UHN Princess Margaret Cancer Centre

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Poster Presentations Session #1

WEDNESDAY APRIL 29th , 2026
2:00-3:30 PM
PGRL Gallery

(updated April 28, 2026)

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Functional modulation of p53 via combinatorial modifications
Authors and institutional affiliation: Dan Lu, Harvard Medical School, Boston, USA
Email of submitting/first author: dan_lu@hms.harvard.edu
Training program first author is enrolled in: Senior Postdoc/Visiting Scientist (I am a senior postdoc equivalent position although my official title was re-defined in order to help me maintain visa status in the USA following changes in federal regulation in 2025)
Year of training: 8 (8th year equivalent of a postdoc)
Abstract: The tumor suppressor p53 integrates diverse cellular stresses to elicit distinct transcriptional and cell fate responses. A central but unresolved question is how p53 discriminates among different upstream signals to generate specific downstream outputs. One mechanism to encode the signaling logic is thought to occur via differential post-translational modifications (PTMs) of p53; however, technical limitations have previously prevented comprehensive characterization of *entire* modification patterns on *individual* p53 molecules. Here, we applied a novel intact-protein mass spectrometry approach (I2MS) that circumvents enzymatic fragmentation, enabling direct detection of overall PTM patterns on single p53 to decipher its modification form (modform). Using I2MS, we resolved distinct p53 PTM signatures induced by different activation modes, including DNA damage, MDM2 inhibition (Nutlin) and quantified the distribution of all p53 modforms. We found specific acetylation and phosphorylation sites that were uniquely enriched by different stressors. Guided by these data, we generated combinatorial p53 PTM-mimetic mutants to interrogate how specific modification patterns regulate transcriptional output. We demonstrated that defined PTM combinations selectively activate distinct subsets of p53 target genes, resulting in divergent cell fate decisions such as cell cycle arrest versus apoptosis. Leveraging this mechanistic insight, we developed a therapeutic strategy using self-amplifying RNA (saRNA) encoding selected p53 PTM-mimetic variants. Delivery of these saRNAs enabled single copies of p53 PTM-mimetic RNA to be rapidly amplified within any cells with initial uptake of the saRNA, therefore p53 protein levels to also accumulate which caused selective killing across multiple cancer cell lines in vitro. In vivo mice tumor studies are currently underway with the results imminent. Collectively, this work establishes PTM patterning as a critical determinant of p53 signaling and gene regulation specificity, as well as introducing tunable RNA-based approaches as a promising p53 therapeutic avenue; opening new directions for fundamental understanding and targeted manipulation of the p53 network.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Designed Ankyrin Repeat Proteins as a novel tool to reactivate p53 in cancer
Authors and institutional affiliation: Philipp Münick, Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe University Frankfurt Dimitrios-Ilias Balourdas, Institute of Pharmaceutical Chemistry, Goethe University Frankfurt Julianne S. Funk, Institute of Molecular Oncology, Universities of Giessen and Marburg Lung Center (UGMLC) Büşra Yüksel, Institute of Biophysical Chemistry, Goethe University Frankfurt and IMPRS on Cellular Biophysics Danai Mavridi, Institute of Pharmaceutical Chemistry, Goethe University Frankfurt Justin Heftel, Institute of Biophysical Chemistry, Goethe University Frankfurt and IMPRS on Cellular Biophysics Andreas Plückthun, Department of Biochemistry, University of Zurich Thorsten Stiewe, Institute of Molecular Oncology, Universities of Giessen and Marburg Lung Center (UGMLC) Baki Akgül, Institute of Virology, University Hospital Cologne Andreas C. Joerger, Institute of Pharmaceutical Chemistry, Goethe University Frankfurt Volker Dötsch, Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe University Frankfurt
Email of submitting/first author: muenick@bpc.uni-frankfurt.de
Training program first author is enrolled in: Postdoctoral Fellow at the Institute of Biophysical Chemistry and the Center for Biomolecular Magnetic Resonance at Goethe University Frankfurt
Year of training: First year postdoctoral fellow

Abstract:

The tumor suppressor p53 is inactivated in over 50% of human cancers, due to mutations in its DNA-binding domain (DBD). Some of the mutations do not only inactivate p53 but destabilize the DBD causing conformational changes or unfolding, exposing aggregation-prone regions. These mutants adopt the wild-type conformation at lower temperatures, but get destabilized at higher ones resulting in partial or complete unfolding and loss of function.

Another inactivation mechanism involves high-risk human papillomavirus (HPV) strains, responsible for ~4.5% of cancers worldwide. The viral E6 protein forms a complex with E6AP ligase, binding the p53 DBD and inducing ubiquitin-dependent degradation. Blocking this interaction could reactivate p53, preventing oncogenesis.

In order to reactivate p53 in cancer, we selected Designed Ankyrin Repeat Proteins (DARPins) against the wild-type and mutant p53 DBD. Characterization of the selected DARPins revealed that DARPin C10 recognizes the same epitope as the HPV-E6 protein, thereby blocking the HPV-E6 induced degradation of p53 leading to transcriptional reactivation of p53 and induction of apoptosis in HPV-positive cells. Furthermore, DARPin C10 binds to certain temperature-sensitive p53 cancer mutants, thereby stabilizing them and restoring their transcriptional activity. By using rational design, we significantly improved the affinity of C10 towards the p53 DBD and thereby increased its potency as a reactivator of p53. The application of a stabilizing DARPin represents a novel therapeutic strategy for cancers caused by high-risk HPV strains or harboring p53 mutations.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:

p53 regulates intracellular iron levels and enhances phagocytic clearance of bacteria and tumor cells in macrophages

Authors and institutional affiliation:

Alvin Guo, Duke-NUS Medical School
Yoko Itahana, Duke-NUS Medical School
Sabhashina Radha Krishnan, Duke-NUS Medical School
Calista, Tsinghua University
Agne Juozulynaite, Duke-Kunshan University
Rati Khaing Hnin, Republic Polytechnic
Belicia Sok Yee Chan, Republic Polytechnic
Koji Itahana, Duke-NUS Medical School

Email of submitting/first author:

alvin.guo@duke-nus.edu.sg

Training program first author is enrolled in:

Postdoctoral training

Year of training:

11 years

Abstract:

Immune regulation is an emerging non-canonical function of p53. Recent studies have highlighted the essential role of p53 in macrophages for anti-pathogenic and anti-tumor immunity. However, the underlying mechanisms are not fully understood. Here, transcriptomic screening and pathway analysis revealed a p53-mediated regulation of the iron uptake and transport pathway. This led us to discover a novel p53 target gene, *SLC40A1*. Activation of p53 through MDM2 inhibitor RG7388 induced SLC40A1 mRNA and protein expression in a p53-dependent manner. *SLC40A1* encodes ferroportin (FPN), an iron exporter. This prompted us to quantify intracellular iron levels, and we found that activation of p53 reduced intracellular iron levels in wild-type macrophages, but not in p53-knockout macrophages. Depletion of either p53 or FPN resulted in the accumulation of intracellular iron. These data indicated that the p53-FPN axis controls iron export in macrophages. To assess the functional significance of the p53-FPN iron export pathway, we focused on phagocytosis, an important process for macrophages to engulf and kill pathogens and cancer cells. To evaluate phagocytosis, macrophages were co-cultured with pHrodo *E. coli* bioparticles or Saos-2 cancer cells. We found that the loss of either p53 or FPN significantly impaired the phagocytic uptake of both bioparticles and cancer cells. Similarly, iron loading suppressed the phagocytic activity. These data suggested that the p53-FPN iron export pathway promotes bacterial or tumor clearance. To test this *in vivo*, luciferase-expressing *E. coli* were injected intraperitoneally into mice. Bacteria clearance was significantly faster in wild-type mice than in p53-knockout mice. Currently, the evaluation of tumor clearance *in vivo* is ongoing. Taken together, our findings identify the p53-FPN iron export pathway as a critical mechanism regulating phagocytosis and contributing to anti-bacterial and anti-tumor responses in macrophages.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:
Authors and institutional affiliation: David Hoyos, Memorial Sloan Kettering Cancer Center Arnold J. Levine, Institute for Advanced Study Benjamin D. Greenbaum, Memorial Sloan Kettering Cancer Center
Email of submitting/first author: David Hoyos
Training program first author is enrolled in: Ph.D. program in Computational Biology
Year of training: Year 3 of Graduate Program
Abstract: Purpose: Pathogenic germline *TP53* alterations drive Li-Fraumeni Syndrome (LFS), an autosomal dominant cancer predisposition syndrome. However, it is not appreciated how the various mutant *TP53* alleles relate to fitness advantages for cancer cells in LFS in terms of oncogenic advantage and immune vulnerability. Materials and Methods: We derive a unified theoretical “free fitness” framework of the rate-limiting processes underlying the fitness advantages of *TP53* mutations inspired from statistical physics. We determine LINE-1 activity in The Cancer Genome Atlas (TCGA) using the TotalReCall algorithm developed in the Greenbaum laboratory across approximately 4,000 short-read whole genome sequencing samples. We conduct a spatial analysis of LFS breast cancers (N=40) and do matched DNA-based T cell receptor (TCR) sequencing in a subset (N=24) LFS breast cancer (LFSBC) samples from tumor-infiltrating lymphocytes. Results: We demonstrate that our free fitness theory predicts the strength of mutant p53-associated cancer phenotypes such as oncogenic LINE-1 retrotransposon activity and immune surveillance in LFS cancers. We show that LINE-1 RNA abundance and somatic retrotransposition activity are predictable from our free fitness theory, discovering that

LINE-1 is active in LFS cancers present in TCGA. In addition, we find that the immune system is actively surveilling LFSBC as early as Ductal Carcinoma *In Situ* (DCIS) via spatial proximity to cancer cells. Notably, upon sequencing tumor-specific infiltrating lymphocytes, we find that there are shared TCR motifs in LFSBC, indicating a shared immune target across individuals with LFS.

Conclusions: We demonstrate that multiple features of LFS cancers are predicted from the free fitness quantities associated with the germline *TP53* variant, including oncogenic LINE-1 retrotransposon activity and immune surveillance. This data suggests that drugs such as Reverse Transcriptase Inhibitors and immunotherapy may be productive in treating LFS cancers.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Mutant p53 shapes an alternative splicing landscape in cancer - research into the mechanism and therapeutic possibilities

Authors and institutional affiliation: Weronika Wojtyś 1, Akanksha Jaiswair 1, Adrianna Porębska 2, Wojciech Kaźmierczak 3, Tomasz Olesiński 3, Dawid Walerych 1, Magdalena Oroń 1

1. Mossakowski Medical Research Institute PAS, Warsaw, Poland
2. Warsaw Medical University, Warsaw, Poland
3. National Cancer Institute, Warsaw, Poland

Email of submitting/first author: moron@imdik.pan.pl

Training program first author is enrolled in: post-doctoral program

Year of training: *7 year after PhD*

Abstract: Gain-of-function (GOF) p53 mutants contribute to oncogenesis mainly through interactions with transcription factors and regulation of gene expression. We found that mutant p53 also affects alternative splicing, beyond changes in splicing factor expression. This project investigated the mechanisms of splicing regulation by mutant p53 in common human cancers and explored the altered splicing landscape for therapeutic targets.

We performed differential gene expression and splicing analyses of RNA-seq data from colon, lung, pancreatic, and head and neck cancer cell lines with mutant p53 knockout or control. Although many splicing-related genes were deregulated, no single gene was common across most of studied cell lines. Using proximity ligation assays, we showed that mutant p53 promotes interaction between SRPK1 kinase and SR splicing factors (SRSF1, SRSF3, SRSF6), increasing their phosphorylation and activity. RNA immunoprecipitation demonstrated altered binding of these SR proteins to mRNAs of previously validated target genes, in the presence of mutant p53. Splicing of FYN, USO1, CEP170, and MFSD8 was affected by mutant p53. Overexpression of alternative isoforms of these genes altered proliferation, colony formation, migration, and invasiveness of cancer cells. Further, we explored therapeutic opportunities. Combining an SRPK1 inhibitor with a p53 reactivator significantly reduced cancer cell viability, with further reduction upon addition of pathway-specific

inhibitors. Effectiveness of drug combination was confirmed on patients derived organoids and *in vivo* in Zebrafish model.

In summary, GOF p53 mutants influence the alternative splicing profile in lung, colon, pancreatic, and head and neck tumors through interactions with the splicing factors SRSF1, SRSF3, and SRSF6, as well as the kinase SRPK1. The resulting isoforms of proteins, such as FYN, USO1, CEP170, and MFSD8, may influence oncogenesis increasing proliferation, migration and invasion potential of cancer cells. The combination of SRPK1 kinase inhibitors with p53 reactivators is a promising new therapeutic strategy worthy of further investigation.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Understanding how the proteostasis network shapes the mutational landscape of *TP53* in cancer

Authors and institutional affiliation: **Paulina Rios**[1,2,3], Diego Detrés[1,2,3], Samuel Gould[1,2,3], Ondine Atwa[1,2,3], Charles Whittaker[2], Matthew D. Shoulders[2,4,5], Francisco J. Sánchez-Rivera[1,2,3]

1. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA
2. David H. Koch for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, MA, USA
3. MIT Center for Precision Cancer Medicine, Massachusetts Institute of Technology, Cambridge, MA, USA
4. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA
5. Broad Institute of MIT and Harvard, Cambridge, MA, USA

Email of submitting/first author: paulinar@mit.edu

Training program first author is enrolled in: Doctorate training in Biology at MIT

Year of training: 3

Abstract: The proteostasis network (PN) regulates the synthesis, folding, and degradation of native proteins to ensure cellular homeostasis. Mutations can lead to the production of aberrant proteins with a distinct reliance on the PN for functional stabilization. This phenomenon is commonly observed in cancer, suggesting that the PN could influence the oncogenic mutational tolerance of human tumors. However, the molecular mechanisms by which the PN shapes the mutational tolerance and aberrant protein landscape in cancer remain poorly understood. Here, we integrated high-throughput base editing (BE) with chemical modulation of the PN to identify oncogenic proteins that exhibit PN-dependent mutational tolerance. Among our candidates, we identified specific mutant p53 proteins that exhibit distinct PN-dependent fitness phenotypes in human cancer cells. To more broadly examine whether the non-random distribution of *TP53* missense mutations in human cancer may reflect positive selection for unstable mutant proteins with increased chaperone dependence, we conducted a BE tiling mutagenesis screen across the entire *TP53* gene in human cancer cells. Pairing this method with chemical modulation of the PN and MDM2 allowed us to identify functionally-distinct mutant p53 proteins that may rely on PN-mediated structural stabilization and folding for their cellular maintenance. To investigate this further, we developed a computational pipeline that integrates high-throughput mutagenesis data with thermodynamic stability predictions to systematically model the biophysical impact of missense mutations on protein stability. Using this pipeline, we found that concomitant inhibition of the PN and MDM2 selects for *TP53* mutations that destabilize the protein. Altogether, these results suggest that the PN shapes the cancer mutational landscape by

promoting the folding and stabilization of structurally aberrant proteins, including those harboring destabilizing mutations.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Activating p53^{Y220C} with a Mutant-Specific Small Molecule
Authors and institutional affiliation: Xijun Zhu, Stanford University Woong Sub Byun, Stanford University Dominika Ewa Pieńkowska, University of Bonn Kha The Nguyen, Stanford University Jan Gerhartz, University of Bonn Qixiang Geng, Stanford University Tian Qiu, Stanford University Jianing Zhong, Stanford University Zixuan Jiang, Stanford University Mengxiong Wang, Stanford University Roman C. Sarott, Stanford University Stephen M. Hinshaw, Stanford University Tinghu Zhang, Stanford University Laura D. Attardi, Stanford University School of Medicine Radosław P. Nowak, University of Bonn Nathanael S. Gray, Stanford University
Email of submitting/first author: s49dpien@uni-bonn.de
Training program first author is enrolled in: University of Bonn Graduate Doctoral Program
Year of training: 2

Abstract:

Purpose

Cancer is a complex and highly dreaded disease characterized by the uncontrolled growth and spread of abnormal cells, often driven by genetic mutations. The most frequently mutated gene in human cancers is the tumor suppressor gene *TP53*, which encodes the transcription factor and tumor suppressor protein p53. Tumor suppressor functions of wild-type p53 are lost in many mutant p53 proteins, leading to uncontrolled cellular proliferation. Currently, there are no approved therapies that directly target mutant p53 and restore its wild-type activity. One such mutant, $p53^{Y220C}$,detected in approximately 100,000 patients per year, has emerged as a tractable target for small-molecule “correctors.”

Materials & Methods

We synthesized and characterized a small-molecule chemical inducer of proximity, termed TRanscriptional Activator of p53 (TRAP-1), designed to engage mutant $p53^{Y220C}$ and the transcriptional co-regulator BRD4 in a ternary complex. Biochemical, cellular, and structural approaches were employed to evaluate ternary complex formation, transcriptional activation, and downstream cellular outcomes. Structural insights were obtained using X-ray crystallography and cryo-electron microscopy (cryo-EM).

Results

TRAP-1 formed a stable ternary complex with $p53^{Y220C}$ and BRD4, leading to potent activation of mutant p53. Treatment of $p53^{Y220C}$-expressing cell lines with TRAP-1 resulted in rapid upregulation of p21 and other p53 target genes and induced cellular senescence and apoptosis. Negative control compounds that are unable to form a ternary complex lack these activities, demonstrating the necessity of chemically induced proximity for the observed pharmacology.Crystal structure of mutant p53 protein bound with B-1 linker (functionalised p53 binder) was solved at reslution of 2.63 Å and cryo-EM analysis resolved the structure of the $p53^{Y220C}$ tetramer bound to DNA at 3.2 Å resolution, providing mechanistic insight into restored transcriptional activity.

Conclusions

This study establishes TRAPs as a novel class of bifunctional small molecules capable of restoring the transcriptional activity of mutant p53. The approach described in this work provides mechanistic detail on how chemically induced proximity can be leveraged to restore p53 function and contributes to the development of new mutant specific p53-directed therapeutics.

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: p53, citrullination and ELF2 in the control of promoter selectivity

Authors and institutional affiliation:

Giulia Pantella[1,2], Alexandra Indeglia[1,3], Andrea Valdespino[1,4], Hsin-Yao Tang[1,5], Annaliese faustino[5], Maureen E. Murphy[1]

[1]Program in Molecular and Cellular Oncogenesis, The Wistar Institute, Philadelphia PA 19104, USA
[2]Graduate Group in Cellular and Molecular Biology, University of Bologna, Bologna Italy
[3]Graduate Group in Biochemistry and Molecular Biophysics, the University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[4]Cell and Molecular Biology Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[5]Proteomics and Metabolomics Shared Resource, The Wistar Institute, Philadelphia, PA 19104, USA

Email of submitting/first author: gpantella@wistar.org

Training program first author is enrolled in:

Cellular and Molecular Biology PhD program, University of Bologna, Bologna Italy

Year of training: Third year

Abstract: TP53 encodes p53, a central tumor suppressor and transcription factor whose activity is finely regulated by multiple post-translational modifications (PTMs). While these modifications are known to influence p53 stability and activity, the mechanisms by which they control promoter selectivity remain to be clarified. Citrullination is a PTM that consists of the irreversible conversion of arginine to citrulline, thereby altering protein structure and function. Recently, we described that peptidyl arginine deiminase type 4 (PADI4), a direct transcriptional target of p53, catalyzes citrullination of the p53 C-terminal domain, redirecting p53 binding from canonical target promoters toward genomic regions enriched for ETS transcription factor motifs. Moreover, citrullinated p53 was found to be specifically associated with the ETS family transcription factor ELF2. To elucidate the molecular basis of this interaction, we used recombinant p53 and ELF2 in the presence or absence of PADI4 and stabilized their interaction with an EDC cross-linking agent, followed by mass spectrometry to map the citrullinated p53–ELF2 interaction domains. To extend our findings and assess the functional relevance of p53, PADI4, and ELF2, we transiently silenced ELF2 or PADI4 in U2OS cells with Nutlin-induced p53, then analyzed cell cycle distribution by propidium iodide (PI) staining and flow cytometry. Interestingly, silencing of either ELF2 or PADI4 independently blocked G1 arrest without altering p21 expression, suggesting that ELF2 and PADI4 function within the same p53-dependent pathway and regulate G1 arrest through a mechanism independent of the canonical p53–p21 axis. To further investigate how ELF2 influences the DNA binding and transcriptional activity of citrullinated p53, we are performing ChIP-sequencing and RNA-sequencing in U2OS cells in the presence or absence of ELF2 and PADI4. Together, these experiments support a model in which citrullination promotes a functional interaction between p53 and ELF2, redirecting p53 to a distinct subset of target genes, and identify ELF2 as a key mediator of citrullinated p53 function.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Targeted Delivery of DARPins via LNPs for Treatment of p53 Related Cancers
Authors and institutional affiliation: Justin Heftel, Institute of Biophysical Chemistry, Goethe University Frankfurt and IMPRS for Cellular Biophysics Tümay Telatar, Institute of Virology, University Hospital Cologne Philipp Münick, Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe University Frankfurt Büşra Yüksel, Institute of Biophysical Chemistry, Goethe University Frankfurt and IMPRS for Cellular Biophysics Danai Mavridi, Institute of Pharmaceutical Chemistry, Goethe University Frankfurt Martin Hufbauer, Institute of Virology, University Hospital Cologne Baki Akgül, Institute of Virology, University Hospital Cologne Andreas C. Joerger, Institute of Pharmaceutical Chemistry, Goethe University Frankfurt Volker Dötsch, Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe University Frankfurt
Email of submitting/first author: heftel@em.uni-frankfurt.de
Training program first author is enrolled in: International Max Planck Research School (IMPRS) for Cellular Biophysics PhD Program
Year of training: 2nd year PhD student

Abstract:
Implicated in a range of developmental diseases and cancers, p53 is a critical but challenging target to drug. It is inactivated in the majority of human cancers, largely due to mutations in the DNA-binding domain (DBD), and it can be inactivated by HPV via E6 ligase triggered degradation. Designed Ankyrin Repeat Proteins (DARPins) present a promising therapeutic for p53-related cancers, including our C10 DARPin, which stabilizes temperature sensitive mutants of p53 and inhibits the HPV-induced degradation of p53.
Already proven effective as a vaccine delivery system, mRNA-LNPs are a potential delivery mechanism for treating a wide variety of diseases. With this, much work is being done to augment the LNPs to specifically target certain cells.
We are establishing a mRNA-LNP based targeted delivery system to deliver the C10 DARPin and other DARPins to relevant p53-related cancer cells, specifically AML myeloblasts and lung and cervical cancer tumor cells. We demonstrate the effectiveness of LNPs in transfecting target cells for delivery of DARPin mRNA. We also present preliminary data indicating the effectiveness of the C10 DARPin in inhibiting HPV-induced degradation of p53 across several high-risk HPV strains. We plan to present preliminary data investigating selective targeting of mRNA-LNPs to relevant cell types via conjugated targeting moieties, namely DARPins selected for cell surface receptors of target cancer cells.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Predicting the age-of-onset for paediatric cancer in children with germline variants of *TP53* regulators and wild-type *TP53*

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Chen K, University of Toronto
Majeed Grant S, University of Toronto
Laverty B, University of Toronto
Kissoondoyal A, Hospital of Sick Children
Shlien A, University of Toronto
Malkin D, University of Toronto

Email of submitting/first author:
kairen.chen@mail.utoronto.ca

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*
MSc Student in the Institute of Medical Science at the University of Toronto

Year of training: *Example: PGY 3*
2nd Year

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose: Approximately 1-2% of pediatric cancer patients harbor *TP53* pathogenic or likely pathogenic (PLP) germline variants (GV). However, *TP53* function is regulated by other genes, and the collective impact of GVs in genes that regulate *TP53* remains unknown. Our lab previously demonstrated that (epi)genetic events enable more precise tumour onset prediction in *TP53* pathogenic GV carriers; here, we extend this framework to patients having wild-type *TP53* and GVs in genes that regulate *TP53*. In this study, we use germline whole genome sequencing (WGS) data from a high-risk pediatric cancer cohort to develop machine learning (ML) models that predict age-of-onset, thereby enabling the identification of genes that collectively influence age-of-onset in concert with wild-type *TP53* and the quantification of their effects.
Methods: WGS data from the SickKids Cancer Sequencing (KiCS) program's cohort of poor-prognosis childhood cancer patients (n = 333) were analyzed. After grouping germline variants by gene and pathogenicity according to ACMG guidelines, these features were used as input to a random forest, with hyperparameters explored via grid search and evaluated using cross-validation, to classify the age-of-onset.
Results and Conclusion: To understand the genomic contributors underlying these ML predictions of age-of-onset, we examined age-of-onset distributions stratified by individual genes. This revealed shifts that, while not statistically significant at the individual gene level, collectively suggest differences in age-of-onset distributions between carriers and non-carriers. For example, the presence of mutant Gap Junction Beta 2 (GJB2) exhibited a shift toward later age-of-onset compared to non-carriers. GJB2 has been reported to suppress expression of *TP53* and is mutated in 6.9% of all KiCS patients making it the most frequently altered gene regulating *TP53*. These shifts suggest that combinations of GVs in genes that regulate wild-type *TP53* could confer cancer predisposition, potentially informing the consideration of clinical surveillance for such combination carriers.

