# DNA Pitt Crew

Fall 2024

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

University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

Genome Stability Program GSP

## Note from Genome Stability Program Leader, Patricia Opresko, PhD

Members of the Genome Stability Program at UPMC Hillman Cancer Center had a very productive year. This past June, Dr. Robert Ferris announced that he accepted the position of Executive Director of the UNC Lineberger Comprehensive Cancer Center and UNC Health System Chief of Oncology Services. Under Dr. Ferris's leadership, UPMC Hillman Cancer Center thrived and received a prestigious two-year "merit extension" to its NCI support grant, which received its best score ever in the exceptional range during the most recent NCI grant renewal and site visit in 2020. Soon after Dr. Ferris was first appointed Director of our cancer center in 2017, he created the Genome Stability Program as one of three basic cancer research programs. We are grateful to Dr. Ferris for his exceptional leadership and vision, under which the GSP was created and has thrived. He will be greatly missed, and we wish him all the best in his new position. A nationwide search will soon be underway to identify a new Director.

I also want to take this opportunity to thank Dr. Bennett Van Houten for his outstanding leadership and dedication to the GSP. Dr. Van Houten joined UPMC Hillman in 2008 as leader of the Molecular and Cellular Cancer Biology Program, which was the precursor to the GSP and the Cancer Biology Program, which was established by Dr. Ferris in 2018. He then continued as co-leader of the GSP. Dr. Van Houten has contributed extensively over the years to the recruitment and mentorship of junior faculty, and to fostering outstanding science and collaborations. His exceptional accomplishments were recently recognized by being named a 2023 American Association for the Advancement of Science (AAAS) Fellow. Dr. Van Houten is passing the torch to a new GSP co-leader, for which a search is underway, while he focuses on his outstanding science. We thank him for his leadership.

Over the past year, GSP members were incredibly busy publishing exciting science in high impact journals, which are highlighted in the following pages. We participated in welcoming and interviewing candidates for our new Oncology Graduate Program, and thank Drs. Bakkenist and Aird for their extraordinary efforts in establishing this PhD program, and Dr. Ferris for his valuable support. Members of the GSP search committee worked with Interim Director Dr. Jeremy Rich and Director Dr. Ferris to welcome and interact with faculty candidates to grow the GSP.

We thank Sarah O'Melia for all her efforts in arranging visits and itineraries, and GSP members for meeting with the candidates and attending their seminars. As a result of these efforts, we are very excited to welcome three new faculty members: Dr. Tatiana Moiseeva, Dr. Logan Myler, and Dr. Orlando Scharer. We thank Drs. Ferris and Rich for their support.

Finally, last May, we enjoyed a very successful Rush to Crush event to raise money for cancer research at UPMC Hillman and thank all those who participated and who donated to this important cause. We hope you enjoy this edition.

Sincerely, Patricia Opresko, PhD

## Inside this Issue:



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## Faculty Spotlight: Tatiana Moiseeva, PhD

*Tatiana Moiseeva, PhD*

Tatiana Moiseeva, 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 investigating the mechanism of the initiation of DNA replication in human cancer and non-disease cells, as well as understanding the impact of replication proteins perturbations in cancer on cell cycle and DNA replication. Dr. Moiseeva completed her bachelor's and master's degrees in biophysics at St. Petersburg State Polytechnical University (St. Petersburg, Russia). She then worked on her PhD under the supervision of Dr. Nikolai Barlev at the Institute of Cytology, Russian Academy of Sciences (St. Petersburg, Russia). Her PhD project was focused on the catalytic activities of proteasomes and proteasome subunits and their regulation by various stresses, including DNA damage induced by doxorubicin. As a result, she published three first-author articles as well as multiple collaborative manuscripts. After successfully defending her PhD in 2010, seeking to gain international experience, Dr. Moiseeva obtained a short-term fellowship funding from EMBO to visit University of Leicester and investigate the potential role of proteasome in regulating splicing under Dr. Ian Epperon's mentorship. As a result of this fellowship, Dr. Moiseeva published a co-first author article, and decided to pursue postdoctoral opportunities abroad. She joined the laboratory of Dr. Chris Bakkenist in 2013 to work on ATM and ATR kinases and their roles in regulating DNA replication after DNA damage, as well as under unperturbed conditions. As a postdoctoral associate, Dr. Moiseeva identified an ATR-dependent signaling mechanism that prevents excessive firing of dormant origins in the absence of DNA damage in S-phase. She also demonstrated that preliminary DNA damage completely blocks ATRi-induced origin firing, making the sequence of treatment – [ATRi, DNA damage] vs. [DNA damage, ATRi] – critical for the outcome. She then described a RIF1/CDK1-dependent molecular mechanism that inhibits dormant origins during normal replication. Working on ATRi-induced origin firing sparked Dr. Moiseeva's interest in the mechanism of replication initiation in human cells, and when she started her own lab at Tallinn University of Technology (Tallinn, Estonia) in 2019, she decided to use ATR inhibition as a tool to investigate the molecular mechanism of origin firing in human cells. Despite the COVID pandemic during the first years of her independent research, Dr. Moiseeva was able to recruit three PhD students and multiple undergraduate and master's students to join her lab in Tallinn, publish, and obtain a prestigious grant from the Estonian Research Council. She then moved back to UPMC Hillman Cancer Center and the Department of Pharmacology and Chemical Biology in February 2024. Dr. Moiseeva is supervising her three Tallinn-based PhD students, Sameera Vipat, Syed Shahid Musvi, and Naga Raviteja Chavata, remotely, as well as actively recruiting for her new Pittsburgh-based lab. The first paper from the Moiseeva lab was published in *Nature Communications* and described the non-catalytic role of DNA polymerase epsilon in replication initiation in human U2OS cells. It demonstrated that in these cells, POLE catalytic subunit is dispensable for the assembly of the replicative helicase, which is a key difference from the well-studied yeast system. This result confirmed the need to re-evaluate the findings from model organisms in human cells and investigate the mechanism of human-origin firing in detail. The lab is currently working to identify novel replication initiation factors in transformed and non-cancerous human cells, investigate specific non-catalytic roles various replication factors in origin firing, and study the cancer-associated perturbations in replication initiation, with the goal of advancing cancer therapies.

