DNA Pitt Crew

Fall 2023

The latest news and updates from the UPMC Hillman Cancer Center Genome Stability Program

University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

Patricia Opresko, PhD

Bennett Van Houten, PhD

Genome Stability Program GSP

Note from the Genome Stability Program Co-leaders, Patricia Opresko, PhD, and Bennett Van Houten, PhD

This Summer and Fall have been busy months for the Genome Stability Program Members at the UPMC Hillman Cancer Center, and we are excited to present the Fall 2023 edition of the DNA Pitt Crew newsletter.

We held an annual mini symposium organized by and for the trainees in GSP on June 20, 2023. This year, Ragini Bhargava, PhD, and Daniela Muoio, PhD, hosted our keynote lecturer Roger Greenberg, MD, PhD from the University of Pennsylvania. We also hosted several guests who made Pitt stops giving lectures on their recent work and interacting with faculty and trainees.

UPMC Hillman Cancer held its annual retreat September 26-27, 2023. The retreat included short talks and 130 posters by faculty and trainees. The Ronald B. Herberman, MD Lectureship was given by George Coukos, MD, PhD from Ludwig Cancer Research and University Hospitals of Canton Vaud (CHUV) Lausanne, Switzerland. He spoke about "Designing effective T cell therapy for solid tumors".

The GSP continues to publish high impact work, as noted in the Hot Papers and Cool Science sections, which discusses impactful studies, including: 1) the role of bone marrow leptin receptor and stem cell maintenance (*Blood*); 2) the Rtf1 subunit of the Paf1 complex stimulates H2B ubiquitination during transcription (*PNAS*) ; 3) small molecule electrophilic nitroalkenes inhibit RAD51-mediated homologous recombination of double-strand breaks (*Redox Biol.*); 4) Large T antigen of Merkle cell virus and SV40 polyomavirus melt DNA origins without ATP-dependent helicase activity (*PNAS*); and 5) Erk and C-Myc signaling in host-derived tumor endothelial cells is essential for solid tumor growth (PNAS).

The GSP continues to recruit new faculty and we were delighted that radiation oncologists Yvonne Mowery, MD, PhD and Serah Choi, MD, PhD have recently joined the GSP to provide strong bench to beside support in cancer treatment.

We wish everyone a productive and healthy 2024!

Inside this issue:

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Faculty Spotlight: Elise Fouquerel, PhD

Elise Fouquerel, PhD

Contributed by Elise Fouquerel, PhD

Elise Fouquerel, PhD, is an Assistant Professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh and a member of the Genome Stability Program at UPMC Hillman Cancer Center. Her research is focused on understanding the roles of ADP-ribose transferases enzymes PARP1 and PARP2 in preserving the integrity of the human genome when exposed to oxidative and replication stresses.

Dr. Fouquerel completed her bachelor's degree in biological and biochemical analyses at the University of Caen (Normandy, France) and then continued her graduate education at The Henri Poincaré University in Nancy (Loraine, France), where she obtained a master's degree in Enzymology and Structural Biology and a second master's degree in Microbiology and Molecular Biology. She then pursued with a PhD under the mentorship of Dr. Valerie Schreiber at the University of Strasbourg (Alsace, France). Her thesis work was focused on deciphering the roles of the enzyme poly(ADP-ribose) glycohydrolase in the response to DNA damage and replication stress and yielded three first author papers and a collaborative methods article. During a DNA repair meeting in the Netherlands, Elise met Dr. Robert Sobol and decided to join his lab at the University of Pittsburgh for her postdoctoral journey to pursue her studies in the DNA repair and poly(ADP-ribose) transferase fields. In Dr. Sobol's lab, she focused on the roles of PARP1 in the repair on alkylated DNA lesions and impact of PARP1 activity on the cell metabolism. She demonstrated that PARP1 overactivation not only deplete cellular NAD^+ pools but also that the ADP-ribose can target hexokinase 1 to shut down its activity, thereby preventing glycolysis. This work was published in *Cell Reports* in 2014.

As her interest in the ART enzyme and DNA repair fields grew stronger, she became fascinated by telomeres, genomic loci where DNA repair mechanisms are differently regulated than in the rest of the genome. She was particularly curious to uncover the roles of DNA-dependent ART enzymes in these mechanisms. Thus, she decided to join Dr. Patricia Opresko's lab for a second postdoc, where she focused on deciphering the mechanisms driving telomere dysfunction upon oxidative stress. In 2016, she received funding from NIEHS in the form of K99/R00 Pathway to Independence Award, which focused on deciphering the roles and interactions of PARP1 and PARP2 at telomeres when subjected to oxidative and replicative stresses. This award paved the way for her current research addressing the roles of PARP enzymes in the preservation of genome integrity.

Elise started her independent position as an Assistant Professor in December 2018 in the department of Biochemistry and Molecular Biology at Thomas Jefferson University and Sydney Kimmel Medical College. Her lab then moved back to the University of Pittsburgh School of Medicine and Department of Pharmacology and Chemical Biology in February 2022.

The Fouquerel lab is composed of two postdoctoral associates, Drs. Daniela Muoio and Rim Nassar, and two PhD students, Natalie Laspata and Lily Thompson. Their research currently investigates three main projects: (1) they recently demonstrated that PARP1 binds R-loops and contributes to prevent R-loop associated genome instability (Laspata et al., 2023, *NAR*). They are now building upon this exciting discovery to gain a deeper understanding of the mechanisms involved in the binding of PARP1 to R-loops and the targets of PARP1 activity at R-loop formation sites. This project was spear headed by Natalie Laspata, who successfully defended on November 9 this year. (2) In a project led by Dr. Muoio and currently under revision in *Nature Communications*, they reported a role for PARP2 in driving telomere fragility through mediating Break-Induced Replication upon replication stress. (3) Finally, the Fouquerel lab received a MIRA R35 award to investigate the impact of oxidative stress on centromere function and genome integrity.

Natalie Laspata, Lily Thompson, Daniela Muoio, Elise Fouquerel, PhD & Rim Nassar

Outside the lab, Elise spends a lot of time outdoors on hikes with her husband and their Great Pyrenees, Poppy. She also enjoys gardening, nature photography and spends the rest of her energy practicing CrossFit.