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Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: *Beyond p53: MDM2 Functions at CpG Islands to Modulate Chromatin and Transcription*

Authors and institutional affiliation:

Paulsohn M-B, University Medical Center Göttingen, Germany
Dickmanns A, University Medical Center Göttingen, Germany
Gerber S, University Medical Center Göttingen, Germany
Prokakis E, University Medical Center Göttingen, Germany
Dobbelstein M, University Medical Center Göttingen, Germany

Email of submitting/first author:

maj-britt.paulsohn@med.uni-goettingen.de

Training program first author is enrolled in:

Molecular Medicine PhD Program of the Georg-August-University Göttingen, Germany

Year of training:

PhD candidate in year 4

Abstract:

Purpose
MDM2 is a key regulator of cell fate, best known for targeting the tumor suppressor p53. However, accumulating evidence indicates that MDM2 also exerts p53-independent functions in chromatin regulation and genome maintenance. Previous studies identified MDM2 as a chromatin-associated factor interacting with Polycomb repressor complexes, but its recruitment mechanisms and functional consequences in p53-proficient cells remain incompletely understood. Here, we aim to further define the chromatin-associated interactome of MDM2 and its impact on epigenetic regulation, transcriptional control, and genomic maintenance.

Materials and Methods
CRISPR-Cas9 genome editing (KO cell lines), sequencing (RNA-seq, ChIP-seq), qRT-PCRs, chromatin immunoprecipitations (ChIP), protein interaction studies (Co-IP), immunoblot analyses

Results
Genome-wide analysis revealed that MDM2 associates with more than 50 % of CpG island in both p53-proficient and p53-deficient human cancer cell lines. This chromatin recruitment is mediated by direct interactions with the CpG-island binding histone demethylases KDM2A and KDM2B, and is further modulated by the Polycomb-associated factor PHF19. MDM2 occupancy correlates with altered histone methylation states, particularly H3K36me2/3, and with transcriptional regulation of defined gene clusters, including HOX genes. These findings suggest a broader role for MDM2 in coordinating chromatin states linked to transcriptional control.

Conclusions
Our data identify a chromatin-based MDM2-KDM2/PHF19 axis that targets CpG islands and potentially integrates epigenetic regulation with p53 signaling. Given the enrichment of transcriptional start sites within the CpG islands, this pathway may represent a global regulator of chromatin plasticity, with implications for DNA replication, repair and cell fate decisions.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: **Elucidating Mechanisms of TP53-mediated Leukemia Immune Escape**

Authors and institutional affiliation: Manuel Enrique Contreras, Massachusetts Institute of Technology; Grace Johnson, Massachusetts Institute of Technology; Jon Acosta, Massachusetts Institute of Technology; Sam Gould, Massachusetts Institute of Technology; Francisco Sánchez-Rivera, Massachusetts Institute of Technology; Michael Hemann, Massachusetts Institute of Technology

Email of submitting/first author: **meca@mit.edu**

Training program first author is enrolled in: **MIT Biology PhD Program**

Year of training: **GY 3**

Abstract: *Suggested format: Purpose, Materials and Methods, Results, Conclusions*

Purpose: The immune system is a potent barrier to malignant cellular transformation, yet many humans develop and unfortunately succumb to cancer. In the context of acute leukemias, such as acute myeloid leukemia (AML) and B-cell acute lymphoblastic leukemia (B-ALL), immunotherapies have been widely used and achieved cures in some cases. Despite this, specific genetic subsets of leukemia have been documented to lead to worse treatment outcomes, and the roles of many of these mutations in immunotherapy resistance remain elusive. Leukemias with mutations in *TP53* are a notable example of a disease subset known to lead to worse treatment outcomes, even when treated with immunotherapy. Thus, we hypothesize that *TP53* mutations promote immune evasion and immune suppression in the leukemia microenvironment, contributing to therapy resistance. **Methods:** We developed a novel *in vivo* screening approach using an immunogenic murine B-ALL model combined with high-throughput base editing to screen thousands of patient-derived mutations to identify immune-evasive genetic variants. This immunogenic B-ALL model was also leveraged to test the role of specific *Trp53* mutations in disease progression under strong immune pressure. **Results:** *Trp53* mutations, among many others, were associated with immune escape in our screen. Certain *Trp53* variants were particularly enriched in leukemia populations retaining antigen expression. Studies of individual *Trp53* mutations showed significantly fewer leukemia-specific T-cells in the bone marrow compared to isogenic controls. Interestingly, mutant *Trp53* escaping populations retained antigen expression, whereas *Trp53* wild type leukemias predominantly escaped through stochastic antigen silencing. **Conclusions:** Together, these findings suggest *Trp53* mutations can alter the response to T-cell based anti-leukemia immunity in our models, contributing to immune escape and therapy failure. Ongoing studies aim to further define the molecular pathways underlying this phenotype and to determine its relevance across additional leukemia contexts and treatment modalities.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:

A stapled peptide inhibitor of MDM2 enables pharmacological activation of p53 in zebrafish

Authors and institutional affiliation:

Pavitra Kannan, Karolinska Institutet
Dilraj Lama, Karolinska Institutet
Sania Kheder, Karolinska Institutet
Martin Krkoska, Karolinska Institutet
Filip Mihalic, Uppsala University
Kim Kobar, Children's Hospital of Eastern Ontario Research Institute / University of Ottawa
Zdenek Andrysik, Masaryk University
Lars Bräutigam, Karolinska Institutet
Susanne Lindström, Karolinska Institutet
Per Jemth, Uppsala University
Jason Berman, Children's Hospital of Eastern Ontario Research Institute / University of Ottawa
David P Lane, Karolinska Institutet

Email of submitting/first author:

pavitra.kannan@ki.se

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Swedish Cancer Society Radiotherapy Fellowship

Year of training: *Example: PGY 3*

Y3

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose
Measuring the activity of full-length p53 is essential for understanding its dysregulation in cancer and non-cancer conditions such as aging and diabetes. Zebrafish (*Danio rerio*) are a powerful model system for studying p53 activity in vivo due to the relative conservation of p53 structure and function between zebrafish and humans. However, because p53 is rapidly degraded under normal conditions, its activity in zebrafish has traditionally been visualized using methods that induce apoptosis or have substantial off-target effects, limiting the ability to study p53-specific responses. Here, we investigated whether pharmacological inhibitors of Mdm2 could be used to selectively activate p53 in zebrafish.

Methods
To assess the binding affinity of small-molecule and stapled peptide Mdm2 inhibitors to zebrafish Mdm2, we used biophysical binding assays and molecular dynamics simulations. The transcriptional activity of p53 was then measured in inhibitor treated zebrafish embryos using real-time quantitative PCR and RNA sequencing. Induction of apoptosis was evaluated using acridine orange staining. To test p53 dependency, assays were performed in embryos carrying wild-type p53, a transcriptionally inactive p53 mutation, or mutant p53.

Results
Small-molecule Mdm2 inhibitors exhibited weak binding to zebrafish Mdm2, likely due to an amino acid variation within the zebrafish Mdm2 binding pocket. In contrast, the stapled peptide exhibited high binding affinity to zebrafish Mdm2 and induced transcriptional activation of p53 target genes in a p53-dependent manner. RNA sequencing revealed upregulation of the p53 signaling pathway and downregulation of DNA replication pathways. Treatment with the stapled peptide did not result in detectable increases in apoptosis in zebrafish embryos.

Conclusions
Despite species-specific differences in p53–Mdm2 binding, we found that a stapled peptide inhibitor of Mdm2 activates p53 in zebrafish without overt toxicity. This tool provides a useful approach to pharmacologically activate p53 and visualize its activity in zebrafish models of cancer and non-cancer canditions.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Inhibition of the epitranscriptomic writer METTL3 is a therapeutic vulnerability specific for p53-deficient Pancreatic Ductal Adenocarcinomas

Authors and institutional affiliation:

Kha The Nguyen, Stanford University
Tianyu Zhao, Stanford University
Seth Tadamichi Kohno, Stanford University
Laura D. Attardi, Stanford University

Email of submitting/first author: tknguyen@stanford.edu

Training program first author is enrolled in: Postdoctoral scholar in Radiation Oncology, School of Medicine, Stanford University

Year of training: *3*

Abstract:

Pancreatic Ductal Adenocarcinoma (PDAC) is a very lethal cancer, with a 5-year survival rate of approximately 13%. In PDAC, *TP53* is one of the most frequently mutated genes, with mutations occurring in ~75% of cases, reflecting the critical role of p53 in suppressing PDAC. Therefore, there is an urgent need to develop effective precision therapies for this deadly disease, especially those targeting *TP53*-mutated PDAC cells without affecting surrounding normal cells. In previous studies from our laboratory, we identified the METTL3 methyltransferase 3, N6-adenosine-methyltransferase complex subunit, as a new p53-interacting protein. The p53-METTL3 interaction is important for co-transcriptionally installing m6A modifications on p53 target gene transcripts, and METTL3 thus amplifies p53 tumor suppressor activity. In contrast, when p53 is inactivated, we discovered a surprising synthetic lethal effect of inhibiting METTL3 in oncogene-expressing fibroblasts. Here, we

propose to test the therapeutic potential of METTL3 inhibition in *p53* null PDACs. We found that knocking down *Mettl3* using shRNAs inhibits growth of *p53* null but not *p53* wild-type mouse PDACs grown as xenografts in mice. Notably, attenuating METTL3 expression suppressed *TP53*-null PDAC tumors in immunocompetent mice but not in immunodeficient mice, suggesting a role for the immune system in tumor growth inhibition. RNA-seq analysis revealed that *Mettl3* knockdown induces signatures reflecting an innate immune response in *TP53*-null PDAC cells. We are currently exploring the underlying connection between *Mettl3* knockdown and a tumor suppressive innate response, and these findings will be presented. Collectively, our findings suggest a new approach for treating *p53* null PDAC tumors, by targeting METTL3 to augment the innate immune response to eliminate cancer cells.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Distinct genomic remodeling in Li-Fraumeni Syndrome breast cancer

Authors and institutional affiliation:
Nabamita Boruah, Ph.D, University of Pennsylvania
Renyta Moses, University of Pennsylvania
Ryan Hausler, University of Pennsylvania
Heena Desai, University of Pennsylvania
Gregory Kelly, University of Pennsylvania
Anupma Nayak MD, University of Pennsylvania
Kara N. Maxwell MD, University of Pennsylvania

Email of submitting/first author: Nabamita.Boruah@Pennmedicine.upenn.edu

Training program first author is enrolled in: Post-doctoral trainee at Hem/Onc Department of Penn Medicine, University of Pennsylvania, Philadelphia in Kara Maxwell, MD's lab.

Year of training: Fourth Year

Abstract:

Purpose: Pathogenic germline variants (PGVs) in *TP53* cause Li-Fraumeni syndrome (LFS), a hereditary multicancer predisposition disorder. Among females with LFS, breast cancer (BC) is the most common malignancy, affecting 80–90% of carriers at an early age and frequently exhibiting HER2 positivity. Notably, LFS-associated BC displays a high burden of short aneuploid amplified segments (SAAS), suggesting distinct *TP53*-driven genomic mechanisms that remain poorly understood.

Materials and Methods: To investigate genotype–phenotype correlations underlying these features, we performed whole-genome sequencing (WGS) and targeted sequencing of invasive ductal carcinoma (IDC), ductal carcinoma in situ (DCIS), and matched adjacent and contralateral normal breast tissues from individuals with LFS. Data were compared with early-onset non-LFS BC and tumors from The Cancer Genome Atlas (TCGA) harboring either wild-type or somatic *TP53* mutations.

Results: Across hormone receptor (HR) subtypes, expected increases in genomic instability were observed in triple-negative versus ER+ non-LFS BC. However, overall instability measures were similar or lower in LFS-BC. In ER+ tumors, microsatellite instability and aneuploidy scores were significantly reduced in LFS-BC compared to non-LFS BC. In contrast, LFS-BC showed increased segmental allelic imbalance with high-level focal amplifications, particularly in HER2+ tumors. These amplified regions were significantly shorter than those in non-LFS BC and frequently encompassed oncogenes such as *ERBB2*, defining a SAAS phenotype. Unlike sporadic *TP53*-mutant BC in TCGA, which exhibited global aneuploidy and homologous recombination deficiency, LFS-BC accumulated focal amplifications. WGS revealed predominant chromosomal instability (CN9) and chromothripsis (CN5) signatures with minimal whole-genome doubling. Amplicon Architect identified extrachromosomal DNA (ecDNA) in 14/19 tumors, including *ERBB2*-containing ecDNA in DCIS and HER2+ IDCs.

Conclusions: LFS-associated breast cancers are defined by focal, high-level amplifications within SAAS and frequent ecDNA formation, revealing a germline *TP53*-driven unique mechanism of oncogene amplification and structural genome evolution.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Nutlin-3a-mediated IL17RB repression leads to sustained p53 responses via modulating cellular level of p21 and Bcl-2

Authors and institutional affiliation:
Alessandro Provvido, Can Sabancelebi, Shiva Shastri, Joseph Sachakov, Olivia Hu, Ivana Vancurova, and Yan Zhu
Department of Biological Sciences, St. John's University, Queens, NY 11439, USA

Email of submitting/first author:
zhuy1@stjohns.edu

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Nova Southeastern University Kiran C. Patel College of Allopathic Medicine

Year of training: *Example: PGY 3*

Medical School Year 2

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose
Dysregulation of the interleukin-17 (IL-17) cytokine family and its receptors is implicated in inflammation and cancer. Interleukin-17 receptor B (IL17RB) has been shown to promote tumorigenesis; however, its regulatory mechanisms remain unclear. This study investigated whether IL17RB is regulated by the tumor suppressor p53 and examined the functional consequences of this regulation in cancer cells.
Materials and Methods
Human cancer cell lines were treated with nutlin-3a to activate p53. IL17RB expression was examined at protein and transcriptional levels using proteasome inhibition, p53 depletion, and p21 dependency assays. IL17RB overexpression and depletion approaches were used to assess the role of IL17RB in nutlin-3a-induced inhibition of cell proliferation and migration. The effects of IL17B ligand treatment on p53 downstream targets, including p21 and Bcl-2, were analyzed. Bioinformatic analyses compared IL17RB expression in tumor and normal tissues and evaluated correlations between IL17RB, p21, and Bcl-2 in tumor samples.

Results
IL17RB was identified as a transcriptional repression target of p53. Nutlin-3a markedly reduced IL17RB expression in multiple cancer cell lines, an effect not rescued by proteasome inhibition. p53 depletion partially restored IL17RB expression, and p53-mediated repression was partially p21-dependent. Functionally, IL17RB depletion enhanced, whereas IL17RB overexpression attenuated, nutlin-3a-induced inhibition of cell proliferation and migration. Consistently, IL17RB depletion increased, while IL17RB overexpression reduced, nutlin-3a-induced p21 expression. IL17B treatment weakened nutlin-3a-mediated p21 induction and Bcl-2 repression. Bioinformatic analyses revealed elevated IL17RB expression in prostate and colon tumors compared with normal tissues, with weak but significant positive correlations with Bcl-2 and negative correlations with p21 in colon tumors.
Conclusions
These findings identify IL17RB as a novel p53 repression target and demonstrate that p53-mediated downregulation of IL17RB contributes to the tumor suppressive function of p53. By limiting IL17RB signaling, p53 sustains its antiproliferative responses, uncovering a functional link between inflammatory signaling and p53-dependent tumor suppression.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Elucidating the role of PADI4 in p53-mediated tumor suppression

Authors and institutional affiliation:
Andrea Valdespino[1,2], Alexandra Indeglia[1,3], Giulia Pantella[1], Anneliese Faustino[1], Hsin-Yao Tang[1], Maureen E. Murphy[1]
[1]Program in Molecular and Cellular Oncogenesis, The Wistar Institute, Philadelphia PA 19104, USA
[2]Graduate Group in Cancer Biology, the University of Pennsylvania Perelman School of Medicine, Philadelphia PA 19104, USA
[3]Graduate Group in Biochemistry and Molecular Biophysics, the University of Pennsylvania Perelman School of Medicine, Philadelphia PA 19104, USA

Email of submitting/first author: avaldespino@wistar.org

Training program first author is enrolled in: Graduate group in Cancer Biology, the University of Pennsylvania

Year of training: 4

Abstract:
Although *TP53* is the most mutated gene in human cancer, the mechanisms of p53 mediated tumor suppression have not been fully elucidated. We identified the p53 target gene, *PADI4*, to be one of only three target genes showing impaired transactivation by all cancer risk associated p53 variants studied in our lab. PADI4 is a calcium dependent regulator of citrullination, which is the process of deaminating arginine to the non-natural amino acid citrulline, and overexpression of PADI4 results in a tumor suppressive phenotype both *in vitro* and *in vivo*. Furthermore, the tumor suppressive phenotype observed in our animal studies was found to be T-cell dependent. Using mass spectrometry and ChIP-seq, we identified citrullination as a novel post translational modification on p53 that results in the relocalization of p53 from canonical p53 binding sites to ETS binding motifs. On a protein level, induction of PADI4 correlates with strong induction of citrullinated histone H3. However, our ChIP-sequencing resulted in a loss of peaks associated with citrullinated H3 upon co-induction with p53. Using immunofluorescence and cell fractionation, we observe that p53 and PADI4 cooperate to evict citrullinated histones from the nucleus in a gasdermin E-dependent manner. We performed RNA-sequencing on PADI4-induced and

p53-stabilized cells in the presence and absence of gasdermin E and found that cytosolic citrullinated H3 results in upregulation of pathways involved in T-Cell receptor signaling, NK-mediated cytotoxicity, and Toll-like receptor signaling. The findings from this study highlight the need to reassess the role of PADI4 in cancer and has the potential to uncover new pathways that regulate tumor suppression by p53.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Ras–MAPK pathway convergence defines early therapeutic vulnerability in p53-deficient pediatric high-grade glioma
Authors and institutional affiliation: • Tymofyeyeva A, The Hospital for Sick Children • Desai K, The Hospital for Sick Children • Javier M, The Hospital for Sick Children • Bullivant G, Lifescience Dynamics, previously The Hospital for Sick Children • Forst J, The Hospital for Sick Children • Svergun N, previously The Hospital for Sick Children • Ye V, St. Michael's Hospital, previously The Hospital for Sick Children • Kushida M, The Hospital for Sick Children • Yu C, The Hospital for Sick Children • Whetstone H, The Hospital for Sick Children • Ward R, Takeda Canada, previously The Hospital for Sick Children • Dirks PB, The Hospital for Sick Children
Email of submitting/first author: anna.tymofyeyeva@sickkids.ca
Training program first author is enrolled in: Developmental, Stem Cell & Cancer Biology (DSCB) program.
Year of training: Second year of graduate school.

Abstract:

Purpose: Pediatric high-grade gliomas (pHGGs) are the leading cause of cancer-related mortality in children and remain largely incurable. While molecular profiling has revealed extensive intratumoural heterogeneity in end-stage disease, this complexity obscures the earliest tumour-initiating events that arise during neurodevelopment. Loss of TP53 function is a central event in pediatric gliomagenesis, yet how p53 deficiency shapes early tumour evolution and therapeutic vulnerability remains poorly defined. We hypothesized that defining the earliest molecular and cellular states of p53-deficient gliomagenesis would reveal targetable vulnerabilities that are lost during malignant progression. **Materials and Methods:** We developed a novel pHGG model using Nestin-Cre;$Trp53^{fl/fl}$ mice exposed embryonically to the alkylating mutagen *N*-ethyl-*N*-nitrosourea (ENU). This system models p53 loss in neural progenitors in the context of an environmental insult and enables longitudinal analysis of premalignant, early, and end-stage lesions. Tumours and precursor lesions were analyzed using transcriptomic profiling, histopathology, and pathway-level analyses. Cell lines derived from early- and late-stage tumours were used for functional drug sensitivity assays. **Results:** This model reproducibly generated high-grade gliomas that resemble human pHGG. Longitudinal profiling revealed progressive increases in genetic complexity, stem-like features, and immune remodeling during tumour evolution, while early lesions exhibited relative molecular homogeneity. Recurrent activating Braf mutations emerged at the earliest detectable stages and were frequently retained in advanced tumours, paralleling the early appearance of BRAFV600E mutations in pediatric gliomas. Correspondingly, RAS–RAF–MAPK pathway activation was evident prior to malignant progression. Functionally, cell lines from early-stage, p53-deficient Braf-mutant lesions displayed significantly greater sensitivity to BRAF inhibition compared to lines from end-stage tumours and patient-derived pHGGs, indicating loss of therapeutic vulnerability with tumour evolution. **Conclusions:** These findings define the temporal molecular evolution of p53-deficient pediatric gliomagenesis *in vivo* and identify early MAPK pathway activation as a developmentally constrained, targetable vulnerability. Our results suggest that therapeutic strategies focused exclusively on end-stage pHGG may overlook critical windows for intervention and highlight the importance of early interception in p53-driven pediatric brain tumours.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5 pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: **Advancing the Precision Medicine Paradigm for TP53 Y220C-Mutant Cancers with Rezatapopt**

Authors and institutional affiliation: ***Masha V. Poyurovsky***, *PMV Pharmaceuticals, Inc*
Erin Michaud1, Lizhong Xu1, Hong Yang1‡, Wenhui Li1‡, Mary Kate McBrayer1, Yvonne Sun1, Christopher Mulligan1, Josh Battaglia1‡, Melissa Dumble1, Anna Puzio-Kuter1

[1]PMV Pharmaceuticals, Inc, Princeton NJ, USA

Email of submitting/first author: *mpoyurovsky@pmvpharma.com*

Training program first author is enrolled in: *NA / Industry Presentation*

Year of training: *PGY10*

Abstract: *Approx. 300 words Suggested format: Purpose, Materials and Methods, Results, Conclusions*

Purpose: The TP53 Y220C mutation, occurring in approximately ~1% of solid tumors, creates a surface pocket that thermally destabilizes the p53 protein, leading to loss of tumor suppressor function. Rezatapopt (PC14586) is a first-in-class, small-molecule reactivator designed to bind this pocket and restore wild-type (WT) p53 conformation. Rezatapopt has demonstrated promising single-agent clinical activity; ongoing studies systematically identify rational combination strategies to maximize therapeutic potential.

Materials and Methods: Structure-guided medicinal chemistry yielded rezatapopt with high affinity for Y220C (SC_{150} = 9 nM). To identify cooperative pathways, a high-throughput combination screen of 1,746 FDA-approved and clinical-stage agents was performed using Y220C-mutant cell lines (NUGC-3, T3M-4). Top candidates, including inhibitors of the PI3K/AKT/mTOR and MAPK pathways, as well as standard-of-care therapies (e.g., paclitaxel, gemcitabine, bevacizumab), were evaluated in vitro and in vivo using xenograft models.

Results: Rezatapopt restored WT transcriptional function (including CDKN1A and MDM2) and induced tumor regression in Y220C-mutant models. PI3K-pathway inhibition (e.g., alpelisib) enhanced apoptotic signaling and induced deeper tumor regressions in vivo compared to monotherapy. In addition, combining rezatapopt with bevacizumab, an anti-angiogenic therapy widely used in ovarian and other cancers, yielded significant tumor growth inhibition in xenografts, supporting an actionable combination. Clinically, in the Phase 1/2 PYNNACLE trial (NCT04585750), rezatapopt monotherapy achieved objective responses in patients harboring a TP53 Y220C mutation across several tumor types, including ovarian, breast, endometrial, lung and head and neck cancers.