In her free time, Dr. Moiseeva enjoys hiking with her husband, playing with her cats Milky and Red, practicing yoga and arm balances, as well trying out various arts and crafts.

## Trainee Spotlight: Mariarosaria De Rosa, PhD

*Mariarosaria De Rosa, PhD*

Mariarosaria De Rosa graduated with her PhD in Molecular Medicine and Medical Biotechnology from the University of Naples "Federico II", Italy, in the laboratory of Dr. Avvedimento. During her PhD research, she studied the interplay between DNA repair and gene expression regulation and contributed to unveiling RNA-mediated stabilization of chromatin loops in estrogen-induced genes and the role of BER enzymes in TGF-β1-induced epithelial-to-mesenchymal transition. She then led a study on DNA damage in Huntington's disease, finding that DNA damage signatures and telomere shortening in peripheral blood mononuclear cells are predictive biomarkers of disease progression in premanifest patients. Thanks to being awarded a travel grant during her PhD, she further expanded her interest in telomere biology while working at Stony Brook University in the lab of Dr. Bruce Demple. Dr. De Rosa then joined the Opresko group in January 2019, excited to exploit the newly developed TRF1-FAP system to study the repair of oxidative damage at telomeres. While learning new cellular biology tools and techniques specific to the telomere field, she contributed to a study published in Nature Structural and Molecular Biology that uncovered the role of telomeric 8-oxoguanine in inducing cellular senescence in non-diseased cells. Simultaneously, she investigated the roles of the BER glycosylases OGG1 and MUTYH in telomeric 8oxoG-induced senescence. Dr. De Rosa presented her findings at various conferences over the years, including the Environmental Mutagenesis and Genomics Society (EMGS) and the Cold Spring Harbor (CSHL) Telomere meeting, and she received Best Poster Awards at the 7th US-EU conference on Endogenous DNA Damage and Repair in Stony Brook and the University of Pittsburgh Postdoctoral Data and Dine. Her manuscript on this study is currently undergoing revision while she is collaborating on other two projects in the lab and leading a separate study about the role of MUTYH in the maintenance of telomere stability in cancer cells. In February, Dr. De Rosa was awarded a K99/R00 from the NIHES entitled "Investigating roles for oxidative guanine damage in transcription regulation," which will support her in the transition to an independent investigator and will allow her to delve into her long-time interest in understanding how oxidative stress and oxidative guanine damage modulate gene expression.

When not in the lab, Dr. De Rosa is socially very active. She was elected Communications Chair of the University of Pittsburgh Postdoctoral Association (UPPDA) for three consecutive years, which allowed her to organize informational and networking events for Pitt postdocs and to represent the University of Pittsburgh at the National Postdoctoral Association (NPA) meeting in Chicago in 2022. Outside of academia, Mariarosaria loves spending time with her cats Luna and Artemis. She also enjoys taking care of her summer garden and baking homemade pizza and bread; these latter are the perfect "carb loading" for her runs on the Pittsburgh bridges and hills while she trains for the next marathon and to fuel her kayaking on PA lakes.

## Pitt Stops: Special Events and Visiting Speakers

### Peter McHugh, PhD

**Professor in Department of Oncology**
**Director, Oncology Laboratories at WIMM**
**University of Oxford**

*Visit: February 8th & 9th, 2024*

Contributed by Bennett Van Houten, PhD

*Peter McHugh, PhD*

Professor McHugh presented a lecture, "Potential for targeting nucleases for the treatment of cancer," in which he spoke about an interesting family of nucleases involved in genome stability that contain a metallo-betalactamase (MBL) fold. These include SNM1A, SNM1B/Apollo, and SNM1C/Artemis. The SNM1A nuclease is remarkable in that it can digest through a DNA interstrand crosslink allowing efficient interstrand cross-link repair. One of the most complicated DNA repair pathways in human cells. After introducing the audience to these nucleases, he described how SNM1A plays a critical role in

## Pitt Stops: Special Events and Visiting Speakers (continued)

removing dirty ends from complex DNA breaks (Nat. Comm. 2024). He presented data that SNM1A can digest through 8-oxoG, whereas exonuclease 1 cannot. He also presented data that SNM1A, accumulates at sites of DNA double-strand breaks adjacent to 53BP1, and is also often found at replication forks due to its PIP box. He then described his laboratory's progress on these nucleases' structure and function analysis with small molecule inhibitors to improve efficacy in killing tumor cells with chemotherapeutic compounds that crosslink DNA (*Nucleic Acids Res. 2021*). His outstanding seminar elicited a warm reception from the audience and generated an exciting question-and-answer session.

*Raluca Gordan, PhD*

### Raluca Gordan, PhD

**Associate Professor**
**Biostatistics & Bioinformatics, Division of Integrative Genomics**
**Duke University**

*Visit: February 19th, 2024*

Contributed by Patricia Opresko, PhD

On February 19th, Dr. Raluca Gordon presented the exciting seminar, "A surprising role for transcription factor proteins in increasing mutagenesis in cancer genomes." Dr. Gordon is an Associate Professor in Biostatistics & Bioinformatics in the Division of Integrative Genomics at Duke University. Her lab is focused on investigating how transcription factors are recruited to the genome and how their recruitment impacts other cellular processes at the genomic level, using high-throughput sequencing and bioinformatic approaches. She described how her lab made the very interesting discovery that transcription factors can cause mutations at their binding sites in the genome by interfering with DNA repair enzymes. She described work published in Nature, demonstrating how DNA mismatches cause by DNA replication errors, can lead to high affinity binding by certain transcription factors, and thereby may interfere with mismatch repair. She also described work published in PNAS showing that ETS binding sites in promoters are hot spots for UV damage and mutations in melanoma. Finally, she shared unpublished data further supporting a role for transcription factors in competing with DNA repair proteins. We greatly enjoyed her visit and the time she spent meeting with our faculty and trainees.

