cumulative incidence of tSTS was 0.036% (95% CI 0.027-0.48) and angiosarcomas was 0.019% (95% CI 0.012-0.029). Compared with patients who received mastectomy + no/unknown radiotherapy, patients receiving mastectomy + radiotherapy were at an elevated risk, though this was not statistically significant (HR 2.18, 95% CI 0.71-6.70). Compared with

	patients who received mastectomy + no/unknown radiotherapy, patients who received BCS without radiotherapy (HR 3.04, 95% CI 1.19-7.76) or with radiotherapy (HR 7.01, 95% CI 3.55-13.84) were at elevated risk of developing tSTS. Nearly all angiosarcomas occurred among patients treated with BCS + radiotherapy. These results indicate that the greatest risk of tSTS is observed after BCS + radiotherapy, suggesting the need for heightened awareness of tSTS.
Abstracts	• **Whelan G**, Gierach G, Veiga L, Hardell K, Lang J, Patil S, Schonfeld S, Berrington de Gonzales A, Vo J. Association of surgery and radiotherapy with risk of thoracic soft tissue sarcoma among older survivors of breast cancer. American Society of Clinical Oncology Annual Meeting, Chicago, IL; June 2-6, 2023. *J Clin Oncol.* 2023; (suppl 16; abstr e24063)
Professional Meetings	• American Society of Clinical Oncology (ASCO) Annual Meeting, Chicago, IL; June 2-6, 2023.