Conclusions: Rezatapopt is a potent and clinically active allele-selective p53 Y220C reactivator. These findings provide a mechanistically grounded and clinically relevant framework for the development of combination therapies. Both standard-of-care strategies (e.g., bevacizumab) and pathway-targeted approaches (e.g., PI3K inhibition) represent rational paths for clinical trials in patients with TP53 Y220C-mutant cancers.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Decoding p53(R249S)-mutated liver cancer: from developmental origin to targeted therapies

Authors and institutional affiliation:

Megan E. Fisher[1,2]
Yu-Wen Huang[2]
Mo-Fan Huang[1,2]
Ruiying Zhao[2]
Dung-Fang Lee[1,2]

1. The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences
2. Department of Integrative Biology and Pharmacology, McGovern Medical School, The University of Texas Health Science Center at Houston

Email of submitting/first author: megan.e.fisher@uth.tmc.edu

Training program first author is enrolled in:
The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences

Year of training:
3

Abstract:

Purpose: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and remains a major global health challenge due to its rising incidence and limited therapeutic options. One of the most recurrent mutations in HCC is the p53(R249S) hotspot mutation, yet its direct oncogenic impact is difficult to isolate in traditional cancer cell lines, which harbor numerous co-occurring mutations. This study aims to define the mechanistic role of p53(R249S) in early tumorigenic events using a genetically controlled model and to identify actionable downstream effectors for therapeutic intervention.
Materials and Methods: To investigate the mutation in a physiologically relevant context, we generated a human pluripotent stem cells (hPSC) line carrying only the p53(R249S) mutation and differentiated these cells into hepatocyte-like cells. We performed RNA sequencing followed by pathway enrichment analyses to identify signaling networks perturbed by mutant p53. FOXM1 emerged as a leading candidate effector and was further validated through gene expression profiling. To assess therapeutic potential, we treated HCC cell lines with thiostrepton, a FOXM1 inhibitor, and measured cellular viability along with expression of FOXM1 target genes.
Results: Transcriptomic analysis revealed that p53(R249S) induces broad dysregulation of cell cycle-related pathways. FOXM1 was significantly upregulated in mutant hepatocyte-like cells, and its target genes were enriched among differentially expressed genes. In HCC cell lines, thiostrepton treatment markedly reduced cell viability and consistently decreased expression of FOXM1-regulated genes, supporting its activity against the FOXM1 pathway.
Conclusions: These findings identify a previously uncharacterized p53(R249S)-FOXM1 oncogenic axis that may contribute to HCC initiation and progression. Our hPSC-based platform provides a tractable system for dissecting mutant p53-driven mechanisms for testing targeted therapies. Ongoing work includes determining thiostrepton efficacy in vivo and integrating computational genomic analyses to further define how p53(R249S) activates FOXM1. Overall, this study highlights FOXM1 as a promising therapeutic target for p53-mutant HCC.

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Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Unraveling how distinct p53 hotspot mutations dictate immunotherapy response in breast cancer

Authors and institutional affiliation:
Annemieke Bouwman[1,#] Antoinette van Weverwijk[1,#] Max Wellenstein[1,#] Danique Duits[1], Tisee Hau[1], Kim Vrijland[1], Onno Bleijerveld[2], Liesbeth Hoekman[2], Reuven Agami[3], Wilbert Zwart[3,4], Kimberly Bonger[5] and Karin de Visser[1,6]

1 – Division of Tumor Biology and Immunology, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands;
2 - Proteomics Facility, Netherlands Cancer Institute, Amsterdam, The Netherlands;
3 - Division of Oncogenomics, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands;
4 - Laboratory of Chemical Biology and Institute for Complex Molecular Systems, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands;
5 – Department of Chemical Biology & Immunology, Leiden Institute of Chemistry, Leiden University, The Netherlands
6 - Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands.

# - these authors contributed equally

Email of submitting/first author: a.bouwman@nki.nl

Training program first author is enrolled in: Onderzoeksschool Oncology Amsterdam (OOA), Netherlands Cancer Institute

Year of training: Year 4

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Inter-patient heterogeneity in immune cell composition and function is a major challenge in the development of novel breast cancer immunotherapies. The tumor suppressor gene *TP53* is one of the most frequently mutated drivers of breast cancer, and accumulating evidence suggests that specific hotspot mutations confer gain-of-function properties beyond simple loss of p53 activity. In our work, we demonstrate that distinct *TP53* mutations drive the establishment of different tumor immune microenvironments, resulting in either “hot” (immune-enriched) or “cold” (immune-suppressed) tumors. Analysis of the TCGA breast cancer dataset confirms that different *TP53* hotspot mutations are associated with distinct immune phenotypes, highlighting the clinical relevance of mutation-specific immune modulation. Using mouse models, we further show that tumors driven by hot mutants respond more effectively to PD-1 blockade, whereas cold mutants fail to do so. To uncover how specific *TP53* hotspot mutations drive different immune phenotypes, we employed a multi-omics approach. Proteomic profiling revealed enrichment of interferon (IFN) signaling pathways in hot mutants, particularly type I IFNs, suggesting cold mutants suppress IFN signaling or hot mutants upregulate it. In addition, tumors driven by hot p53 mutations secrete elevated levels of eosinophil attractant eotaxin-1, and present higher levels of intratumoral eosinophils that could contribute immunotherapy response. We hypothesize that distinct *TP53* hotspot mutations dictate immune phenotypes and immunotherapy response through different molecular mechanisms. This work aims to provide mechanistic insight into mutation-specific immune modulation and highlights the importance of considering *TP53* mutations in the design of breast cancer (immuno)therapies to enable personalized treatment strategies. (249 words)

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20th International p53 Workshop- Abstract Submission

Title of study/project:

Opposing transcriptional outcomes through the p53 isoform lens

Authors and institutional affiliation:

Annika Wylie, Ph.D.
Assistant Professor and Cancer Prevention Institute of Texas (CPRIT) Scholar
Southern Methodist University, Dallas TX

Email of submitting/first author:

annikaw@smu.edu
annika.wylie@gmail.com

Abstract:

p53 genes are broadly conserved throughout the animal kingdom and function as transcriptional activators that respond to stress. In addition, we showed that p53 exerts conserved functions to downregulate genes such as retrotransposons, a class of mobile genetic elements broadly implicated in human disease. However, the mechanisms of p53 repression are not understood and are generally assumed to be indirect. Here, we investigate synthetic and native *cis*-regulatory elements in *Drosophila* to examine opposing features of p53 mediated transcriptional control *in vivo*. We show that transcriptional repression by p53 operates continuously through canonical DNA binding sites that confer p53-dependent transactivation at earlier developmental stages. p53 transrepression is correlated with local H3K9me3 chromatin marks and occurs without the need for stress or Chk2. In sufficiency tests, two p53 isoforms qualify as transrepressors and a third qualifies as a transcriptional activator. Targeted isoform-specific knockouts dissociate these opposing transcriptional activities, highlighting features that are dispensable for transactivation but critical for repression and for proper germ cell formation. Additional factors that contribute to p53 tissue-specific responses will be discussed. Together, these results demonstrate that certain p53 isoforms function as constitutive tissue-specific repressors, raising important implications for tumor suppression by the human counterpart.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Early rhabdomyosarcoma priming in mice with germline *TP53* mutations

Authors and institutional affiliation: *Example: Kissoondoyal Ashby, The Hospital for Sick Children*

Quaglietta R. Paula, The Hospital for Sick Children; University of Toronto

Laverty Brianne, The Hospital for Sick Children; University of Toronto

Malkin David, The Hospital for Sick Children; University of Toronto

Email of submitting/first author: **ashby.kissoondoyal@sickkids.ca**

Training program first author is enrolled in: **Postdoctoral Fellowship**

Year of training: **Year 3**

Abstract: *Approx. 300 words*

Purpose: Li-Fraumeni syndrome (LFS), associated with germline *TP53* mutations, confers a markedly increased risk of early childhood cancers, including rhabdomyosarcoma (RMS). Growing evidence in LFS suggests that malignant transformation in LFS begin during embryonic development. However, the developmental mechanisms linking *TP53* dysfunction to tumour-permissive cellular states remain poorly defined. Using LFS-associated RMS (LFS-RMS) as a model, we tested whether germline *Trp53* mutations reprogram early myogenesis to establish muscle states primed for tumorigenesis.

Methods: We performed single-nucleus RNA sequencing (snRNA-seq) on LFS-RMS tumours (N = 5), matched distal muscle (N = 5), and healthy embryonic (days 10, 12, 14 and 16; N = 64) and postnatal muscle (days 60, 120, 210 and 300; N = 64) tissues from male and female *Trp53*$^{R172H/+}$ (R172H) and wild-type littermates. We derived an LFS-RMS transcriptional signature, conducted Gene Ontology enrichment, and integrated deep (800x) whole-exome sequencing (WES) of matched samples to assess genomic alterations accompanying developmental transcriptional changes.

Results: We identified a myogenic LFS-RMS transcriptional signature enriched for WNT signaling, RNA metabolic pathways, and glycolytic reprogramming. This signature was significantly elevated at the peak of embryonic myogenesis (E12) and associated with delayed myogenic maturation in R172H embryos. WES revealed dynamic shifts in genomic mutational burden during embryonic development in R172H mice. Integrated snRNA-seq and WES analyses uncovered overlapping temporal windows of transcriptional dysregulation and genomic instability during key pre- and postnatal stages of muscle development. Moreover, myogenic cells harboring the LFS-RMS signature persisted postnatal muscle.

Conclusions: We have generated the first developmental LFS atlas, integrating snRNA-seq and deep WES across embryonic and postnatal stages, and tumours. We demonstrate germline *Trp53* mutations reprogram myogenesis early in development, establishing persistent tumour-priming states. By defining

when and where tumour-permissive programs arise, our work provides a foundational framework for early detection, prevention, and intervention strategies in LFS.

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: ***TP53*** **Role in Modulating Immune Surveillance in Early Breast Cancer**

Authors and institutional affiliation:

Łasut-Szyszka B*, Department of Microbiology, Tumor and Cell Biology, Karolinska Insitute, Stockholm, Sweden
Kirk A*, Department of Microbiology, Tumor and Cell Biology, Karolinska Insitute, Stockholm, Sweden
Zerdes I, Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden
Sifakis E, Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden
Bergh J, Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden
Wang P, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany
Sayed S, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany
Buchholz F, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany
Foukakis T, Department of Oncology-Pathology, Karolinska Institute, Stockholm, Sweden
Selivanova G*, Department of Microbiology, Tumor and Cell Biology, Karolinska Insitute, Stockholm, Sweden

Email of submitting/first author: **barbara.lasutszyszka@ki.se**

Training program first author is enrolled in: ***post doc training***

Year of training: ***II year***

Abstract:

Purpose
The tumor microenvironment (TME) and immune cells are critical regulators of cancer progression. The tumor suppressor p53 plays a central role in maintaining cellular homeostasis and modulating immune responses. Mutations in *TP53* occur in approximately 50% of all cancers and in ~30% of breast cancers, particularly in triple-negative and HER2-positive subtypes. Beyond loss of tumour-suppressive function, mutant p53 may acquire gain-of-function properties that influence immune surveillance. The aim of this study is to assess the impact of *TP53* status on immune regulation in breast cancer.
Materials and Methods
We analyzed the clinicopathological and gene expression data from the METABRIC cohort of early-stage breast cancer (n = 1,980), with *TP53* mutational status available for 820 patients. We evaluated the immune cell composition and immune-related gene expression in relation to *TP53* status and breast cancer subtype. We validated our findings using

publicly available datasets, as well as gene expression data obtained upon mutant *TP53* correction by base editing in cancer cell lines.

Results

Certain types of breast cancers harboring *TP53* mutations displayed enrichment of immune cell subpopulations and increased expression of some immune checkpoint molecules and pro-inflammatory cytokines. The impact of *TP53* status varied across breast cancer subtypes. A subset of mutant *TP53* tumours exhibited increased infiltration of pro-inflammatory immune cells, including T cells, compared with wild-type *TP53* tumours. These observations were corroborated by *in vitro* analysis of gene expression upon correction of *TP53* mutation by base editing.

Conclusions

These findings suggest that *TP53* mutations may act as a molecular switch between immunosuppressive and immunologically active TMEs by promoting chronic inflammation. Ongoing *in vivo* studies aim to further elucidate the role of mutant versus wild type *TP53* in immune surveillance across breast cancer subtypes.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Pediatric pan-cancer characterization of transposable elements and their modulation by germline *TP53* variants
Authors and institutional affiliation: Laverty B, The Hospital for Sick Children Yadahalli S, The Hospital for Sick Children Majeed S, The Hospital for Sick Children Kissoondoyal A, The Hospital for Sick Children Raiti L, The Hospital for Sick Children Gong A, The Hospital for Sick Children Alon N, The Hospital for Sick Children Kashif S, The Hospital for Sick Children Solovyov A, Memorial Sloan Kettering Cancer Center Davidson S, The Hospital for Sick Children Li Y, The Hospital for Sick Children Layeghifard M, The Hospital for Sick Children Shlein A, The Hospital for Sick Children Malkin D, The Hospital for Sick Children Subasri V, University Health Network
Email of submitting/first author: b.laverty@mail.utoronto.ca
Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program* Medical Biophysics PhD at the University of Toronto
Year of training: *Example: PGY 3* *6*

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Transposable elements (TEs) are repetitive regions that are silenced through epigenetics. Although TE activation is a feature of development and adult cancers, their role in pediatric malignancies is poorly understood. Approximately 15% of pediatric cancers arise in the context of hereditary cancer predisposition, such as Li-Fraumeni Syndrome, caused by germline *TP53* (g*TP53*) variants. *TP53* binds LINE1 elements to suppress their transcription, and adult tumors with somatic *TP53* mutations exhibit elevated TE activity. These findings suggest g*TP53* variants disrupt TE regulation, predisposing tissues to malignant transformation. To investigate this, we characterized the germline and tumour TE landscape across a pediatric pan-cancer cohort and evaluated the impact of g*TP53* variants.

We identified TEs in 456 germline and 380 tumour samples. Germline ALU, LINE1, SVA elements were called three tools, while tumor LINE1 elements were called with two. We excluded 96% of germline and 23% of tumor TEs classified as common (>3% of gnomAD or our cancer-free cohort (n=166)).

We observed no difference in germline TE burden between g*TP53* carriers and non-carriers, prompting us to examine insertion-site patterns. A support vector machine trained on TE distribution predicted g*TP53* status with an AUPRC of 0.74, suggesting g*TP53* variants influence the position of germline insertions. In a subset of cancer patients, germline TEs disrupted immune-related regulatory elements (FDR < 0.05). Preliminary evidence suggests these immune pathways are altered in germline blood and fibroblast DNA, indicating a systemic effect.

Half of tumours harboured at least one insertion, with epithelial-origin cancers containing more TEs, reflecting adults-onset cancers. As TEs are active in brain development we analyzed an additional 102 medulloblastoma samples and found 97% contained no insertions. Unlike adult tumours, somatic or germline *TP53* variants did not increase LINE1 insertion burden.

We found g*TP53* variants do not increase germline or tumour TE burden but strongly influence the positional distribution of germline insertions. This work enhances our understanding of *TP53*-associated cancers to guide future therapeutics.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Investigating the cooperation of p53 gene dosage and mutant *Kras* in driving non-small cell lung cancer
Authors and institutional affiliation: Mitheera V – Department of Genetics Aatish Thennavan – Department of Systems Biology Xiaojie Yu – Department of Genetics Celine Shuet Lin Kong – Department of Pulmonary Medicine Jichao Chen – Department of Pulmonary Medicine Guillermina Lozano – Department of Genetics The University of Texas MD Anderson Cancer Center, Houston, TX, USA
Email of submitting/first author: mv@mdanderson.org
Training program first author is enrolled in: The University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences (Cancer Biology and Genetics & Epigenetics Dual PhD Program)
Year of training: *4th Year PhD Student*
Abstract: Non-small cell lung cancer (NSCLC) is underlined by inter- and intratumoral heterogeneity in tumors and the associated tumor microenvironment (TME). *KRAS* and *TP53* are the most frequently altered genes in human NSCLC. Mouse models with an activating $Kras^{G12D/+}$ mutation and *Trp53* deletion (KP) have been historically used to model tumoral heterogeneity and evolution. However, in NSCLC patients with KRAS mutations, loss-of-heterozygosity (LOH) of the *TP53* allele is a common occurrence. **Hence, the role of p53 haploinsufficiency in sculpting NSCLC progression and evolution remains understudied.** Therefore, we hypothesized that p53 gene dosage exacerbates *Kras*-initiated NSCLC by driving **tumoral and TME heterogeneity**. To test this hypothesis, we performed single-cell multiomics on tumor-bearing lungs from mouse models with $Kras^{G12D/+}$ *(K)* and $Kras^{G12D/+}$*;* $Trp53^{Flox/+}$ *(*$KP^{+/-}$*)* alleles *(K* = 21,361 cells; $KP^{+/-}$ = 22819 cells*)* and performed a meta-analysis utilizing publicly available *KP* single-cell data. Leveraging these models, we show *Trp53* gene dose constrains tumor burden in *K* and $KP^{+/-}$ lungs, which parallels the dose-dependent survival rates observed in Kras-initiated NSCLC

patients. From single-cell multi-omics analyses, we identified unique cellular states associated with *Trp53* dosage arising in *K*, *KP*$^{+/-}$ and *KP* tumors. In addition, we also show a decrease in the CD8+ T cells and an increased infiltration of *Siglecf*+ neutrophils and immunosuppressive monocyte derived-macrophages in *KP*$^{+/-}$ compared to *K* TME. Ongoing characterization efforts include validating the identified tumor cell states and TME populations *in situ* and testing the prognostic potential of the NSCLC signatures identified in this study using human NSCLC clinical data. Completion of this study will reveal novel molecular dependencies which will aid in the development of treatment regimes in the clinic for NSCLC patients with *KRAS* and *TP53* alterations.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Decoding Functional Consequences of TP53 Mutations through Single-Cell RNA Sequencing

Authors and institutional affiliation:
Bhuiyan N, University of Edinburgh
Guirardel L, University of Edinburgh
Kudla G, University of Edinburgh

Email of submitting/first author:
nabid.bhuiyan@ed.ac.uk

Training program first author is enrolled in:
Genetics and Molecular Medicine (PhD) (2021-Current)

Year of training:
Year 4

Abstract:
Mutants in TP53 result in loss of function, dominant-negative effects, or gain-of-function phenotypes that promote tumorigenesis. Understanding how specific TP53 mutants alter gene expression and cellular pathway remains a challenge due to the diversity of mutations and their context-dependent effects across cell types and signalling networks.

To systematically measure the functional consequences of TP53 mutations, I developed a high-throughput platform combining saturation mutagenesis, barcoded genome integration, and single-cell RNA sequencing (scRNA-seq). A saturation mutagenesis library of TP53 was linked to unique DNA barcodes, enabling precise identification of each variant. Using the HEK293 Flp-In T-REx system and MaxCyte electroporation, I achieved single-copy, inducible integration of individual TP53 variants, ensuring consistent genomic context and expression control. Following induction of TP53 variant expression, pooled mutant libraries were either exposed or unexposed to X-ray irradiation. Single-cell transcriptomes were generated using Parse Biosciences' Split-Pool Ligation-based Transcriptome sequencing (SPLiT-seq). Pathway-level analyses comparing wild-type and mutant domains were performed, and missing data were inferred using predictive modelling.

I obtained transcriptome profiles for 3107 p53 variants across 37183 cells, representing 40% of possible single amino acid changes in p53. Differential expression analysis revealed distinctions between wild-type and mutant groups. X-ray exposure induced marked transcriptional shifts, while mutations in the DNA-binding domain predominantly exhibited loss-of-function characteristics. In contrast, variants within the C-terminal regulatory domain retained or enhanced p53 pathway activity relative to wild-type. Gene set enrichment and hallmark analyses identified further mutation-specific pathway differences.

In addition to the p53 work, I conducted a small pilot scRNA-seq experiment using 26 transcription factor (TF) sequences. This identified TF-specific differential gene expression, and preliminary Gene Ontology analysis linked the top genes to relevant biological processes.

This approach provides a scalable, quantitative framework to functionally annotate TP53 mutations in an isogenic background. It demonstrates the feasibility of coupling saturation mutagenesis with scRNA-seq to uncover mutation-specific phenotypes and pathway alterations. Future work will expand this platform to explore additional transcription factors and assess responses to stress and drug treatments.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **Non-canonical p53 functions in DNA replication and recombination as a novel tool for variant classification**

Authors and institutional affiliation:
Rebecca Jansche
Universitätsklinik Ulm

Prof. Lisa Wiesmüller
Universitätsklinik Ulm

Email of submitting/first author: **rebecca.jansche@uni-ulm.de**

Training program first author is enrolled in:
Rebecca Jansche (First Author) is enrolled in the IGRADU PhD Program from 2021-2026.

Year of training:
Final year of training (4. year).

Abstract:

Aim/ Introduction:
The increasing number of variants of unknown significance (VUS) in high-risk genes poses an ever-growing challenge for patient management. Functional assays gained importance among criteria used to predict the pathogenicity of hereditary breast and ovarian cancer (HBOC). Currently, *TP53* variant classification considers canonical functions exclusively. Previous work revealed non-canonical functions in DNA replication, therefore we analyzed *TP53* variants from German HBOC patients regarding non-canonical functions to establish an independent classification tool.

Material and Methods:
For the analysis of non-canonical functions, 43 *TP53* variants (including 23 VUS) were expressed in p53-negative K562 cells, patient-derived lymphocytes were immortalized. Spontaneous recombination-mediated bypass of replication barriers was analyzed by flow cytometric quantification of EGFP-positivity in cells carrying a genomically integrated EGFP reporter**.** The DNA fiber spreading assay was used to investigate replication dynamics. Proximity ligation assay (PLA) was applied to detect functional complexes in p53-dependent replication control.

Results:
Recombination measurements achieved a clear discrimination between benign/likely benign and pathogenic/likely pathogenic variants, and revealed significant correlations with data on canonical functions as well as AlphaFold Missense Prediction scores. Fiber spreading assay resulted in a more heterogeneous picture reflecting the multifactorial nature of this readout. Separation-of-function (SOF) analysis identified overlapping sets of *TP53* variants with SOF between canonical and non-canonical functions as well as between recombination and replication. 3D structure analysis and PLA of SOF variants unveil impact on DNA- and/or polymerase iota binding as well as dimer-dimer formation.

Conclusion:
Our results demonstrate that recombination measurements are suitable to aid classification of *TP53* variants. Together with data by Funk et al. 2025. Nature Genetics our data question the heavy weight given to the yeast transactivation assay in current guidelines. Importantly, we provide the first assay to take non-canonical functions of p53 into account, therefore reducing circularity of canonical functions included in the classification process.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Investigating the role of the p53 target gene *Zmat3* in tumor suppression using *in vivo* and *in vitro* models of lung adenocarcinoma

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Bolle C,M. Stanford University
Mabene A,R. Stanford University
Boutelle A,M. Stanford University
Bieging-Rolett K. Stanford University
Attardi L,D. Stanford University

Email of submitting/first author: bolle@stanford.edu

Training program first author is enrolled in: Stanford University Cancer Biology PhD Program

Year of training: *Example: PGY 3*
4th year

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Lung cancer is the global leading cause of cancer deaths. The tumor suppressor and transactivator p53 is mutated in 50% of lung adenocarcinomas (LUADs), yet how it serves as a tumor suppressor is not completely understood. Our lab established the RNA-binding protein ZMAT3 as a key mediator of p53's tumor suppressive function. ZMAT3 is a zinc finger protein known to bind RNA and impact alternative splicing, yet little is known about ZMAT3's mechanism of action in LUAD and which aspects of p53-mediated tumor suppression can be attributed to ZMAT3. Research into this crucial target gene of p53 has the potential to open up new avenues for therapeutics by identifying critical targets of ZMAT3 regulation. To understand the mechanisms of ZMAT3 action in LUAD, we sought to investigate the impact of *Zmat3* inactivation on gene expression and tumor development using a mouse LUAD model driven by oncogenic *Kras* expression. We initiated LUAD in *Kras*$^{LSL\text{-}G12D/+;}$*Rosa26*$^{LSL\text{-}tdTomato/LSL\text{-}tdTomato}$ (KT), *Kras*$^{LSL\text{-}G12D/+;}$ *Zmat3*$^{flox/flox}$; *Rosa26*$^{LSL\text{-}tdTomato/LSL\text{-}tdTomato}$ (KZT) and *Kras*$^{LSL\text{-}G12D/+;}$ *Trp53*$^{flox/flox}$; *Rosa26*$^{LSL\text{-}tdTomato/LSL\text{-}tdTomato}$ (KPT) mice using Adenovirus-Cre, and tumors were collected for RNA-sequencing and histological analysis. We conducted analysis of lung histology, which display a trend for

increased tumor burden (tumor area/total lung area) upon inactivation of *Zmat3*, intermediate between wild-type and p53-deficient mice. We are currently analyzing the RNA-seq data to identify how *Zmat3* loss impacts RNA homeostasis and gene expression, to determine which gene expression programs are disrupted upon loss of ZMAT3 regulation. Comparison of gene expression programs regulated by ZMAT3 with those regulated by p53 will reveal ZMAT3-dependent and ZMAT3-independent pathways downstream of p53. As a complementary approach, we utilized murine LUAD cells in which p53 can be reactivated by Adenoviral-Cre expression. We used these cells in paired RNA-seq and eCLIP-seq experiments to identify RNA transcripts bound and regulated by ZMAT3. Preliminary analysis suggests that cell migration and motility, extracellular matrix and transitional cell programs are upregulated in ZMAT3-deficient cells. Overlap of *in vivo* and *in vitro* data will ultimately reveal the most critical ZMAT3 effectors in tumor suppression. By investigating how ZMAT3 functions downstream of p53's tumor suppressive roles, we hope to uncover vulnerabilities that can be targeted therapeutically in LUAD.