*David Pellman, MD*

### David Pellman, MD

**Margaret M. Dyson Professor of Pediatric Oncology**
**Dana-Farber Cancer Institute**
**Harvard Medical School**

*Visit: March 19th, 2024*

Contributed by Bennett Van Houten, PhD

On March 19, Dr. David Pellman delivered an exceptional seminar entitled, "Mechanisms Driving Rapid Evolution of Cancer Genomes". Dr. Pellman is the Margaret M. Dyson Professor of Pediatric Oncology at the Dana-Farber Cancer Institute and an HHMI Investigator at Harvard Medical School. He is well known for his pioneering work elucidating mechanisms of cell divisions and how errors during cell division can drive alterations in cancer genomes and has published several recent reports in Nature. His lab discovered and described the mechanism of chromosome shattering and stitching back together termed "chromothripsis," which leads to thousands of chromosomal alterations. Dr. Pellman described the powerful method his lab developed called "Look-Seq." Which combines live cell imaging and single-cell genome sequencing to discover how cell division errors cause genomic alterations in cancer cells. He presented data showing how they compare the genomic sequences of two daughter cells and how chromothripisis is a major source of extra-chromosomal DNA. He described long-term consequences

## Pitt Stops: Special Events and Visiting Speakers (continued)

of making and breaking chromosome bridges for genomic alternations and how these events can lead to copy number gains in cancer. We thank Dr. Pellman for taking the time to share his exciting work with us and to meet with our UPMC Hillman faculty.

### Anna Pluciennik, PhD

**Assistant Professor**
**Department of Biochemistry and Molecular Biology**
**Thomas Jefferson University**

*Visit: April 15th & 16th, 2024*

Contributed by Elise Fouquerel, PhD

*Anna Pluciennik, PhD*

Dr. Pluciennik obtained her PhD from the University of Lodz (Poland) and trained as a Postdoctoral researcher in the lab of Nobel Prize awardee Dr. Paul Modrich at Duke University Medical Center and contributed to the characterization of the mismatch repair pathway (MMR). Her research interests lie in the understanding of the molecular pathways that mediate the pathology of neurodegenerative disorders and cancer, with a focus on DNA repair processes in the central nervous system (CNS), and the effect of aging on these activities. She has recently published in PNAS on the role of FAN1 nuclease in the removal of triple repeat extrusions.

During her visit with the GSP group, Dr. Pluciennik delivered a fantastic and very engaging seminar entitled, "Crosstalk between DNA repair systems in genome instability." She presented exciting data on the FAN1 and its role in the mismatch repair pathway. She also shared unpublished work of a beautiful cryo-EM structure of a FAN1 mutant. This is a significant accomplishment as it gives a detailed understanding of the contribution of this mutation in the occurrence of Huntington's disease.

*Julian Stingele, PhD*

### Julian Stingele, PhD

**Professor**
**Gene Center and Department of Biochemistry**
**Ludwig-Maximilians-Universität München, Germany**

*Visit: May 3rd, 2024*

Contributed by Bennett Van Houten, PhD

Dr. Stingele presented an exciting lecture, "Nucleic acid-protein crosslinks and their resolution" showing a lot of new and unpublished data. One of the largest DNA obstacles to overcome for normal cellular processes to proceed are lesions in which proteins are covalently bound to DNA or RNA. Chemotherapeutic agents, toxicants like formaldehyde, and even metabolites of ethanol can produce these lesions. After briefly discussing the fate of protein-RNA crosslinks and their effects on translation (Mol Cell. 2023), his talk focused on protein-DNA crosslinks. After discussing the structure and function of SPRTN, a metalloprotease, that when mutated can result in Ruijs-Aalfs syndrome, he presented data on how ubiquitylation of SPRTN stimulates its activity to digest protein-DNA cross-links to allow for further processing by DNA repair enzymes. He discussed a new sequencing approach to measure DNA-protein crosslinks DPC-seq across the genome and showed using a CRISPR screen. Is a direct role of CSA and CSB in processing these lesions ( Nat Cell Biol. 2024). The GSP greatly enjoyed his visit and outstanding presentation.

## Pitt Stops: Special Events and Visiting Speakers (continued)

**We enjoyed a series of seminars from outstanding postdoctoral fellows in the field of genome stability.**

**Logan Myler, PhD**

Dr. Myler was a Postdoctoral fellow in Dr. Titia de Lange's lab at Rockefeller University. On Wednesday, December 6th, 2023 he presented a lecture entitled, "Dissecting molecular mechanisms of DNA repair as a basis for precision oncology."

**Madison Adolph, PhD**

Dr. Adolph was a Postdoctoral fellow in Dr. David Cortez's lab at Vanderbilt University. She delivered a seminar on Wednesday, January 10th, 2024 entitled, "Roles of single-stranded DNA binding proteins in the preservation of genome stability."

**Feng Tang, PhD**

Dr. Tang was a Postdoctoral fellow in Dr. Yinsheng Wang's lab at UC Riverside, presented a seminar on January 31st, 2024 entitled, "Chemical biology approaches for assessing genome instability."


*UPMC Hillman Cancer Center*

**Alessandra Brambati, PhD**

Dr. Brambati was a postdoctoral fellow in Dr. Agnel Sfeir's lab at Memorial Sloan Kettering Cancer Center, presented a seminar on Wednesday, March 27th, 2024 titled, "Balancing act: exploring the role of non-standard DSB repair pathways in maintaining genome stability and diversity."

**Joshua Heyza, PhD**

Dr. Heyza was a postdoctoral fellow in Dr. Jens Schmidt's lab at Michigan State University, delivered a seminar titled, "Defining mechanisms of DNA double-strand break repair using live-cell single-molecule imaging" on Monday, April 29th, 2024.

## Scientific Conference Highlights and Awards

*Bennett Van Houten, PhD*

**Bennett Van Houten, PhD, Named AAAS Fellow**

Bennett Van Houten, PhD, co-director of the Genome Stability Program at UPMC Hillman Cancer Center, and Richard M. Cyert Professor of Molecular Oncology and professor of pharmacology and chemical biology in the University of Pittsburgh School of Medicine, is among the 502 scientists, engineers and innovators elected as a 2023 American Association for the Advancement of Science (AAAS) Fellow.

