DNA Pitt Crew

Spring 2023

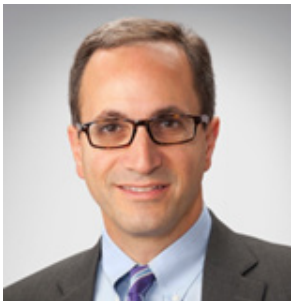
The latest news and updates from the UPMC Hillman Cancer Center Genome Stability Program

University of Pittsburgh

UPMC | HILLMAN CANCER CENTER

Genome Stability Program GSP

Note from Director Robert L. Ferris, MD, PhD



UPMC Hillman Cancer Center embarked on two important milestones this spring. For the first time in our more than 30-year history as a National Cancer Institute-designated Comprehensive Cancer Center, we are joining an elite group of other cancer centers invited to apply for a two-year extension for the renewal of this prestigious designation.

The other milestone was our inaugural Rush to Crush Cancer fundraiser for research at UPMC Hillman. There was an impressive lineup of events and an even more impressive show of support from our community. This included a survivors' walk with more than 400 participants and a bike ride with over 750 bikers. UPMC Hillman leadership is grateful for the support of volunteers, spectators, walkers, and riders who participated in the event to make it a success. We are inspired by this event and all the work of those throughout our cancer center who work every day to reduce the burden of cancer.

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

We are excited to present the Spring 2023 edition of the DNA Pitt Crew newsletter, which provides recent information about UPMC Hillman Cancer Center (HCC) Genome Stability Program (GSP) members, new recruits, and some of the exciting scientific accomplishments from this program. We have been fortunate these past few months to enjoy visits from outstanding scientists in genome stability, as described in the DNA Pitt Stop section. GSP members and trainees shared their work in talks and posters at recent conferences, including the Gordon Research Seminar/ Conference on DNA Repair and the US-EU Conference on Endogenous DNA Damage. This edition includes four highlights of recent high impact publications and scientific discoveries: 1) identifying TPP1 promoter variants in melanoma that promote telomere elongation (*Science*); 2) defining a novel role for RAD51AP1 in regulating the alternative lengthening of telomeres pathway (*Molecular Cell*); 3) uncovering a role for ferroptosis of tumor neutrophils in immune suppression in cancer (*Nature*); and 4) discovering that UVA irradiation from UV nail polish dryers induces signatures of 8-oxoguanine damage (*Nature Communications*). We also highlight and congratulate junior faculty members Drs. Fouquerel and Nechemia-Arbely on their first senior author publications. Finally, we thank the members of the Rush to Crush Cancer Genome Stability team and their captain Dr. Fouquerel, for volunteering and fundraising, and for their perseverance in enduring Pittsburgh hills and rain on their bike rides! We are grateful to all the donors who support cancer research.

Inside this issue:

Contact Us:

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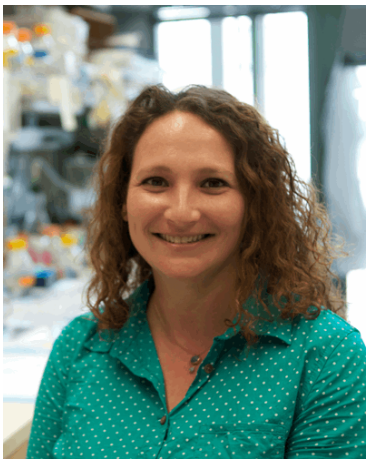


Yael Nechemia-Arbely, PhD

Faculty Spotlight: Yael Nechemia-Arbely, PhD

Contributed by Yael Nechemia-Arbely, PhD

Yael Nechemia-Arbely, PhD, is an Assistant Professor 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 structure, function, and maintenance of epigenetically defined human centromeres and their role in guarding the genome.

Dr. Nechemia-Arbely completed her bachelor's degree in Basic Medical Sciences at the Hebrew University of Jerusalem (Israel). She then continued her graduate education at the same university. Her graduate thesis, under the mentorship of Jonathan Axelrod, PhD, and Eithan Galun, MD, focused on the role of IL-6 classic and trans-signaling in tissue injury and repair. Her work showed that treatment of mice with HyperIL-6 (a fusion protein consisting of IL-6 fused to its soluble receptor (sIL-6R)) can prevent kidney damage and death as a result of acute kidney injury, prolonging the life span of the treated mice.

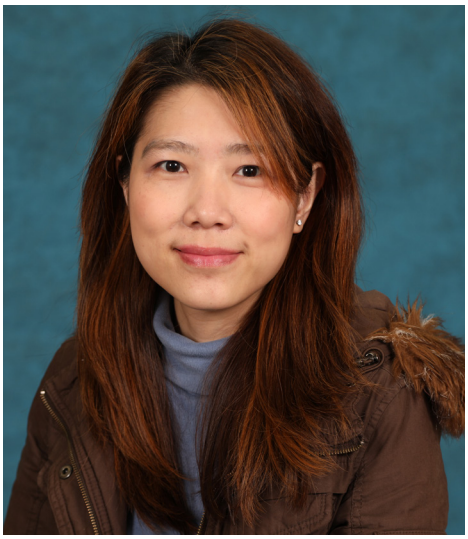
In a second project, Dr. Nechemia-Arbely showed that IL-6 trans-signaling, induced by HyperIL-6, promotes entry of hepatocytes into the cell cycle, boosts hepatocyte mitosis (cell division) and accelerates liver regeneration following partial hepatectomy. It was during this second project that she became fascinated with observing mitotic cells and decided to focus on mitosis for her postdoctoral studies. Dr. Nechemia-Arbely joined the lab of Don Cleveland, PhD, at the Ludwig Institute for Cancer Research, University of California, San Diego, where she studied human centromeres - the central genetic element responsible for accurate chromosome segregation during mitosis.

Steel City Chromatin Club

The Nechemia-Arbely lab focuses on human centromeres epigenomics, a challenging endeavor that is complicated by the highly repetitive a-satellite DNA located at human centromeres. Dr. Nechemia-Arbely's lab is tackling this challenging centromeric DNA and the histones and proteins bound to it by using novel epigenomics tools such as DiMelo-seq - a long-read, single-molecule method for mapping protein–DNA interactions genome wide. An additional focus of her lab is understanding whether CENP-A, a centromeric histone H3 variant, is capable of precisely and stably specifying human centromere position throughout cellular proliferation. To investigate the positional stability of human centromeres as cells proliferate, her lab uses a human fibroblast cell line that harbors a neocentromere (epigenetic stable acquisition of a new centromere at a new chromosomal site). Lastly, her lab is interested in understanding how CENP-A overexpression, seen in several types of cancer, affects the stability of the human genome. Her recent work demonstrated remarkable plasticity in the position of centromeric CENP-A and site of kinetochore assembly between different individuals while highlighting the requirement for precise CENP-A maintenance across the cell cycle to preserve unique epigenetic centromere identities.

Outside of the lab, Dr. Nechemia-Arbely is busy with her three girls. She loves ballet shows and enjoys arts and crafts and exploring nature while hiking with her family. Yael has recently organized a highly successful Steel City Chromatin Club which meets every two months and featured two presentations from faculty or trainees who's work impacts the structure and function of chromatin.

Lior Lumerman, Dr. Nechemia-Arbely, Poulomi Nath and Megan Mahlke receiving the UPMC Hillman Cancer Center 2021 Junior Scholar award for Basic Cancer Research.



Pattra Chun-on, MD, MPH

Trainee Spotlight: Pattra Chun-on, MD, MPH

Contributed by Jonathan K. Alder, PhD, and Pattra Chun-on, MD, MPH

Pattra received her Doctor of Medicine from the Faculty of Medicine Siriraj Hospital, Mahidol University in Thailand. She practiced as an internal medicine physician at Chulabhorn Cancer Center, providing medical care and treatment to non-cancer and cancer patients with medical conditions. During this time, she served as the chief of the general medical doctor unit and chief of occupational health environment and safety, responsible for patient services, health care providers' safety, and worker health surveillance. These responsibilities brought her to pursue her Master of Public Health and PhD degrees in Environmental and Occupational Health at the University of Pittsburgh.