Trainee Spotlight: Matt Schaich, PhD

Matt Schaich, PhD

Contributed by Matt Schaich, PhD

Matt graduated with his PhD in 2020 from the University of Kansas Medical Center in the laboratory of Dr. Bret Freudenthal. During his PhD research, he studied how DNA polymerases guard genomic integrity by selecting the proper nucleotide for insertion using X-ray crystallography and enzymology. Improper nucleotide insertion can result in a mismatch and mutagenesis on one hand; on the other hand, several nucleotide analogs currently in the clinic use polymerase insertion into the genome to produce their therapeutic effect. His studies initiated with pol β, the polymerase involved in base excision repair, and then extended to telomerase reverse transcriptase in collaboration with the Opresko group at Pitt. Using a detailed structure-function analysis, they identified a variant of telomerase that inserts ribonucleotides just as efficiently as deoxyribonucleotides, which in a biological context, would extend telomere sequences composed of RNA.

Matt then joined the Van Houten group in January 2021, excited to move from the static world of 3D structure to the more dynamic world of single-molecule studies – by fluorescently tagging molecules they can be watched in real time as they bind and scan DNA for damage. Matt joined the Van Houten lab only a few weeks before a new single-molecule scope arrived at UPMC Hillman Cancer Center, where he was supported by a Hillman Oncology Fellowship, the C-trap correlative optical tweezers and fluorescent microscope from Lumicks. Matt quickly became a primary user of this system and currently acts as the C-trap facility user director.

After joining the Van Houten lab, Matt was instrumental in developing a new single-molecule method for the C-trap, termed the Single-molecule analysis of DNA-binding protein from nuclear extracts (or SMADNE). This system allows researchers to directly determine single-molecule kinetics of a DNA binding protein without the need for purification. Using the system, the Van Houten group has been able to study over 30 different proteins and variants on the C-trap system. Aside from this method development paper (published in *NAR* in 2023), Matt has been prolifically involved on other projects in the lab since joining in 2021– so far Matt has been coauthor for five other research articles, with one other manuscript currently in revision supported by a F32 from NIEHS. Outside of the lab, Matt enjoys playing piano and violin duets with his wife Laurel, as well as exploring the latest restaurants and breweries in Pittsburgh. Matt submitted his K99/R00 pathway to independence proposal this fall. If that goes well, he will be in the market to secure his own independent faculty position.

Pitt Stops: Special Events and Visiting Speakers

Nimrat Chatterjee, PhD

Nimrat Chatterjee, PhD

Assistant Professor
University of Vermont

Visit June 28, 2023

Contributed by Patricia Opresko, PhD

Dr. Nimrat Chatterjee delivered an engaging research seminar entitled "Targeting translesion synthesis-dependent cancer resistance and genome instability." She talked about her exciting work on translesion protein REV1 and its ability to interact with numerous translesion (TLS) DNA polymerases to facilitate bypass of DNA lesions, often causing mutations. By targeting the interface of REV1 with TLS polymerases, Dr. Chatterjee and colleagues developed an exciting new drug that inhibits REV1 termed JH-RE-06. They found this drug enhances cisplatin treatment of cancer cells and mouse xenograft models, and she shared some new data elucidating the mechanism. The goal is to prevent the development of

therapy resistant cancer cells by inhibiting TLS-induced mutagenesis. She also talked about exciting new work investigating the role of DNA damage response and translesion synthesis pathways in host responses to viral infections, including the SARS-CoV-2 virus responsible for the COVID-19 pandemic. During her visit, she met with many of our faculty to discuss various projects, and found new opportunities for collaboration.

Pitt Stops: Special Events and Visiting Speakers (continued)

Orlando Scharer, PhD

Orlando Scharer, PhD

Associate Director, Center for Genomic Integrity
Institute for Basic Science
Ulsan National Institute of Science and Technology

Visit: June 25-June28, 2023

Contributed by Bennett Van Houten, PhD

Dr. Scharer presented a lecture, entitled, "Understanding and targeting nucleotide excision repair" in which he spoke on two topics. In the first part of his seminar, Dr. Scharer summarized his recent published work on role of XPA-RPA interface in nucleotide excision repair (NER) (*Proc Natl Acad Sci U S A*. 2022 Aug 23;119(34): e2207408119). He, in collaboration with Dr. Walter Chazin at Vanderbilt, found that there are two critical interaction surfaces of XPA-RPA in the preincision NER complex. In the second part of his talk, he presented unpublished work on the mechanism of how the compound, trabectedin, inhibits transcription-coupled NER in such a manner as to kill NER proficient cells due to an accumulation of aborted incision products initiated by XPF, but not completed by XPG. He and his team were able to map trabectedin-induced single strand breaks across the genome using a new technique called TRABI-seq to map highly expressed genes and transcription start sites. Surprisingly, this genomic approach revealed divergent transcriptions into the gene body on the transcribed strand and roughly 200 bp on the non-transcribed strand. It is not known if these aborted TCR-NER complexes could act to block further transcription and DNA replication. These exciting results could be used for precision oncology in the treatment of tumors in conjunction with cisplatin.

Scientific Conference Highlights and Awards

Natalie Laspata

Awards

- Fouquerel's lab PhD student Natalie Laspata was awarded a travel award to go to the EMGS meeting in Chicago and won the 2nd place Best Poster Award. She defended her PhD thesis in November.
- Dr. Daniela Muoio also got an EMGS travel Award.
- Dr. Peter Di received a National Institute of Allergy and Infectious Diseases award, developing a novel class of peptide antibiotics targeting carbapenem-resistant gram-negative organisms.
- Dr. Ragini Bhargava from the O'Sullivan lab won best poster award at the Gordon Research Conference on Epigenetics in August.

GSP Mini Symposium

06/20/2023 - The Assembly, Pittsburgh
Contributed by Ragini Bhargava, PhD & Daniela Muoio, PhD

Roger Greenberg, MD, PhD

In June 2023, the Genome Stability Program (GSP) hosted its highly anticipated annual mini-symposium, featuring Roger Greenberg, MD, PhD. This event was jointly chaired by postdocs Ragini Bhargava, PhD from the O'Sullivan Lab and Daniela Muoio, PhD, from the Fouquerel Lab.

The mini-retreat shined a spotlight on the research of eight outstanding GSP trainees. Each trainee delivered captivating talks, setting the stage for the highlight of the day: the keynote address by Dr. Roger Greenberg.

Dr. Greenberg holds the prestigious J. Samuel Staub Professorship in the Department of Cancer Biology at the Perelman School of Medicine at the University of Pennsylvania and is known for his groundbreaking contributions to the field of genome stability. Dr. Greenberg's lab has been at the forefront of exploring how cells respond to DNA damage and chemotherapeutics. Moreover, his lab maintains a keen interest in unraveling the intricate molecular mechanisms governing recombination at telomeres in cancers, especially those reliant on the Alternative Lengthening of Telomeres (ALT) pathway for their propagation.