Being recognized by AAAS for scientifically and socially distinguished achievements is one of the most distinct honors in the scientific community. A tradition dating back to 1874, election as a AAAS Fellow is a lifetime honor, and all Fellows are expected to maintain the highest standards of professional ethics and scientific integrity.

AAAS honored Van Houten as a fellow for "outstanding contributions to the field of DNA damage and repair, particularly the development of several novel methods for understanding the choreography of the DNA repair processes." Van Houten and his lab study the formation and repair of DNA damage in nuclear and mitochondrial genomes, paying particular interest to the structure and function of proteins that mediate nucleotide excision repair and the role of oxidative stress in human disease.

## Scientific Conference Highlights and Awards (continued)

**GSP Faculty & Lab Members Awarded**

- Karen Arndt, PhD, lab

  Graduate student, Tasniem Fetian, won the Leo B. and Teresa Y. Wegemer Endowed STEM Fellowship award for 2024.

- Yael Arbely, PhD, lab

  Dr. Arbely is a Co-Organizer of an International Conference: The 8th Dynamic Kinetochore EMBO Workshop, to be held in Switzerland in June 17-20, 2024. She is also an invited discussion leader at the 2024 Centromere Biology Gordon Research Seminar (GRS), July 27-28, 2024, and preceding the GRC, at the University of Southern Maine in Portland, Maine.

- Elise Fouquerel, PhD, lab

  PhD student, Lily Thompson was awarded a F31 fellowship from the NIEHS. She received the TJU travel award to attend the EMBO Dynamic Kinetochore Conference in Switzerland in June 2024 and a $1500 travel award from the Health and Environmental Sciences Institute to attend a future meeting. Dr. Rim Nassar also received the $1500 travel award from the Health and Environmental Sciences Institute to attend a future meeting. Dr. Daniela Muoio had her first author paper accepted for publication in Nature Communications, and she received the EMGS travel award to attend the national meeting in September in Palm Springs.

- Yvonne Mowery, MD, PhD, lab

  Ashlyn Rickard, PhD, a postdoctoral associate, won an award for one of the Best Poster Presentations at the 2024 University of Pittsburgh Postdoctoral Research Symposium. Her presentation was entitled, "Evaluating the therapeutic potential of the small molecule ATR inhibitor BAY 1895344 and radiotherapy in head and neck cancer." Dr. Rickard was also awarded the Hillman Postdoctoral Fellowship for Diversity in Innovative Cancer Research in November 2023, and was selected as a 2023 Hillman Early-Career Fellow for Innovative Cancer Research.

- Patricia Opresko, PhD, lab

Libby Childs, PhD student, department of Human Genetics, was awarded an NIEHS Diversity Supplement. Theresa Heidenreich, PhD student, Molecular Genetics and Developmental Biology Program, was awarded a portion of the John S. Lazo Cancer Pharmacology departmental fellowship.

## Hot Papers


1. Yang X, Wang Z, Samovich SN, Kapralov AA, Amoscato AA, Tyurin VA, Dar HH, Li Z, Duan S, Kon N, Chen D, Tycko B, Zhang Z, Jiang X, Bayir H, Stockwell BR, Kagan VE, Gu W. "PHLDA2-mediated phosphatidic acid peroxidation triggers a distinct ferroptotic response during tumor suppression." *Cell Metab*. 2024;36(4):762-77.e9. Epub 20240202. doi: 10.1016/j.cmet.2024.01.006. PubMed PMID: 38309267.
2. Xu M, Senanayaka D, Zhao R, Chigumira T, Tripathi A, Tones J, Lackner RM, Wondisford AR, Moneysmith LN, Hirschi A, Craig S, Alishiri S, O'Sullivan RJ, Chenoweth DM, Reiter NJ, Zhang H. "TERRA-LSD1 phase separation promotes R-loop formation for telomere maintenance in ALT cancer cells." *Nat Commun*. 2024;15(1):2165. Epub 20240309. doi: 10.1038/s41467-024-46509-z. PubMed PMID: 38461301; PMCID: PMC10925046.
3. Johnson SA, Paul T, Sanford SL, Schnable BL, Detwiler AC, Thosar SA, Van Houten B, Myong S, Opresko PL. "BG4 antibody can recognize telomeric G-quadruplexes harboring destabilizing base modifications and lesions." *Nucleic Acids Res*. 2024;52(4):1763-78. doi: 10.1093/nar/gkad1209. PubMed PMID: 38153143; PMCID: PMC10939409.
4. Lee J, Lee J, Sohn EJ, Taglialatela A, O'Sullivan RJ, Ciccia A, Min J. "Extrachromosomal telomere DNA derived from excessive strand displacements." *Proc Natl Acad Sci U S A*. 2024;121(19):e2318438121. Epub 20240502. doi: 10.1073/pnas.2318438121. PubMed PMID: 38696464; PMCID: PMC11087782.