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: In Search of a Stratified Toronto Protocol using Statistical Mechanics

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Neches C, Memorial Sloan Kettering Cancer Center, Harvard University
Hoyos D, Tri-Institutional PhD Program in Computational Biology and Medicine
Greenbaum B, Halvorsen Center for Computational Oncology at Memorial Sloan Kettering Cancer Center

Email of submitting/first author: nechesc1@mskcc.org

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Harvard College, double concentration in Physics and History & Literature

Year of training: *Example: PGY 3*

Third-year undergraduate

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

The aims of this project are to (1) identify the relative binding strength of wild-type-mutant p53 pairs, (2) identify the relative binding strength of these pairs to DNA, and (3) to identify which tissues present with these different pairs. Using *in silico* mutagenesis via BioPython, we created a sequence library. Then, we used AlphaFold3 to create a comprehensive structural database of the individual mutant proteins – this resulted in 94,192 structures. Due to the instability of mutant p53 proteins, these are the first complete models of p53 mutant proteins. Using the co-folding function of Boltz-2, we then modeled the wild-type-mutant pairs bound to the p53 target DNA motifs. We then quantified the quality of binding using free energy (kcal/mol). From this, we were able to develop a spectrum for likely severity of LFS phenotypes, where we set wild-type-wild-type binding as the standard, and measure deviations from that. By determining which mutant-wild-type pairs diverge most from the wild-type, we can determine different risk levels within Li Fraumeni Syndrome. This, in turn, allows us to match mutant pairs to the tissues in which they most frequently occur. We can then suggest a more targeted monitoring program, wherein specific at-risk variants are monitored more carefully in their tissues of risk.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Targeting the p53 cancer mutants Y220C, Y220N, and Y220S with the small-molecule stabilizer rezatapopt
Authors and institutional affiliation: Danai Mavridi, *Goethe University Frankfurt & Structural Genomics Consortium (SGC)* Julianne S. Funk, *Philipps-University Marburg* Dimitrios-Ilias Balourdas, *Goethe University Frankfurt & Structural Genomics Consortium (SGC)* Andreas Krämer, *Goethe University Frankfurt & Structural Genomics Consortium (SGC)* John Spencer, *University of Sussex, Brighton* Volker Dötsch, Goethe University Frankfurt Thorsten Stiewe*, Philipps-University Marburg* Andreas C. Joerger, *Goethe University Frankfurt & Structural Genomics Consortium (SGC)*
Email of submitting/first author: mavridi@pharmchem.uni-frankfurt.de
Training program first author is enrolled in: PhD candidate on Onassis Foundation Fellowship in Biochemistry
Year of training: 2nd
Abstract: The cavity-creating p53 cancer mutation Y220C, which accounts for an estimated 125,000 new cancer cases per year, serves as an excellent paradigm for the development of mutant p53 reactivators. Several molecules that reactivate this thermolabile cancer mutant by targeting the mutation-induced crevice have been developed, and one of them, rezatapopt, is currently in clinical trials. The less frequently occurring Y220N and Y220S mutations are even more destabilizing than Y220C but create a similar surface crevice, raising the question of whether cancer patients with these mutations might also benefit from rezatapopt treatment. Here, we show that rezatapopt also binds to the Y220N and Y220S mutants, with nanomolar affinity, resulting in a full recovery of wild-type-like stability for the latter. High-resolution crystal structures of all three mutants bound to rezatapopt revealed a conserved binding mode, highlighting key interactions, including multipolar interactions of a fluorine substituent at a chiral center with the protein backbone. Consistent with the biophysical and structural data, rezatapopt reactivated p53 signaling in both Y220C and Y220S mutant cells by restoring the folded conformation and transcriptional activity, leading to anti-proliferative effects and apoptosis, albeit requiring higher compound concentrations in Y220S cells. The Y220N mutant, despite exhibiting high-nanomolar affinity for rezatapopt and substantial stabilization, did not show noticeable effects in cells at the concentrations

tested, as rezatapopt binding resulted in only partial compensation for the mutation-induced loss of stability, for which we provide a structural explanation. Our data suggest that the development of clinical pan-Y220C/N/S reactivators, which could benefit an additional 10,000 patients per year, is challenging but not impossible.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Microenvironmental Control of mutp53-Driven Cell Competition

Authors and institutional affiliation:

D. Dobrović*, University of Trieste, International Centre of Genetic Engineering and Biotechnology (ICGEB), Italy

F. Napoletano*, University of Trieste, ICGEB, Italy

S. Joruiz, ICGEB, Italy

C. Dezi, University of Trieste, ICGEB, Italy

R.Hamad, University of Trieste, ICGEB, Italy

S.Piazza, ICGEB, Italy

A. Rustighi, University of Trieste, ICGEB, Italy

S. Torrini, ICGEB, Italy

L. Braga, ICGEB, Italy

L. Fava, University of Trento, Italy

G. Del Sal, University of Trieste, ICGEB, IFOM ETS, Italy

Email of submitting/first author:
daria.dobrovic@phd.units.it

Training program first author is enrolled in:
PhD in Molecular Biomedicine, University of Trieste

Year of training:
PhD, year 2

Abstract:

Tumor development is driven by the accumulation of (epi)-genetic alterations that deregulate cell growth, together with the selection of cells capable of adapting to dynamic microenvironmental conditions such as hypoxia, nutrient fluctuations, and mechanical stress. Aging, the major risk factor for cancer, and its associated features including chronic inflammation and extracellular matrix (ECM) remodeling, generate permissive tissue environments that favor the expansion and evolution of preneoplastic clones harboring oncogenic mutations. Among these alterations, missense mutations in the tumor suppressor TP53 (mutp53) are highly prevalent.

Preneoplastic clones can colonize tissues through cell competition a process whereby "fitter" cells eliminate neighbouring less fit cells. However, the mechanisms governing cell competition remain poorly understood. Elucidating these mechanisms may uncover novel vulnerabilities in tumor initiation.

We previously demonstrated that microenvironmental cues, including mechanical signals, critically regulate stabilization/transcriptional activity of mutp53 (Ingallina et al., *Nat. Cell Biol.* 2018, Tombari et al *Nat. Commun.* 2023). Based on this, we hypothesized that microenvironmental cues promote mutp53 stabilization/function in preneoplastic cells, driving clonal expansion through cell competition. We generated ad hoc cellular models using CRISPR/Cas9 genome editing and ex vivo models derived from $p53^{WM}$ mice (Zhang et al., *Nat. Commun.* 2018), enabling the tracking of competing mutp53 and normal cells. We found that mutp53-expressing human breast epithelial cells acquire a competitive advantage over co-cultured normal cells in a context-dependent manner. Notably, increased ECM stiffness selectively enhanced the fitness of mutp53 cells. Transcriptomic profiling of competitive mutp53 cells identified a mutp53-dependent gene program associated with mechano-transduction and clonal dominance.

Conclusions:

We identified a mutp53-dependent gene program that promotes the expansion of preneoplastic cells at the expense of normal cells under conditions of increased ECM stiffness.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Designer p53 as a tool to understand cell fate decisions

Authors and institutional affiliation:

Emma Kinloch, Francis Crick Institute and Kings College London
Karen Vousden, Francis Crick Institute
Manuel Muller, Kings College London

Email of submitting/first author:
emma.kinloch@crick.ac.uk

Training program first author is enrolled in:
Joint Francis Crick Institute-Kings College London Studentship

Year of training:
Year 2

Abstract:

The tumour suppressor p53 plays a central role in orchestrating cell-fate decisions in response to cellular stress, yet the mechanisms that enable p53 to determine cellular life and death remain uncertain. Among the many factors proposed to modulate p53 activity, post-translational modifications (PTMs) are thought to play a key part. Although extensive phosphorylation, ubiquitination, and acetylation sites have been mapped across the N- and C-terminal regulatory domains of p53, it is still not fully understood how individual PTMs, or defined combinations of them, steer cells towards cell-cycle arrest, apoptosis, or alternative stress responses. A major limitation in the field so far has been the lack of methods to generate p53 proteins carrying precisely defined, site-specific modifications and the absence of reliable approaches to introduce these modified p53 variants into cells in their functional tetrameric form.

This project integrates protein semi-synthesis with the development of protein transduction techniques to investigate how p53 PTMs govern cellular life and death decisions. Using synthetic

peptide chemistry and recombinant protein expression, ‘designer’ p53 species have been generated that carry precisely installed phosphorylated residues within the activation-linked N-terminal regulatory region. After refolding, these modified proteins are refolded into native tetramers and display sequence-specific DNA binding. The variants will be introduced into p53-null human cancer cells via an optimised intracellular delivery system and used to enable direct assessment of how defined phosphorylation events influence p53 stability, target-gene selectivity, and downstream pathway activation, including cell cycle arrest and apoptosis.

By disentangling the contributions of specific PTMs of p53 from the global cell stress response, this works aims to clarify whether, and how, PTMs are able to encode p53’s cell-fate determining functions.

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Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Altered p53 Family Expression and Acquired Resistance to MAPK-Targeted Therapy in Melanoma

Authors and institutional affiliation:

***Slade, N**.; Horvat, A.; Josić, J.; Vlašić, I., Tadijan, A., Supina Pavić, C.*

Laboratory for Protein Dynamics, Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb

Email of submitting/first author:
slade@irb.hr

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Year of training: *Example: PGY 3*

Abstract:

Purpose: The identification of BRAF as a driver mutation in melanoma enabled the development of targeted therapies, revolutionizing patient treatment. Alongside immunotherapy, patients with BRAFV600E mutation are commonly treated with combined BRAFi/MEKi therapy, while those harboring NRAS Q61 mutations, an alternative activating alteration within the MAPK pathway, are primarily treated with immunotherapy or MEK inhibition. Despite significant clinical benefits, most patients eventually develop therapeutic resistance, and the underlying molecular mechanisms are still not understood. In addition to reactivation of the MAPK pathway or activation of the PI3K/AKT signaling pathway, increasing evidence suggests that dysregulation of the p53 pathway contributes to acquired resistance in both BRAF- and NRAS-mutated melanoma. The aim of this study was to investigate the involvement of p53 family members in the development of resistance to BRAF/MEK–targeted therapy in metastatic melanoma.
Materials & Methods: Using primary BRAF- or NRAS-mutated melanoma cell lines that differ in their sensitivity to BRAFi/MEKi or MEKi, respectively, we analyzed p53 family expression. Gene expression was assessed by RT-qPCR, while protein levels, localization, and protein–protein interactions were examined by Western blot analysis, immunofluorescence and co-immunoprecipitation.
Results: We observed diverse expression patterns of p53 family isoforms that correlated with acquired resistance to BRAFi/MEKi. Specifically, elevated levels of p53γ, Δ40p53, and Δ133p53 isoforms, as well as ΔNp73, were detected in resistant melanoma cell lines. Depletion of these potentially inhibitory isoforms will help us to better understand their contribution to the development of therapeutic resistance.
Conclusions: Our findings demonstrate that melanoma exhibits imbalanced p53 family isoform expression, which may influence therapeutic response. Understanding the functional roles of these isoforms may uncover novel therapeutic targets and suggest new strategies to overcome MAPK inhibitor resistance in melanoma.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Altered T cell activation and impaired effector function in *Trp53*-R172H mice

Authors and institutional affiliation:

Fischer NW, Genetics & Genome Biology Program, The Hospital for Sick Children
Giovino C, The Hospital for Sick Children, University of Toronto
Ong N, The Hospital for Sick Children, University of Toronto
Quaglietta P, The Hospital for Sick Children, University of Toronto
Tikhonova A, Princess Margaret Cancer Centre, University of Toronto
Malkin D, The Hospital for Sick Children, University of Toronto

Email of submitting/first author: nick.w.fischer@gmail.com

Training program first author is enrolled in: Program in Genetics & Genome Biology

Year of training: Early Career Researcher Year 7

Abstract:

Purpose: To investigate how germline mutant p53 influences anti-tumor immune activation and effector function. p53 plays an important role in modulating T cell proliferation in response to antigen stimulation and controls expression of the negative checkpoint regulator VISTA. However, how immune-intrinsic p53 dysfunction alters T cell activation thresholds and shapes anti-tumor immunity in vivo remains poorly defined.

Materials & Methods: Tumor-challenged Trp53-R172H and wild-type mice were analyzed by multiparameter flow cytometry to characterize T cell phenotypes in tumors and immune organs. Ex vivo stimulation assays were performed on isolated lymphocytes to assess activation, proliferation, cytotoxic capacity, and expression of inhibitory and differentiation markers. Thymic responses were additionally examined following irradiation stress.

Results: Following irradiation, thymocytes from Trp53-R172H mice exhibited reduced expression of the inhibitory checkpoint molecule VISTA, accumulation of double-positive cells, and loss of CD3-high thymocytes. Splenic T cells displayed heightened activation and proliferation. Despite increased ex vivo expansion, tumor-infiltrating mutant p53 T cells had impaired cytotoxic function and dysregulated PD-1 expression.

Conclusions: Germline mutant p53 is associated with heightened immune activation but impaired effector function during tumor challenge. These findings indicate that p53 regulates immune activation and functional competence, and that immune-intrinsic p53 dysfunction contributes to tumor progression in Trp53-mutant settings.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Dissecting the roles of two different mutant P53 proteins in the development and sustained growth of tumours using novel genetically-engineered mouse models

Authors and institutional affiliation:

*Bhandari. R., Walter and Eliza Hall Institute**^
Wang. Z., Anhui Medical University*
Lieschke. E., Francis Crick Institute*
Kueh. A., Olivia Newton-John Cancer Research Institute
Chen. Y., Walter and Eliza Hall Institute
Tai. L., Anhui Medical University
Whelan. L., Walter and Eliza Hall Institute
Chen. T., Walter and Eliza Hall Institute
Sutherland. K., Walter and Eliza Hall Institute
Diepstraten. S., Walter and Eliza Hall Institute
Atkin-Smith. G., Walter and Eliza Hall Institute
Doerflinger. M., Walter and Eliza Hall Institute
Herold. M., Olivia Newton-John Cancer Research Institute
Strasser. A., Walter and Eliza Hall Institute
Kelly. G., Walter and Eliza Hall Institute

*co-first authors
^presenting author

Email of submitting/first author: Reet Bhandari: bhandari.r@wehi.edu.au

Training program first author is enrolled in: Medical Biology PhD program

Year of training: 3rd year PhD student

Abstract:

The tumour suppressor gene TP53 (Trp53 in mice) is frequently mutated in various cancers and is associated with aggressive disease, therapy resistance and poor prognoses. While most tumour suppressors frequently have truncating or loss-of-function (LOF) mutations, TP53 predominantly carries “hotspot” missense mutations. Therefore, specific TP53 mutations are hypothesized to contribute to tumorigenesis through dominant-negative (DNE) or gain-of-function (GOF) mechanisms, in addition to LOF effects. Understanding these mechanisms is critical for designing new therapies against mutant TP53 cancers.
To study the relative contributions of these putative mutant TP53 mechanisms to tumorigenesis, we developed unique “switchable” mouse models, whereby the Trp53 gene can be switched from WT to mutant to drive tumorigenesis and switched back to a WT or Trp53KO state using Flpe and Cre recombinases, respectively. These models carry either the R246Q or R172H mutation (R248Q and R175H in humans) allowing us to characterize different Trp53 mutations’ contribution to tumorigenesis and whether their restoration to WT or removal impacts tumour remission.
We found that the Trp53R246Q mice primarily developed thymic lymphomas, akin to Trp53KO mice, and with similar latency. Conversely, many Trp53R172H mice developed sarcomas, suggesting mutation-specific GOF effects during tumorigenesis. The Trp53 switchable mouse models were crossed to the Eμ-Myc mouse model of aggressive lymphoma. “Switching” from Trp53R246Q to Trp53WT in established lymphoma cells led to apoptosis and ablated lymphoma expansion in vivo. By contrast, removal of the mutant protein by converting the lymphoma cells into a Trp53KO state did not impact tumour growth, suggesting that this mutant does not significantly contribute any GOF effects to ongoing tumour expansion. Ongoing experiments are assessing the Trp53R172H mutant in sustaining sarcoma and lymphoma growth. These data will resolve the debate surrounding the roles of different TRP53 mutants in tumour development and expansion and inform therapeutic strategies for mutant TP53 cancers.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **Optimization of small molecule ligand of p53-Y220C**

Authors and institutional affiliation:
Suyin Pan, University of Southampton
Matthias Baud, University of Southampton
Joseph Stephenson Clarke, University of Southampton
Patrick J. Duriez, University of Southampton
Andreas C. Joerger, Goethe University

Email of submitting/first author:
s.pan@soton.ac.uk

Training program first author is enrolled in: *PhD in medicinal chemistry, year 4*

Year of training: *Year 4 of PhD*

Abstract: The p53 tumor suppressor is central to the natural physiological defense against cancer, and mutations of p53 lead to cancer progression. p53-Y220C is the ninth most common cancer mutation. The Y220C mutation at the surface of the DBD makes the mutated protein unstable at physiological temperature, leading to the loss of p53 tumor suppressor function. In this work, we will describe the optimization of small molecule pharmacological chaperones of p53-Y220C. By combining molecular design, docking studies, organic synthesis, and *in vitro* biophysical evaluation, we focused on improving the properties of previously reported small molecule leads and their binding affinity to the surface of p53-Y220C, while also reducing their molecular weight. We will describe our investigation of heterocyclic aromatic derivatives, whose core scaffold acts as an isosteric replacement for the benzothiazole present in previous lead series developed in the Baud group. We will discuss our design strategy for isosteric core replacement and subsequent substitution, including how the new heterocyclic aromatic core engages and/or retains key hydrogen bonds with structural water networks in the protein pocket. Through iterative rounds of rationale design, synthesis, and biophysical characterisation, we identified a new derivative displaying low nM affinity ($K_d \approx 700$ nM) and higher ligand efficiency (LE = 0.21) compared to previous series, while maintaining high aqueous solubility (> 1.5 mM). Importantly, this new lead series is accessible in only a few operationally simple and scalable synthetic steps (≈50% overall yield), and possesses a range of still unexplored exit

vectors for future derivatisation. These features will later allow rapid SAR development and discovery of even more potent derivatives. This is a major milestone on this project, which (following further confirmation), promises to unlock new families of potent and cell active derivatives for future cellular assessment in a variety of p53-Y220C driven cancer.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Modifications of the p53 DNA Binding Domain Regulate Sequence Specificity

Authors and institutional affiliation:
Hailey Shankle, Thomas Jefferson University
Khushali Vyas, Thomas Jefferson University
Tisha Kalpesh Desai, Thomas Jefferson University
Theodhora Qyshkollari, Thomas Jefferson University
Samantha Barnada, Thomas Jefferson University
Hideharu Hashimoto, Thomas Jefferson University
Erik Debler, Thomas Jefferson University
Steven McMahon, Thomas Jefferson University

Email of submitting/first author: hailey.shankle@jefferson.edu

Training program first author is enrolled in: PhD in Biochemistry and Molecular Pharmacology

Year of training: 5th year PhD

Abstract:

Approx. 300 words
Suggested format: Purpose, Materials and Methods, Results, Conclusions

The tumor suppressor p53 is a sequence-specific transcription factor responsible for regulating gene expression and consequent cell fate decisions. A central characteristic of this process is p53 binding as a tetramer to its regulatory DNA motifs of the consensus sequence (RRRCWWGYYY(N)RRRCWWGYYY), which is unusually long and tolerates remarkable degeneracy. p53 utilizes distinct DNA binding modes at response elements (REs) linked to different cell fates. For example, REs of A/T-rich pro-survival genes are recognized with high affinity, while G/C-rich cell death REs are recognized with lower affinity. Under cellular stress, p53 undergoes post-translational modifications, which control the activity of p53 in response to DNA damage. One example is the acetylation of K120, a DNA contact residue at the tip of the flexible L1 loop of the p53 DNA binding domain. DNA damage activates signaling pathways that culminate in the acetylation of K120, which in turn correlates with the induction of apoptosis. Published crystal structures suggest acetylation of K120 causes retraction of the L1 loop, as acetylation expands the conformational space of this residue.

	This potentially enables a different binding mode of p53. Here, we utilize a p53 mutant with two L1 loop substitutions: S121F and V122G (FG), as a tool to better understand sequence-specific nuances that p53 utilizes to determine cell fate, as these mutations impart conformational flexibility to the L1 loop. In the absence of DNA, the L1 loop of the FG p53 mutant adopts a recessed conformation, opposite to WT p53. This mutation-based retraction of the L1 loop allows FG p53 to acquire a distinct binding pattern at known p53 target genes. While FG p53 shows similar binding to wild-type p53 at some previously identified p53 REs, this mutant better recognizes and binds at sequences with spacers between RE half-sites and degenerate neighboring 1.5x half-sites. This raises the exciting hypothesis that p53 with a retracted L1 loop (resulting from either the FG p53 mutations or from DNA-damage-induced acetylation of K120), allows for permissive binding at a new set of neighboring degenerate REs to drive distinctive gene expression patterns and cell fate decisions.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

UHN Princess Margaret Cancer Centre

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Poster Presentations Session #2

THURSDAY APRIL 30th , 2026
2:00-3:30 PM
PGRL Gallery

(updated April 28, 2026)

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: ***Robust activation of p53 as an innovative strategy to enhance the sensitivity of cancer cells to the FAS receptor ligand***

Authors and institutional affiliation:

Łasut-Szyszka B*, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland
Drzyzga A, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland
Smolarczyk R, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland
Jakubowska P, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland
Gdowicz-Kłosok A, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland
Krześniak M, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland
Rusin M, Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland

Email of submitting/first author: **barbara.lasut-szyszka@gliwice.nio.gov.pl**

Training program first author is enrolled in: -

Year of training: -

Abstract:

Purpose
The p53 protein activates the pro-apoptotic *FAS*, which encodes the death receptor for Fas ligand (FasL). Cancer cells exposed to FasL alone undergo apoptosis only to a limited extent. Robust apoptosis is induced only when FasL exposure is preceded by treatment with a novel drug combination consisting of actinomycin D and nutlin-3a (ActD+Nut3a). Nutlin-3a can be replaced by its derivative, idasanutlin. This effect is striking, as within only 5 hours, FasL at a low concentration (20 ng/mL) eliminates the majority of cancer cells. Sensitization of cancer cells by the proposed drug combination is entirely dependent on *TP53* status, yet highly universal across cancer cell lines of diverse origins, including lung and breast cancers, melanomas, and osteosarcomas. Importantly, this effect is markedly reduced in normal human fibroblasts and epithelial cells. These promising results provide a strong rationale for further preclinical studies. The main aim of this project is to determine whether the ActD+Idasanutlin (ActD+Ida) combination sensitizes cancer cells to FasL-induced apoptosis in an *in vivo* model.

Materials and Methods

An *in vivo* xenograft model was employed using Balb/c nude mice injected with human lung cancer A549-luc2 cells. Four experimental groups were established: control, FasL alone, ActD + Idasanutlin, and ActD + Idasanutlin + FasL. The treatment regimen was administered twice. Tumor growth was monitored over time and tumors were subsequently analyzed by hematoxylin and eosin staining to assess tumor architecture, as well as by immunohistochemistry to evaluate cell death (TUNEL assay), tumor vascularization and immune cell infiltration.

Results

Treatment with the combination of ActD + Idasanutlin + FasL resulted in significant inhibition of tumor growth and extensive tumor cell death *in vivo*. Tumor tissues displayed a high proportion of TUNEL-positive cells.

Conclusions

These findings indicate that *TP53* plays a critical role in sensitizing cancer cells to FasL-induced apoptosis and may represent a promising strategy for the development of novel therapeutic approaches targeting tumors with wild-type *TP53*.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **Exploring the Role of Microbiota in Cancer Development in Li-Fraumeni Syndrome.**

Authors and institutional affiliation:

Ong W. Y. Noel, University of Toronto
Giovino Camilla, University of Toronto
Fischer Nicholas, The Hospital for Sick Children
Quaglietta R. Paula, University of Toronto
Kissoondoyal Ashby, The Hospital for Sick Children
Malkin David, University of Toronto

Email of submitting/first author: **noel.ong@mail.utoronto.ca**

Training program first author is enrolled in: *First author is PhD Candidate in University of Toronto, Department of Medical Biophysics*

Year of training: **Year 6**

Abstract:

Purpose:
Li-Fraumeni Syndrome (LFS), an inherited cancer predisposition syndrome caused by germline *TP53* mutations, confers high risk of early-onset malignancies. Microbial factors contribute to a substantial proportion of cancers, but the role of the gut microbiome in LFS-associated tumour susceptibility remains poorly understood. This study aimed to determine whether gut microbiota and/or microbiota-derived metabolites contribute to tumour growth in a mutant p53 background, and to identify metabolic and inflammatory mechanisms underlying this effect.