While pursuing a Master's degree, she focused on the environmental perspective of environmental health science and risk assessment. In her PhD degree, she is interested in cancer cell biology and translational medicine. During Pattra's rotation in the Opresko lab, Dr. Patricia Opreko and her lab members were passionate about telomere biology. Fortunately, Dr. Opresko introduced Pattra to Dr. Jonathan Alder to learn more about telomere disorders, and the cellular mechanisms that cause clinical phenotypes. Then Pattra joined the Alder lab as a graduate student

In the Alder Lab, Pattra has focused on telomere maintenance mechanisms in cancer. Her project involves somatic mutations in TERT and TPP1 promoters and their roles in telomere maintenance, specifically in cutaneous melanoma. Since joining the Alder lab, Pattra has co-authored one journal article in the *American Journal of Respiratory Cell and Molecular Biology* and a first-author publication in *Science*. Outside of the lab, Pattra is a mother of a little girl and has also been involved in the Doctoral Student Organization, where she served in an executive board position from 2018-2019. Pattra successfully defended her thesis entitled "Recurrent TPP1 Promoter Mutations Drive Telomere Maintenance in Melanoma" on April 7th, earning a PhD from the Department of Environmental and Occupational Health in the University of Pittsburgh School of Public Health.

Pattra plans to continue researching telomere biology and telomere-related diseases in Boston, where she will work as a Postdoctoral Fellow in Dr. Suneet Agarwal's lab at Boston Children's Hospital. She will learn more in-depth about telomere-related gene mutations, which could be translated into clinical medicine.

Pitt Stops: Special Events and Visiting Speakers

Tatiana Moiseeva, PhD

Tatiana Moiseeva, PhD

Principal Investigator
Department of Chemistry and Biotechnology
Tallinn University of Technology in Estonia

Virtual visit, Mar 21, 2023.
Contributed by Patricia Opresko, PhD

Dr. Tatiana presented a virtual seminar entitled "Deciphering the mechanisms of DNA replication initiation in human cells", and met with many of the GSP faculty. In 2019, she joined the Tallinn University of Technology faculty. In her seminar, she described her recent *Nature Communication* manuscript, which was awarded the prestigious article of the year in the field of natural, and health sciences by Tallin University. She introduced the study by describing how DNA polymerase eplison (PolE) is essential for replication, and how PolE deficiencies lead to developmental abnormalities and cancer. Her group developed an innovative auxin-inducible degron system to deplete PolE, and examine consequences for DNA replication. They discovered that PolE-deficient cells could assemble the CMG DNA helicase replication initiation complex. However, her team discovered that a non-catalytic domain of PolE was required to continue successful replication initiation. Her seminar was followed by an engaging Q&A session about her exciting work and discoveries.

Pitt Stops: Special Events and Visiting Speakers (continued)

Barbara van Loon, PhD

Deputy Leader for Research and Innovation Department of Clinical and Molecular Medicine Faculty of Medicine and Health Sciences at the Norwegian University of Science and Technology, Trondheim, Norway

In person visit: November 11, 2022.
Contributed by Ben Van Houten, PhD

Karen Arndt, Barbara van Loon, Elise Fouquerel and Ben Van Houten, enjoying the city lights from mount Washington.

Professor van Loon presented a fascinating lecture on "Non-canonical Functions of AAG DNA Glycosylase in Gene Expression and Associated Impacts on Behaviour". After discussing the implications of her recent work in which her lab showed that the alkyladenine glycosylase (AAG) binds to chromatin and forms a complex with RNA polymerase (pol) II. This interaction occurs through direct contact with Elongator and results in transcriptional co-regulation. Importantly, at co-regulated genes, aberrantly methylated bases accumulate towards the 3'end in regions where they see an accumulation of AAG and the second base excision repair (BER) protein APE1. These results provide insights into genome stability maintenance in actively transcribing chromatin and reveal roles of aberrantly methylated bases in regulation of gene expression. Dr. van Loon then discussed some unpublished work on the role of BER proteins on normal function of neurons derived from iPSCs and the role of E3 ubiquitin ligases in rare human neurological syndromes. Finally, she presented data showing that AAG KO mouse models alterations in neurodevelopment especially in the hippocampal regions. She described how AAG KO mice are less anxious and have alterations in spatial learning, leading to decreased anxiety and more rapid learning. Professor van Loon's pioneering work is well cited, and she recently received the prestigious 2021 Anders Jahre Young Researcher Award in 2021.

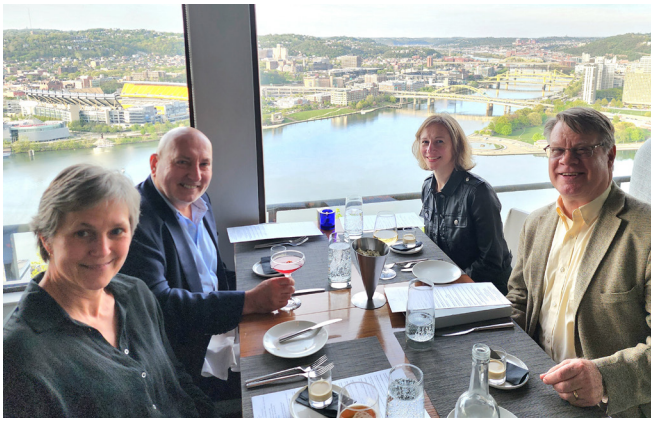
Yinsheng Wang, PhD

Distinguished Professor of Chemistry and the Donald T. Sawyer Endowed Founder's Chair in Chemistry at the University of California, Riverside. Director of the National Institute of Environmental Health Sciences (NIEHS) Environmental Toxicology Graduate Program

In person visit, Dec 12, 2022.
Contributed by Ben Van Houten, PhD

Here is Yinsheng Wang Pictured doing one of his favorite hobbies - hiking and enjoying nature.

Professor Wang presented an outstanding lecture, " Chemical Biology of DNA Damage and Repair". He first described his work on a toxic metabolite, methylglyoxal that is generated in high amounts in diabetic patients and produces the N(2)-(1-carboxyethyl)-2'-deoxyguanosine (N(2)-CEdG) DNA adduct and its mutagenic bypass in bacteria and mammalian cells. He then described DNA pol eta interacting proteins using the APEX approach, and discovered that DNA pol eta helps recruit DHX9, a helicase to quadruplex sequences in cells. Finally, he discussed how his laboratory uses quantitative mass spectrometry (MS) to follow protein and DNA adducts resulting from oxidative stress, and how he is combining MS with genomic approaches to map thymine glycol residues at base pair resolution in the genome. He has found that these lesions accumulate in heterochromatic regions.



Carola Neumann, Stephen West, Patty Opresko and Ben Van Houten, having dinner.

Stephen West, PhD

Senior Group Leader
The Francis Crick Institute in London, England

In person visit, April 27, 2023.
Contributed by Ben Van Houten, PhD

We were delighted to host Dr. Steve West, who is a Principal Group leader at the Francis Crick Institute and is internationally recognized for his seminal contributions to our understanding of the molecular mechanisms of homologous recombination during DNA double-strand break repair. Dr. West presented an outstanding lecture that discussed two topics: 1) the structure and function of the BCDX2 complex (RAD51B-D and XRCC2); and how targeting the enzyme, deoxyribonucleoside monosphate N-glycosidase (DNPH1) published in *Science* (372:156-165, 2021) BRCA-deficient cells to PARP inhibitors. His exciting and novel cryo-EM high resolution structures revealed the assembly of the BCDX2, and how the RAD51B binding to ADP, is dependent upon the RAD51C ATPase. His study of DNPH1 revealed that the action of the glycosylase SMUG1 on hydroxymethyl-deoxy-Uridine (hmdU) helps cause synthetic lethality in the presence of PARPi through replication fork collapse, DNA strand break formation and apoptosis. Furthermore, PARPi resistant BRCA1-deficient cells could be sensitized to killing by treating cells with low doses of hmdU and DPNH1 inhibitors.