At the GSP retreat, Dr. Greenberg delivered an enlightening talk entitled, "Emerging Vulnerabilities in Homologous Recombination," where he shared insights and exciting findings at the intersection of DNA repair and the immune system.

Following his keynote lecture, the event departed from tradition, substituting the usual poster session with a dynamic flash talk session, which again highlighted the exceptional work carried out by undergraduate, graduate, and postdoctoral trainees of the GSP.

Dr. Greenberg actively engaged throughout the day, posed insightful questions, and fostered stimulating discussions. The event was an immersive and enriching celebration of the research being carried out by the members of the program, and we are looking forward to the next retreat!

Hot Papers

1. Ao W, Kim HI, Tommarello D, Conrads KA, Hood BL, Litzi T, Abulez T, Teng PN, Dalgard CL, Zhang X, Wilkerson MD, Darcy KM, Tarney CM, Phippen NT, Bakkenist CJ, Maxwell GL, Conrads TP, Risinger JI, Bateman NW. "Metronomic dosing of ovarian cancer cells with the ATR inhibitor AZD6738 leads to loss of CDC25A expression and resistance to ATRi treatment." *Gynecol Oncol*. 2023;177:60-71. Epub 20230826. PubMed PMID: 37639904.
2. Ellison MA, Namjilsuren S, Shirra MK, Blacksmith MS, Schusteff RA, Kerr EM, Fang F, Xiang Y, Shi Y, Arndt KM. "Spt6 directly interacts with Cdc73 and is required for Paf1 complex occupancy at active genes in Saccharomyces cerevisiae." *Nucleic Acids Res*. 2023;51(10):4814-30. PubMed PMID: 36928138; PMCID: PMC10250246.
3. Groslambert J, Prokhorova E, Wondisford AR, Tromans-Coia C, Giansanti C, Jansen J, Timinszky G, Dobbelstein M, Ahel D, O'Sullivan RJ, Ahel I. "The interplay of TARG1 and PARG protects against genomic instability." *Cell Rep*. 2023;42(9):113113. Epub 20230906. PubMed PMID: 37676774.
4. Jang S, Raja SJ, Roginskaya V, Schaich MA, Watkins SC, Van Houten B. "UV-DDB stimulates the activity of SMUG1 during base excision repair of 5-hydroxymethyl-2'-deoxyuridine moieties." *Nucleic Acids Res*. 2023;51(10):4881-98. PubMed PMID: 36971122; PMCID: PMC10250209.
5. Karapetyan L, Iheagwara UK, Olson AC, Chmura SJ, Skinner HK, Luke JJ. "Radiation dose, schedule and novel systemic targets for radio-immunotherapy combinations." *J Natl Cancer Inst*. 2023. Epub 20230622. PubMed PMID:

Hot Papers (continued)

37348864.

6. Longarini EJ, Dauben H, Locatelli C, Wondisford AR, Smith R, Muench C, Kolvenbach A, Lynskey ML, Pope A, Bonfiglio JJ, Jurado EP, Fajka-Boja R, Colby T, Schuller M, Ahel I, Timinszky G, O'Sullivan RJ, Huet S, Matic I. "Modular antibodies reveal DNA damage-induced mono-ADP-ribosylation as a second wave of PARP1 signaling." *Mol Cell*. 2023;83(10):1743-60. e11. Epub 20230427. PubMed PMID: 37116497; PMCID: PMC10205078.
7. Mielko Z, Zhang Y, Sahay H, Liu Y, Schaich MA, Schnable B, Morrison AM, Burdinski D, Adar S, Pufall M, Van Houten B, Gordân R, Afek A. "UV irradiation remodels the specificity landscape of transcription factors." *Proc Natl Acad Sci U S A*. 2023;120(11):e2217422120. Epub 20230308. PubMed PMID: 36888663; PMCID: PMC10089200.
8. Rose AM, Goncalves T, Cunniffe S, Geiller HEB, Kent T, Shepherd S, Ratnaweera M, O'Sullivan RJ, Gibbons RJ, Clynes D. "Induction of the alternative lengthening of telomeres pathway by trapping of proteins on DNA." *Nucleic Acids Res*. 2023;51(13):6509-27. PubMed PMID: 36940725; PMCID: PMC10359465.
9. Yu YP, Liu S, Ren BG, Nelson J, Jarrard D, Brooks JD, Michalopoulos G, Tseng G, Luo JH. "Fusion Gene Detection in Prostate Cancer Samples Enhances the Prediction of Prostate Cancer Clinical Outcomes from Radical Prostatectomy through Machine Learning in a Multi-Institutional Analysis." *Am J Pathol*. 2023;193(4):392-403. Epub 20230118. PubMed PMID: 36681188; PMCID: PMC10123524.

Featured: Translational Research

LepR$^+$ niche cell–derived AREG compromises hematopoietic stem cell maintenance under conditions of DNA repair deficiency and aging.

The cross talk between extrinsic niche-derived and intrinsic hematopoietic stem cell (HSC) factors controlling HSC maintenance remains elusive. In this study Dr. Du and colleagues demonstrated that amphiregulin (AREG) from bone marrow (BM) leptin receptor (LepR$^+$) niche cells is an important factor that mediates the cross talk between the BM niche and HSCs in stem cell maintenance. They discovered that mice deficient of the DNA repair gene *Brca2*, specifically in LepR$^+$ cells (LepR-Cre;*Brca2*$^{fl/fl}$), exhibited increased frequencies of total and myeloid-biased HSCs. Furthermore, HSCs from LepR-Cre;*Brca2*$^{fl/fl}$ mice showed compromised repopulation, increased expansion of donor-derived, myeloid-biased HSCs, and increased myeloid output. *Brca2*-deficient BM LepR$^+$ cells exhibited persistent DNA damage–inducible overproduction of AREG. Ex vivo treatment of wild-type HSCs or systemic treatment of C57BL/6 mice with recombinant AREG impaired repopulation, leading to HSC exhaustion. Conversely, inhibition of AREG by an anti–AREG-neutralizing antibody or deletion of the Areg gene in LepR-Cre;*Brca2*$^{fl/fl}$ mice rescued HSC defects caused by AREG.

Impact: Mechanistically, AREG activated the phosphoinositide 3-kinases (PI3K)/AKT/ mammalian target of rapamycin (mTOR) pathway, promoted HSC cycling, and compromised HSC quiescence. They further showed that BM LepR$^+$ niche cells from other DNA repair–deficient and aged mice also showed persistent DNA damage–associated overexpression of AREG, which exerts similar negative effects on HSC maintenance. Therefore, Dr. Du has defined an important factor that regulates HSCs function under conditions of DNA repair deficiency and aging.