Methods:
Trp53R172H/WT (mutant) mice and wildtype littermates were treated with an antibiotic cocktail to deplete gut microbiota prior to subcutaneous implantation of MC38 colon adenocarcinoma cells. To isolate the role of microbial metabolites, filtered faecal transplant (FFT) from mutant mice was performed to wildtype recipients. Faecal bile acids were quantified by LC-MS, intestinal NF-κB activation assessed by immunoblotting, inflammatory cytokines measured by ELISA, and gut permeability evaluated using FITC-dextran assays.

Results:
Antibiotic treatment significantly reduced tumour size in mutant mice but had no effect in wildtype controls, indicating a microbiome-dependent tumour-promoting effect of mutant p53. FFT increased tumour growth in wildtype recipients, implicating soluble microbial metabolites from mutant mice promote tumour growth. Untreated mutant mice exhibited elevated faecal bile acids that correlated positively with tumour mass and were reduced by antibiotics. Changes in bile acids were accompanied by increased intestinal NF-κB activation, elevated pro-inflammatory cytokines, systemic inflammatory signatures, and increased gut permeability, all of which were attenuated following microbial depletion.

Conclusion:
Our findings reveal a previously underappreciated mutant p53-microbiome axis in tumourigenesis. Mutant p53-driven upregulation of the mevalonate pathway may expand the reservoir of bile acids, which are then metabolized by the microbiota into pro-inflammatory species that activate NF-κB signalling and promote tumour growth. This work positions mutant p53 as a regulator of host-microbiome metabolic crosstalk and suggests looking beyond tumour cell-intrinsic pathways for LFS tumour surveillance.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:
Metabolic reprogramming in Li-Fraumeni Syndrome underlies the pre-cancer niche and cancer predisposition

Authors and institutional affiliation:

Quaglietta P.R., Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids), Toronto, Canada; Institute of Medical Science, The University of Toronto, Toronto, Canada

Kissoondoyal A., Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids), Toronto, Canada

Ong N.WY., Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids), Toronto, Canada; Department of Medical Biophysics, The University of Toronto, Toronto, Canada

Fischer N.W., Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids), Toronto, Canada

Malkin D., Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids), Toronto, Canada; Department of Medical Biophysics, The University of Toronto, Toronto, Canada; Division of Haematology/Oncology, SickKids, Toronto, Canada.; Department of Paediatrics, The University of Toronto, Toronto, Canada

Email of submitting/first author:
paula.quaglietta@mail.utoronto.ca

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*
PhD, Institute of Medical Science Program

Year of training: *Example: PGY 3*
5th year PhD Candidate

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Li-Fraumeni Syndrome (LFS) is a hereditary cancer predisposition syndrome associated with germline mutations in *TP53* (mutp53). Mutp53 abrogates canonical tumor-suppressive functions, including DNA repair, metabolism, and apoptosis. The accumulation of these effects, along with clonal expansion of metabolically reprogrammed cells, can enhance cell survival and adaptation to stress conditions, priming a pre-cancerous niche. We hypothesize that mutp53 alters metabolism to promote a pre-cancerous primed state. Moreover, metabolic interventions can reverse this state to reduce cancer onset in LFS.

LFS (*Trp53*$^{+/R172H}$) mice and wild-type (WT) littermates were followed across four age cohorts (60, 120, 210, 300 days) and treated with metformin-supplemented drinking water (1mg/mL) or left untreated. At endpoint, plasma, muscle, liver, kidney, spleen, thymus, and brain tissues were collected. Flow cytometry immune profiling was performed on spleen and thymus. All tissues were analyzed by LC-MS/MS untargeted proteomics and Seahorse metabolic assays. All statistics were performed in R.

Longitudinal immune profiling revealed similar splenic and thymic lymphocyte proportions between LFS and WT mice across development. However, LFS mice exhibited exaggerated developmental changes in expression of metabolism-associated exhaustion markers KLRG1 and PD-1 in $CD4^+$, $CD8^+$, and $CD4^+CD8^+$ double-positive T cells. Metformin treatment rescued this phenotype in splenic exhausted double-positive T cells. Proteomic profiling revealed coordinated dysregulation of metabolic and developmental pathways in LFS mice across immune and non-immune tissues, and the effect of longitudinal metformin on the pre-cancer niche. Ongoing validation using Seahorse metabolic assays and *in vitro* studies with patient-derived cells supports a pre-cancer niche phenotype across tissue and cellular contexts, enabling dissection of cell-type-specific contributions to LFS pre-cancer priming.

We demonstrated that metabolic reprogramming occurs systemically in LFS to promote a cancer-primed state. Moreover, pharmacologic metabolic modulation can rescue these changes, suggesting potential a potential cancer interception or treatment for LFS patients to revert the cancer priming phenotype.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:
p53 coordinates nucleotide metabolism and replication fidelity to enforce chromosomal stability

Authors and institutional affiliation:

Banerjee R, University of Toronto
Aquino, B, University of Toronto
Cosper, PF, University of Wisconsin, Madison
Kirsch DG, University of Toronto

Email of submitting/first author:
risha.banerjee@uhn.ca

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Medical Biophysics Graduate program

Year of training: *Example: PGY 3*

3

Abstract:

Purpose:
Chromosomal instability (CIN), or chromosome missegregation during mitosis, is a hallmark of aggressive, p53-mutant cancers. While p53 canonically eliminates cells with high CIN, the upstream mechanisms by which it restrains CIN remain understudied. Preliminary data from our lab suggests that CIN occurs early following p53 loss and is preceded by impaired nucleotide metabolism and replication stress. Here, we investigate how p53 suppresses CIN via regulation of nucleotide homeostasis and associated metabolites.

Materials and methods:
Mouse embryonic fibroblasts (MEFs) were generated from WT, $p53^{fl/fl}$, $p53^{LSL\text{-}25,26/fl}$ (Transactivation domain (TAD) 1 mutant), and $p53^{LSL\text{-}25,26,53,54/fl}$ (TAD1+2 mutant) mice. CIN was quantified as frequency of chromosome missegregation events during mitosis using immunofluorescence microscopy. Nucleotide and metabolite abundance was measured by LC-MS; replication stress was assessed using DNA fiber assays. Western blotting, ELISA, and RT-qPCR measured enzyme activity, protein abundance, and RNA expression respectively. Lung tumors were generated in $Kras^{LSL\text{-}G12D/+};p53^{fl/fl}$, $Kras^{LSL\text{-}G12D/+};p53^{LSL\text{-}25,26/fl}$,

and $Kras^{LSL\text{-}G12D/+}$;$p53^{LSL\text{-}25,26,53,54/fl}$, mice by intra-nasal Adeno-Cre delivery. CIN and gene expression were assessed histologically.

Results:

p53 KO MEFs have increased CIN, faulty replication fork fidelity, altered dNTP abundance, and low expression of Rrm2b, a p53 target involved in nucleotide pool homeostasis. Conversely, $p53^{25,26/-}$ mutant MEFs, which cannot transactivate most canonical p53 targets but retain tumor-suppressive ability, show reduced CIN, preserved replication fork fidelity, and basal nucleotide pools. $p53^{25,26,53,54/-}$ mutants phenocopy p53-deficient MEFs. Exogenous nucleotide supplementation in p53 KO MEFs reduced CIN and DNA damage, implicating nucleotide pool imbalance in CIN onset following p53 loss. This was also observed *in-vivo*: $Kras^{G12D/+}$;$p53^{-/-}$ tumors have reduced Rrm2b expression and elevated CIN and aneuploidy compared to $Kras^{G12D/+}$;$p53^{25,26/-}$ lung tissue.

Conclusions:

Collectively, these data support a model where p53 coordinates nucleotide metabolism and replication fidelity to enforce chromosomal stability. Loss of p53-dependent nucleotide homeostasis predisposes to CIN, while partial retention of transactivation alleviates this phenotype both *in-vitro* and *in-vivo.*

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Variation at the R181 residue of p53 confers loss of p53 DNA binding cooperativity with the retention of mitochondrial-associated apoptosis

Authors and institutional affiliation:
Moses R, University of Pennsylvania; Indeglia A, University of Pennsylvania; Levine A, Dana-Farber Cancer Institute; Hausler R, University of Pennsylvania; Kelly G, University of Pennsylvania; Miller S, Fox Chase Cancer Center; Anez I, Dana-Farber Cancer Institute; Heller M, University of Pennsylvania; Delgado R, University of Pennsylvania; Orr C, University of Pennsylvania; Kohlmann W, Huntsman Cancer Institute; Naumer A, Huntsman Cancer Institute; Vagher J, Huntsman Cancer Institute; Cahill S, Dana-Farber Cancer Institute; Maese L, Huntsman Cancer Institute; Karanicolas J, Fox Chase Cancer Center; Garber J, Dana-Farber Cancer Institute; Murphy M, Wistar Institute; Maxwell K, University of Pennsylvania

Email of submitting/first author: Renyta.Moses@pennmedicine.upenn.edu

Training program first author is enrolled in: Cell and Molecular Biology (Cancer Biology) Graduate Program at the University of Pennsylvania

Year of training: PhD Candidate Year 5

Abstract:

Purpose

p53 primarily acts as a transcription factor and binds to target sites on DNA cooperatively as a tetramer. This cooperative binding is mediated by salt-bridge interactions between p53 residues E180 and R181 from two different p53 monomers. Variants at the R181 residue are one of the most identified *TP53* variants by germline genetic testing, however the mechanism by which these variants disrupt p53 tumor suppression is not understood.

Materials and Methods

To investigate the clinical phenotype of p53 R181-variant carriers, we compared cancer incidence, type, and age of onset between families with R181H/C and families with other *TP53* variants in three independent academic centers. To elucidate the mechanism of R181-mutant mediated tumorigenesis, we performed multi-omic and other functional analyses on CRISPR knock-in hemizygous R181H and R181C HCT116, MCF7, and LNCaP cancer cell lines compared to wild-type and knock-out controls.

Results

We show that families with *TP53* p.R181H and p.R181C variants have an attenuated cancer risk phenotype compared to patients with hotspot loss of function *TP53* variants. Despite this clinical phenotype, we find that p53 R181H and R181C variants have significantly diminished ability to transactivate a set of ~300 known p53 target genes. This loss of transactivation ability does not occur through defects in p53 structure or oligomerization, but through reduced cooperative binding to DNA. Despite the complete loss of p53's transcriptional function, we observe residual apoptotic activity in R181H and R181C mutant cells when treated with DNA-damaging agent 5-fluorouracil. We show that the R181 variants retain transcription-independent apoptosis by localizing to the mitochondria and interacting with proapoptotic BAK to induce cytochrome c release and apoptosis.

Conclusions

We report the first separation of function DNA binding domain p53 mutation that results in retention of transcription-independent p53 functions despite loss of p53 transactivation activity, resulting in a reduced penetrance phenotype.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Mechanisms of p53-mediated transcriptional regulation of transposable elements

Authors and institutional affiliation:

Schwarz R, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Germany
*Riege K,*Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Germany
Fischer M, Institute of Biochemistry and Cell Biology, Medical Faculty, Otto von Guericke University, Germany
Hoffmann S, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Germany

Email of submitting/first author:

Robert.Schwarz@leibniz-fli.de

Training program first author is enrolled in:

Postdoc at Leibniz Institute on Aging - Fritz Lipmann Institute (FLI)

Year of training:

second year

Abstract:

The transcription factor and tumor suppressor p53, also known as the guardian of the genome, has been suggested to restrict transposable element (TE) expression. Tumors harboring mutant p53 show increased TE expression levels compared to wild-type p53 tumors. Mechanistically, it has been suggested that p53 directly represses the expression of TEs, particularly LINE-1 retrotransposons. However, most studies of p53-regulated TE expression have been limited to family-level analyses, a few specific TE loci, or exogenous consensus versions of TE families. These approaches have naturally hampered systematic in-depth expression analyses of individual TE instances. Here, we investigated the occurrence of p53 binding sites in TEs and the locus-specific regulation of TEs by p53 on a genome-wide scale. We used p53 binding sites derived from ChIP-seq data with predicted p53 response elements. Our data show that approximately half of all p53 binding sites are located within TEs and that several of these binding sites contribute to promoters and enhancers. Using a locus-specific TE expression analysis strategy, we identified more than 18,000 significantly differentially regulated individual TEs upon p53 activation by Nutlin-3a, with a similar number of up and down-regulated TEs. Importantly, our data suggest that the direct binding of p53 is strongly associated with upregulation of TEs, including LINE-1 instances. This finding is consistent with the general transactivator function of p53, although it contradicts previous studies on TE regulation by p53. We used ATAC-seq and CUT&Tag to identify p53-mediated changes in the chromatin structure at TEs. Our data reveal that p53 makes the DNA accessible and establishes active chromatin states at TEs. Using CAGE-seq data, we discovered that some transcription start sites (TSSs) drive the

expression of entire islands of TEs, enabling transcription factors such as p53 to regulate multiple TEs through one shared promoter.

Taken together, our study maps a comprehensive landscape of p53-regulated TEs in the human genome and highlights the function of p53 as a transactivator of TEs through chromatin remodeling.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Mutant p53 confers chemoresistance by activating KMT5B-mediated DNA repair pathway in nasopharyngeal carcinoma

Authors and institutional affiliation:

Haidan Luo, *The University of Texas Health Science Center at Houston, USA and Sun Yat-Sen University, China*
Mo-Fan Huang, *The University of Texas Health Science Center at Houston, USA*
An Xu, *The University of Texas Health Science Center at Houston, USA*
Donghui Wang, *The University of Texas Health Science Center at Houston, USA and Sun Yat-Sen University, China*
Julian A. Gingold, *Einstein/Montefiore Medical Center, USA*
Jian Tu, *The University of Texas Health Science Center at Houston, USA*
Ruoyu Wang, *The University of Texas Health Science Center at Houston, USA*
Zijun Huo, *The University of Texas Health Science Center at Houston, USA*
Yen-Ting Chiang, *The University of Texas Health Science Center at Houston, USA and China Medical University, Taiwan*
Kuang-Lei Tsai, *The University of Texas Health Science Center at Houston, USA*
Jie Su, *Accutar Biotech, USA*
Danielle A. Bazer, *Stony Brook University, USA*
Mien-Chie Hung, *China Medical University and Asia University, Taiwan*
Canmao Xie, *Sun Yat-Sen University, China*
Yubiao Guo, *Sun Yat-Sen University, China*
Dung-Fang Lee, *The University of Texas Health Science Center at Houston, USA*
Huiling Yang, *Sun Yat-Sen University, China*
Ruiying Zhao, *The University of Texas Health Science Center at Houston, USA*

Email of submitting/first author:

Ruiying.zhao@uth.tmc.edu; luohaid@mail2.sysu.edu.cn

First author: Haidan Luo

Training program first author is enrolled in:

PhD program in Molecular Medicine at the Sun Yat-sen University

Year of training:

PGY 3

Abstract:

Purpose:

Nasopharyngeal carcinoma (NPC) is prevalent in East and Southeast Asia and is commonly treated with radiotherapy combined with chemotherapy, including 5-fluorouracil (5-FU). However, therapeutic efficacy is often limited by the development of chemoresistance. Although p53 mutations contribute to 5-FU resistance in several cancers, their role in NPC remains poorly defined. This study aimed to investigate the contribution of mutant p53 to 5-FU resistance in NPC and to explore potential therapeutic strategies to overcome this resistance.

Materials & Methods:

NPC cell lines and tumor samples harboring the p53(R280T) mutation were analyzed to assess drug response and gene expression changes. DNA damage, cell viability, and tumor growth assays were used to evaluate 5-FU sensitivity. Chromatin immunoprecipitation and gene expression analyses were performed to identify p53(R280T) target genes. Loss-of-function studies were conducted to assess the role of KMT5B in chemoresistance. A compound screening approach was used to identify regulators of KMT5B expression, followed by in vitro and in vivo evaluation of combination therapy.

Results:

We identified p53(R280T) as a gain-of-function mutation that promotes 5-FU resistance in NPC by upregulating DNA repair genes. Mutant p53 directly binds to the promoter of the DNA repair–associated gene KMT5B, leading to its increased expression. Depletion of KMT5B restored 5-FU–induced DNA damage and enhanced drug sensitivity. Curcumin was identified as an effective down regulator of KMT5B, and combined treatment with 5-FU and curcumin significantly suppressed NPC tumor growth.

Conclusions:

These findings reveal a previously unrecognized role of mutant p53-driven DNA repair in NPC chemoresistance and suggest that targeting the p53–KMT5B axis may represent a promising strategy to enhance 5-FU efficacy in NPC patients.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **The role of p53 in early primary invasion**

Authors and institutional affiliation:

Zou L.#* , *Humaney M.**, *Zhang L.#*, *Su B.#*& *Ben Neriah Y.**, .

#Shanghai Institute of Immunology, Department of Immunology and Microbiology, Shanghai Jiao Tong University School of Medicine

**The Lautenberg Center for Immunology and Cancer Research, Hebrew University of Jerusalem*

Email of submitting/first author: lang.zou@mail.huji.ac.il

Training program first author is enrolled in: PhD in biomedical science

Year of training: *PhD 6th year*

Abstract:

Purpose: Loss of the tumor suppressor p53 is strongly associated with metastatic progression and poor prognosis in colorectal cancer, yet its role in restraining the earliest stage of invasion remains unclear. This study investigates how p53 loss enables epithelial cells to acquire invasive and tumor-initiating properties during primary invasion.

Materials and Methods: We used an inducible, intestinal-specific CKIα and p53 double-knockout (DKO) mouse model to capture early invasive events in vivo. Cellular states and lineage transitions were examined by high-definition spatial transcriptomics, with spatial proximity analyses to assess tumor–microenvironment interactions.

Results: Combined loss of p53 and CKIα caused rapid epithelial destabilization and enrichment of $Prox1^{+}$ epithelial cells invading the villous lamina propria. These cells exhibited strong tumor-initiating capacity ex vivo, indicating a stem-like invasive state normally suppressed by p53. Spatial analyses identified a non-canonical stem-like population distinct from $Lgr5^{+}$ intestinal stem cells. Cell trajectory inference demonstrated that $Prox1^{+}$ cells originate from differentiated villus epithelium, revealing a p53-dependent barrier to

epithelial plasticity. Spatial profiling further uncovered coordinated remodeling of the tumor microenvironment, with enrichment of myeloid cells adjacent to invading Prox1^{+} cells, suggesting that p53 loss promotes invasion through both epithelial reprogramming and microenvironmental signaling.

Conclusions: These findings reveal a previously unappreciated role for p53 in suppressing primary invasion by constraining epithelial plasticity and tumor–microenvironment communication. Loss of p53 activates a Prox1-associated early primary invasion that highlight new opportunities for early prognosis and clinical interventions.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: MDM2 binds and suppresses RNA polymerase III to restrain the innate immune response to cytosolic DNA.

Authors and institutional affiliation:

Sabrina Weber, Department of Molecular Oncology, University Medical Center Göttingen
Valentina Manzini, Department of Molecular Oncology, University Medical Center Göttingen
Hannah Schotte, Department of Molecular Oncology, University Medical Center Göttingen
Helene Dietrich, Department of Molecular Oncology, University Medical Center Göttingen
Prof. Matthias Dobbelstein, Department of Molecular Oncology, University Medical Center Göttingen

Email of submitting/first author:

Sabrina.weber@med.uni-goettingen.de

Training program first author is enrolled in:

I am part of the training program of molecular medicine at the University Medical Center Göttingen

Year of training: *4th PhD year*

Abstract:

The oncoprotein MDM2 is widely recognized as the principal negative regulator of p53, thereby controlling the expression of numerous RNA polymerase II (Pol II)-dependent genes involved in cell cycle arrest and apoptosis. Beyond this canonical role, MDM2 also engages in transcriptional regulation independently of p53, for instance through interactions with polycomb repressor complexes. Here, we identify RNA polymerase III (Pol III) as an additional target of MDM2 function. Using two complementary chemical tools – a small-molecule MDM2 antagonist (MI-1061) that releases p53 and augments MDM2 expression, and a PROTAC degrader (MD-224) that simultaneously activates p53 and promotes MDM2 ubiquitination and proteasomal destruction – we dissected the role of MDM2 in cells with amplified MDM2. Elevated MDM2 suppressed the transcription of Pol

III-dependent genes encoding tRNA or 5S ribosomal RNA. Mechanistically, we found the aminoterminal domain of MDM2 associated with the catalytic subunit of Pol III, revealing a molecular link between MDM2 and the Pol III machinery. Because Pol III also acts as a cytosolic DNA sensor that converts DNA into double-stranded RNA to trigger RIG-I–TBK1–IRF3–dependent signaling, we asked whether MDM2 modulates this pathway. Indeed, enhanced MDM2 expression markedly attenuated the induction of innate immunity genes such as *CXCL10*, *OAS1*, and *MX1* following transfection with Poly(dA:dT). Similarly, DNA damage induced by a radiomimetic agent activated Pol III–dependent innate signaling, and this was again blunted by high MDM2 levels, resulting in enhanced cell survival. Taken together, our findings establish MDM2 as a previously unrecognized suppressor of Pol III–dependent transcription in response to cytosolic DNA. This expands the repertoire of MDM2 functions beyond repression of p53 and Pol II activity, positioning MDM2 as a broad regulator of transcription and innate immunity.

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Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: **Structure-Based Rational Design of a High-Affinity DARPin for Reactivating p53 Function**

Authors and institutional affiliation:
Yüksel B, IMPRS Max Planck Institute for Biophysics; Goethe University Frankfurt
Münick P, Goethe University Frankfurt
Heftel J, IMPRS Max Planck Institute for Biophysics; Goethe University Frankfurt
Mavridi D, Goethe University Frankfurt
Joerger A, Goethe University Frankfurt
Takizawa Y, The University of Tokyo
Kurumizaka H, The University of Tokyo
Volker Dötsch, Goethe University Frankfurt

Email of submitting/first author: buesra.yueksel@biophys.mpg.de

Training program first author is enrolled in: International Max Planck Research School (IMPRS) of Max Planck Institute for Biophysics, 2022-2027

Year of training: 3

Abstract: Mutations in the DNA binding domain (DBD) of p53 and its degradation by viral proteins, such as HPV E6, lead to the loss of its vital functions, thereby promoting cancer formation. We identified a DARPin, designated c10, which specifically targets the p53-DBD and can reactivate p53 function in both mutation-induced and viral degradation scenarios. However, the affinity of c10-DARPin to the p53-DBD is relatively low at physiological temperatures, making it unsuitable for therapeutic applications.
To enhance the affinity of c10-DARPin, we employed a structure-based rational design approach. This involved a series of biophysical characterizations to identify the optimal binder. Isothermal titration calorimetry (ITC) was performed at various temperatures to assess the thermodynamic parameters of the c10-DARPin-p53-DBD interaction. Differential scanning fluorimetry (DSF) was used to evaluate the thermal stability of the complexes. X-ray crystallography was conducted to obtain high-resolution structural data, elucidating the molecular interactions between c10-DARPin and the p53-DBD.
From these comprehensive biophysical analyses, the best binder was selected based on its affinity, stability, and structural compatibility. This optimized c10-DARPin variant was then evaluated in cell culture assays to compare its efficacy. Specifically, we conducted HPV degradation assays, transactivation (TA) assays, quantitative PCR (qPCR), and western blot analyses using endogenous cell lines to assess the reactivation of p53 function. Additionally, we investigated the impact of c10-DARPin binding on p53 tetramerization and DNA binding using

Cryo-EM analysis of the p53-c10-nuclesome complex. This approach allowed us to gain detailed insights into the structural and functional effects of c10-DARPin on p53, paving the way for its potential therapeutic application while our structure-based rational design strategy significantly enhanced the affinity.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Early molecular signatures of *TP53* loss of heterozygosity in Li-Fraumeni Syndrome

Authors and institutional affiliation:

Stack H, University of Toronto; The Hospital for Sick Children
Kissoondoyal A, The Hospital for Sick Children
Malkin D, University of Toronto; The Hospital for Sick Children

Email of submitting/first author:

hailey.stack@sickkids.ca

Training program first author is enrolled in:

University of Toronto PhD in Medical Biophysics

Year of training:

Year 1

Abstract:
Li-Fraumeni syndrome (LFS) is a hereditary cancer-predisposition disorder caused by germline *TP53* mutations. Mutant p53 disrupts DNA-damage repair and cell-cycle control, leading to an elevated cancer risk of ~40% by adolescence and nearly 100% over a lifetime. Recent work showed that 86% of LFS tumors exhibit loss of the wild-type (WT) *TP53* allele (loss of heterozygosity (LOH)), an alteration not observed in healthy tissues. Importantly, LOH appears to arise years before tumor diagnosis, likely during prenatal or early postnatal development, implicating it as an early driver of precancer evolution in LFS. While *TP53* LOH is well characterized in sporadic cancers with somatic *TP53* mutations, its role in LFS tumor initiation remains poorly understood. To address this, we leverage LFS patient-derived dermal fibroblasts collected either years before or after cancer diagnosis. Using droplet digital PCR, we quantify WT and mutant *TP53* allelic ratios over time in culture. Fibroblasts collected after a patient's cancer diagnosis show a significant enrichment of the mutant allele, suggesting possible WT *TP53* loss, compared fibroblasts obtained before diagnosis maintained stable allelic balance. Single-cell RNA sequencing will be applied to uncover transcriptional differences between cells with or without allelic imbalance. This will enable identification of changes in gene-expression and biological pathways altered before, during, and after WT *TP53* loss. These LOH-associated signatures will then be mapped to a

Trp53$^{R172H/+}$ LFS mouse model across embryonic, postnatal, and tumor stages, determining when and where these precancer signatures emerge *in vivo*. Collectively, this project will generate the first allelic–transcriptomic map of TP53 LOH in LFS fibroblasts, providing insight into the earliest steps of precancer evolution. By uncovering pathways uniquely disrupted across LOH states, we aim to identify mechanistic drivers of early tumor susceptibility and highlight potential windows for cancer prevention or interception in LFS.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Harnessing Mutational Signatures to Pinpoint The Development Of Chemoresistance in Metastatic and Relapsed Osteosarcoma

Authors and institutional affiliation:

- Blay, S. Hospital for Sick Children
- Layeghifard, M. Hospital for Sick Children
- Davidson, S. Hospital for Sick Children
- Choi, T. Hospital for Sick Children
- Schwertschkow, A. Hospital for Sick Children
- Papaemmanuil, E. Memorial Sloan Kettering Cancer Center
- Cowley, M. Children's Cancer Institute Australia
- Spector, L. University of Minnesota
- Onel, K. Roswell Park Comprehensive Cancer Center
- Alexandrov, L. University of California San Diego
- Wunder, J. Mount Sinai Hospital
- Andrulis, I. Mount Sinai Hospital
- Gladdy, R. Mount Sinai Hospital
- Villani, A. Hospital for Sick Children
- Malkin, D. Hospital for Sick Children
- Shlien, A. Hospital for Sick Children

Email of submitting/first author:
sasha.blay@sickkids.ca

Training program first author is enrolled in:
PhD program, Department of Laboratory Medicine & Pathobiology, University of Toronto

Year of training:
5

Abstract:

Chemoresistance remains a major clinical barrier in pediatric osteosarcoma, yet the genomic events underpinning its emergence remain poorly defined. For my graduate studies, I leveraged whole genome sequencing of 218 osteosarcoma tumour samples from 176 patients across 8 pediatric cancer cohorts, integrating longitudinal and post-treatment samples representing advanced disease to reconstruct evolutionary trajectories from diagnosis to metastasis or relapse.

