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Application Summary

Competition Details

Competition Title: 2022 TAM Predoctoral Student Pilot Award
Category: Internal Funding Opportunity
Cycle: 2022
Submission Deadline: 11/4/2022 11:59 PM

Application Information

Submitted By: [REDACTED]
Application ID: [REDACTED]
Application Title: Comparative analysis on the functional role and mechanisms of distinct p53 mutations in promoting metastasis in esophageal squamous cell carcinoma (ESCC)
Date Submitted: 11/4/2022 2:12 PM

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Do you self-identify as individual with a disability: [REDACTED]
Do you self-identify as individual from a disadvantaged background: [REDACTED]

Application Details

Proposal Title

Comparative analysis on the functional role and mechanisms of distinct p53 mutations in promoting metastasis in esophageal squamous cell carcinoma (ESCC)

Comparative analysis on the functional role and mechanisms of distinct p53 mutations in promoting metastasis in esophageal squamous cell carcinoma (ESCC)

Esophageal cancer is a highly aggressive cancer and is the 6th leading cause of cancer mortality worldwide, with esophageal squamous cell carcinoma (ESCC) being the most prevalent subtype [1, 2]. Especially, ESCC patients with distant metastasis have a poor clinical outcome with 5-year relative survival rate of 5% [3]. Mutations of the tumor suppressor *TP53* (human homolog of mouse *Trp53*) that encodes p53, are detected frequently in many cancer types, including ESCC, which in turn correlate generally with poor survival and high metastatic rates [4]. Indeed, previous studies on ESCC and esophageal adenocarcinoma (EAC) identified that more than 80% of the patients have a mutation in *TP53*, mostly in the DNA-binding domain [5, 6].

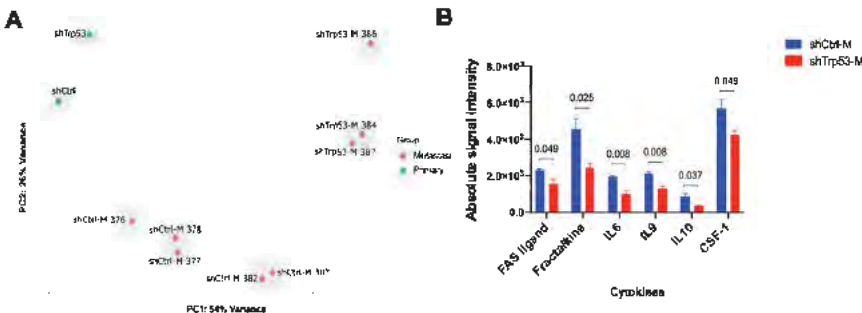


Figure 1: *Trp53^{R172H}* alters transcriptomic signature and cytokine secretion in ESCC cells during lung metastasis. (A) Principle component analysis (PCA) demonstrated distinct gene expression profiles depending upon p53 status. **(B)** Representative results of the cytokine array conducted on conditioned media from shCtrl-M (n=5) and shTrp53-M (n=3) metastatic cells. n=3, Student's unpaired t-test. Error bars represent SEM.

shTrp53-M cells. We identified six cytokines (FAS ligand, Fractalkine, interleukin 6 (IL-6), IL-9, IL-10 and colony stimulating factor 1 (CSF-1)) with enhanced secretion in *Trp53^{R172H}*-dependent manner in metastatic cells, suggesting their potential involvement in mediating metastasis (Fig. 1b).

To date, our studies of mutant p53 have focused primarily on *Trp53^{R172H}* mutation. However, it is critical to recognize that not all p53 mutant proteins are equivalent and this has significant implications for conceptual and therapeutic perspectives. Indeed, many binding partners of p53 interact only with a subset of mutant p53 proteins and some target genes have been identified in the context of only specific missense *TP53* mutations [8]. Similarly, certain missense *TP53* mutations correlate with different pro-oncogenic properties and tumor growth when compared to others, indicating distinct phenotypic characteristics of these mutations [9, 10]. In accordance, our analysis of ESCC and EAC patient datasets reveal that specific p53 mutations are associated with markedly differential overall survival rates (Fig. 2, TCGA, Nature 2017): Patients with *TP53^{R273H}* and *TP53^{R282W}* have a median 5-year overall survival of 32.23 and 5.53 months, respectively. In contrary to such significant differences, a comprehensive comparison of different missense p53 mutations in tumorigenesis and tumor metastasis has never been conducted extensively, which necessitates investigation of whether these specific p53 mutations have gain-of-function properties and which downstream genes mediate metastasis in this context. Therefore, we wish to determine how pro-oncogenic activities and mechanisms regulated by mutant p53 differ or are similar, and we **hypothesize** that different DNA-contact and conformational p53 mutations, will have specific epigenetic/transcriptomic signatures, which can lead to differences in pro-tumorigenic properties. We will pursue this hypothesis with the help of the **Single Cell Analysis Core** (see letter by Peter Sims, PhD) and through the following interrelated **Specific Aims:**