## Hot Papers (continued)

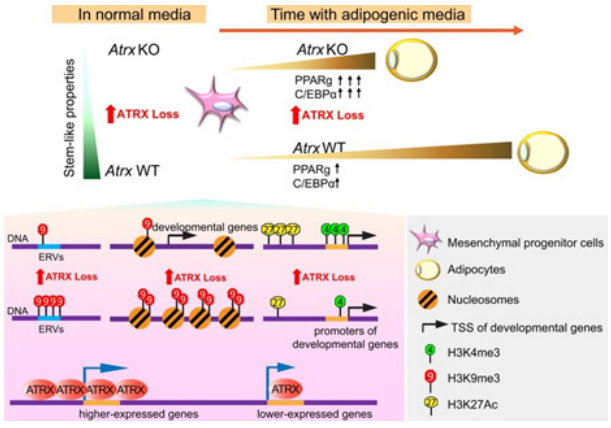

5. Karimian K, Groot A, Huso V, Kahidi R, Tan KT, Sholes S, Keener R, McDyer JF, Alder JK, Li H, Rechtsteiner A, Greider CW. "Human telomere length is chromosome end-specific and conserved across individuals." Science. 2024;384(6695):533-9. Epub 20240411. doi: 10.1126/science.ado0431. PubMed PMID: 38603523.
6. Malekzadeh H, Surucu Y, Chinnapaka S, Yang KS, Arellano JA, Samadi Y, Epperly MW, Greenberger JS, Rubin JP, Ejaz A. "Metformin and adipose-derived stem cell combination therapy alleviates radiation-induced skin fibrosis in mice." *Stem Cell Res Ther*. 2024;15(1):13. Epub 20240108. doi: 10.1186/s13287-023-03627-7. PubMed PMID: 38185658; PMCID: PMC10773046.
7. Sullivan DI, Bello FM, Silva AG, Redding KM, Giordano L, Hinchie AM, Loughridge KE, Mora AL, Königshoff M, Kaufman BA, Jurczak MJ, Alder JK. "Intact mitochondrial function in the setting of telomere-induced senescence." *Aging Cell*. 2023;22(10):e13941. Epub 20230908. doi: 10.1111/acel.13941. PubMed PMID: 37688329; PMCID: PMC10577573.
8. Odhiambo DA, Pittman AN, Rickard AG, Castillo RJ, Bassil AM, Chen J, Ravotti ML, Xu ES, Himes JE, Daniel AR, Watts TL, Williams NT, Luo L, Kirsch DG, Mowery YM. "Preclinical Evaluation of the ATR Inhibitor BAY 1895344 as a Radiosensitizer for Head and Neck Squamous Cell Carcinoma." *International Journal of Radiation Oncology, Biology, and Physics*. 2024 Apr 1;118(5):1315-1327.


## Featured Translational Research

**ATRX guards against aberrant differentiation in mesenchymal progenitor cells.**

Contributed by Bennett Van Houten

Alterations in the tumor suppressor ATRX are recurrently observed in mesenchymal neoplasms. ATRX has multiple epigenetic functions including heterochromatin formation and maintenance and regulation of transcription through modulation of chromatin accessibility. In this study, Dr. Nacev and colleagues show in murine mesenchymal progenitor cells (MPCs) that Atrx deficiency aberrantly activated mesenchymal differentiation programs. This includes adipogenic pathways where ATRX loss induced expression of adipogenic transcription factors and enhanced adipogenic differentiation in response to differentiation stimuli. These changes are linked to loss of heterochromatin near mesenchymal lineage genes together with increased chromatin accessibility and gains of active chromatin marks. They also observed depletion of H3K9me3 at transposable elements, which are derepressed including near mesenchymal genes where they could serve as regulatory elements. Finally, they demonstrated that loss of ATRX in a mesenchymal malignancy, undifferentiated pleomorphic sarcoma, results in similar epigenetic disruption and de-repression of transposable elements.




**Impact:** These results reveal a role for ATRX in maintaining epigenetic states and transcriptional repression in mesenchymal progenitors and tumor cells and in preventing aberrant differentiation in the progenitor context.

**Funding:** This work was supported by Damon Runyon Cancer Research Foundation (CI-124-23; NIH (NCI) [K08CA245212 (B.A.N.), P30CA008748, P30CA047904, P50CA217694]; Connective Tissue Oncology Society.

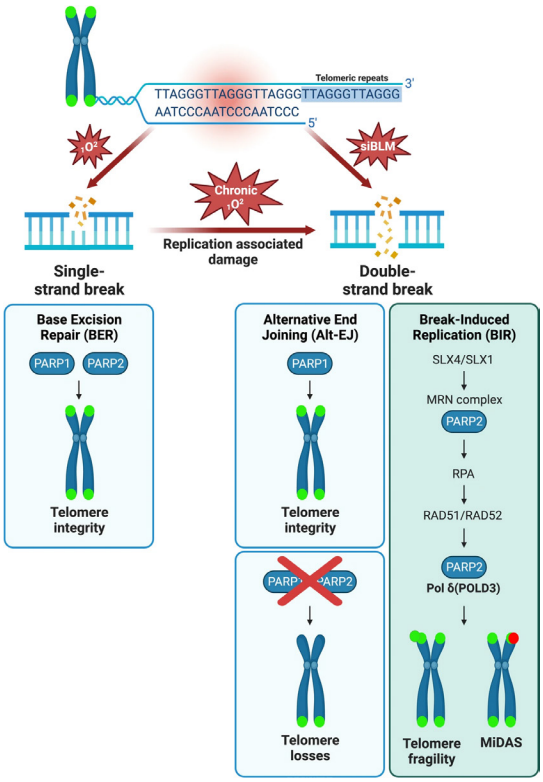
**Source:** Fang Y, Barrows D, Dabas Y, Carroll TS, Singer S, Tap WD, Nacev BA. *Nucleic Acids Res*. 2024. PMID: 38477352.

*Figure Legend: Model for ATRX-dependent chromatin and gene regulation in MPCs. Atrx KO MPCs demonstrate reduced stem-like properties, such as slower proliferation, reduced colony formation and downregulated mesenchymal stemness gene expression.*

## Cool Science

### PARP2 promotes break induced replication-mediated telomere fragility in response to replication stress.

Contributed by Patricia Opresko




PARP1 and PARP2 are DNA-dependent ADP-ribosyl transferase (ARTs) enzymes with Poly(ADP-ribosyl)ation activity that are triggered by DNA breaks, and have overlapping functions in base excision repair. Both enzymes are targets of PARP inhibitor drugs used in cancer therapy. In this study, Dr. Fouquerel and colleagues discovered a novel role for PARP2 in responding to replication stress at telomeres that is distinct from PARP1's role. This team found that PARP2 promotes DNA replication stress-induced break induced replication (BIR) at telomeres to prevent telomere loss following chronic induction of oxidative DNA lesions and BLM helicase depletion. PARP2 promotion of BIR led to the production of fragile telomeres, which can arise from BIR pathways. During this process, PARP2, but not PARP1, promotes DNA end resection, strand invasion and BIR-dependent mitotic DNA synthesis by orchestrating POLD3 recruitment and activity. This study identified a novel role for PARP2 in responding to DNA replication stress.