Cynthia Burrows, PhD

Distinguished Professor of Chemistry
Thatcher Presidential Endowed Chair of Biological Chemistry
University of Utah

In person visit, February 13-15, 2023.
Contributed by Patricia Opresko, PhD

Cynthia Burrows, PhD

Dr. Burrows gave the UPMC Hillman Cancer Center Basic & Translational Research Seminar entitled "Repair of Oxidative Damage in Promoter vs. Telomere G-Quadruplex". She is well known for her seminal work on DNA damage and repair, especially oxidative DNA lesions.
She talked about her lab's discovery that 8-oxoguanine has epigenetic properties and that its formation and processing in gene promoters can regulate gene expression in response to oxidative stress. She presented evidence that some gene promoters are enriched for G-quadruplex forming sequences, but are also prone to 8-oxoguanine formation. Her Elegant biochemical and transcription reporter studies revealed that base excision repair enzyme APE1 can bind to abasic sites, arising from 8-oxoguanine excision, in G-quadruplexes but its cleavage activity is inhibited. Instead, APE1 recruits transcription factors to upregulate gene expression. Finally, she presented some very interesting evidence for how differences in G-quadruplex conformations in promoter versus telomeric sequences influence APE1 binding and activity. Her talk was very engaging, and she explained complex chemistry in a highly accessible manner. Finally, she met with GSP trainees after her lecture during an informal lunch to share her experience and career advice.

Pitt Stops: Special Events and Visiting Speakers (continued)

Joe Nassour, PhD

Joe Nassour, PhD

Research Associate
Jan Karlseder's laboratory
Salk Institute for Biological Studies

In person visit, January 3-5, 2023.
Contributed by Patricia Opresko, PhD

Dr. Nassour presented a lecture entitled "Autophagy and innate immunity in telomere-mediated tumor suppression". Dr. Nassour described his exciting work on the role of autophagy in cellular bypass from telomere drive replicative crisis, which was published in two manuscripts in *Nature* and provided the basis for his NCI K99/R00 award. He talked about his work showing that telomere shortening and dysfunction can lead to crisis, which triggers autophagy to prevent premalignant cells with unstable telomeres from replicating. He found telomere dysfunction led to cytoplasmic DNA species that activated the cGAS-STING signaling pathway. He then discussed his work uncovering the mechanism of autophagy activation. He found the innate immune sensor Z-DNA binding protein 1 (ZBP1) is activated upon binding telomere transcripts (TERRA) from dysfunctional telomeres. Interaction of this complex with the mitochondria activates a MAVS-dependent interferon response to eliminate cells with dysfunctional telomeres. Dr. Nassour's outstanding talk was very well received, and he met with several faculty members at HCC during his visit.

Scientific Conference Highlights and Awards

Awards

Congratulations to Dr. Samantha Sanford on her NCI F32CA275287 award entitled "Investigating how chemotherapeutic thiopurines inhibit telomerase elongation of telomeres"

7th US-EU Conference on Endogenous DNA Damage

Dr. Mariarosaria De Rosa received an award for Best Poster Presentation at the 7th US-EU Conference on Endogenous DNA Damage and Repair held at Stony Brook University, NY in November 6-9. The award was received in presence of 2015 Nobel Laureate in Chemistry Tomas Lindahl, who paved the way for the studies on DNA damage and repair mechanisms.

2023 Mammalian DNA Repair Gordon Research Conference

February 4 - 10, 2023 – Ventura, CA

Congratulation to Gordon Research Conference (GRC) chair Patty Opresko and Vice Chair Roger Greenberg (University of Pennsylvania) for a highly engaging and successful conference on innovative science in the DNA damage and repair field. The main conference was preceded by a trainee-led one and half day conference (GRS). Many of the members of the GSP participated in both and delivered outstanding talks and/or poster presentations.

Gordon Research Seminar presentations:

- Matt Schaich, PhD (Van Houten lab) gave a talk entitled "Single-molecule analysis of DNA-binding proteins from nuclear extract (SMADNE)".
- Sanjana Thosar (Opresko lab) gave a talk entitled "Telomeric 8-oxoG promotes replication stress-driven ALT".
- Natalie Laspata (Fouquerel lab) gave a talk entitled "PARP1 associates with R-loops to promote their resolution and genome stability".
- Ryan Barnes, PhD (Opresko lab) gave a talk entitled "Oxidative and replicative stress at telomeres".
- Ben Van Houten, PhD gave a talk entitled "Exploring how proteins find and repair damaged DNA".
- Natalie Laspata (Fouquerel lab); Sripriya Raja & Matt Schaich (Van Houten lab) Ryan Barnes & Sanjana Thosar (Opresko lab) all presented posters.

Patricia L. Opresko, PhD is being honored with the Dr. Bernard F. Fisher Chair for Breast Cancer Discovery Science

Dr. Opresko was recently honored with the Dr. Bernard F. Fisher Chair, a newly formed chair position being launched this year. She was nominated for this appointment because of her highly innovative and impactful work that builds on an extraordinary and sustained record of scientific accomplishment advancing our understanding of how telomeres protect the genome and prevent cancer and premature aging. Her work also focuses on how environmental exposures compromise telomeres' protective function.

Dr. Opresko is co-leader of UPMC Hillman Cancer Center's Genome Stability Program, professor of Environmental and Occupational Health in the School of Public Health, and professor in the Department of Pharmacology and Chemical Biology in the School of Medicine at Pitt. She is also co-chair of the working group on environmental risk factors for cancer in western Pennsylvania and a member of the working group on cancer and aging, both at UPMC Hillman Cancer Center.

Dr. Opresko is author of more than 95 peer-reviewed publications in top-tier journals that include *Molecular Cell, Cell Reports, Nature Structural & Molecular Biology, Nature Communications, PNAS, and Nucleic Acids Research*. Her H-index is 46 (Google Scholar), which speaks to the impact of her work. She is recognized nationally and internationally as an expert and has been invited to give numerous talks at universities, research institutes, and scientific conferences, including keynote and distinguished scientist seminars. She is an active member of the scientific community, and as a testament was recently elected as Vice President and upcoming President of the 380-member Environmental Mutagenesis and Genomics Society (EMGS). She was elected to Chair the 2023 Gordon Research Conference on DNA Repair. Dr. Opresko is also an exceptional mentor and lecturer; her trainees have received NIH K and F grants, travel awards, presentation awards, and been selected to give talks at numerous conferences. Please join in congratulating Dr. Opresko for receiving this prestigious recognition.