Genome raw sequencing files were processed in-house using established pipelines, or when unavailable, mutation and copy number estimation Mutect2 and PURPLE output were used. Subclonal evolution was reconstructed using PycloneVI and PairTree. Mutational signatures were extracted with SigProfileExtractor at both the bulk and subclonal level.

To define early tumour-shaping events, I assessed mutational burden, structural variation, and copy number alterations. Tumour suppressor inactivation, particularly *TP53*, and whole-genome doubling were common and often truncal, preceding clonal diversification. Chromothripsis and kataegis were pervasive, while homologous recombination deficiency was rare. Copy number analyses revealed recurrent deletions in *STAG2*, *ATRX*, and *RB1*, often biallelic and early events, highlighting the dominant role of tumour suppressor loss in early osteosarcoma pathogenesis.

Platinum-associated mutational signatures (SBS31/35) were detected in 47 of 111 treatment-exposed tumours, predominantly in metastatic and relapse samples. These signatures marked chemoresistant subclones, which exhibited clonal bottlenecks, increased cancer cell fractions, and convergent emergence across distinct metastatic sites, implying parallel evolution under therapeutic pressure. Whole genome duplication and elevated ploidy were enriched in SBS+ tumours, suggesting a permissive role for chromosomal instability in resistance development.

Finally, proof-of-concept experiments using circulating tumour DNA (ctDNA) from plasma demonstrated that liquid biopsy can robustly recapitulate platinum-associated signatures found in bulk tumour and detect additional resistant clones not evident in tissue samples. Ongoing work investigates whether ctDNA can serve as a non-invasive biomarker for chemoresistance and track resistant clones over time.

Collectively, these findings reconstruct osteosarcoma's mutational history and identify genomic hallmarks of chemoresistance, with implications for precision surveillance and early detection of relapse.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project:
Elucidating the p53 transcriptome in the Pancreas for Novel PDAC Therapy

Authors and institutional affiliation:

Shunbin Xiong[1], Mitheera V[1], Xiaoping Su[2], Vinod Pant[1], Guillermina Lozano[1]
[1]Department of Genetics, [2] Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA 77030

Email of submitting/first author:

sxiong@mdanderson.org

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026.*
N/A

Year of training:

N/A

Abstract:

p53 activation elicits a range of cellular outcomes, including apoptosis, cell cycle arrest, ferroptosis, and metabolic reprogramming. Previous work from our lab using a mouse model of conditional *Mdm2* deletion to induce global p53 activation revealed only seven common p53 target genes across the pancreas, heart, intestine, ovary, and kidney. These findings underscore the tissue- and cell-type-specific nature of the p53 transcriptional program and provide a mechanistic basis for the diverse cellular responses to p53 activation observed across different tissues.

Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal cancers worldwide, with a 5-year overall survival rate of approximately 10% and a median survival of only 10 months. *TP53* is mutated in ~75% of PDAC cases, and these mutations often correlate with the progression from precursor lesions to invasive carcinoma. Consequently, reactivation of p53 represents an attractive therapeutic strategy for advanced PDAC. However, no mutant p53–reactivating therapies are currently approved for clinical use, highlighting an unmet need to target p53-mediated tumor suppressive pathways in PDAC.

In the pancreas, conditional deletion of *Mdm2* induces acinar-to-ductal metaplasia (ADM) concomitant with activation of 135 unique p53 target genes. To delineate pancreas-specific p53 target genes and pathways in a longitudinal manner, we conditionally deleted *Mdm2* at multiple time points. To generate a comprehensive single-cell atlas of p53 targets in the pancreas, we performed single-cell RNA sequencing (scRNA-seq) and applied cell lineage normalization to ensure robust representation of all pancreatic cell types. Given that PDAC arises from pancreatic ductal epithelial cells that emerge following ADM, we have elucidated pancreatic acinar- and ductal-specific p53 target signatures. To validate our single-cell findings, we overexpressed p53 in primary p53-deficient PDAC cell lines and assessed the expression of the identified target genes. Further functional analyses will involve mechanistically assessing the role of identified p53 target genes in attenuating PDAC progression.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Using fluorescent reporter mice and intravital imaging to investigate the p53 response to oncogene activation driving tumour development

Authors and institutional affiliation:
Annabella F Thomas[1,2], Elizabeth Lieschke[1], Lin Tai[1], Andrew Kueh[1], Edwin Hawkins[1,2], Marco J Herold[1,2], Georgia Atkin-Smith[1,2], Gemma L Kelly[1,2], Andreas Strasser[1,2].

1The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia
[2]The Department of Medical Biology, The University of Melbourne, Melbourne, Victoria, Australia

Email of submitting/first author:

thomas.a@wehi.edu.au

Training program first author is enrolled in:
First author is a full-time postdoctoral researcher at the WEHI in Melbourne, Australia.

Year of training: 1st year of postdoctoral research

Abstract:

The *p53* gene is mutated in ~50% of human cancers and these mutations often contribute to poor responses to cancer therapy. The p53 protein is a master regulator of several cellular responses to diverse stresses, such as activation of oncogenes or DNA damage. It functions as a homo-tetrameric transcription factor that directly transcriptionally regulates ~500 genes, some of which suppress tumorigenesis. This includes *p21*, which is required for p53-mediated induction of cell cycle arrest/cell senescence, and *Puma*, which is critical for p53-mediated apoptosis induction. Deregulated over-expression of the transcription factor c-MYC promotes tumorigenesis by causing aberrant growth and proliferation of cells. As a safeguard against tumorigenesis, deregulated

MYC over-expression can trigger apoptosis through the activation of p19Arf which then activates p53, leading to the expression of pro-apoptotic PUMA.

To investigate the p53-mediated cellular responses promoting tumour suppression, we created two reporter mouse lines in which GFP is knocked into the *p21* locus behind an IRES, or in which the *Puma* coding region was replaced with tdTomato. We also inter-crossed these p21 and Puma reporter mice to explore why certain cells undergo cell cycle arrest/senescence after p53 activation, while others undergo apoptotic cell death. We validated these novel reporter mice through comprehensive flow cytometry analysis and intravital imaging of live mice, revealing reporter activity in diverse immune cell types in various organs, including the bone marrow calvarium.

To investigate the p53-response in a tumorigenic setting, reporter mice were crossed to the *Eμ-Myc* transgenic mouse model, where c-MYC is over-expressed in B lymphoid cells. Flow cytometry analysis of B cell subsets revealed an increase in *Puma* but not *p21* reporter expression, compared to control B lymphoid cells that do not over-express c-MYC. Furthermore, intravital imaging of these mice also revealed a significant increase in *Puma* but not *p21* reporter activity in cells within the bone marrow. Ongoing studies aim to elucidate which p53 target genes, in addition to *Puma*, are induced in response to *MYC* oncogene expression in B lymphoid cells to effect tumour suppression. This will contribute to the understanding of tumour initiation and tumour suppressive responses and may reveal new targets for cancer therapy.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **Structural and functional characterization of the p53–β-catenin interactome**
Authors and institutional affiliation: **Jakov Vuk, Medical University of Graz, Austria** **Benjamin Bourgeois, Medical University of Graz, Austria** **Hermann Habacher, Medical University of Graz, Austria** **Georg Krainer, University of Graz, Austria** **Tobias Madl, Medical University of Graz, Austria**
Email of submitting/first author: **jakov.vuk@medunigraz.at**
Training program first author is enrolled in: **PhD program in Biomolecular Structures and Interactions (BioMolStruct)**
Year of training: **First year of PhD**
Abstract: The tumor suppressor protein p53, a cell-cycle arrest and apoptosis regulator, often called “the guardian of the genome”, and β-catenin, a core component of canonical Wnt signaling pathway, which drives cell proliferation, are dysregulated and highly mutated in many cancers (e.g. colorectal cancer). The two signaling pathways, among other regulators, converge through MDM2, a key negative regulator of p53, creating a molecular network that contributes to uncontrolled proliferation and resistance to apoptosis. Despite their clinical relevance, the mechanisms underlying the coordinated dysregulation of p53 and Wnt signaling remain poorly understood, and the molecular details of the crosstalk between p53 and β-catenin are not fully resolved. We hypothesize that β-catenin directly modulates p53 function by forming a ternary complex with MDM2. Using a divide-and-conquer approach and employing state-of-the-art NMR spectroscopy and biophysical techniques such as ITC (Isothermal Titration Calorimetry), FCS (Fluorescence Correlation Spectroscopy), and FP (Fluorescence Polarization) we localized the binding interfaces between p53 and β-catenin, and determined quantitative binding

affinities and thermodynamic parameters. These findings were validated *in cellulo* with co-immunoprecipitation.

Our binding studies identified the minimal complex of the p53–β-catenin interface, comprising the transactivation domain 2 (TAD2) region of p53 and the N-terminal part of the β-catenin armadillo domain (ArmN). Notably, our data suggest that MDM2 binds β-catenin and forms ternary complex with p53.

Together, these results establish TAD2 of p53 as the key region for driving the interaction between p53 and β-catenin. Furthermore, MDM2 act as a potential modulator of this interaction. Therefore, this project lays the foundation for the rational design of modulators targeting the p53–β-catenin interface, with the potential to restore p53 tumor-suppressor activity in Wnt-driven cancers or attenuate aberrant Wnt activity in p53-compromised tumors.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Decoding Developmental Vulnerabilities in Sonic Hedgehog Medulloblastoma Tumorigenesis

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Escorcia Dominguez J, The Hospital for Sick Children & University of Toronto
Dirks P, The Hospital for Sick Children & University of Toronto

Email of submitting/first author:
juan.escorciadominguez@sickkids.ca

Training program first author is enrolled in:

Developmental, Cancer, and Stem Cell Biology Program at The Hospital for Sick Children.
Department of Molecular Genetics at the University of Toronto.

Year of training: *Example: PGY 3*

Direct-entry PhD, year 2.

Abstract:

Purpose
Medulloblastoma (MB) is the most common pediatric brain tumour. Sonic Hedgehog (SHH)-subgroup MB constitutes one-third of all cases, and is divided into four subgroups. From these, the pediatric SHH-MB (SHH-α) is the deadliest. It is characterized by MYCN amplifications and TP53 inactivating mutations, and represents very high-risk MB. While the granule lineage has been implicated in SHH-α, the specific cell type that undergoes transformation remains unknown. Additionally, the precise developmental window during which transformation occurs has not yet been defined.

Materials and Methods
Our group developed a protocol to differentiate embryonic hindbrain neuroepithelial stem cells (hbNES) into functional granule neurons. This system accurately

recapitulates the granule neuron lineage, the fundamental cell family underlying cerebellar development. We introduced driver mutations into the hbNES in an inducible manner. By controlling when these mutations occur as the cells mature, we seek to investigate how different developmental stages have distinct vulnerabilities to transformation. We assessed the functional differences in culture through proliferation assays, and gene expression changes via quantitative PCR (qPCR) and immunocytochemistry (ICC).

Results

Firstly, TP53 was knocked-out (KO) in hbNES using CRISP-Cas9 technology. TP53 deletion was validated using Western blotting, and a nutlin-3-based assay. Secondly, a Tet-OFF construct was introduced into the AAVS1 locus via homology-directed repair to overexpress MYCN under inducible control. The construct was validated using Sanger sequencing and ICC. Finally, cells underwent differentiation along the granule lineage. Differences at the RNA and protein level were assessed between wild-type and mutant cells through qPCR and ICC, respectively, illustrating the effects of driver mutations at different developmental stages.

Conclusions

Medulloblastoma is the most common malignant pediatric brain tumour, and SHH-α has the poorest prognosis. Ultimately, elucidating the initial steps and developmental vulnerabilities driving tumour initiation will help identify new therapeutic targets. Moreover, our increasing understanding will enable earlier screening and diagnosis, advancing precision care for pediatric MB patients.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:
Deep CRISPR mutagenesis characterizes the functional diversity of *TP53* mutations

Authors and institutional affiliation:
Funk JS[1], Klimovich M[1], Drangenstein D[1], Pielhoop O[1], Hunold P[1], Borowek A[1], Noeparast M[1], Pavlakis E[1], Neumann M[1], Balourdas D-I[2,3], Kochhan K[1], Merle N[1], Bullwinkel I[1], Wanzel M[1], Elmshäuser S[1], Teply-Szymanski J[4], Nist A[5], Procida T[6], Bartkuhn M[6,7], Humpert K[1,8], Mernberger M[1], Savai R[6,9,10,11], Soussi T[12,13], Joerger AC[2,3] and Stiewe T[1,5,6,8,9]

[1]Institute of Molecular Oncology, Marburg University, Marburg, Germany
[2]Institute of Pharmaceutical Chemistry, Goethe University, Frankfurt am Main, Germany
[3]Buchmann Institute for Molecular Life Sciences and Structural Genomics Consortium (SGC), Frankfurt am Main, Germany
[4]Institute of Pathology, Marburg University, Marburg University Hospital, Marburg, Germany
[5]Genomics Core Facility, Marburg University, Marburg, Germany
[6]Institute for Lung Health (ILH), Justus Liebig University, Giessen, Germany
[7]Biomedical Informatics and Systems Medicine, Justus Liebig University, Giessen, Germany
[8]Bioinformatics Core Facility, Marburg University, Marburg, Germany
[9]Universities of Giessen and Marburg Lung Center (UGMLC), German Center for Lung Research (DZL), Giessen, Germany
[10]Cardio-Pulmonary Institute (CPI), Giessen, Germany
[11]Lung Microenvironmental Niche in Cancerogenesis, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
[12]Equipe «Hematopoietic and Leukemic Development», UMRS_938, Sorbonne Université, Centre de Recherche Saint-Antoine, Paris, France
[13]Dept of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Clinical Genetics, Uppsala University Hospital, Uppsala, Sweden

Email of submitting/first author:
julianne.funk@uni-marburg.de

Training program first author is enrolled in:
DZL (Deutsches Zentrum für Lungenforschung) Academy

Year of training:
Second Year Postdoc

Abstract:
The *TP53* mutational landscape harbors more than 2,000 described missense mutations and is altered in approximately half of all human cancers. Precise functional interpretation of these variants is essential for clinical decision-making and personalized oncology.
A saturation genome-editing strategy based on CRISPR-mediated homology-directed repair was used to generate a library of 9,225 *TP53* variants in cancer cells, covering 94.5% of all cancer-associated mutations. This high-resolution dataset enables accurate discrimination between benign and pathogenic variants through quantitative fitness measurements, achieving substantially higher sensitivity than previous large-scale *TP53* screens.
The variant map reveals a broad continuum of functional outcomes, including partially impaired mutants and rare variants that remain responsive to pharmacological reactivation. In addition to protein-level defects, the analysis uncovers splicing alterations and nonsense-mediated mRNA decay as important contributors to *TP53* dysfunction, highlighting mechanisms frequently underrepresented in routine variant assessment.
These findings demonstrate the value of saturation genome editing for comprehensive functional annotation of *TP53*. The resulting dataset provides a powerful resource for improving *TP53* variant classification in clinical genetics and supports more refined approaches to personalized cancer therapy.

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Exploring the prenatal genetic determinants of tumour risk in Li-Fraumeni syndrome: sequencing of newborn screening dried blood spots

Authors and institutional affiliation:
Tanvi Anandampillai, Hospital for Sick Children
Laura Raiti, Hospital for Sick Children
Anita Villani, Hospital for Sick Children
Adam Shlien, Hospital for Sick Children
David Malkin, Hospital for Sick Children
Yiming Wang, Hospital for Sick Children

Email of submitting/first author:
tanvi.anandampillai@sickkids.ca

Training program first author is enrolled in:
Genetics and Genome Biology

Year of training: 1

Abstract:

Exploring the prenatal genetic determinants of tumour risk in Li-Fraumeni syndrome: sequencing of newborn screening dried blood spots

Li-Fraumeni Syndrome (LFS), caused by germline *TP53* mutations, is an inherited cancer predisposition syndrome with a lifetime cancer risk approaching 100%. LFS is linked to a broad spectrum of early-onset cancers, including but not limited to brain cancer, breast cancer, osteosarcomas and soft-tissue sarcomas. There is broad phenotypic heterogeneity in the age of onset and tumour subtype, even in affected family members with identical *TP53* variants. We have previously shown that this heterogeneity is associated with other germline genomic/epigenomic variants and somatic variants. Surprisingly, loss of heterozygosity (LOH) of *TP53*, a key driver of tumorigenesis, is predicted to arise years before tumour diagnosis. Considering the early onset of tumours in LFS, it is likely that the LOH of *TP53* and other somatic variants arose prenatally with further clonal evolution after birth, which may dictate postnatal cancer risks in these patients.

To explore the origin of these prenatal cancer driver mutations, we performed genome sequencing and comprehensive cancer panel sequencing (864 cancer-associated genes) of

newborn screening dried blood spots (NBS DBS) from 20 LFS patients and 5 healthy controls. We focused on *TP53* LOH and somatic cancer driver mutations, accompanied by global mutational landscape analysis. These findings will be compared to sequencing data from matched postnatal germline and tumour samples, readily accessible for 7 LFS patients in our cohort. Our study has the potential to impact surveillance practices and uncover new links between prenatally acquired mutations and pediatric cancers, revealing novel approaches for early diagnosis, prevention, and interception.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:

Design of Pharmacological Chaperones for Mutated p53

Authors and institutional affiliation:

*Syme T, University of Southampton, Bioinformatics Institute A*STAR Singapore*
Baud M, University of Southampton
*Verma C, Bioinformatics Institute A*STAR Singapore*

Email of submitting/first author:

ts8g23@soton.ac.uk

Training program first author is enrolled in:

PhD

Year of training:

3

Abstract:

Purpose

The thermally destabilising Y220C mutation in p53 inactivates the protein at physiological temperatures, disrupting crucial functions such as tumour suppression. However, this mutation also presents an opportunity for therapeutic intervention by creating a transient surface pocket that can be targeted by small molecules. This work aims to develop and evaluate an active-learning (AL) framework to prioritise compounds for alchemical free energy calculations, reducing computational expense while accelerating the identification and optimisation of hit and lead ligands targeting p53-Y220C.

Materials & Methods

We developed an iterative AL workflow to benchmark combinations of molecular representations, machine-learning models, and acquisition strategies for relative binding free energy (RBFE) calculations. Candidate molecules were generated via reaction-based enumeration, with an emphasis on synthetically accessible transformations. In each iteration, a subset of compounds was selected for RBFE evaluation, and the resulting affinity change estimates were used to update the predictive model. Performance of the different strategies was assessed against random selection using top-k recall metrics under fixed computational budgets representative of practical RBFE campaigns.

Results

Across multiple independent runs, the AL strategy consistently identified high-affinity ligands more efficiently than random selection. The approach enabled faster and more directed exploration of chemical space while avoiding the prohibitive computational cost of exhaustive RBFE screening, and without compromising the robustness of free energy predictions. Selected top predicted binders found with these methods were synthesised and experimentally validated.

Conclusions

These results demonstrate that active learning can substantially improve the efficiency of RBFE-driven ligand discovery for challenging p53 mutants such as Y220C. By reducing computational cost while maintaining effective identification of affinity-enhancing ligand modifications, this framework enables more tractable chemical space exploration and provides a scalable strategy for integrating physics-based free energy methods into early-stage drug discovery.

Please email your submission to us at XX. Please use the following subject heading:
Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Title of study/project: **Promoter G-Quadruplexes as Structure-Encoded Switches of p53 Family Activity: Selective Enhancement of Partial-Function Mutants**

Authors and institutional affiliation:

Brázda V.[1, 2], Kratochvilová L[1, 2], Vrtalová L. [L1, 2], Monti P.[3], Rieko O.,[4] Inga A.[5]

1 Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, 61200 Brno, Czech Republic
2 Faculty of Chemistry, Brno University of Technology, 612 00 Brno, Czech Republic
3 Neuro-oncology and Mutagenesis, IRCCS Azienda Ospedaliera Metropolitana, 16132 Genoa, Italy
4 Laboratory of Fundamental Oncology, National Cancer Center Research Institute, Tokyo, Japan
5 Laboratory of Transcriptional Networks, Department of Cellular, Computational and Integrative Biology, CIBIO, University of Trento, via Sommarive 9, 38123 Trento, Italy

Email of submitting/first author: **vaclav@ibp.cz, vabdna@gmail.com**

Abstract:

Background: Non-canonical DNA secondary structures, particularly G-quadruplexes (G4s), are enriched near transcription factor response elements and can modulate gene expression. How these structures tune the activity of p53 family proteins—including wild-type (WT) p53 and clinically relevant partial-function mutants—remains an open question with clear therapeutic implications. To define how native and engineered promoter G-quadruplex-forming sequences (G4FS) shape transactivation by p53 family proteins, and to determine whether G4 topology provides a context-dependent advantage to mutant p53 relative to WT p53.

Methods: We combined (i) an isogenic yeast luciferase reporter platform harboring human promoter-derived or modeled G4FS adjacent to p53 response elements (REs), with (ii) chromatin immunoprecipitation in myeloid leukemia cell lines profiling target occupancy of WT p53 and oncogenic mutants (R175H, Y220C, M237I, R248Q, R273H, R282W), and (iii) biophysical assays confirming G4 formation. Transactivation was quantified across expression ranges and contrasted with NF-κB family proteins to assess specificity.

Results: Promoter G4FS consistently increased transactivation by partial-function p53 family variants (e.g., cancer-associated p53 mutants; ΔN-p63α), while attenuating or re-wiring WT p53 responses depending on expression level. Notably, PQS upstream of the RE boosted p53-R282W-driven reporter expression at low p53 abundance, whereas it reduced WT p53 output; at high expression, PQS presence decreased reporter activity irrespective of G4 position. ChIP revealed a significant association of WT p53 and R282W target sites with putative G4 sequences, aligning occupancy with the observed functional outcomes. In contrast, NF-κB transactivation was predominantly inhibited by G4FS in a promoter-dependent manner, underscoring TF-specific topology effects.