SPECIFIC AIM 1. To compare key characteristics of isogenic tumor cells that harbor wild-type p53, p53 null or distinct DNA-contact/conformational p53 mutations.

Research Strategy: To elucidate the functional role of p53 mutations in specific cancer types, we are generating isogenic cell lines: We have introduced two DNA contact mutations (R273C and R282W) into cells isolated from Tamoxifen-treated *K5Cre^{ERT2};Rosa26tdTomato^{Isl1/Isf1}* (KT) mouse model and primary human esophageal cell lines that we have established in the lab [11, 12]. In parallel, we are currently working on establishing isogenic cells with two conformational p53 mutations (R175H and G245D). For this purpose, we are using cytosine base editing technology, which allows us to express these missense *TP53* mutations endogenously that can be considered to be a better approach than ectopically expression to study epigenetic/transcriptomic changes of distinct missense mutations and variants [13, 14]. The introduced mutations are verified through sequencing the exon,

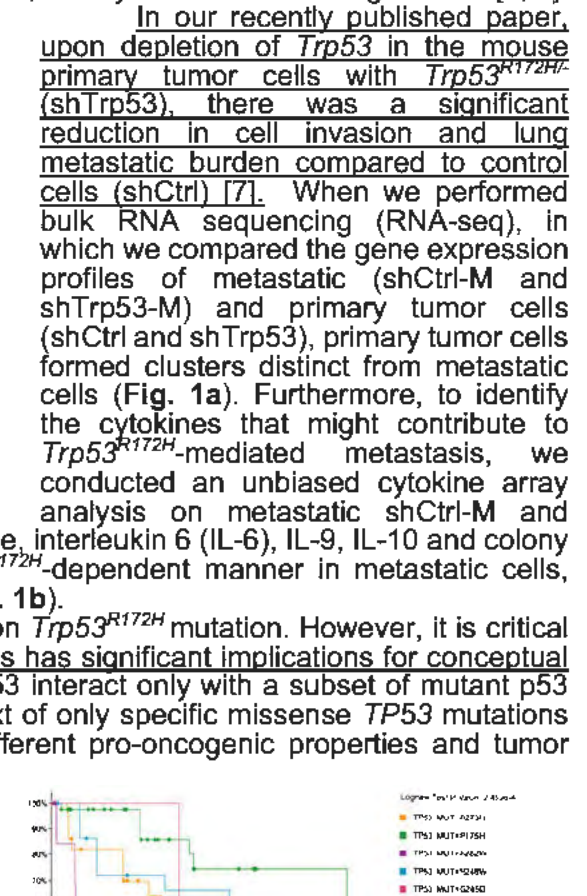


Figure 2: TCGA analysis on overall survival rate of esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) patients with various *TP53* mutations.

TP53 MUT	N	Median (95% CI)	5-year OS (%)
TP53 MUT G172H	30	15	32.23 (17.28 - NA)
TP53 MUT G273C	44	9	52.55 (36.63 - NA)
TP53 MUT R273H	24	13	5.53 (14.30 - NA)
TP53 MUT R282W	35	14	11.64 (0.45 - NA)
TP53 MUT G245D	1	1	22.70