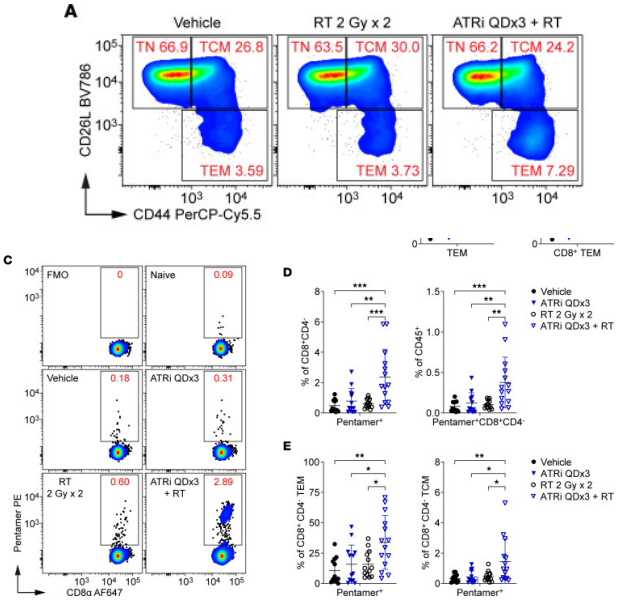
Hot Papers

1. 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*, Mar 17:gkad180.
2. Gallant JN, Chen SC, Ortega CA, Rohde SL, Belcher RH, Netterville JL, Baregamian N, Wang H, Liang J, Ye F, Nikiforov YE, Nikiforova MN, Weiss VL. "Evaluation of the Molecular Landscape of Pediatric Thyroid Nodules and Use of a Multigene Genomic Classifier in Children." *JAMA Oncol*, 8(9):1323-7.
3. 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*, Mar 27:gkad206.
4. Klimas A, Gallagher BR, Wijesekara P, Fekir S, DiBernardo EF, Cheng Z, Stolz DB, Cambi F, Watkins SC, Brody SL, Horani A, Barth AL, Moore CI, Ren X, Zhao Y. "Magnify is a universal molecular anchoring strategy for expansion microscopy." *Nat Biotechnol*, Epub 2023/01/02.
5. Liu JB, Baugh KA, Ramonell K, McCoy KL, Karslioglu-French E, Morariu EM, Ohori NP, Nikiforova MN, Nikiforov YE, Carty SE, Yip L. "Molecular Testing Predicts Incomplete Response to Initial Therapy in Differentiated Thyroid Carcinoma without Lateral Neck or Distant Metastasis at Presentation: Retrospective Cohort Study." *Thyroid*, Epub 2023/03/27.
6. 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*, 120(11):e2217422120.
7. 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*, gkad150.
8. Sannino S, Manuel AM, Shang C, Wendell SG, Wipf P, Brodsky JL. "Non-essential amino acid availability influences proteostasis and breast cancer cell survival during proteotoxic stress." *Mol Cancer Res*, Mcr-22-0843.
9. Schaich MA, Schnable BL, Kumar N, Roginskaya V, Jakielski RC, Urban R, Zhong Z, Kad NM, Van Houten B. "Single-molecule analysis of DNA-binding proteins from nuclear extracts (SMADNE)." *Nucleic Acids Res*, Epub 2023/03/02.
10. Tyurina YY, Kapralov AA, Tyurin VA, Shurin G, Amoscato AA, Rajasundaram D, Tian

H, Bunimovich YL, Nefedova Y, Herrick WG, Parchment RE, Doroshow JH, Bayir H, Srivastava AK, Kagan VE. "Redox phospholipidomics discovers pro-ferroptotic death signals in A375 melanoma cells in vitro and in vivo." *Redox Biol*, 61:102650.

11. Yu W, Chen Y, Putluri N, Osman A, Coarfa C, Putluri V, Kamal AHM, Asmussen JK, Katsonis P, Myers JN, Lai SY, Lu W, Stephan CC, Powell RT, Johnson FM, Skinner HD, Kazi J, Ahmed KM, Hu L, Threet A, Meyer MD, Bankson JA, Wang T, Davis J, Parker KR, Harris MA, Baek ML, Echeverria GV, Qi X, Wang J, Frederick AI, Walsh AJ, Lichtarge O, Frederick MJ, Sandulache VC. "Evolution of cisplatin resistance through coordinated metabolic reprogramming of the cellular reductive state." *Br J Cancer*, Epub 2023/04/04.
12. 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*, 193(4):392-403.
13. Zou H, Poore B, Brown EE, Qian J, Xie B, Asimakidou E, Razskazovskiy V, Ayrapetian D, Sharma V, Xia S, Liu F, Chen A, Guan Y, Li Z, Wanggou S, Saulnier O, Ly M, Fellows-Mayle W, Xi G, Tomita T, Resnick AC, Mack SC, Raabe EH, Eberhart CG, Sun D, Stronach BE, Agnihotri S, Kohanbash G, Lu S, Herrup K, Rich JN, Gittes GK, Broniscer A, Hu Z, Li X, Pollack IF, Friedlander RM, Hainer SJ, Taylor MD, Hu B. "A neurodevelopmental epigenetic programme mediated by SMARCD3-DAB1-Reelin signalling is hijacked to promote medulloblastoma metastasis." *Nat Cell Biol*, 25(3):493-507.
14. 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 USA*, 120(1): e2211927120.

Featured: Translational Research



The schedule of ATR inhibitor AZD6738 can potentiate or abolish antitumor immune responses to radiotherapy.

Inhibitors of the DNA damage signaling kinase ATR increase tumor cell killing by chemotherapies that target DNA replication forks but also kill rapidly proliferating immune cells including activated T cells. Nevertheless, ATR inhibitor (ATRi) and radiotherapy (RT) can be combined to generate CD8+ T cell-dependent antitumor responses in mouse models. To determine the optimal schedule of ATRi and RT, we determined the impact of short-course versus prolonged daily treatment with AZD6738 (ATRi) on responses to RT (days 1-2). Short-course ATRi (days 1-3) plus RT caused expansion of tumor antigen-specific, effector CD8+ T cells in the tumor-draining lymph node (DLN) at 1 week after RT. This was preceded by acute decreases in proliferating tumor-infiltrating and peripheral T cells and a rapid proliferative rebound after ATRi cessation, increased inflammatory signaling (IFN-β, chemokines, particularly CXCL10) in tumors, and an accumulation of inflammatory cells in the DLN. In contrast, prolonged ATRi (days 1-9) prevented the expansion of tumor antigen-specific, effector CD8+ T cells in the DLN, and entirely abolished the therapeutic benefit of short-course ATRi with RT and anti-PD-L1. Our data argue that ATRi cessation is essential to allow CD8+ T cell responses to both RT and immune checkpoint inhibitors.

Funding: This work was supported by NIH grants R01CA236367 and R01CA26617 (to CJB). This project used the Animal Facility, Cancer Pharmacokinetics and Pharmacodynamics Facility, and Cytometry Facility that are supported in part by award P30CA047904 from the NIH.

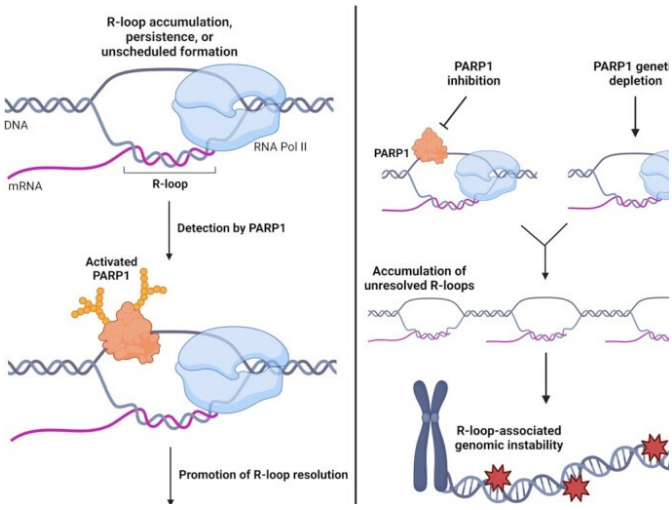
Source: Vendetti FP, Pandya P, Clump DA, Schamus-Haynes S, Tavakoli M, diMayorca M, Islam NM, Chang J, Delgoffe GM, Beumer JH, Bakkenist CJ. "The schedule of ATR inhibitor AZD6738 can potentiate or abolish antitumor immune responses to radiotherapy." *JCI Insight*. 2023, **8**(4) e165615.