Funding: This work is supported by the National Institutes of Health (NIH), National Heart, Lung, and Blood Institute (grant R01HL151390) (to W.D). This project used the Hillman Animal Facility, which is supported, in part, by NIH, National Cancer Institute (grant P30CA047904). W.D is a 2021- 22 Hillman Senior Fellow for Innovative Cancer Research at the University of Pittsburgh.

Source: Wu L, Lin Q, Chatla S, Amarachintha S, Wilson AF, Atale N, Gao ZJ, Joseph J, Wolff EV, Du W. *Blood*. 2023 Nov 2;142(18):1529-1542. PMID: 37584437; PMCID: PMC10656728.

Featured: Translational Research (continued)

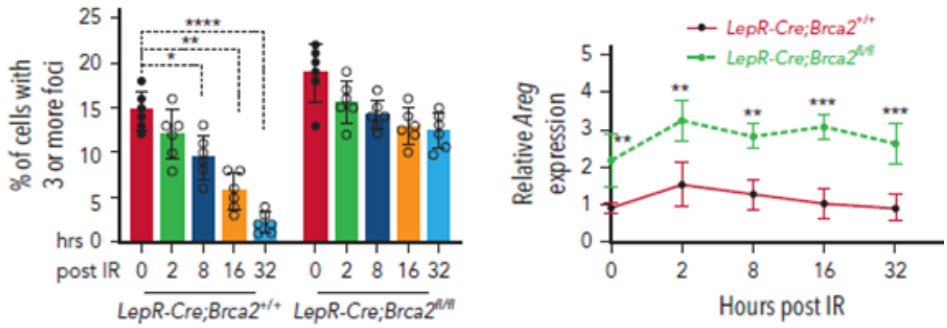


Figure Legend: Persistent DNA damage. BM CD45-LepR+ cells isolated from LepR-Cre;Brca2+/+ or LepR-Cre;Brca2fl/fl mice after IR (300 cGy) were subjected to immunofluorescence staining for γ-H2AX foci (left) and qPCR analysis for Areg messenger RNA levels (right) at 0, 2, 8, 16, and 32 hours after IR (n = 6).

Cool Science

Paf1 complex subunit Rtf1 stimulates H2B ubiquitylation by interacting with the highly conserved N-terminal helix of Rad6.

The coupling of histone modifications to transcription elongation plays an important role in regulating the accuracy and efficiency of gene expression. The RNA polymerase II (RNAPII)-associated Paf1 transcription elongation complex (Paf1C) is required for the ubiquitylation of histone H2B, and achieves this via its Rtf1 subunit which interacts with ubiquitin conjugase Rad6. In this study, Dr. Ardnt and colleagues sought to understand how Rad6 is targeted to its histone substrates. Using in vitro cross-linking followed by mass spectrometry, they defined the primary contact surface between the histone modification domain (HMD) of Rtf1 and Rad6. This allowed them to identify and characterize a separation-of-function mutation in S. cerevisiae RAD6 that greatly impaired the RAD6-HMD interaction and H2BK124 ubiquitylation, but not other Rad6 functions. Their results support a model in which a specific interface between a transcription elongation factor and a ubiquitin conjugase guides substrate selection toward a highly conserved chromatin target in regulating gene expression.

Impact: Disrupting the coordination between transcription and histone modifications can deleteriously affect gene expression and genome architecture. This study provides insights into how a conserved domain in Paf1C, which is necessary and sufficient for Paf1C-mediated stimulation of H2B ubiquitylation, interacts with RAD5 ubiquitin conjugase thereby guiding its specificity. RAD6 dysregulation has been implicated in cancer.

Funding: R01GM52593 and R35GM141964 (to K.M.A).

Source: Fetian T, McShane BM, Horan NL, Shodja DN, True JD, Mosley AL, Arndt KM (GS). *Proc Natl Acad Sci U S A*. 2023. 20:e2220041120. PMID: 37216505, PMCID: PMC10235976.

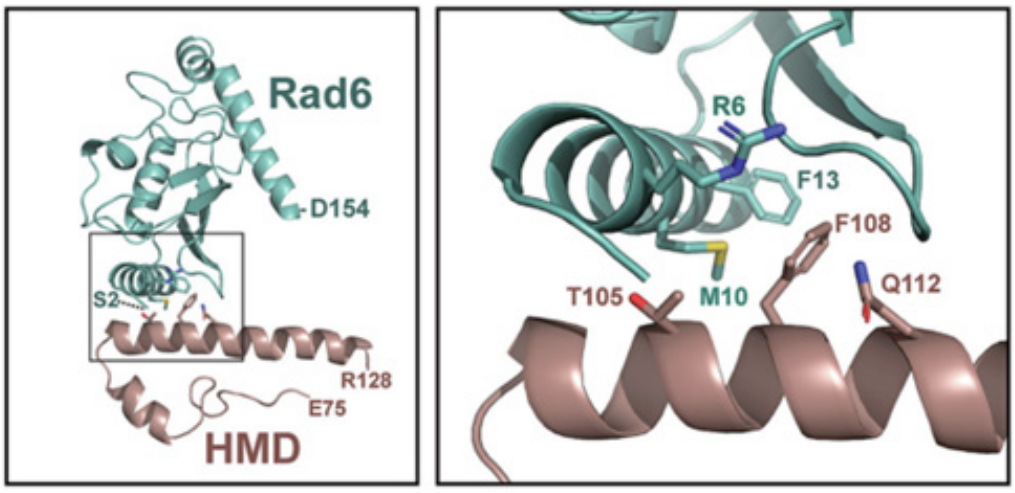


Figure Legend: Cross-linking-instructed model of the Rad6-HMD interface using crystal structures of Rad6 (PDB ID: 1AYZ) and Rtf1 HMD (PDB ID: 5E8B). Docking was done using ClusPro 2.0. The Inset in the left panel is expanded on the right.

More Cool Science

Small molecule nitroalkenes inhibit RAD51-mediated homologous recombination and amplify triple-negative breast cancer cell killing by DNA-directed therapies.