**Impact:** This study provides evidence that inhibition of PARP2 in cancer cells that experience high replication stress may be useful for depleting telomeres to halt cellular replication.

**Funding:** This work was supported by an NIH MIRA R35 award (R35GM142982) and start-up funding from UPMC Hillman Cancer Center.

**Source:** Muoio D, Laspata N, Curry C, Darkoa-Larbi S, Uttam S, Fouquerel E. *Nat Communications*. 2024. PMID: 38565848.

*Figure Legend: During replication stress, PARP1 promotes Alt-EJ to preserve telomere integrity while PARP2 orchestrates the BIR pathway during which it promotes DNA end resection and mitotic DNA synthesis by regulating PolD3 recruitment and activity. PARP2-dependent BIR triggers telomere fragility. Absence of PARP1 and PARP2 triggers telomere loss (created with BioRender.com).*

### A molecular index for biological age identified from the metabolome and senescence-associated secretome in humans.

Contributed by Bennett Van Houten

Unlike chronological age, biological age is a strong indicator of health of an individual. To define a high-resolution signature of biological age, Dr. Gurkar and associates analyzed metabolome, circulating senescence-associated secretome (SASP)/inflammation markers and the interaction between them, from a cohort of healthy and rapid agers. The balance between two fatty acid oxidation mechanisms, β-oxidation and ω-oxidation, associated with the extent of functional aging. Furthermore, a panel of 25 metabolites, Healthy Aging Metabolic (HAM) index, predicted healthy agers regardless of gender and race. HAM index was also validated in an independent cohort. Causal inference with machine learning implied three metabolites, β-cryptoxanthin, prolylhydroxyproline, and eicosenoylcarnitine as putative drivers of biological aging. Multiple SASP markers were also elevated in rapid agers. Together, these findings reveal that a network of metabolic pathways underlie biological aging, and the HAM index could serve as a predictor of phenotypic aging in humans.

**Impact:** Cancer increases with age; this study has developed a tool based on measuring 25 metabolites that predict healthy biological aging.

**Funding:** This work was supported in part by the Pittsburgh Claude D. Pepper Older Americans Independence Center (P30 AG024827- Pilot Grant A.U.G.). A.U.G. is supported by R00 AG049126, NIH- R01HL161106, U54AG075931, National Academy of Medicine (Catalyst grant), AFAR/Hevolution and RK Mellon Foundation grant. This project used the UPCI Cancer Biomarkers Facility: Luminex Core Laboratory that is supported in part by award P30CA047904. The WRAP cohort in this publication was supported by the National Institute on Aging

## Cool Science (continued)

of the National Institutes of Health under Award Numbers R01AG054047 and RF1AG054047.

**Source:** Hamsanathan S, Anthonymuthu T, Prosser D, Lokshin A, Greenspan SL, Resnick NM, Perera S, Okawa S, Narasimhan G, Gurkar AU. *Aging Cell*. 2024;23(4):e14104 PMID: 38454639.

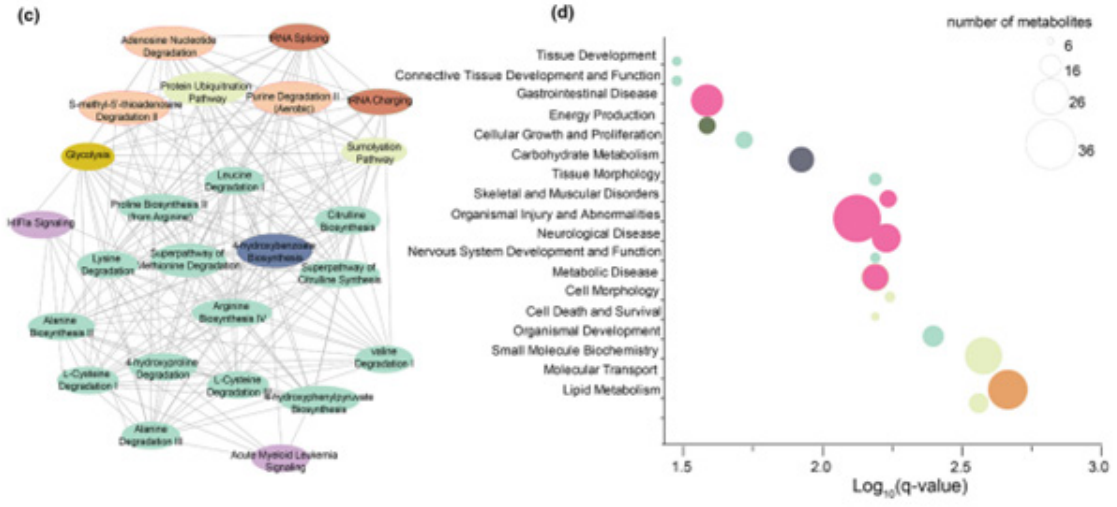
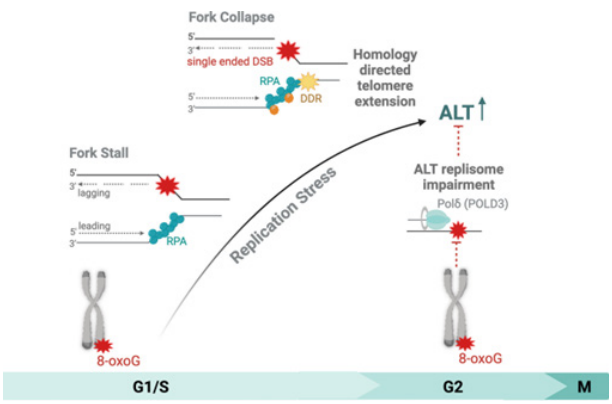



*Figure Legend: (c) Pathway-enrichment analysis of pathways in rapid agers shows alterations in amino acid (AA) biosynthesis. (d) Major disease and biofunction pathways associated with predictors of rapid agers. Pathways are represented in y-axis and the size of the bubble indicates the number of metabolites identified in each pathway.*