Figure Legend: (A-E) CT26 tumor-bearing mice were treated with ATRi on days 1-3 (ATRi QDx3), RT on days 1-2 (RT 2 Gy x 2), ATRi QDx3 + RT, or vehicle, and tumor-draining lymph nodes (DLNs) were immunoprofiled at day 9. (A) Representative cytograms depicting CD62L and CD44 expression on CD8+ T cells. Activated and naive CD8+ T cell subsets were defined as effector memory (Tem; CD44hiCD62Llo), central memory (Tcm; CD62LhiCD44hi), or naive (Tn; CD62LhiCD44lo). (C-E) Tumor antigen-specific CD8+ T cells were labeled with AH1 Pentamer. (C) Representative cytograms depicting Pentamer+ CD8+ T cells. Fluorescence-minus-one (no Pentamer) and naive (negative, no tumor) controls shown. (D) Quantitation of Pentamer+ CD8+ T cells as percentages of CD8+CD4- cells or CD45+ immune cells. (E) Quantitation of Pentamer+ CD8+ Tem and Tcm cells as percentages of CD8+CD4- Tem and Tcm cells.

Papers of Note from Junior Faculty

Congratulations to Drs. Fouquerel and Nechemia-Arbley on their first senior author publications from their labs!
Laspata N, Kaur P, Mersaoui SY, Muoio D, Liu ZS, Bannister MH, Nguyen HD, Curry C, Pascal JM, Poirier GG, Wang H, Masson JY, **Fouquerel E.** PARP1 associates with R-loops to promote their resolution and genome stability. *Nucleic Acids Res*. 2023, 51(5): 2215-37.

This study demonstrates that PARP1 binding to RNA-DNA hybrid R-loops activates its ADP-ribosylation activity, and prevents the accumulation of unresolved R-loops. The authors reveal that PARP1 is a novel sensor for R-loops and a suppressor of R-loop-associated genomic instability.



Funding: National Institute of Health [R00ES027028]; UPMC Hillman Cancer Center and the Sydney Kimmel Cancer Center at Thomas Jefferson University (to E.F.); FDN-388879 (to J.Y.M.); CIHR MOP-418863 (to G.P.); CIHR PJT-173370 (to J.M.P.); P. Edward Evans Foundation; American Society of Hematology Scholar Award the 2022 AACR Career Development Award to Further Diversity, Equity, and Inclusion in Cancer Research, which is supported by Merck [22-20-68-NGUY]; National Institutes of Health's National Center for Advancing Translational Sciences [KL2TR002492 and UL1TR002494]; NHLBI [R01HL163011 to H.D.N.]; Thomas Jefferson University and Sidney Kimmel Cancer Center (to E.F.); Masonic Cancer Center, University of Minnesota (to H.D.N); J.Y.M. is a Tier I Canada Research Chair in DNA repair and Cancer Therapeutics; S.Y.M. is a FRQS postdoctoral fellow; E.F. is an Innovative Cancer Research Program Hillman fellow

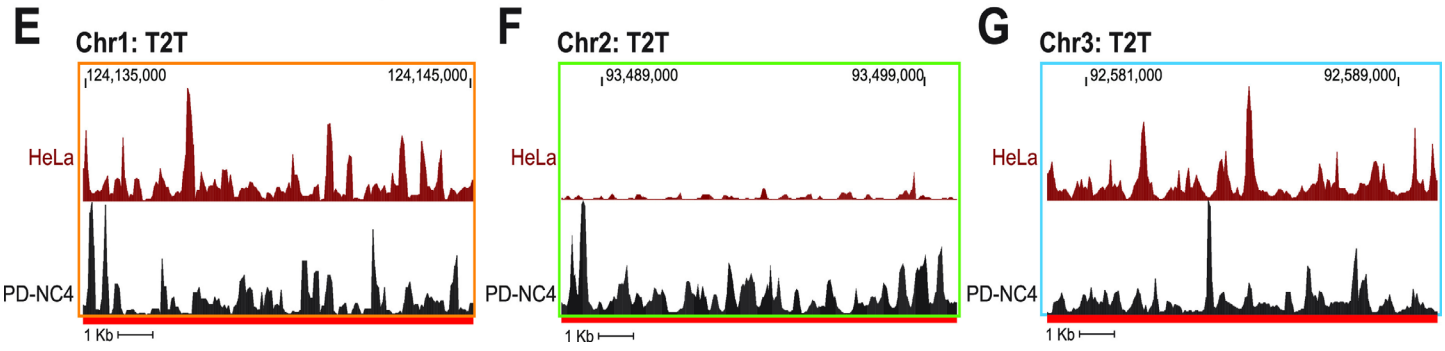
Figure Legend: PARP1 association with R-loop structures induces its Poly(ADP-ribosyl)ation activity. PARP1 genetic depletion or inhibition leads to R-loop persistence and triggers genome instability.

Mahlke MA, Lumerman L, Ly P, **Nechemia-Arbely Y.** "Epigenetic centromere identity is precisely maintained through DNA replication but is uniquely specified among human cells." 2023, *Life Sci Alliance*, 6(3).

This study uses the recently released Telomere - to - Telomere (T2T) genome assembly to map centromeric histone variant CENP-A. The authors show that despite CENP-A dilution during DNA replication, CENP-A is precisely reloaded onto the same sequences within the daughter centromeres, maintaining unique centromere identity among human cells.

Funding: This work was supported by a grant (R35GM142717) from the NIH to Y Nechemia-Arbely.

Figure Legend: (E, F, G) High-resolution view of CENP-A reads in HeLa (maroon) and PD-NC4 (black) cells when mapped to the T2T assembly at the same centromeres shown in (B, C, D). Color-coded panels represent the colored locations indicated in (B, C, D). Scale bar, 1 Kb.



Cool Science

TPP1 promoter mutations cooperate with TERT promoter mutations to lengthen telomeres in melanoma.

Overcoming replicative senescence is an essential step during oncogenesis, and the reactivation of TERT through promoter mutations is a common mechanism. TERT promoter mutations are acquired in about 75% of melanomas but are not sufficient to maintain telomeres, suggesting that additional mutations are required. We identified a cluster of variants in the promoter of ACD encoding the shelterin component TPP1. ACD promoter variants are present in about 5% of cutaneous melanoma and co-occur with TERT promoter mutations. The two most common somatic variants create or modify binding sites for E-twenty-six (ETS) transcription factors, similar to mutations in the TERT promoter. The variants increase the expression of TPP1 and function together with TERT to synergistically lengthen telomeres. Our findings suggest that TPP1 promoter variants collaborate with TERT activation to enhance telomere maintenance and immortalization in melanoma.

Impact: Their findings suggest that TPP1 promoter variants cooperate with TERT activation to enhance telomere maintenance and immortalization in melanoma.

Funding: National Institute of Health [R35CA209974], [R01HL135062]

Source: Chun-On P, Hinchie AM, Beale HC, Gil Silva AA, Rush E, Sander C, Connelly CJ, Seynnaeve BKN, Kirkwood JM, Vaske OM, Greider CW, Alder JK. "TPP1 promoter mutations cooperate with TERT promoter mutations to lengthen telomeres in melanoma." *Science*. 2022, **378**(6620): 664-8.