Conclusions: G-quadruplexes act as structure-encoded switches that differentially tune p53 family transactivation, selectively amplifying weaker or partial-function variants while constraining WT activity in a dose-dependent manner. These findings highlight DNA topology as a critical determinant of p53 signaling and suggest structure-aware strategies—such as G4-targeting ligands or promoter engineering—to functionally "rescue" mutant p53 and refine classification of TP53 variants.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: A New Layer of p53 Control: The TIRR Pathway

Authors and institutional affiliation:
Susan Kilgas[1], Klara Kuret Hodnik[2], Niek Van Wietmarschen[1], Samantha Schreiter[1], Jędrzej Chrzanowski[3], Wojciech Fendler[3], Jernej Ule[4], Dipanjan Chowdhury[1,5]
[1] Division of Radiation and Genome Stability, Department of Radiation Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
[2] National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia
[3] Department of Biostatistics and Translational Medicine, Medical University of Lodz, Lodz, Poland
[4] The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK; Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, Queen Square, London WC1N 3BG, UK; National Institute of Chemistry, Hajdrihova 19, 1001 Ljubljana, Slovenia
[5] Correspondence: Dipanjan_Chowdhury@dfci.harvard.edu

Email of submitting/first author: susan_kilgas@dfci.harvard.edu

Training program first author is enrolled in: Postdoctoral Fellowship; Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Year of training: Postdoctoral Fellow (Year 4)

Abstract:

Purpose:
Restoring tumor suppressor p53 activity remains a central goal in cancer therapy, yet current approaches are limited by toxicity and lack of selectivity. While p53-dependent transcriptional programs are well characterized, how the stability of p53 target transcripts is selectively controlled remains poorly understood. Here, we investigate the RNA-binding protein TIRR (Tudor Interacting Repair Regulator) as a modulator of p53 target transcript stability and explore its potential as a selective node shaping p53 pathway output.

Methods:
We integrate genome-wide RNA-binding (iCLIP-seq), transcriptomic profiling (RNA-seq), nascent transcription analysis (PRO-seq), RNA stability assays, and functional perturbation approaches to define how TIRR regulates a subset of p53 target transcripts and influences tumor suppressive outcomes.

Results:
We identify TIRR as a bimodal regulator of p53 signaling. Consistent with prior work, TIRR limits 53BP1-dependent p53 transactivation without altering p53 protein stability. In parallel, we uncover a distinct post-transcriptional function in which TIRR binds a subset of p53 target transcripts, preferentially at 3′ untranslated regions (3′UTRs), and promotes their destabilization. Mechanistically, TIRR associates with components of the RNA exosome complex, linking selective RNA binding to transcript destabilization and degradation.

Conclusions:
This work reveals a dual role for TIRR in transcriptional and post-transcriptional regulation of the p53 pathway, integrating 53BP1-dependent and -independent mechanisms to shape p53 target gene output. By coupling RNA binding to selective transcript destabilization via the RNA exosome, TIRR defines a previously underappreciated layer of p53 regulation and highlights a potential avenue for selective, non-toxic p53 reactivation in cancer therapy.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: DARPins for recognition and degradation of p53 mutants

Authors and institutional affiliation:

Käthe Lehnhoff[1], Philipp Münick[1], Birgit Dreier[2], Jonas V. Schaefer[2], Christian Osterburg[1], Andreas Plückthun[2], and Volker Dötsch[1]

[1] Institute of Biophysical Chemistry and Center for Biomolecular Magnetic Resonance, Goethe University Frankfurt, Germany

[2] Department of Biochemistry, University of Zurich, Switzerland

Email of submitting/first author: lehnhoff@bpc.uni-frankfurt.de

Training program first author is enrolled in:
PhD student at the Institute of Biophysical Chemistry, Goethe University Frankfurt

Year of training: PhD Year 2

Abstract:

The transcription factor p53 is a key tumor suppressor in human somatic cells. In half of all cancers, p53 is inactivated due to mutations, which are predominantly clustered in the DNA-binding domain (DBD). These mutations can remove essential DNA interaction sites or destabilize the DBD, leading to conformational changes or unfolding of the protein. Mutant p53 can exert a dominant-negative effect on the wild-type protein and other proteins, including p53 family members.

To provide a novel tool for detection and degradation of p53 mutants in mammalian cells as an alternative to conventional antibodies, we selected a DARPin that targets destabilized p53 DBD mutants. We demonstrated in pull-down assays and immunofluorescence staining, that the selected DARPin C6 selectively binds to destabilized DBD mutants without

interacting with the wild-type protein or DNA contact mutants in mammalian cell lines. Interestingly, DARPin C6 binds to destabilized isoforms harboring deletions within the DBD, too. Through the fusion of the DARPin with an E3 ligase, we generated bioPROTACs to induce the targeted degradation of destabilized DBD mutants and identified DARPin C6-SPOP as the most efficient bioPROTAC for various mutants. Furthermore, in H1299 cells co-expressing p53 wild-type and mutant protein, the selective degradation of the mutant using DARPin C6-SPOP reactivated the wild-type.

These results demonstrate the potential of DARPins as versatile tools in research and therapeutics for the specific detection and degradation of p53 mutants.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Unraveling mechanisms of RFX7 regulation

Authors and institutional affiliation:
Schwab K, University Hospital, Magdeburg
Özcan G, University Hospital, Jena and Leibniz Institute on Aging, Jena
Schenk T, University Hospital, Jena
Hoffmann S, Leibniz Institute on Aging, Jena
Fischer M, University Hospital, Magdeburg

Email of submitting/first author:
katjana.schwab@med.ovgu.de

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

In Germany, it is not common to be enrolled in dedicated training programs.

Year of training: *Example: PGY 3*
Early Postdoc (first year)

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose: We previously showed that the tumor suppressor p53 activates the transcription factor RFX7 in response to p53-activating stress signals and that this activation is associated with an unknown post-translational modification (PTM) of RFX7. Here, we elucidate the PTM underlying RFX7 regulation.

Materials and Methods: U2OS *RFX7*-knockout cells were reconstituted with doxycycline-inducible FLAG–RFX7, and p53 was activated using Nutlin-3a. RFX7 phosphorylation was assessed by electrophoretic mobility shifts on immunoblots and validated using a dephosphorylation assay. To identify differentially phosphorylated amino acids, phosphoproteomics was performed comparing Nutlin-3a and control-treated cells. To identify proteins that interact with hypo- or hyper-phosphorylated RFX7, co-immunoprecipitation was combined with mass

spectrometry analysis (coIP–MS). RFX7 activity was inferred by analyzing RFX7 target gene expression by RT–qPCR and immunoblotting (e.g., PDCD4, PIK3IP1, MXD4, and DDIT4) in response to established kinase inhibitors.
Results: We find that the lower-migrating form of RFX7 reflects a hypo-phosphorylated state. Thus, RFX7 is kept inactive by phosphorylation. Upon p53 activation, RFX7 becomes hypophosphorylated. Phosphoproteomics reveals at least a dozen RFX7 sites that are phosphorylated when RFX7 is inactive. CoIP–MS identified candidate RFX7 interacting proteins. Importantly, we identified kinases that enriched for binding to inactive, hyperphosphorylated RFX7. Using established kinase inhibitors, we validated a kinase as being critical for RFX7 phosphorylation and inactivation.
Conclusions: These data suggest a model whereby RFX7 activity is largely regulated by phosphorylation. p53 signaling impairs RFX7 phosphorylation and thereby activates RFX7 and RFX7 target gene expression. The kinase that inactivates RFX7 reveals a potential role of RFX7 in multiple cancer-relevant pathways.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: **EXOp53 – An Extracellular Vesicle Based Platform for Functional p53 Replacement In Vivo.**

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Ksenia Magidey, AnserBio
Bracha Sreibman, AnserBio
Albina Lin, AnserBio
Ishai Luz, The Shraga Segal Department of Microbiology, Immunology & Genetics, Faculty of Health Sciences, Ben-Gurion University
Tomer Cooks, The Shraga Segal Department of Microbiology, Immunology & Genetics, Faculty of Health Sciences, Ben-Gurion University
Alex Tendler, AnserBio

Email of submitting/first author:

kseniam@anser-bio.com

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

High-Resolution Extracellular Vesicle Characterization Using CytoFLEX Nano

Year of training: *Example: PGY 3*

2025

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

EXOp53 – An Extracellular Vesicle Based Platform for Functional p53 Replacement In Vivo

Purpose

The tumor suppressor p53 is lost or functionally impaired in majority of human cancers, motivating efforts to restore p53 activity therapeutically. Extracellular vesicles (EVs) have emerged as drug delivery platform due to their capacity to transport bioactive cargo and modulate intracellular signaling. Here, we present a p53 replacement strategy based on EVs derived from chicken corneal epithelial cells, which harbors high levels of wild type p53.

Materials & Methods

EXOp53 EVs were purified using qEV and concentrated using 100-kDa TFF. EVs were characterized by flow cytometry following fixation and permeabilization for intravesicular p53 detection or

fluorescently labeled (Aco490) for uptake and biodistribution studies. In vitro potency was evaluated using Annexin V/PI assay in multiple cancer cell lines harboring p53 mutations. In vivo efficacy was assessed in Nude mice bearing Colo320DM xenografts (dominant-negative p53 mutation, DNE), with daily treatment initiated at tumor volumes of ~100 mm^3.

Results

Intravesicular flow cytometry demonstrated robust endogenous p53 content, with >60% p53-positive EVs across three independent batches. Uptake studies in HCT116 p53-knockout cells revealed nuclear accumulation of delivered p53 within 2-6 hours, accompanied by a sustained reduction in MDM2 levels. Biodistribution analyses showed preferential accumulation of EVs in tumors as early as 2 hours post-injection, with minimal uptake in other organs.

EXOp53 treatment selectively suppressed proliferation of cancer cells by inducing apoptosis and cell-cycle arrest. In vivo, monotherapy significantly inhibited tumor growth in Colo320DM xenografts without adverse effects on body weight. Tumor-derived cancer cells isolated from treated mice retained cell-cycle arrest when cultured ex vivo. Immunohistochemical analysis further revealed lymphocyte infiltration, with a significant increase in $CD8^+$ T-cell presence in EXOp53-treated tumors.

Conclusions

These data demonstrate that EXOp53 enables tumor-targeted delivery of functional p53 and exerts antitumor activity in DNE p53 models supporting its potential as a novel p53 replacement therapeutic strategy.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Elucidating the mechanism of viral mimicry induction following p53 loss

Authors and institutional affiliation:

Mackenzie Wienke, MD Anderson Cancer Center
Kevin Wanniarachchi, MD Anderson Cancer Center
Ella Adamson, Princess Margaret Cancer Centre: University Health Network
Matt Maitland, Princess Margaret Cancer Centre: University Health Network
Junwoo Lee, MD Anderson Cancer Center
Brian Raught, Princess Margaret Cancer Centre: University Health Network
Charles Ishak, MD Anderson Cancer Center

Email of submitting/first author: mawienke@mdanderson.org

Training program first author is enrolled in: UT Health Houston MD Anderson Cancer Center Graduate School of Biomedical Sciences

Year of training: Pre-Candidacy PhD Student: Year 2

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

High grade serous ovarian carcinoma (HGSOC) is one the deadliest gynecological cancers with over 95% of patients having a mutation in *TP53*. In these cancers, it is observed that transposable elements (TEs) become dysregulated which are normally transcriptionally silenced to prevent mutagenic consequences. If TEs become accessible, their transcription can produce double stranded RNAs (dsRNAs) which resemble viral dsRNA genomes, and activate an anti-viral response known as 'viral mimicry'. Previous studies (Ishak *et. al*, 2025), suggest that the loss of p53 leads to induction of 'viral mimicry'. In this project, we seek to understand the molecular mechanism of p53-mediated dsRNA silencing to improve our understanding of repetitive elements role in HGSOC initiation.

To study direct effects of p53 on silencing TEs, we performed BioID experiments and identify transcriptional regulatory proteins that interact with p53. We will then conduct co-immunoprecipitation experiments to determine direct binding partners with endogenous p53 and GST-tagged p53. From identified p53 binding partners, we will perform genetic knockouts to analyze the roles of prioritized candidates towards silencing dsRNAs and 'viral mimicry'.

To investigate the indirect effects of p53 on epigenetic regulation of dsRNA, we will analyze existing RNA-seq datasets to evaluate changes in gene expression and CUT&RUN data to analyze p53 binding sites – at transcriptional genes commonly found to regulate TEs – following p53 loss. We will analyze DNA methylation at TEs in *Trp53*$^{+/+}$ and *Trp53*$^{-/-}$ genotypes to determine changes in repressive epigenetics marks following p53 loss.

Our expected results are that p53 regulates epigenetic or transcriptional regulators – either directly through our BioID experiments or indirectly through our sequencing data – to suppress TEs. We expect this study will uncover unknown mechanisms by which p53 regulates TEs whose regulation is lost during initiation of HGSOC. We expect these results will improve our understanding of the contributions of TE dysregulation towards HGSOC initiation.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project:
WNT Signaling Variants as Modifiers of *TP53*-Driven Cancer Susceptibility in Li-Fraumeni Syndrome

Authors and institutional affiliation:

Driscoll M, Department of Medical Biophysics, University of Toronto. Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids).

Quaglietta P, Department of Medical Biophysics, University of Toronto. Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids).

Malkin D, Department of Medical Biophysics, University of Toronto. Genetics and Genome Biology Program, The Hospital for Sick Children (SickKids). Department of Pediatrics, Division of Hematology/Oncology, SickKids.

Email of submitting/first author:

Maddy.driscoll@mail.utoronto.ca

Training program first author is enrolled in:

Medical Biophysics Graduate Program at the University of Toronto

Year of training:

Year 2

Abstract:

Li-Fraumeni Syndrome (LFS) is a cancer predisposition syndrome associated with germline *TP53* mutations, leading to significantly increased lifetime cancer risk. Individuals with LFS display striking clinical heterogeneity, including variation in tumour onset, aggressiveness, and metastasis, highlighting the need to understand genetic modifiers of cancer susceptibility.

Recent work from our lab suggests that variation in Wnt signalling contributes to this clinical diversity. Wnt pathway hyperactivation promotes oncogenesis by stabilizing β-catenin, activating transcriptional programs that support tumour initiation and progression. Whole-genome sequencing on a multi-institutional LFS cohort identified Wnt-pathway variants associated with decreased cancer risk and improved survival. These variants are predicted to dampen β-catenin signalling, suggesting a novel mechanism to counterbalance *TP53*-driven oncogenesis.

To investigate the impact of genetic modifiers in LFS, we are developing a multi-omic atlas of patient-derived dermal fibroblasts, representing diverse clinical presentations: wild-type (n=5), clinically unaffected LFS carriers (n=7), and LFS individuals with known malignancies (n=6). Using these 18 cell lines, genomic and transcriptomic sequencing will be integrated with proteomic analyses to link genetic variation to transcriptional and protein-level signalling changes in a *TP53*-deficient context.

Preliminary proteomic analyses show increased expression of the primary Wnt receptor in LFS cells compared to wild-type, suggesting baseline priming towards Wnt activation. Gene set enrichment analysis found an enrichment of β-catenin, Lef1, and Myc transcriptional programs in LFS cells, consistent with activated Wnt signalling states. Together, these findings support hyperactive Wnt signalling as a defining feature of the LFS cellular environment and a contributor to *TP53*-driven oncogenesis.

This study will explore biological heterogeneity among LFS patients, supporting future research into Wnt modifiers as therapeutic interventions. Beyond the scope of this project, the LFS fibroblast multiomic atlas is a novel resource for the LFS and *TP53* research communities, providing cross-platform comparisons of molecular activities across distinct clinical outcomes.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: Targeted Prevention as a Novel Approach to Therapy-Related Myeloid Neoplasms

Authors and institutional affiliation:
Rondeau V, Chan D, Campo G, Solangi A, Jahangiri S, Jin L, Wong G, Dhir A, Seneviratne S, Tse A, Vanner R
Princess Margaret Cancer Center, Toronto, ON, CA

Email of submitting/first author: vincent.rondeau@uhn.ca

Training program first author is enrolled in: Princess Margaret Cancer Centre

Year of training: 5th year post-doctoral fellow

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose: Chimeric Antigen Receptor T cell (CART) therapy is an effective treatment for relapsed hematological cancers, but whose safety is limited by frequent therapy-related myeloid neoplasms (t-MN). t-MN comprise a group of myelodysplastic syndromes and leukemias arising following chemotherapy or radiation. Many t-MNs arise via transformation of pre-leukemic hematopoietic stem cells (HSCs) with clonal hematopoiesis (CH) driver mutations, and CH driver mutations in the TP53 gene (TP53-CH) are particularly high risk. Targeting the transformation from CH to t-MN may represent a promising approach to prevent t-MN; however, the transforming factors are unknown. We hypothesize that TP53-CH HSCs have unique dependencies under selective pressure which can be targeted to prevent the transformation from CH to t-MN.

Materials and Methods: We knocked out (KO) key functional domains in TP53 or the control pseudogene OR2W5 from purified umbilical cord blood human hematopoietic stem and progenitor cells (HSPCs; defined as $CD34^{+}CD38^{-}$) using CRISPR-Cas9. Fludarabine and

cyclophosphamide are the most common chemotherapies used in CAR-T conditioning regimens and were used in combination.

Results: Our preliminary data highlight that genotoxic stress confers a selective advantage to TP53-mutant HSPCs. Combination of fludarabine and cyclophosphamide profoundly impaired the colony forming ability of OR2W5-KO HSPCs and their reconstitution ability in xenograft mice while such treatment had minimal effect on TP53-KO HSPCs. Bulk RNA-sequencing analysis revealed downregulation in gene expression programs related to apoptosis, quiescence and DNA repair in TP53-KO HSPCs compared to OR2W5-KO HSPCs after treatment. Functional assays confirmed that TP53-KO HSPCs displayed higher viability, enhanced proliferative status and exacerbation of DNA damage compared to OR2W5-KO HSPCs following treatment. Transcriptomics and proteomics analysis highlighted a downregulation of cyclin-dependent kinase inhibitor p21 in treated TP53-KO HSPCs, suggesting that the use of CDK inhibitors, to mimic p21 activity, might represent a promising therapeutic approach to target TP53-KO HSPCs during lymphodepleting chemotherapy.

Conclusions: In summary, our data highlight a selective advantage to TP53-mutant clones accumulating DNA damage under chemotherapy used in CAR-T myeloablation and suggest that inhibiting CDK activity might represent a novel putative strategy to prevent transition of TP53-CH into t-MN.

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Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Examining the roles of *Gtse1* and *Eda2r* as physiological effectors of p53 activity

Authors and institutional affiliation:

Vinod Pant[1], Sydney M. Moyer[1], Mitheera V[1], Amanda R. Wasylishen[1], Akshita Mirani[1], Natalie Fowlkes[2], and Guillermina Lozano[1]

[1]Department of Genetics, [2]Department of Veterinary Medicine and Surgery, The University of Texas MD Anderson Cancer Center, Texas, 77030, USA

Email of submitting/first author: vpant@mdanderson.org

Abstract:

TP53 encodes a transcription factor that regulates hundreds of genes involved in diverse cellular processes. In our previous work, we identified a conserved p53-dependent transcriptional signature that included two previously under-characterized targets, *Gtse1* and *Eda2r*. *Gtse1* (G2 and S phase–expressed protein 1) encodes a microtubule-associated protein whose expression is induced in a p53-dependent manner, whereas *Eda2r* encodes a transmembrane receptor in the TNF-receptor superfamily that is known to induce apoptosis. To investigate the physiological roles of these genes as downstream effectors of p53, we generated mouse alleles for *Gtse1* and *Eda2r* and compared their phenotypes with those of previously characterized *Cdkn1a* (*p21*) and *Bbc3* (*Puma*)-null models. Notably, unlike *Cdkn1a*- and *Bbc3*-null mice, which display no overt phenotype, mice expressing a truncated *Gtse1* variant (*Gtse1Δ7*) or lacking *Eda2r* exhibited defects in spermatogenesis and liver abnormalities, respectively. Furthermore, in a conditional *Mdm2* deletion model that drives constitutive p53 activation resulting in tissue disintegration and gastrointestinal toxicity, *Gtse1* and *Cdkn1a*, both regulators of the cell

cycle emerged as key p53 effectors, whereas *Bbc3* and *Eda2r* had no impact on the phenotype. Collectively, our data demonstrate that p53 safeguards tissue homeostasis during stress primarily through cell-cycle regulation, highlighting this pathway as a central mechanism of its tumor-suppressive function.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **A mathematical perspective on the robustness of p53 oscillations**

Authors and institutional affiliation:
Dr. Wouter Jongeneel,
KTH Royal Institute of Technology and Digital Futures,
Stockholm, Sweden.

Email of submitting/first author:
wouterjo@kth.se

Training program first author is enrolled in: Received a personal grant (2.4 MSEK, 2 years) from Digital Futures for the project 'programmability of cells', this project is all about the development of mathematical tools towards better understanding the oscillatory behaviour of p53. The project is housed at KTH Stockholm, in particular, at the control theory (DCS) and mathematics (TDA) divisions. Importantly, the project is part of a larger project with expert biologists (from Stockholm University) at SciLifeLab.

Year of training: MSc TU Delft (November 2019), PhD EPFL (June 2024) followed by 1 year of postdoctoral research at UCL. The current project started at KTH in November 2025.

Abstract:
Experiments have shown that the period (not the amplitude) of p53 oscillations exhibit a certain form of robustness. Extending work by Golubitsky, Stewart and coworkers on homeostasis of equilibria to the case of limit cycles, we are able to zoom in on structural features of the p53 genetic regulatory network that accommodate robustness of the period. Additionally, we discuss what is different for MDM2, again, aligned with experimental results. At last, towards better positioning the mathematical theory itself, we point to new experiments.

Please email your submission to us at p53workshop2026@uhn.ca . Please use the following subject heading: Abstract- p53 International workshop

20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Functional Annotation of p53 Variants Using in vivo CRISPR Screening and Nurse-Led Clinical Translation

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Adway Kadam[1,2], Adèle Usmanov[1,3], Daniel Schramek[4,5], Sampath Loganathan*[1,2,4,6], YiQing Lü*[1,3,4,5,6]

1. Cancer Research Programme, Research Institute of McGill University Health Centre
2. Department of Otolaryngology, Head and Neck Surgery, McGill University
3. Ingram School of Nursing, Faculty of Medicine and Health Sciences, McGill University
4. Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital
5. Department of Molecular Genetics, University of Toronto
6. Rosalind and Morris Goodman Cancer Institute, McGill University

*. Corresponding.

Email of submitting/first author:
yiqing.lu@mail.mcgill.ca

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Member of the Daniel Schramek team at University of Toronto, Molecular Genetics, and Mount Sinai Hospital

Year of training: *Example: PGY 3*

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Functional annotation of genetic variants lags far behind discovery in the post-genomic era. This gap is critical for the tumour suppressor p53 (*TP53*) as it is the most frequently mutated gene in human cancers. While hotspot mutations are well-studied, the clinical landscape is dominated by Variants of Unknown Significance (VUS). These rare mutations remain uncharacterised and require rapid functional validation to determine pathological potential. We developed a high-throughput, parallel *in vivo* gene-editing platform to systematically identify p53 VUS that may act as causal oncogenic drivers.

To identify cancer-promoting variants, we employ parallel CRISPR screens in the mouse delivered via ultrasound-guided *in utero* injection. Our approach utilises a base-editing library saturating the p53 genomic region, incorporating biochemically optimised Cas variants (CsIsrB, CwIsrB, and IscB) fused with APOBEC/TadA. As an alternative approach, we employ Cas12a-based prime editing to model the top 2,000 clinically frequent VUS. These tools are coupled with Cas12a-based modulation of endogenous genes and Cre recombinase to activate a PIK3CA-Notch-based tumour-prone background. This modular toolset allows us to investigate p53 within diverse genetic contexts, mimicking the synergistic signalling found in human Head and Neck Squamous Cell Carcinoma (HNSCC). Following tumour growth, DNA is subjected to amplicon and next-generation sequencing to quantify enriched p53 mutations, which are cross-referenced with TCGA patient data to distinguish pathogenic drivers from silent passengers.