Nitro fatty acids (NO2-FAs) are lipid signaling molecules generated from metabolic and inflammatory reactions between conjugated diene fatty acids and nitric oxide or nitrite-derived reactive species. NO2-FAs react with protein cysteine thiolates to induce posttranslational protein modifications that can impact protein function. Dr. Neumann and colleagues previously published that nitroalkene (E) 10-nitro-octadec-9-enoic acid (CP-6) induces DNA damage in a TNBC xenografts by inhibiting homologous-recombination (HR)-mediated repair of DNA double-strand breaks (DSB). In the current study, Dr. Neumann and collaborators report that CP-6 specifically targets RAD51 Cys319, a residue that is essential for RAD51-mediated HR repair of DSBs. A nitroalkene library screen identified two nitroalkenes, a non-natural fatty acid CP-8 and a dicarboxylate ester CP-23 that showed superior TNBC cell killing compared to CP-6, and synergism with three different PARP inhibitors and γ-IR. CP-8 and CP-23 inhibited γ-IR-induced RAD51 foci formation and HR in a GFP-reporter assay, but did not affect benign human epithelial cells. In vivo, only CP-8 showed promising anticancer activities alone and combined with the PARP inhibitor talazoparib in an HR-proficient TNBC mouse model. Preliminary preclinical toxicology analysis suggests CP-8 is safe. This study supports CP-8 as a novel anticancer molecule for treating cancers sensitive to homologous recombination-mediated DNA repair inhibitors.

Impact: This study supports the use of CP-8 as a single agent and as a non-toxic cotreatment with DNA-damaging anticancer therapy. This study provides additional evidence for nitroalkenes such as CP-8 as a new potential drug class of high clinical relevance in anticancer therapy.

Funding: R56CA233817 (to C.A.N), Congressionally Directed Medical Research Programs Breast Cancer Research Program BC180467 (to C.A.N, B.A.F).

Source: Hong L, Braden DC, Zhao Y, Skoko JJ, Chang F, Woodcock SR, Uvalle C, Casey A, Wood K, Salvatore SR, Asan A, Harkness T, Fagunloye A, Razzaghi M, Straub A, Spies M, Brown DD, Lee AV (CB), Schopfer F, Freeman BA (CB), Neumann CA (GS). *Redox Biol*. 2023. 66:102856. PMID: 37633047; PMCID: PMC10472314.

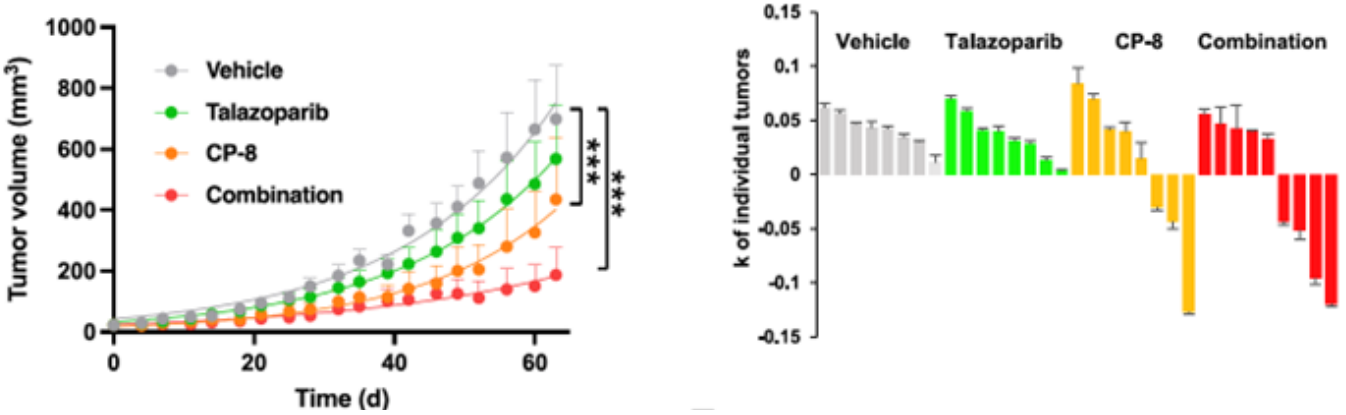


*Figure Legend: CP-8 inhibits in vivo TNBC tumor growth. A. MM231 xenograft treatment with CP-8 and talazoparib showed tumor reduction with CP-8 alone and in combination with talazoparib. Treatment start: day 0. Vehicle: 7.5% DMSO+61.7% PEG +30.8% saline. Vehicle n = 8, talazoparib n = 8, CP-8 n = 8 and combination n = 9. Statistical analysis by Two-way ANOVA, multiple comparisons ***: p < 0.0001. B. k values of the growth curve for each tumor volume over time (day 0 to day 63).*

More Cool Science: Inter-programmatic collaboration

Unlicensed origin DNA melting by MCV and SV40 polyomavirus LT proteins is independent of ATP-dependent helicase activity.

Cellular eukaryotic replication initiation helicases are first loaded as head-to-head double hexamers on double-stranded (ds) DNA origins and then initiate S-phase DNA melting during licensed (once per cell cycle) replication. Merkel cell polyomavirus (MCV) large T (LT) helicase oncoprotein similarly binds and melts its own 98-bp origin but replicates multiple times in a single cell cycle. To examine the actions of this unlicensed viral helicase, the Chang/Moore laboratories examined multimerization of MCV LT molecules as they assembled on MCV DNA origins using real-time single-molecule microscopy in collaboration with the Van Houten laboratory.. MCV LT formed highly stable double hexamers having 17-fold longer mean lifetime (τ, >1,500 s) on DNA than single hexamers. Unexpectedly, partial MCV LT assembly without double-hexamer formation was sufficient to melt origin dsDNA as measured by RAD51, RPA70, or S1 nuclease cobinding. DNA melting also occurred with truncated MCV LT proteins lacking the helicase domain, but was lost from a protein without the multimerization domain that could bind only as a monomer to DNA. SV40 polyomavirus LT also multimerized to the MCV origin without forming a functional hexamer but still melted origin DNA. MCV origin melting did not require ATP hydrolysis and occurred for both MCV and SV40 LT proteins using the nonhydrolyzable ATP analog, adenylyl-imidodiphosphate (AMP-PNP). LT double hexamers formed in AMP-PNP, and melted DNA, consistent with direct LT hexamer assembly around single-stranded (ss) DNA without the energy-dependent dsDNA-to-ssDNA melting and remodeling steps used by cellular helicases.

Impact: Polyomavirus LT viral helicases recruit the host cell replisome machinery to replicate their viral DNA. This study showed that differences between viral and cellular replication helicase DNA melting mechanisms may explain how viral DNA can be rapidly and repeatedly amplified during a single cell cycle. The team reported that viral LTs melt origin DNA through their multimeric binding energy and do not require helicase activity for initial dsDNA melting and double-hexamer assembly.