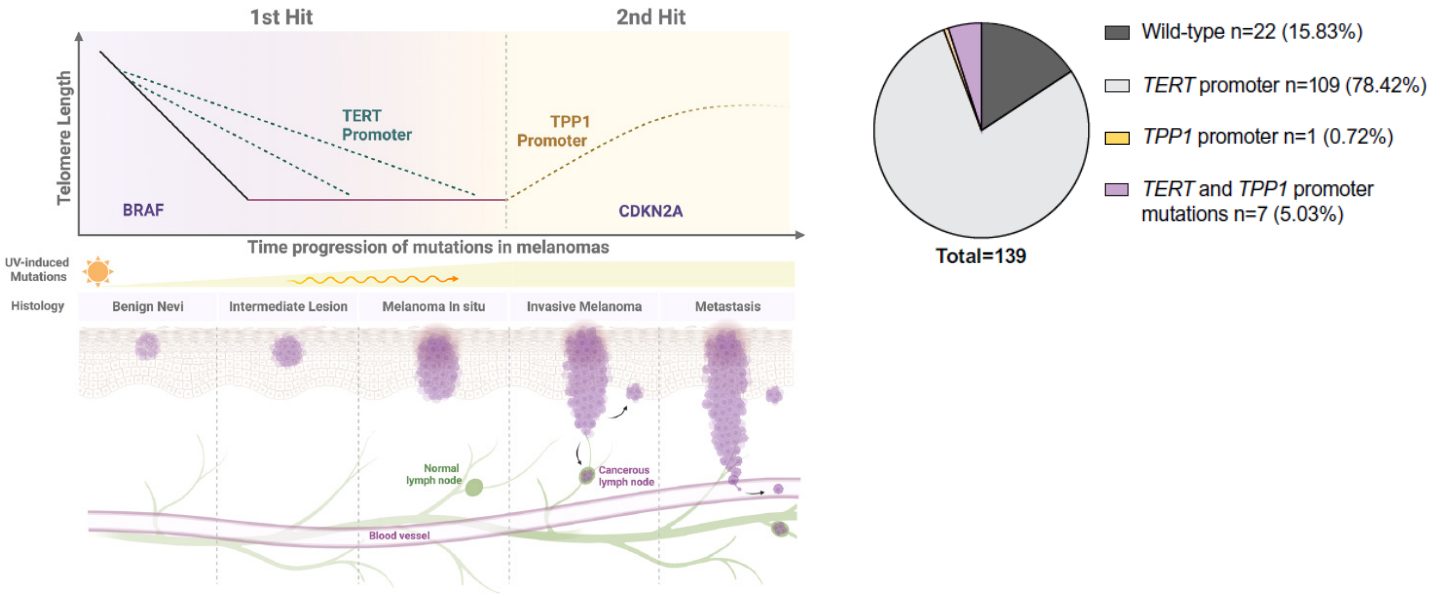


Figure legend. A) Model for melanogenesis in the context of UV-induced mutagenesis. TERT promoter mutations can extend replicative capacity by lengthening the shortest telomeres but do not prevent bulk telomere shortening and crisis (solid purple line). More cell divisions (dotted line) increase the chances of acquiring a 2nd hit prior to crisis. TPP1 promoter variants as a 2nd hit act synergistically with TERT promoter mutations to lengthen bulk telomeres, and may enable telomere maintenance and immortalization. B) Proportion of cutaneous melanomas that had TERT, TPP1, or TERT + TPP1 variants.

More Cool Science

RAD51AP1 regulates ALT-HDR through chromatin-directed homeostasis of TERRA.

Alternative lengthening of telomeres (ALT) is a homology-directed repair (HDR) mechanism of telomere elongation that controls proliferation in subsets of aggressive cancer. Recent studies have revealed that telomere repeat-containing RNA (TERRA) promotes ALT-associated HDR (ALT-HDR). Here, we report that RAD51AP1, a crucial ALT factor, interacts with TERRA and utilizes it to generate D- and R-loop HR intermediates. We also show that RAD51AP1 binds to and might stabilize TERRA-containing R-loops as RAD51AP1 depletion reduces R-loop formation at telomere DNA breaks. Proteomic analyses uncover a role for RAD51AP1-mediated TERRA R-loop homeostasis in a mechanism of chromatin-directed suppression of TERRA and prevention of transcription-replication collisions (TRCs) during ALT-HDR. Intriguingly, we find that both TERRA binding and this non-canonical function of RAD51AP1 require its intrinsic SUMO-SIM regulatory axis. These findings provide insights into the multi-contextual functions of RAD51AP1 within the ALT mechanism and regulation of TERRA.

Impact: Understanding the molecule mediators of ALT activity will help develop new targets for treating ALT tumors, which represent 15% of cancers and include highly aggressive glioblastoma cancers.

Funding: R.J.O., R01CA207209, R01CA262316, R37CA263622, and American Cancer Society #RSG-18-038-01-DMC; S.C.W., S10OD019973; P.S., R35CA241801 and RO1ES007061; A.I.N., GM094231; H.Z, U01CA260851. M.M., French National League against Cancer. K.M.M., Cancer Prevention and Research Institute of Texas #RP220330.

Source: CKaminski N, Wondisford AR, Kwon Y, Lynskey ML, Bhargava R, Barroso-González J, García-Expósito L, He B, Xu M, Mellacheruvu D, Watkins SC, Modesti M, Miller KM, Nesvizhskii AI, Zhang H, Sung P, O'Sullivan RJ. "RAD51AP1 regulates ALT-HDR through chromatin-directed homeostasis of TERRA." *Mol Cell*. 2022, **82**(21):4001-17.

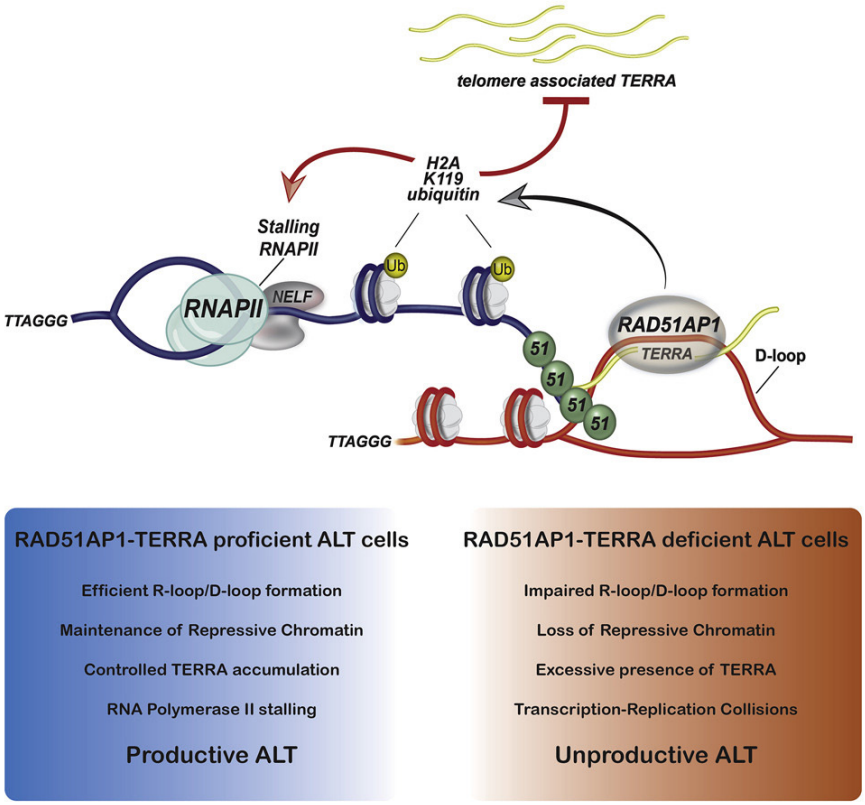
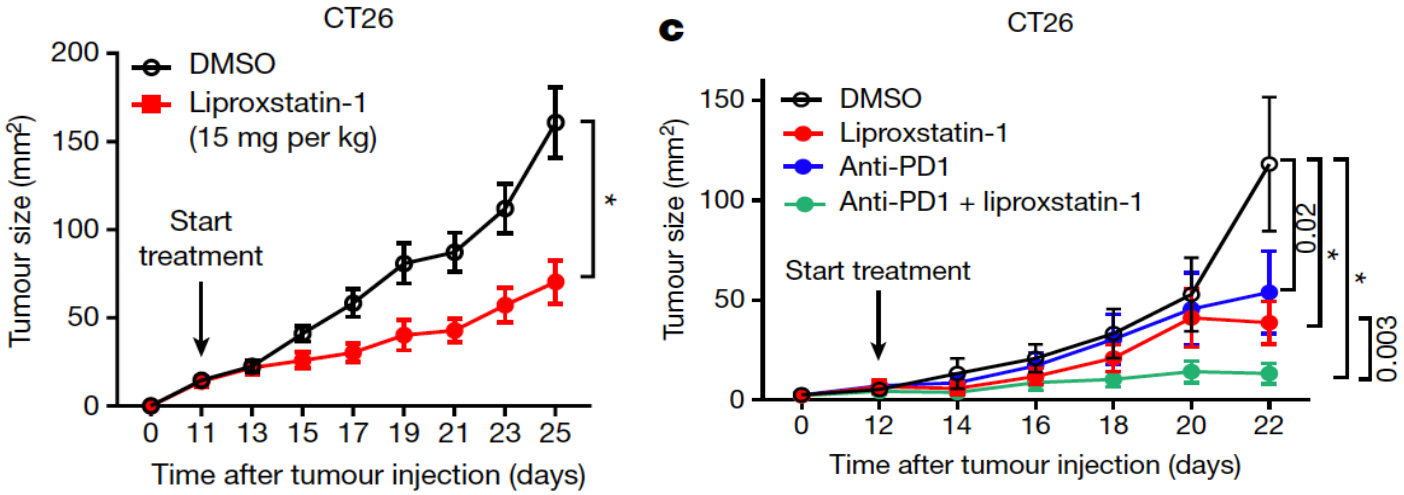