### Oxidative guanine base damage plays a dual role in regulating productive ALT-associated homology-directed repair.

Contributed by Patricia Opresko

Cancer cells achieve telomere maintenance to continue proliferating by upregulating telomerase or the alternative lengthening of telomeres (ALT) pathways via homology-directed repair at DNA breaks in the telomeres. 8-Oxoguanine (8oxoG) is among the most common endogenous DNA lesions, and telomeric TTAGGG repeats are hypersensitive to 8oxoG formation. In an intra-programmatic collaboration with Dr. O'Sullivan's lab, the Opresko lab demonstrated that the targeted formation of 8oxoG at telomeres stimulates ALT activity and homologous recombination at telomeres specifically in ALT cancer cells. Mechanistically, a single induction of 8oxoG damage increases DNA replication stress, as shown by increased telomere fragility and ATR kinase activation in ALT cancer cells, but not in telomerase expressing cancer cells. Furthermore, ALT cells are more sensitive to chronic telomeric 8oxoG damage than telomerase expressing cancer cells, consistent with increased 8oxoG-induced replication stress. However, the production of 8oxoG at telomeres specifically in the G2 cell cycle phase, when ALT telomere elongation occurs, impairs telomeric DNA synthesis. This study demonstrates that a common oxidative base lesion has a dual role in regulating ALT activity depending on when the damage arises in the cell cycle.




**Impact:** These results provide direct evidence that a prevalent oxidative DNA lesion can modulate ALT by impairing replication, which raises the possibility of therapeutically exploiting oxidative stress and damage to target ALT+ cancers.

**Funding:** This work was supported by NIH grants F32AG067710 and K99ES033771 (RPB), F30CA278287 and training award T32GM133332 (AW), R01CA207209 and R01262316 (RJO'S.), R35ES030396 and R01CA207342 (PLO), P30CA047904 (UPMC Hillman Cancer Center Cytometry Facility).

**Source:** Thosar SA, Barnes RP, Detwiler A, Bhargava R, Wondisford A, O'Sullivan RJ, Opresko PL. *Cell Rep*. 2024. PMID: 38194346.

*Figure Legend: Model for telomeric 8oxoG regulation of ALT activity. Oxidative stress leads to 8oxoG damage (red star) particularly*

## Cool Science (continued)

*in G-rich telomeres. When telomeric 8oxoG arises in G1/S cell-cycle phases, it promotes replication fork stalling, which can collapse into a single-ended DSB and stimulates ALT-HDR. However, when telomeric 8oxoG arises in G2 phase, it inhibits the ALT replisome and telomere extension.*

**Deregulated DNA ADP-ribosylation impairs telomere replication.**

Contributed by Patricia Opresko

Although ADP ribosylation of proteins has been well studied, DNA can also be ADP-ribosylated and far less is known regarding how this DNA modification is regulated and how it may contribute to genome stability, epigenetics and immunity. In this study, Dr. O'Sullivan and colleagues show that telomeres are substrates for DNA ADP-ribosylation (DNA-ADPr) that is catalyzed by PARP1 and removed by TARG1 in cells. Mechanistically, they show that DNA-ADPr is coupled to lagging strand DNA synthesis at telomeres, and forms in single-stranded DNA at unligated Okazaki fragments and on the 3' single-stranded telomere overhang. Persistent DNA-ADPr modifications, due to TARG1 deficiency, promotes telomere shortening. In a clever experiment, the team used bacterial DNA ADP-ribosyl-transferase toxin linked to telomeric protein TRF1 to selectively and directly modify DNA at telomeres. With this system they demonstrate that unhydrolyzed DNA-linked ADP-ribose impairs telomere replication and integrity. This study identifed telomeres as chromosomal targets of PARP1 and TARG1-regulated DNA-ADPr and showed that regulation of this modification impacts telomere stability.

**Impact:** This study establishes the critical importance of controlling DNA-ADPr turnover for genome stability. Furthermore, persistent replicative stress and single stranded DNA caused by TARG1 inhibition may benefit strategies that inhibit ADP ribosylation, such as PARP inhibitors, to kill cancerous cells.

**Funding:** This work was supported by NIH grants F30CA278287 and training award T32GM133332 (ARW), R01CA207209 and R01262316 (RJO'S.), R35ES030396 (PLO), K22CA245259 (JM), and P30CA047904.

**Source:** Wondisford AR, Lee J, Lu R, Schuller M, Groslambert J, Bhargava R, Schamus-Haynes S, Cespedes LC, Opresko PL, Pickett HA, Min J, Ahel I, O'Sullivan RJ. *Nat Struct Mol Biol*. 2024. PMID: 38714889.