in which the mutation is introduced. We will also use the monoclonal antibody that detects local and global conformational changes in p53 (Pab240) [15], as well allele-specific antibodies (in collaboration with Dr. Kanaga Sabapathy from National Cancer Center of Singapore (NCCS), which we have obtained through an MTA) [16], and conduct immunohistochemistry on these cells along with a non-target control [16, 17]. Furthermore, p53 will be deleted in the same human cells by CRISPR/Cas9 and mouse cells will be isolated from $K5Cre^{ERT2}; Rosa26^{tdTomato^{Isl/Isl}; p53^{loxP/loxP}}$ (KTP) mice, which will be used for a direct comparison of different missense p53 mutations. Then to assess the functional role of specific p53 mutations, we will use the cell lines generated to analyze proliferation, migration and invasion through *in vitro* WST-1 proliferation, 3D tumoroid formation, transwell migration and Boyden chamber invasion assays, respectively. In parallel, we will assess the consequences of the p53 on tumor progression *in vivo*: We will evaluate primary tumor growth following subcutaneous xenografts and metastatic burden in lungs following lateral tail-vein injection. After assessing tumor growth and metastatic burden, half of each primary tumor and lung tissue with metastatic tumors will be sectioned for histology and the other half will be used in Specific Aim 2. Data from these will provide information as to whether and when different p53 alleles function differentially in the abovementioned studies.

Anticipated outcomes, potential pitfalls and alternative approaches. Previously, we observed a significant reduction in cell invasion *in vitro* and lung metastatic burden *in vivo* upon deletion of $Trp53^{R172H}$ in mouse ESCC cells [7]. Furthermore, the role of various $TP53$ hotspot mutations have been indicated to be selected for and have a role in a range of pro-oncogenic properties in various cancer types [8]. Accordingly, we anticipate that each of the p53 mutations will induce some pro-tumorigenic events compared to wildtype p53 control with some overlapping and also distinct characteristics, in terms of proliferation, tumor growth, migration, invasion and metastasis. One of the pitfalls of our approach is that base editing may not be efficient for some of the point mutations, particularly G245D. If this were the case, we will use alternative approaches such as prime editing or knocking in the point mutation with homologous directed repair (HDR) [18, 19].

SPECIFIC AIM 2. Determine single-cell transcriptomic signature and chromatin accessibility (epigenetic) changes in primary and metastatic tumors harboring different p53 mutations.

Research Strategy. Metastasis is a highly heterogeneous process with diverse array of phenotypic outcomes. To analyze how different p53 mutations contribute to transcriptomic and epigenetic states in primary and metastatic tumors, we will perform single-cell RNA-sequencing (RNA-seq) and assay for transposase-accessible chromatin with high throughput sequencing (ATAC-Seq) (10X Genomics Chromium) on tumors with distinct p53 mutations harvested in Specific Aim 1, and conduct bioinformatic analysis in collaboration with Single Cell Analysis Core. The support from the core will include library preparation, sequencing, demultiplexing and data analysis. These studies will allow us to evaluate the changes in cell subpopulations and tumor heterogeneity, as well as the precise genome-wide alterations in transcripts and chromatin accessibility within the primary and metastatic tumors dependent upon specific p53 mutations. In follow-up experiments, we will perform chromatin immunoprecipitation sequencing (ChIP-seq) on the isolated primary and metastatic tumor samples using the commercially available ChIP-grade p53 antibody (Vector Laboratories, CM5) to examine if p53 binding co-localizes with changes in chromatin accessibility, and to identify putative targets of various p53 mutations.

Anticipated outcomes, potential pitfalls and alternative approaches. Data from these genomic studies will be analyzed by the computational wing of Single Cell Analysis Core and through our collaboration with the bioinformatician Dr. Spyros Karaiskos, PhD, and pathways affected by these different mutant alleles will be provided to us for further exploration of unique and novel targets. Overall, these datasets will allow us to identify the underlying transcriptomic and epigenetic changes of primary and metastatic tumors with the proposed genetic modifications at a single-cell resolution, as well as their putative gene targets. A pitfall of our approach is that ChIP-Seq can have limitations, especially due to fixation step that can potentially mask epitopes or decrease resolution and specificity [20, 21]. As an alternative approach we can utilize cleavage under targets and release using nuclease (CUT&RUN), a novel technique that provides profiling at a higher resolution with which we have optimized [22].