Figure Legend: Shows the cooperative roles of RAD51AP1 and TERRA in generating HR intermediates during ALT-HDR. Proteomic analyses uncover that RAD51AP1 binding of R-loops might serve to maintain chromatin that suppresses TERRA and prevents transcription-replication collisions (TRCs) during ALT-HDR.

More Cool Science

Ferroptosis of tumour neutrophils causes immune suppression in cancer.

Ferroptosis is a non-apoptotic form of regulated cell death that is triggered by the discoordination of regulatory redox mechanisms culminating in massive peroxidation of polyunsaturated phospholipids. Ferroptosis inducers have shown considerable effectiveness in killing tumour cells in vitro, yet there has been no obvious success in experimental animal models, with the notable exception of immunodeficient mice1,2. This suggests that the effect of ferroptosis on immune cells remains poorly understood. Pathologically activated neutrophils (PMNs), termed myeloid-derived suppressor cells (PMN-MDSCs), are major negative regulators of anti-tumour immunity3-5. Here we found that PMN-MDSCs in the tumour microenvironment spontaneously die by ferroptosis. Although decreasing the presence of PMN-MDSCs, ferroptosis induces the release of oxygenated lipids and limits the activity of human and mouse T cells. In immunocompetent mice, genetic and pharmacological inhibition of ferroptosis abrogates suppressive activity of PMN-MDSCs, reduces tumour progression and synergizes with immune checkpoint blockade to suppress the tumour growth. By contrast, induction of ferroptosis in immunocompetent mice promotes tumour growth. Thus, ferroptosis is a unique and targetable immunosuppressive mechanism of PMN-MDSCs in the tumour microenvironment that can be pharmacologically modulated to limit tumour progression.



Impact: Ferroptosis is a unique and targetable immunosuppressive mechanism of myeloid-derived suppressor cells in the tumor microenvironment that can be pharmacologically modulated to limit tumor progression.

Funding: National Institute of Health [R01 CA165065], [T32 DK007780], [P30 CA016520], [U01 AI156924], [R01 CA266342], [R01 CA243142], [2T32DK007780-21], [P30 DK046200], [R01 DK108722], [R01-CA-229803-01], [AI156924], [P30 CA010815,

Source: Kim R, Hashimoto A, Markosyan N, Tyurin VA, Tyurina YY, Kar G, Fu S, Sehgal M, Garcia-Gerique L, Kossenkov A, Gebregziabher BA, Tobias JW, Hicks K, Halpin RA, Cvetesic N, Deng H, Donthireddy L, Greenberg A, Nam B, Vonderheide RH, Nefedova Y, Kagan VE, Gabrilovich DI. "Ferroptosis of tumour neutrophils causes immune suppression in cancer." *Nature*. 2022, **612**(7939): 338-46.

Figure legend. Antitumor effect of ferroptosis inhibition. (Left panel) CT26 tumor growth in mice with DMSO and 15mg per kg liproxstatin-1 treatment (left panel), or with and without anti-PD1 (right panel). Data are mean±s.e.m. Statistical analysis was performed using two-way ANOVA with correction for multiple comparisons.

More Cool Science

DNA damage and somatic mutations in mammalian cells after irradiation with a nail polish dryer.

Ultraviolet A light is commonly emitted by UV-nail polish dryers with recent reports suggesting that long-term use may increase the risk for developing skin cancer. However, no experimental evaluation has been conducted to reveal the effect of radiation emitted by UV-nail polish dryers on mammalian cells. Here, we show that irradiation by a UV-nail polish dryer causes high levels of reactive oxygen species, consistent with 8-oxo-7,8-dihydroguanine damage and mitochondrial dysfunction. Analysis of somatic mutations reveals a dose-dependent increase of C:G>A:T substitutions in irradiated samples with mutagenic patterns similar to mutational signatures previously attributed to reactive oxygen species. In summary, this study demonstrates that radiation emitted by UV-nail polish dryers can both damage DNA and permanently engrave mutations on the genomes of primary mouse embryonic fibroblasts, human foreskin fibroblasts, and human epidermal keratinocytes.

Impact: This study demonstrates that UVA radiation emitted by nail polish dryers can both damage DNA and permanently engrave mutations on the genomes of mammalian cells and is consistent with driver mutations in melanoma. Caution is recommended for a large population using this apparatus.

Funding: National Institute of Health [R01 CA269919], [R01 ES030993], [R01 ES032547], [R35 ES031638]

Source: Zhivagui M, Hoda A, Valenzuela N, Yeh YY, Dai J, He Y, Nandi SP, Otlu B, Van Houten B, Alexandrov LB. "DNA damage and somatic mutations in mammalian cells after irradiation with a nail polish dryer." *Nat Commun*. 2023, **14**(1):276