Funding: This study was supported by NIH Grants R35CA197463 (to P.S.M), R01CA232604 (to Y.C), R35ES031638 (to B.V.H), K99ES033738 (to S.R.H), R01ES031796 and R01ES030335 (to K.A.B), 2P30CA047904 (to the University of Pittsburgh Medical Center, Hillman Cancer Center), and a major equipment grant S10OD032158-01A1 (to B.V.H). This study was also supported by Hillman Postdoctoral Fellowship for Innovative Cancer Research (to M.A.S and S.R.H.) and American Cancer Society Postdoctoral Fellowship (133947-PF-19-132-01-DMC) (to S.R.H)

Source: Wan L, Toland S, Robinson-McCarthy LR, Lee N, Schaich MA, Hengel SR, Li X, Bernstein KA, Van Houten B, Chang Y, Moore PS. *Proc Natl Acad Sci U S A*. 2023;120(30):e2308010120. Epub 20230717. PubMed PMID: 37459531; PMCID: PMC10372695.

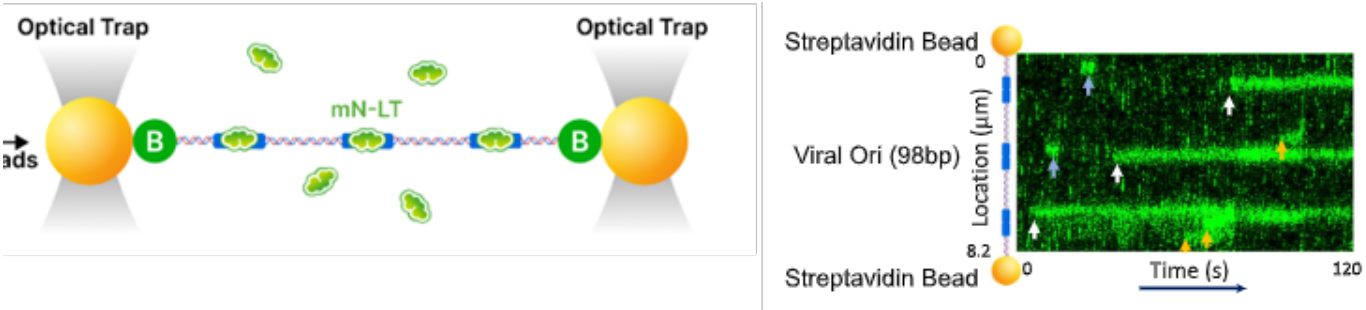


Figure Legend: Watching MCV LT binding to replication origins (ori) in real-time. MCV replication ori containing DNA was suspended between two bead using optical traps of the LUMICKS C-trap instrument and mNEON-MCV LT was flowed into the flow cell and bound at specific sites (left). Kymograph showing binding positions of mNEON-MCV LT binding to MCV ori. Fluorescence intensity and subsequent bleachinig experiments showed that MCV LT binds to ori as two hexameric rings.

More Cool Science

ERK and c-Myc signaling in host-derived tumor endothelial cells is essential for solid tumor growth.

The limited efficacy of the current antitumor microenvironment strategies is due in part to the poor understanding of the roles and relative contributions of the various tumor stromal cells to tumor development. Dr. Liu and colleagues describe a versatile in vivo anthrax toxin protein delivery system allowing for the unambiguous genetic evaluation of indi-vidual tumor stromal elements in cancer. Their reengineered tumor-selective anthrax toxin exhibits potent antiproliferative activity by disrupting ERK signaling in sensitive cells. Since this activity requires the surface expression of the capillary morphogenesis protein-2 (CMG2) toxin receptor, genetic manipulation of CMG2 expression using our cell-type–specific CMG2 transgenic mice allows us to specifically define the role of individual tumor stromal cell types in tumor development. They established mice with CMG2 only expressed in tumor endothelial cells (ECs) and determined the specific contribution of tumor stromal ECs to the toxin's antitumor activity. These results demonstrate that disruption of ERK signaling only within tumor ECs is sufficient to halt tumor growth. They further discovered that c-Myc is a downstream effector of ERK signaling and that the MEK–ERK–c-Myc central metabolic axis in tumor ECs is essential for tumor progression. As such, disruption of ERK–c-Myc signaling in host-derived tumor ECs by our tumor-selective anthrax toxins explains their high efficacy in solid tumor therapy.

Impact: This study describes a versatile and highly cell-type–specific genetic platform based on the anthrax toxin protein delivery system, allowing the rigorous therapeutic assessment of individual tumor stromal cell types in tumor progression. This team's approach provides a powerful tool in understanding the tumor micro-environment, facilitating the rational design of anti-TME strategies in cancer therapy.

Funding: This research was supported by the institutional seed fund (to S.L) and the grant (R01CA254938) (to S.L from the National Cancer Institute, NIH, and in part by Intramural Programs of the National Institute of Allergy and Infectious Diseases (to S.H.L), and National Institute of Dental and Craniofacial Research (to T.H.B), NIH.

Source: Zuo Z, Liu J, Sun Z, Cheng YW, Ewing M, Bugge TH, Finkel T, Leppla SH, Liu S. ERK and c-Myc signaling in host-derived tumor endothelial cells is essential for solid tumor growth. *Proc Natl Acad Sci U S A*. 2023;120(1):e2211927120. Epub 20221227. PubMed PMID: 36574698; PMCID: PMC9910475.