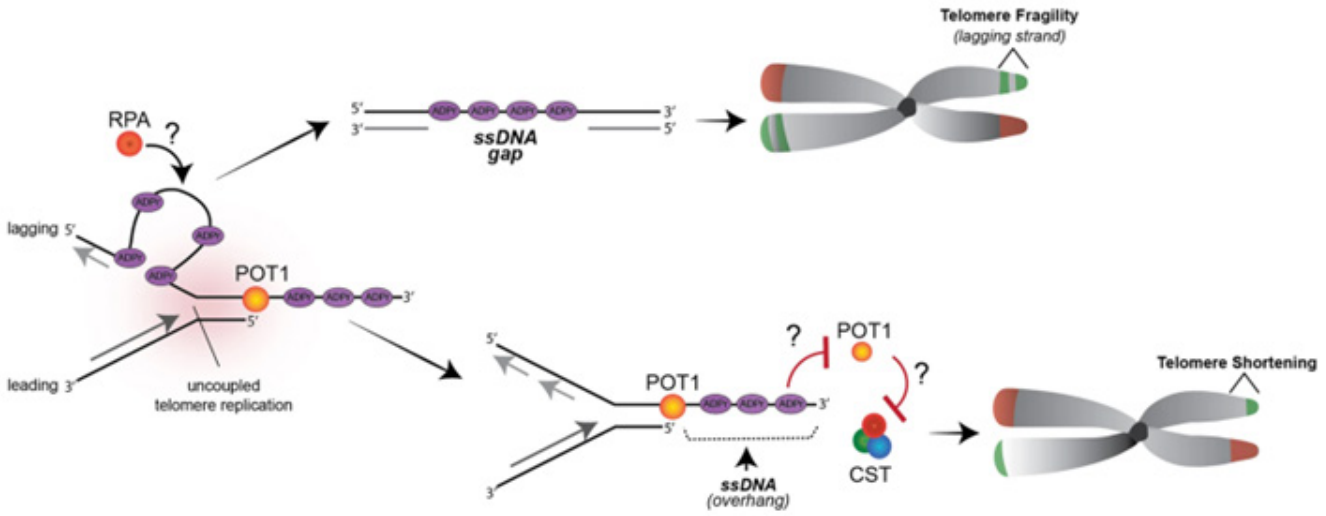



*Figure Legend: Model for how persistent DNA-ADPr impacts telomere replication. Single-stranded DNA at unligated Okazaki fragments stimulates DNA ADP-ribosylation (DNA-ADPr) by PARP1. If TARG1 does not remove the DNA-ADPr, these modifications interfere with telomere replication leading to persistent single stranded gaps that are associated with telomere fragility. Persistent DNA-ADPr on the telomere 3'overhang may disrupt telomere replication by interfering with POT1 binding and/or CST fill-in DNA synthesis, leading to telomere shortening.*

## Faculty and Staff News

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

*Brittani Schnable, PhD*

**Brittani Schnable, PhD**

A graduate student from Dr. Van Houten's lab, Brittani Schnable defended her thesis entitled, "Thymine DNA glycosylase combines sliding, hopping, and nucleosome interactions to efficiently search for 5-formylcytosine," on May 17th, 2024. She earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine. The Van Houten lab uses a single molecule approach to study DNA repair proteins. She used a combination of biochemistry, single molecule fluorescence microscopy, atomic force microscopy, STED, and single particle tracking. to study UV-DDB, glycosylases and their interactions with other repair proteins at 3 different levels: purified proteins, nuclear extracts and whole cells.

**Michelle Lynskey, PhD**

A graduate student from Dr. O'Sullivan's lab, Michelle Lynskey defended her thesis entitled, "HIRA protects against R-loop induced instability in ALT cancer cells," on April 19th, 2024. She earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine. She worked as an undergraduate researcher studying the regulation of planar cell polarity during forebrain, utricular, and cochlear development. Her main focus was on histone chaperones and ALT.

Outside the lab, Michelle is a big basketball fan who loves the Boston Celtics and the University of Virginia Men's Basketball team. In addition to watching and playing basketball, binge watching Love Island and cooking for her friends, Michelle's favorite thing to do is to play with her beloved dog Belly.

*Michelle Lynskey PhD*

*Dennis Braden, PhD*

**Dennis Braden, PhD**

A graduate student from Dr. Neumann's lab, Dennis Braden, defended his thesis entitled, "Dissecting the molecular mechanism of nitroalkene-mediated PARPi sensitization," on June 10th, 2024. He earned a PhD in Molecular Pharmacology from the University of Pittsburgh School of Medicine.

The main interest of the Neumann laboratory is to expand knowledge of cell signaling that is, in part, mediated by oxidation and reducing (redox) reactions as reactive oxygen species (ROS) deregulate the redox homeostasis and promote tumor formation by initiating an aberrant induction of signaling networks that cause tumorigenesis, including breast cancer. To investigate the specific mechanisms underlying redox-induced tumorigenesis, the Neumann laboratory focuses on the redox-induced posttranslational modifications (PTM) of protein cysteines, which are essential in cell signaling.

## Faculty and Staff News (continued)

**Join us in welcoming the new faculty to the Genome Stability Program!**

*Tatiana Moiseeva, PhD*

### Tatiana Moiseeva, PhD

UPMC Hillman Cancer Center welcomes Tatiana Moiseeva, PhD, effective February 1st, 2024. She is an assistant professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh.

At UPMC Hillman, Dr. Moiseeva is developing an independently-funded research program in genome stability, which includes collaborative efforts with basic and clinical faculty across the cancer center. She is primarily interested in the investigation of the initiation in DNA replication in human cells.

### Logan Myler, PhD

UPMC Hillman Cancer Center welcomes Logan Myler, PhD, effective September 1st, 2024. He is an assistant professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh.

He studies the mechanisms of DNA repair and telomere maintenance using a combination of biophysics, biochemistry, and cell biology. He's currently investigating how the shelterin complex structurally binds to telomeric chromatin and prevents the action of various nucleases, helicases, and other signaling proteins to prevent aberrant DNA repair. These mechanisms are critical to prevent genome instability and senescence, which are hallmarks of cancer and aging.

*Logan Myler, PhD*

*Orlando Schärer, PhD*

### Orlando Schärer, PhD

UPMC Hillman Cancer Center welcomes Orlando D. Schärer, PhD, as a senior leader effective October 1st, 2024. He is a professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh.

Orlando D. Schärer has served as Associate Director of the IBS Center for Genomic Integrity and Distinguished Professor at the Department of Biological Science of UNIST in Ulsan, South Korea since 2017. Among recent highlights from his laboratory are the elucidation how DNA repair processes are controlled through protein-protein interactions (PNAS 2022) and how the antitumor drug trabectedin hijacks DNA repair systems to induce DNA breaks to selectively kill tumor cells (*Nat Commun, 2024*).

## Faculty and Staff News (continued)

**We acknowledge all the ways our GSP members fight cancer:**

RUSH TO CRUSH CANCER
Benefitting UPMC Hillman Cancer Center

We would like to say a special thank you to all the Genome Stability Program members who took part in the Rush to Crush Cancer fundraiser in May of 2024. This event not only promotes wellness and fellowship but will also educates about cancer research, inspires hope, and raises funds for cancer research – all with the ultimate goal to help cancer patients. Join us in the future to ride to leave cancer behind!


*From left to right: Logan Myler, Bennett Van Houten, Sam Sanford, Tatiana Moiseeva, Elise Fouquerel, Shikhar Uttam, Patricia & Cammie Opresko.*

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/**