Conclusions and Future Directions: This innovative and comprehensive approach will open up new avenues to elucidate mechanisms involved in pro-oncogenic events mediated by distinct p53 mutations. The proposed study is fueled by interrelationship between the [REDACTED] lab and Single Cell Analysis Core to investigate transcriptomic and epigenetic signatures of emerging primary and metastatic tumor populations mediated by specific p53 alleles in the context of ESCC. Our mechanistic insights pave a forward-looking perspective on our ability to nominate genes/pathways regulated by mutant p53 that might be targeted in our preclinical models and ultimately in phase 1 clinical trials. Especially, our future directions are motivated by the knowledge gap on how unique p53 status impacts metastatic tumor microenvironment (TME). It has been suggested that loss or gain of function mutations in p53 have a critical impact on promoting immunosuppressive TME through modulating the secretion of growth factors, cytokines and chemokines [23-26]. In accordance, our abovementioned RNA-seq data suggested that interferon alpha, gamma and inflammatory response pathways are positively correlated with $Trp53^{R172H}$, and the cytokine array (Fig. 1b) identified cytokines with enhanced secretion in $Trp53^{R172H}$ -dependent manner in metastatic cells. Therefore, the future directions will involve the characterization of the cellular components of the TME of tumors with different p53 mutations through high-resolution and multiplexed approaches. Furthermore, we will identify the major mediators of the crosstalk between tumor cells and cellular components of the TME and examine their role in fostering metastatic abilities in ESCC. Overall, the proposed study and future directions will establish a comparative platform to investigate the intrinsic and extrinsic mechanisms mediated by distinct p53 mutations, and their role in metastatic capabilities.

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[REDACTED]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: [REDACTED]

eRA COMMONS USER NAME (credential, e.g., agency login): [REDACTED]

POSITION TITLE: Graduate Student Research Assistant

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
[REDACTED]				

A. Personal Statement

As evidenced by my academic training, diverse research experiences, numerous awards and honors, and teaching and tutoring positions, I am passionate about learning and sharing my love of biomedical science. Indeed, my well-rounded and curiosity-driven research experiences that led to various publications and poster presentations have prepared me for my current position. Now in my graduate training at Columbia University, I am working to expand my scientific knowledge, increase my critical and innovative thinking, advance and contribute to science, improve my technical lab skills, and participate in teaching and mentorship opportunities.

I am interested in understanding the genetic and molecular mechanisms of cancer metastasis that can be targeted therapeutically. I am co-mentored by Dr. [REDACTED] a [REDACTED] leader in gastrointestinal oncology, and Dr. [REDACTED], a pioneer in the p53 field to investigate how specific p53 mutations foster metastasis in esophageal carcinomas. My scientific contributions since the beginning of my graduate studies have already led to two co-author publications and multiple poster presentations. The [REDACTED] and [REDACTED] labs have extensive experience in utilizing 2D cell culture systems, 3D organoids and genetically engineered mouse models, as well as novel genetic and pharmacological approaches to investigate various pro-tumorigenic properties such as proliferation, migration, invasion and metastasis. Furthermore, I am developing novel gene editing, multiplex immunofluorescence (IF) staining and chromatin profiling protocols to expand technical resources in the lab.

As a PhD student, I had the unique opportunity to become a member of the HICCC Trainee Associate Membership (TAM) program, which offers access to a community of trainees and mentors conducting groundbreaking cancer research, pilot grants, career development and educational events. Through the scientific and technical support, as well as mentorship that I will receive under the TAM Pre-doctoral Student Pilot Award, I will improve my research on comparative analysis of distinct p53 mutations in esophageal cancer.