To bridge the gap between genomic data and patient care, we have integrated a translational arm involving community care. We are training Registered Nurses (RN) and Nurse Practitioners (NPs) to use our functional atlas for improved patient education and increased awareness of rare genetic risks. This training facilitates a direct connection between community care nurses and oncologists, ensuring more effective referrals and the establishment of high-priority follow-up protocols. By identifying high-risk variants early, we enable vigilant monitoring for disease initiation and early signs of relapse in patients harbouring confirmed pathogenic VUS.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: **Beyond Transcription: Translatome and Proteome Dynamics Modulate the p53 Network**

Authors and institutional affiliation: *Fislova A, Faculty of Medicine, Masaryk University*
Andrysik Z, Faculty of Medicine, Masaryk University

Email of submitting/first author: zdenek.andrysik@med.muni.cz

Training program first author is enrolled in: *Early career researcher - junior research group leader*

Year of training: *2nd*

Abstract:

Purpose: The tumor suppressive capacity of the p53 network is unmatched by any other signaling pathway in human cells. Through transactivation of hundreds of target genes, p53 regulates approximately ten cellular functions with anti-oncogenic roles, most notably cell cycle arrest and apoptosis. However, such concentrated control requires tight regulation through numerous feedback loops to maintain dynamic stability at both the cellular and organismal levels. Our recent work suggested that stress-induced inhibition of canonical translation substantially impacts the cellular response to p53 activation, including a remarkably strong apoptotic response in otherwise resistant cells. Since over 80% of genes directly induced by p53 are protein-coding, gene-specific regulation of protein expression may decisively impact the execution of the cellular program triggered by p53 at the transcriptomic level. We therefore decided to investigate how the p53 network is modulated across the transcriptome, translatome, and proteome.

Materials and Methods: Inhibition of canonical translation is characteristic of the Integrated Stress Response (ISR), which can be initiated by stimuli that also activate p53. We used the eIF2α inhibitor nelfinavir to mimic ISR and the MDM2 inhibitor nutlin to activate p53. While each drug individually causes cell cycle arrest, combined treatment with both inhibitors results in rapid cell death in all TP53 wild-type systems tested. To elucidate the mechanistic basis of this phenotypic shift, we performed RNA-seq, Ribo-seq, and mass spectrometry analyses in three colorectal carcinoma cell lines, followed by comprehensive analysis of signaling pathway activity.

Results: Our initial data show that ISR increases induction of the p53 network at the transcriptomic level. However, phenotype-specific regulation emerges at the proteome level. While induction of pro-apoptotic p53 target genes is further amplified, other p53-governed cellular programs are selectively downregulated.
Conclusions: Our data suggest that both the translatome and proteome represent underappreciated regulatory levels for modulating cellular responses to p53 activation.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: RUNX3-p53-MYC interplay determines metastatic outgrowth in gastric cancer
Authors and institutional affiliation: Jung-Won Lee, National University of Singapore Linda Shyue Huey Chuang, National University of Singapore Aashiq Hussain, National University of Singapore Junichi Matsuo, National University of Singapore Nawaphat Jangphattananont, National University of Singapore Supriya Srivastava, National University of Singapore Kazuto Suda, Juntendo University School of Medicine Ming The, National University of Singapore Manabu Takamatsu, Japanese Foundation for Cancer Research Tetsuo Noda, Japanese Foundation for Cancer Research Yoshiaki Ito, National University of Singapore
Email of submitting/first author: Jw_lee@nus.edu.sg / Jung-Won Lee
Training program first author is enrolled in: Postdoctoral Fellowship
Year of training: Year 3

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose

Although RUNX3 suppresses early tumorigenesis, its frequent elevated expression in gastric cancer metastases suggests context-dependent functions. We therefore sought to determine how TP53 status governs a RUNX3-driven transcriptional switch between tumor suppression and metastatic reprogramming, and to establish proof-of-concept for TP53 genotype-guided therapeutic disruption of RUNX in metastasis.

Materials and Methods

RUNX family gene expression was quantified by immunohistochemistry in gastric cancer specimens including metastases. RUNX3 was deleted by CRISPR–Cas9 in metastatic gastric cancer cell lines with TP53 loss-of-function, gain-of-function mutation, or wild-type TP53. Analyses using RNA-seq, ChIP-seq/re-ChIP, co-immunoprecipitation, migration/invasion assays and mouse metastasis models were integrated. RUNX small-molecule inhibitors (Ro5-3335 or AI-10-104) were tested in vitro and in vivo.

Results

RUNX3 was the most frequently expressed RUNX paralog in metastatic lesions and was elevated in 57% of gastric cancer liver metastases. TP53 status dictated RUNX3 binding partners and transcriptional output in metastatic gastric cancer cell lines. With functional p53, RUNX3 reinforced p53-dependent cytostatic and pro-apoptotic pathways and constrained epithelial–mesenchymal transition (EMT) and metastatic outgrowth. With p53 loss or mutation, RUNX3 engaged MYC to drive TGFβ–dependent EMT programs and metastatic colonization. Accordingly, pharmacologic RUNX inhibition suppressed EMT-associated gene expression, migration/invasion and metastatic burden in TP53-defective contexts, but enhanced EMT and metastatic traits in TP53-wild-type cells.

Conclusion

TP53 status determines whether RUNX3 promotes p53-dependent tumor suppression or MYC-driven metastatic programs, thereby predicting benefit versus harm of RUNX inhibition during clinical intervention. Our work supports TP53 genotype–guided targeting of RUNX signaling in metastatic gastric cancer.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project:
Quantitative single-cell analysis of p53 restoration in TP53-null ovarian cancer cells

Authors and institutional affiliation:

M. Dillingham McCullough, Harvard University
D. Miller, Harvard Medical School
R. Tatavosian, Harvard Medical School
A. Grodsky, Harvard Medical School
G. Lahav, Harvard Medical School

Email of submitting/first author:

Meg Dillingham McCullough, mdillingham@g.harvard.edu

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*

Systems, Synthetic and Quantitative Biology PhD Program, Harvard University

Year of training: *Example: PGY 3*

4th year PhD student

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

Purpose: Restoration of wild-type p53 activity is a promising therapeutic strategy for cancers harboring TP53 mutations. While early p53 restoration approaches showed limited success, recent clinical efficacy of a Y220C p53 reactivator has renewed interest in therapeutic p53 restoration. Despite decades of work on p53 restoration, the behavior of restored p53 at the single-cell level remains poorly understood. Because p53 dynamics are known to determine cell fate following stress, we hypothesized that the dynamic behavior of restored p53 may similarly influence fate outcomes during p53 restoration. We therefore quantified p53 dynamics and corresponding fate outcomes in individual cells following controlled induction of p53.

Materials and methods: We engineered a TP53-null ovarian cancer cell line with doxycycline-inducible wild-type p53 fused to a fluorescent reporter. Using multi-day live-cell imaging, we quantified p53 dynamics and corresponding fate outcomes in individual cells following p53 induction.

Results: Increasing levels of restored p53 were associated with a larger fraction of cells undergoing terminal fates. While the predominant effect of p53 restoration in our model system was cell cycle arrest, a subpopulation of cells underwent cell death. Single-cell analysis revealed heterogeneity in restored p53 dynamics, with specific dynamical features (duration and magnitude) associated with cell death.

Conclusion: Restored p53 exhibits heterogeneous dynamics across genetically identical cells that associate with divergent fate outcomes. These findings suggest that the temporal patterns of p53 play an important role in determining cellular outcomes in response to p53 restoration. Decoding, and ultimately controlling, these dynamic features of p53 signaling could provide a rational framework for designing strategies to selectively eliminate cancer cells with non-functional p53, thereby advancing the long-anticipated goal of harnessing p53 for targeted cancer therapy.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project:
Regulation of p53's Dual Roles in DNA Repair and Transcription by Time-Dependent Modifications

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center

Shaffer A, Harvard Medical School
Luchetti A, Niels Bohr Institute, University of Copenhagen
Jessen E, Niels Bohr Institute, University of Copenhagen
Mock C, Harvard Medical School
Miller D, Harvard Medical School
Jensen M H, Niels Bohr Institute, University of Copenhagen
Heltberg M, Niels Bohr Institute, University of Copenhagen
Lahav G, Harvard Medical School

Email of submitting/first author: **airlia_shaffer@hms.harvard.edu**

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*
Postdoctoral Fellow in Systems Biology

Year of training: *Example: PGY 3*
Postdoctoral year 3

Abstract: *Approx. 300 words*
Suggested format: Purpose, Materials and Methods, Results, Conclusions

p53 is best known as a transcription factor that regulates stress-response genes, yet its tumor-suppressive function cannot be fully explained by transcriptional regulation alone. Following ionizing radiation, p53 levels have been shown to oscillate, and these dynamics influence target gene selection. Recent theoretical studies suggest that p53 oscillations may also enhance the efficiency of DNA damage repair, by redistributing repair material between damage sites. However, it is still unknown whether, when, or how p53 interacts with repair condensates.

Using antibody staining to detect chromatin-bound p53, we observe that p53 accumulates at DNA double-strand breaks in discrete foci that colocalize with canonical repair proteins after ionizing radiation. With new methods of statistical image analysis, we find p53 binding to DNA repair condensates is temporally regulated after DNA damage. We show that this time-dependent

recruitment is controlled by post-translational modifications at p53's C-terminal and that perturbing these modifications alters p53 accumulation at repair sites. Ongoing work aims to determine how such interactions influence repair efficiency. Together, these results support a model in which time-dependent modifications act as a regulatory mechanism determining when p53 functions as a direct mediator of repair or as a transcription factor.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st, 2026 (5pm EDT)
Notification of acceptance: February 27, 2026

Title of study/project: **The Origin and Consequences of Heterogeneous p53 Dynamics in Single Cells.**

Authors and institutional affiliation:

David Miller, Harvard Medical School
Nicola Martino, Harvard Medical School; Massachusetts General Hospital; Broad Institute of MIT and Harvard
Roubina Tatavosian, Harvard Medical School
Sara Buhrlage, Harvard Medical School; Dana-Farber Cancer Institute
Seok-Hyun Yun, Harvard Medical; Massachusetts General Hospital; Broad Institute of MIT and Harvard
Galit Lahav, Harvard Medical School

Email of submitting/first author:

David_miller@hms.harvard.edu

Training program first author is enrolled in:

Systems Biology, Harvard Medical School, Postdoctoral fellow 2023-present

Year of training: *Postdoctoral fellow year 3*

Abstract:

The tumour suppressor p53 is a key regulator of cancer prevention and of cancer cell responses to therapy. Acting primarily as a transcription factor in response to stress such as DNA damage, p53 influences cell fate by activating DNA repair, cell cycle arrest, senescence, and apoptosis. Deciphering how p53 leads to these diverse cellular outcomes is crucial for understanding how p53 maintains tissue homeostasis and prevents uncontrolled proliferation under genomic insults, as well as for determining how cancer cells with functional p53 respond to DNA-damaging drugs. Importantly, p53 dynamics (temporal changes in its protein levels) vary between individual cells and shape cell fates. What drives cell-to-cell variability in p53 dynamics and how these variations influence induction of p53 target genes at the single-cell level remains unclear.

To address these questions, we used isogenic cells expressing a fluorescent wild-type p53 live-cell reporter and examined a range of DNA-damaging agents and doses to identify conditions generating heterogenous p53 dynamics. We then focused on a regimen that produced a mixture of oscillatory and non-oscillatory behaviours *in vitro* and *in vivo*. Long-term live-cell imaging revealed that these

distinct p53 dynamics causally drive opposing cell fates, with oscillatory p53 promoting survival and recovery, and non-oscillatory p53 causing prolonged arrest or cell death. We found that the origin of p53 behaviour stems from differences in levels of DNA damage, which occur in a cell cycle-dependent manner. Mechanistically, non-oscillatory p53 exhibits increased stability despite elevated levels of its negative regulator MDM2. We identified a key regulator of p53 turnover whose perturbation can shift the balance between oscillatory and non-oscillatory responses, and thereby alter cell fate distributions. Lastly, to investigate how p53 dynamics shape transcriptional responses, we are developing LASE-seq, which links live-cell imaging to scRNA-seq by optical barcoding. Preliminary data support the feasibility of this approach and suggests that distinct p53 dynamics are associated with different regulation of p53-target genes. This ongoing work will reveal how p53 dynamics are regulated and how they control gene expression programs that underlie cell fate decisions.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Enterobacteria induce colorectal cancer chemoresistance through stromal-mediated repression of p53

Authors and institutional affiliation: *Example: Malkin D, University of Toronto*
Kirsch D, Princess Margaret Cancer Center
Schramek D, Lunenfeld-Tanenbaum RI

Fragkoulis K, Karolinska Institute, Sweden
Łasut-Szyszka B, Karolinska Institute, Sweden and Maria Skłodowska-Curie National Research Institute of Oncology, Poland
Saei AA, Karolinska Institute, Sweden
Aschtgen MS, Karolinska Institute, Sweden
***Peuget S**, Karolinska Institute, Sweden*

Email of submitting/first author:
sylvain.peuget@ki.se (presenting author)

Training program first author is enrolled in: *First Author is required to be enrolled in a training program during 2025-2026. Example: Radiation oncology Residency Program*
Konstantinos Fragkoulis: Tumor Biology and Oncology PhD program at Karolinska Institute

Year of training: *Example: PGY 3*
Year 4

Abstract: *Approx. 300 words Suggested format: Purpose, Materials and Methods, Results, Conclusions*

Purpose: Increasing evidence highlights the role of gut microbiota and intratumoral bacteria in the physiopathology of cancer. Interestingly, several cancer-associated bacteria have been shown to interfere with the host defence against tumorigenesis, including the p53 pathway, thereby impacting tumour progression and response. We recently found that p53 regulation by Enterobacteria impacts colorectal cancer (CRC) genetic evolution and p53 mutation rate. In this study, we investigate the underlying molecular mechanisms of p53 interplay with bacteria within the tumour microenvironment.

Materials and Methods: *in vitro* co-culture systems, quantitative proteomics, CRC patient data analysis.

Results: We identify a paracrine circuit from stromal cells, triggered by bacterial molecules, that suppresses p53 activity in the tumour cells. Specifically, macrophages and fibroblasts stimulated by lipopolysaccharides from *Klebsiella pneumoniae* and other Enterobacteria release extracellular vesicles (EVs) that selectively suppress a subset of p53 targets in wild-type p53 cancer cells, impairing their response to standard chemotherapy. Analysis of CRC patient data reveals that the expression of this gene subset negatively correlates with inflammation and is associated with improved survival.

Conclusions: Altogether, our findings uncover a microbiota-stroma-tumour axis that functionally attenuates wild-type p53 activity and could explain how some CRC tumours can progress while retaining wild-type TP53, potentially contributing to the late emergence of p53 mutations in this cancer.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: p53 ser 18 regulates post- prandial glucose homeostasis.

Authors and institutional affiliation:

Hayla S. Dept Endocrinology, Univ Mass Chan Medical School, Worcester, MA*

Jack S., Columbia College of Dental Medicine, New York, NY

Heather L.A. Dept Endocrinology, Univ Mass Chan Medical School, Worcester, MA

Sithara P., Roger J.D. Dept. PMM, Univ Mass Chan Medical School, Worcester, MA

*PI

Email of submitting/first author: Hayla.sluss@umassmed.edu

Training program first author is enrolled in: *PI is presenting the abstract.*

Year of training:

Abstract:

Purpose

The tumor suppressor p53 integrates stress and metabolic signaling across tissues. While p53 has been implicated in glucose homeostasis, the mechanisms linking p53 signaling to systemic metabolic regulation remain incompletely understood. We investigated whether phosphorylation of p53 at serine 18 (Ser18) regulates postprandial glucose control through adipose tissue metabolic pathways during diet-induced metabolic stress.

Materials and Methods

Wild-type and p53S18A knock-in mice were subjected to high-fat diet (HFD). Glucose tolerance tests (GTT), insulin tolerance tests (ITT), and insulin signaling analyses were performed to assess systemic insulin sensitivity. Adipose tissue transcriptomic profiling was conducted to identify p53-dependent metabolic pathways. Circulating metabolites and hepatic signaling responses were evaluated to determine inter-organ metabolic regulation.

Results

*Under HFD conditions, p53S18A mice exhibited significantly exaggerated glucose excursions during glucose challenge compared with wild-type controls, despite similar systemic insulin resistance as measured by ITT and hepatic AKT signaling. Transcriptomic analysis of adipose tissue revealed marked upregulation of **Folh1**, encoding glutamate carboxypeptidase II, an enzyme involved in folate and one-carbon metabolism. These changes occurred without strong induction of inflammatory cytokines but were accompanied by altered expression of immune surveillance genes. We propose that loss of p53 Ser18 signaling disrupts adipose one-carbon metabolic regulation, altering circulating metabolite pools and hepatic metabolic state. This perturbation delays the suppression of hepatic gluconeogenesis*

following nutrient challenge, resulting in amplified postprandial glucose excursions independent of classical insulin signaling defects.

Conclusions

Our findings identify p53 Ser18 phosphorylation as a key regulatory node linking adipose tissue metabolism to systemic glucose homeostasis. These results suggest that adipose-derived metabolic signals, including one-carbon pathway metabolites, contribute to the regulation of hepatic gluconeogenesis during metabolic stress. This work highlights a previously unrecognized role for p53 in coordinating inter-organ metabolic communication under conditions of chronic nutrient excess.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: **Identification of Immunogenic Tumor Antigens for an mRNA-LNP Interception Cancer Vaccine in Li-Fraumeni Syndrome**

Authors and institutional affiliation:

Mahsa Farjami [1,2], Nicholas Fischer [2], Sunam Mander [2], Joserafael Dimayacyac [2], Uri Tabori [1,2,3,4], David Malkin [1,2,3,4]

1. Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada
2. Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada
3. Division of Haematology Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada
4. Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada

Email of submitting/first author: **mahsa.farjami@sickkids.ca**

Training program first author is enrolled in:

Genetic and Genome biology at sickkids/ Medical Biophysics at UofT

Year of training:

Year 3

Abstract:

Introduction: Li-Fraumeni syndrome (LFS) is a rare hereditary cancer predisposition disorder caused by germline pathogenic variants in the *TP53* gene, conferring a near 100% lifetime risk of developing cancer. Current therapeutic strategies are insufficient to prevent recurrent malignancies in LFS patients. We hypothesize that an LNP-mRNA vaccine targeting immunogenic tumor antigens (TAs), in combination with immune checkpoint inhibitors (ICI therapy) could provide an early tumor interception for LFS patients.

Methods: Tumor-associated antigens (TAs) in LFS tumors were identified using genomic and transcriptomic data from patients at St. Jude Children's Research Hospital and The Hospital for Sick Children. For each patient, tumor-specific somatic variants were detected by comparing whole genome sequencing (WGS) data from tumor and matched germline samples using the GATK Mutect2 variant-calling pipeline. These variants were subsequently annotated with Ensembl's Variant Effect Predictor (VEP). Given that immunogenic TAs bind with high affinity to HLA molecules presented on tumor cells, we assessed the interaction between patient-specific HLA alleles and somatic variants. HLA typing was performed using the OptiType computational tool. Prediction of highly immunogenic neoantigens was conducted in silico using netMHC and pVACseq tools.

Result: Across 46 LFS patients, we identified over 1700 tumor antigens. While the majority of TAs were patient-specific, recurrent mutations were observed in the *ATAD3B* and *TMEM14B* genes. The shared *TMEM14B* variant is seen in 4 patients, 3 of whom are diagnosed with adrenocortical carcinoma. The TA in *ATAD3B* gene is found in 50% of rhabdomyosarcoma cases in our cohort (2 of 4). Repeated TA is seen in 36% of cases and they are shown in figure 2. The prevalence of this TA in the current cohort of ACC patients is 15%. The number of discovered TAs per patient ranged from 4 to >100.

Conclusion: Analysis of WGS of tumor and normal samples enables the identification of common immunogenic TAs in LFS patients. These findings provide a foundational step toward the preclinical evaluation of an off-the-shelf cancer vaccine to intercept LFS malignancies.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: January 31st 2026 (5pm EDT)
Notification of acceptance: By or before February 27, 2026

Title of study/project: Identification of inflammation induced epigenetic drivers of colitis-associated colorectal cancer

Authors and institutional affiliation: *Christopher J. Cowley; Memorial Sloan Kettering Cancer Center*
Louis Faure; Memorial Sloan Kettering Cancer Center
Emilie de Vet; Memorial Sloan Kettering Cancer Center
Daniel Kim; Memorial Sloan Kettering Cancer Center
Elizabeth Benitez; Memorial Sloan Kettering Cancer Center
Ruslan Soldatov; Memorial Sloan Kettering Cancer Center
Karuna Ganesh; Memorial Sloan Kettering Cancer Center

Email of submitting/first author: **cowleyc@mskcc.org**

Training program first author is enrolled in: *Postdoctoral fellow*

Year of training: **year 3**

Abstract: Colorectal cancer (CRC) arising from inflammatory bowel disease (IBD), known as colitis-associated colorectal cancer (CAC), exhibits distinct genetic and clinical characteristics compared to sporadic CRC (sCRC). While sCRC is often driven by early APC mutations, CAC is frequently associated with early TP53 mutations. Additionally, the mutational burden between primary and metastatic CRC tumors are largely identical suggesting that disease progression is epigenetically driven. Metastatic progression in sCRC is delineated from an intestinal stem-like state in the primary tumor that undergoes a hyper-plastic conserved fetal-like reversion enabling tumor cells to metastasize and differentiate into non-canonical (squamous, neuroendocrine and endoderm) cell states. How chronic inflammation from IBD may alter this plasticity is unknown. Recently it is appreciated that tissues encode inflammatory memories through epigenetic rewiring, altering tissue fitness and oncogenic potential. To delineate if tumors from CAC patients have altered epigenetic rewiring, I established a single cell multiome (scRNA and scATAC-seq) and patient derived organoid (PDO) biobank of normal colon epithelium (non-inflamed and inflamed) and primary tumor of CAC patients. Preliminary data from patient samples indicates that primary tumor epithelial cells in CAC patients lose their intestinal stem-like identity and are highly plastic gaining expression of a multitude of unique cell states, a phenomenon only observed within

metastatic sites in sCRC. Concurrently, epithelial cells from non-inflamed, inflamed and primary tumors of CAC patients have an increased signature of interferon signaling. Using scATAC-seq data generated from patient samples and functional interrogation of PDOs through interferon stimulation, I aim to identify transcription factors involved in priming of this oncogenic state in the colonic epithelium through inflammatory experience and factors driving cell state alteration in primary CAC tumors. By systematically dissecting the interplay among inflammation induced epigenetic changes and genetic alterations, I will elucidate how some mutated epithelia progress to cancer, while others do not.

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20th International p53 Workshop- Abstract Submission Template

Submission Deadline: December 31, 2025 (5pm EDT)
Notification of acceptance: January 30, 2026

Title of study/project: **Targeting pro-survival proteins to modulate cell fate following p53 activation**

Authors and institutional affiliation:

Allan Shuai Huang[1,2*], Elizabeth Lieschke[1,2*], Pedro Baldoni[1,2*], Annabella Thomas[1,2] Lauren Whelan[1,2] Julia Marchingo[1,2] Amar Balihodzic[3], Michael Milevskiy[1,2], John E La Marca[1,2], Aisling Ross[1,2], Luuk Heitink[1,2], Pardeep Rajasekhar[1,2], [1,2] Catherine Chang[1], Michael Dengler[3], Gordon Smyth[1,2*], Gemma L Kelly[1,2*] and Andreas Strasser[1,2*]

1 The Walter and Eliza Hall Institute (WEHI), Melbourne, Australia

2 Department of Medical Biology, The University of Melbourne, Melbourne, Australia

3 Medical University of Graz, Division of Oncology, Graz, Austria

* Authors contributed equally

Email of submitting/first author: **huang.s@wehi.edu.au**

Training program first author is enrolled in: **Postdoc training**

Year of training: **Third year postdoc**

Abstract:

The transcription factor TP53 (also called TRP53 in mice or p53 in general and used hereafter) is essential to prevent the development of cancer. Upon activation in response to diverse stresses, such as oncogene expression, DNA damage or nutrient deprivation, p53 orchestrates diverse cellular responses, most prominently cell cycle arrest/cell senescence and cell death by apoptosis, by directly or indirectly activating ~500 target genes. It has been postulated that cell survival vs cell death after p53 activation is decided by selective transcriptional induction of either of the direct p53 target genes, *p21* critical for cell cycle arrest/senescence, or *Puma/Bbc3* to initiate apoptosis. Our analysis using mice co-expressing transcriptional reporters for both *p21* and *Puma* demonstrated that single cells induce expression of both the initiators of cell cycle arrest and for apoptosis regardless of whether they will survive or die. Thus, factors other than induction of *p21* and *Puma* must decide cell outcome after p53 activation. Our proteomic, RNA-sequencing, and functional analyses across multiple cell types revealed a higher ratio of anti-apoptotic to pro-apoptotic proteins, along with transcriptional upregulation of the anti-apoptotic genes *Bcl2l1*, in cells that survive after p53 activation compared with cells that undergo cell death. Increasing pro-survival proteins allowed cells to survive that would normally die after p53 activation. Conversely, blunting the pro-survival functions pharmacologically, led to the death of cells that normally survive after p53 activation. These findings reveal the mechanism by which cell fate – survival or death – is decided after p53 activation and identify a strategy how anti-cancer drug induced cell cycle arrest/senescence in malignant cells can be converted into apoptotic death to enhance therapeutic impact.

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