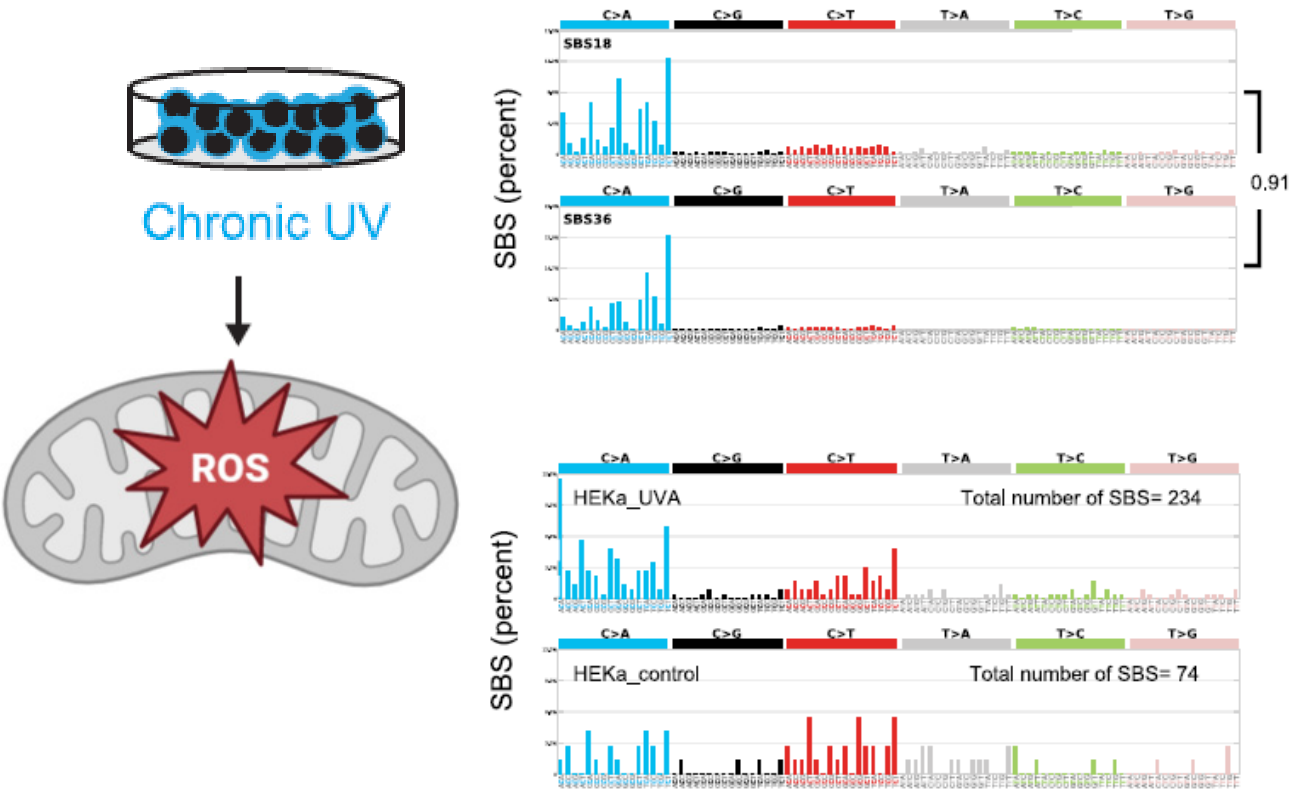
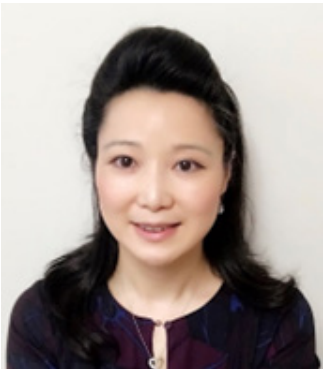


*Figure legend: Chronic UVA irradiation causes mitochondrial dysfunction, increased fluxes of reactive oxygen species and subsequent increases in nuclear somatic mutations. UVA-induced damage cause single base substitutions (SBS) are consistent with two mutational signatures for reactive oxygen species. ***q-value < 0.001; and ****q-value < 0.0001.*

Faculty and Staff News



Jian Yu, PhD

Lin Zhang, PhD

Farewell to Drs. Yu & Zhang

Jian Yu, PhD and Lin Zhang, PhD came to the University of Pittsburgh (UPCI – now, UPMC Hillman Cancer Center) in 2002. Dr. Zhang joined the Department of Pharmacology & Chemical Biology as an assistant professor. He was also a member of the Medical Scientist Training Program (MSTP) and the Physician Scientist Training Program (PSTP) of the University of Pittsburgh/Carnegie Mellon University. Dr. Yu joined the Department of Pathology as an assistant professor and part of the Experimental Pathology Division. She was a preceptor of the Interdisciplinary Biomedical Graduate Training (IBGT) Program at the University of Pittsburgh School of Medicine and of the Medical Scientist Training Program (MSTP) at the University of Pittsburgh. Both Drs. Yu and Zhang were promoted to Professor with Tenure during their 21 years at the University of Pittsburgh and UPMC Hillman Cancer Center and were members of the Genome Stability Program.

Dr. Yu's early work defined p53 transcriptome in colon cancer cells and discovered PUMA as a novel Bcl-2 family member and major mediator of DNA damage and stress-induced mitochondrial damage and death through p53-dependent and independent mechanisms. Her research focused on how programmed cell death used by a multi-cellular organism to selectively remove cells that are no longer needed, damaged, or dangerous. Deregulated cell death leads to a wide variety of human diseases such as chronic inflammation and cancer by compromising tissue repair, remodeling, and regeneration. Focusing on driver-dependent regulation of cell death in the intestinal epithelium, the fastest renewing adult tissue with constant interaction with the immune system and microorganisms. Intestinal renewal, repair, and regeneration are regulated by stem cell intrinsic programs and complex environmental cues to balance cell proliferation, cell death, lineage specification, and with (DNA) damage removal. Dr. Yu and her lab produced over 90 peer-reviewed publications while at Pitt. She taught 10 university courses and served on 12 comprehensive exam and thesis committees. She was a member and Chair of several university committees including UPMC Hillman Cancer Center's (UPCI) Faculty Search Committee. She was also an inaugural member of the UPMC Hillman Cancer Center Women's Initiatives Task Force.

Dr. Zhang's immediate goal of his research is to understand how anticancer drugs kill cancer cells, and more importantly, why they fail so often. Focusing on proteins that control discrete steps of programmed cell death, including PUMA, Bax, BID, SMAC, and Mcl-1, which is directly or indirectly regulated by the most frequently mutated or altered tumor suppressors and oncogenes such as p53, APC, c-Myc and KRAS. Through analyses of these cell death regulators and their associated protein networks under the influence of oncogenic mutations and other alterations, we try to gain a deep understanding of how cell death is initiated and executed in colon cancer cells, why some colon cancer cells are not sensitive to anticancer drugs, and what can be done to restore their sensitivity. The long-term goal is to develop novel strategies and agents to improve colon cancer therapy and prevention. Dr. Zhang and his lab produced over 90 peer-reviewed publications while at Pitt. He taught 10 university courses and served on 29 dissertation committees. He was a member or Chair of several university committees including being Chair of the Hillman Annual Retreat Committee for several years.

Drs. Yu and Zhang along with several of their lab members recently relocated to USC Keck School of Medicine and Norris Comprehensive Cancer Center in Los Angeles, CA.

Ryan Barnes, PhD

Good luck to Ryan in his new position

Ryan Barnes has accepted a tenure track assistant professor position at the University of Kansas Medical Center in the Department of Cancer Biology within the Cancer Center. The Barnes Lab will open this August, and will focus on studying the impact of environmental and physiologic DNA damage on DNA replication and genome stability. Specifically, the lab will study ultraviolet radiation (UVA/UVB) and the oxidative stress it causes in human cells, and this work is funded by his K99/R00 from the NIEHS. The Cancer Center at KUMC was recently designated a Comprehensive Cancer Center by the NCI, making it the only one in the state of Kansas.

Thank you for all the Genome Stability Program members who participated

Congratulations to all GSP members who participated in the inaugural Rush to Crush Cancer events, including Chris Bakkenist, who completed the 60-mile ride, others who completed the 15-mile ride, and those who volunteered for the event! Sam Sanford, Shikar Uttam, Elise Fouquerel, Mariarosaria De Rosa and Ben Nacev completed the 15 mile race, pictured left.

Junior Faculty and Postdoctoral Fellowships

Postdoctoral Fellowships are available through the Hillman Postdoctoral Fellows for Innovative Cancer Research

https://hillmanresearch.upmc.edu/research/hillman-fellows/postdoctoral/

All strong candidates are urged to find a **potential laboratory** of interest and apply.

Closing date is October 31, 2023.

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.

Located in the City of Pittsburgh (routinely ranked as one of the top most livable and affordable U.S. cities), UPMC Hillman (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 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. 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.

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/