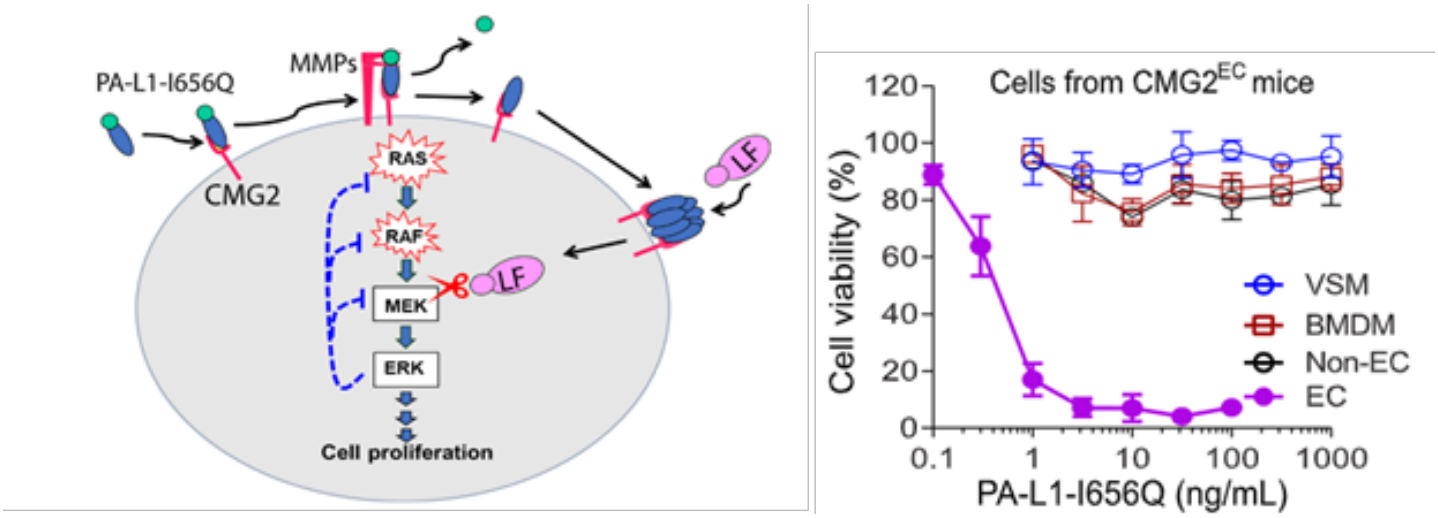


Figure Legend: Anthrax toxin receptor CMG2-based tumor-host genetic platform for assessing tumor ECs. Anthrax toxin protein delivery system as a unique platform for cancer therapy with high specificity. Tumor specificity of PA-L1-I656Q is achieved by engineering the delivering vehicle PA to bind to the CMG2 receptor and rely on tumor-associated proteases (MMPs) for activation. Thus, LF (or LF fusions) can be selectively delivered into tumor cells and tumor stromal cells to inactivate MEK1/2, disrupting the ERK signaling (left). Primary cells from CMG2EC were treated with various concentrations of PA-L1-I656Q in the presence of FP59 (100 ng/mL) for 48 h, followed by an MTT assay evaluating cell viability. Of note, only tumor endothelial cells (EC)s from CMG2EC mice were sensitive to PA-L1/FP59, whereas non-ECs (ICAM2 negative), VSMs, and BMDMs were resistant to the toxin.

Faculty and Staff News

Congratulations to the following students from GSP labs for successfully defending their PhD thesis.

Patricia Opresko & Sanjana Thosar

Sanjana Thosar, a graduate student from Dr. Opresko's lab, defended her thesis entitled, "Oxidative guanine base damage plays a dual role in regulating productive ALT-associated homology directed repair", on August 28th, 2023. She earned a PhD in Molecular Genetics and Developmental Biology from the University of Pittsburgh School of Medicine and is now a Scientist I at Vor Biopharma in Boston, MA.

Hayley Rein, a graduate student from Dr. Bernstein's lab, defended her thesis entitled, "Functional Characterization of RAD51 Paralog Cancer Associated Variants of Unknown Significance," on August 29th, 2023. She earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine, and is now a Clinical Genomic Scientist at Baylor Genetics.

Hayley Rein

Angela Hinchie

Angela Hinchie, a graduate student from Dr. Alder's lab, defended her thesis, "A persistent variant telomere sequence in a human pedigree," on October 10, 2023. She earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine, and is now pursuing postdoctoral opportunities.

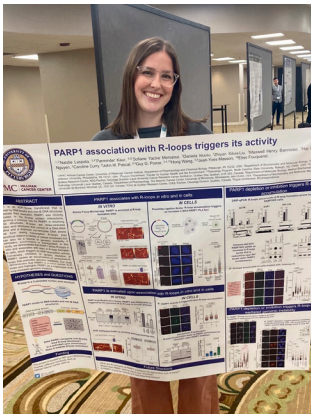
Sripriya Raja, a graduate student from Dr. Van Houten's lab, defended her thesis entitled, "Understanding the role of UV-DDB in the SMUG1 mediated repair of oxidative DNA damage," on September 7, 2023. She earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine and is now a graduate student advisor for the University of Pittsburgh.

Anne Wondisford, a graduate student from Dr. O'Sullivan's lab, defended her these entitled "ADP-ribosylation of telomeres in human cells" on November 21, 2023. She earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine and is completing her MD degree as part of the MSTP. She plans to pursue a course in medical oncology.

Natalie Laspata, a graduate student in Dr. Fouquerel's lab, defended her thesis, "Investigating the role of Poly (ADP-ribose) polymerase 1 (PARP1) in the prevention of R-loop-mediated genomic instability," on November 9, 2023. She earned a PhD in Biochemistry, Structural, & Molecular Biology from the Thomas Jefferson University College of Life Sciences. Her committee nominated her for a PhD Thesis award that will be decided next year.

Brittani Schnable & Sripriya Raja

Roddy O'Sullivan, Anne Wondisford & Roger Greenberg



Natalie Laspata

Faculty and Staff News (Continued)

Michael W. Epperly, PhD

Michael W. Epperly, PhD is retiring December 2023 Professor in Radiation Oncology Department.

As a faculty member in the Department of Radiation Oncology, Dr. Epperly joined the University in 1987. He was actively involved in performing research in radiation biology. In the Center for Medical Counter Measures Against Radiation (CMCR) a grant was awarded to the University of Pittsburgh to Dr. Epperly as a core leader for the Radiobiological Standardization Core and an investigator in Project 1. Dr. Epperly organized and lectured in the radiation biology class for the radiation oncology residents. He also engaged in instructing medical students from the University of Pittsburgh School of Medicine in how to perform radiation biology research. His research involves developing new radiation protectors and mitigators for protection against irradiation-induced damage in vitro and in vivo as well as studying the late effects of irradiation, effects of irradiation on pregnant mice, and development of ALS.

Yvonne Mowery, MD, PhD, DABR

UPMC Hillman Cancer Center welcomed Yvonne Mowery, MD, PhD, DABR Associate Professor of Radiation Oncology - June 1, 2023.

Dr. Mowery will develop an active research program focusing on basic and translational research in head and neck cancer, working closely with other members of the head and neck cancer disease team and developing collaborations throughout UPMC Hillman.

She is joining us from Duke University, where she was the Butler Harris Assistant Professor of Radiation Oncology, Head and Neck Surgery & Communication Sciences. Dr. Mowery was also the Associate Center Director for Radioimmunotherapy at the Duke Cancer Institute Center for Cancer Immunotherapy.

Dr. Mowery is certified in Radiation Oncology by the American Board of Radiology. She is actively involved in preclinical and clinical research related to head and neck cancer and combining radiation therapy with immunotherapy. She is the principal investigator for an investigator-initiated multi-institutional trial evaluating pembrolizumab with a radiosensitizing drug and radiation therapy to treat recurrent head and neck cancer.

Dr. Mowery has authored more than 65 peer-reviewed publications and several book chapters and has presented at numerous national and international conferences. Her research has been funded by foundations, societies, and the National Institute of Dental and Craniofacial Research of the National Institute of Health, including through a K award on overcoming chemoradiation resistance for head and neck cancer. Dr. Mowery is an active member of the scientific community, serving as a reviewer for society meetings, grants, and journals. She has also received several honors and awards for her research and mentoring activities. Dr. Mowery earned her MD and PhD from Duke University's Medical Scientist Training Program.

Faculty and Staff News (Continued)

Serah Choi, MD, PhD

Please join UPMC Hillman Cancer Center welcomed Serah Choi, MD, PhD Associate Professor of Radiation Oncology - September 1, 2023.

Dr. Choi sees patients in the outpatient clinic at Shadyside Hospital in the Department of Radiation Oncology and is developing active research program focusing on basic and translational research in central nervous system (CNS) cancer. She is a member of the UPMC Hillman's Neuro-Oncology Program, working closely within that team as well as with GSP members throughout our cancer center.

Her research interests include targeting glucose transporters in cancers and developing novel therapies for brain tumors. Dr. Choi has over 30 peer-reviewed publications. She has contributed 9 book chapters and numerous presentations at national and international conferences. She is a co-investigator on multiple currently-funded NIH grants. She also serves as a journal reviewer, member of multiple professional societies and national committees, and mentor for medical residents and students.

Dr. Choi is joining us from University Hospitals Seidman Cancer Center/ Case Western Reserve University School of Medicine, where she was an Assistant Professor in the Department of Radiation Oncology since 2018. There, she was also the CNS section leader, co-chair of the UH Seidman Cancer Center Strategy and Execution Subcommittee for Research Systems of Care, and institutional PI for several CNS clinical trials. In addition, from 2018-2022, she served as medical director of the Gamma Knife Radiosurgery Program.

Faculty Recruitment

GSP Faculty Recruitment:

The Genome Stability Program is seeking faculty candidates with strengths in one or more of the following areas: 1) genomics and bioinformatics expertise including CRISPR screens and mutational signature spectra; 2) mechanisms of replication stress during cancer therapy; 3) targeted DNA repair inhibition combined with immune-oncology approaches; and 4) expertise in mass spectrometry to follow adductomics in exposed tissues and tumors. We are seeking exceptional candidates using state-of-the-art approaches and who will join in tenure-track or tenured faculty positions that are commensurate with prior training and experience. A competitive salary and research start-up package will be provided, as well as laboratory and office space within the state-of-the-art Hillman Cancer Center.

Assistant Professor candidates shall have developed skills in research methodology or a related discipline, classroom teaching experience, a strong commitment to collaborating with clinical and/or translational scientists and have the necessary skills for grant writing and producing peer-reviewed publications. There must be a demonstrated potential for external funding. Experience working in data coordinating centers or experience with single center or multi-center trials desired. Post-doctoral experience is required.

Associate Professor candidates shall have a strong record of publications and some extramural grant funding. The candidate shall build and lead an independent research program with extramural grant funding and have a documented record of teaching and mentoring graduate students and postdoctoral fellows.

Professor candidates shall be an established independent investigator with interdisciplinary translational research in molecular structural biology, significant publications, and extramural grant funding. The candidate will have a strong record in teaching and mentoring graduate students, postdoctoral fellows and junior faculty members.

Faculty Recruitment (continued)

Located in the City of Pittsburgh (routinely ranked as one of the top most livable and affordable U.S. cities), UPMC Hillman Cancer Center (previously known as the University of Pittsburgh Cancer Institute) is an NCI-designated Comprehensive Cancer Center with over 300 members; seven research programs in basic, translational, clinical, and population sciences; 12 shared resources that receive funding from our NCI Cancer Center Support Grant, more at https://hillmanresearch.upmc.edu/research/. In 2023, institutional funding base of nearly $143 million. In 2023, the University of Pittsburgh ranked #3 in overall NIH funding. UPMC Hillman Cancer Center serves a catchment area of 29 Western Pennsylvania counties and provides unique opportunities to collaborate with clinical and translational research programs involved in cancer patient care.

To apply for a position, please send your curriculum vitae, a two-page summary of your research plans (together with recommendations) to Patty Opresko, PhD, chair of the GSP Search Committee (plo4@pitt.edu) and Jeremy N. Rich, MD, MHS, MBA, Deputy Director of the UPMC Hillman Cancer Center (richjn@pitt.edu). Applications will be reviewed and evaluated on a continual basis. The University of Pittsburgh is an Affirmative Action/ Equal Opportunity Employer and values equality of opportunity, human dignity and diversity, EOE, including disability/vets.

Prepared by Sarah O'Melia / Edited by Gera Jochum

About the Genome Stability Program

UPMC Hillman Cancer Center's Genome Stability Program works to gain new insights into the molecular pathways that maintain genome integrity and how these processes are altered in cancer cells. The Genome Stability Program works synergistically with other UPMC Hillman Cancer Center programs to translate their novel, basic insights into development of new targets, drug discovery, and recognition of biomarkers to ultimately provide clinical applications for cancer prevention and treatment.

Learn more about the Genome Stability Program at: https://hillmanresearch.upmc.edu/research/programs/genome-stability/

Oncology Graduate Program

The Oncology Graduate Program at the University of Pittsburgh provides comprehensive training opportunities for the next generation of cancer researchers. Located at the UPMC Hillman Cancer Center, one of only 56 National Cancer Institute designated Comprehensive Cancer Centers, students will have access to state-of-the art facilities and train with scientists from the entire spectrum of cutting-edge cancer research and biological basis for care, from the bench to the bedside.

FUNDING

- Stipend: $40,000 for AY 2023-24
- Full tuition remission
- Individual health insurance
- Training grants, travel awards available

CONTACT

- For general information and to learn about our faculty please visit our website.
- For more specific/personal situations or questions please contact oncologygradprogram@pitt.edu

WEBSITE

academics.pitt.edu/programs/oncology-graduate-program

University of Pittsburgh
Graduate Studies
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