B. Positions and Honors

Positions and Employment

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Professional Memberships



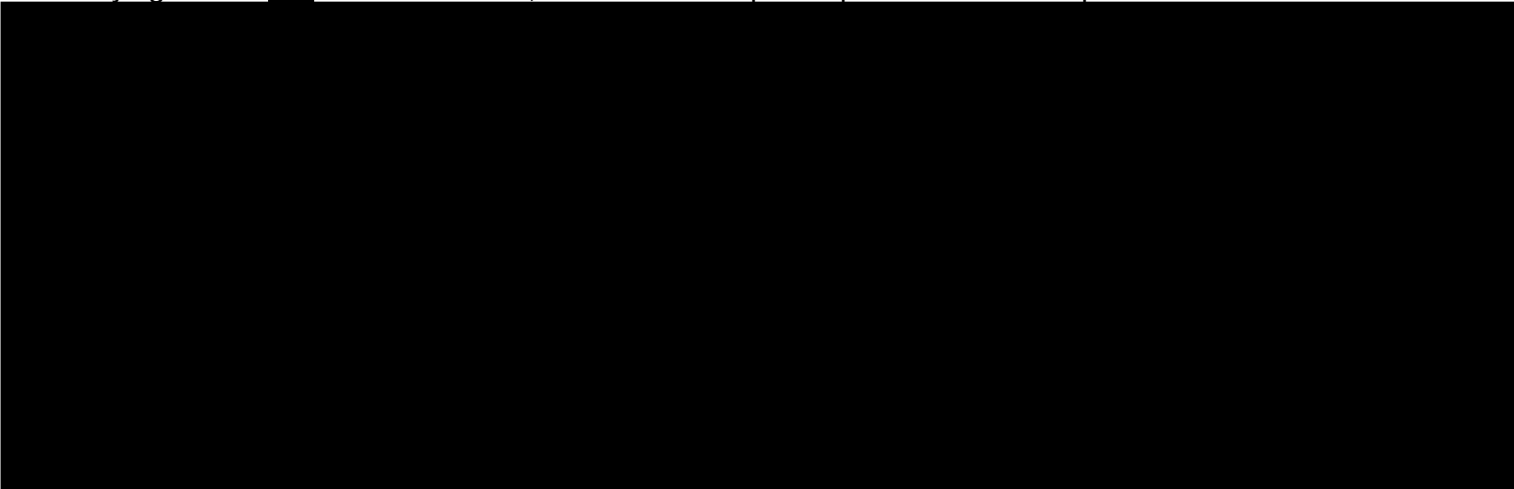
Honors, Scholarships, and Awards



C. Contribution to Science

Undergraduate and Postgraduate Research:

1. I joined to Dr. [REDACTED]' laboratory to study metabolism and aging. His research investigates the understanding of energy substrate and metabolic regulation during health and pathogenesis. In the lab, in collaboration with [REDACTED] I created bioinformatics systems to study the aging process at the physiological, cellular, molecular, and genetic levels, and built biological simulations of metabolic changes that play a role in aging process. As this project opened avenues to understand underlying mechanisms of metabolism, it led to various poster presentations and publication of a review article.



2. I worked in two separate projects in the laboratory of Dr. [REDACTED] at the University [REDACTED]. Her research is internationally recognized for understanding the mechanisms of extracellular matrix (ECM) proteolysis and its role in breast cancer pathogenesis. Under Dr. [REDACTED] mentorship, I investigated the role of matrix metalloprotease 9 (MMP9) on early metastatic dissemination and seeding using the MMTV-PyMT mouse breast cancer model. I also utilized various *in vitro* and *in vivo* approaches to describe how MMP9 suppresses tumor cell migration, invasion, and colony formation. In addition, I had the opportunity to lead a

separate project, which elucidated the conditions under which human glutamate-oxaloacetate transaminase 1 (hGOT1)/oxaloacetate (OxAc)-mediated blood glutamate scavenging (BGS) affects invasion, and whether this inhibits metastasis of triple negative breast cancer (TNBC). Other researchers in the lab continued using the assay that I developed to measure hGOT1 in peripheral blood. My work in the lab led to three publications (two of which are review articles) and one poster presentation.

3. I had an opportunity to work on a project to develop novel amyotrophic lateral sclerosis (ALS) models in the laboratory of Dr. [REDACTED]. His lab studies the development and maturation of motor neurons under normal physiological conditions, as well as during pathogenesis. I generated and optimized *in vitro* ALS models using human induced pluripotent stem cells (iPSCs) to decipher pathogenesis mechanisms and to develop drug screenings based on motor neuron survival assays. As part of the project, I generated motor neuron reporter lines and optimized CRISPR/Cas9 approaches, which led to a published platform for comparison of differentiated motor neurons. This study was particularly exciting as one of the compounds that I verified in our drug screenings with iPSC-derived model system is currently tested in Phase I clinical trials for ALS.

Graduate Research:

4. My current predoctoral research in the [REDACTED] lab uses *in vitro* and *in vivo* approaches to understand mutant *TP53*-mediated mechanisms that promote invasion and metastasis in gastrointestinal cancers. My results indicated the critical role of *TP53*^{R175H}-mediated CSF-1/CSF-1R autocrine signaling in increasing metastatic capabilities in esophageal squamous cell carcinoma (ESCC), and I currently characterize the upstream regulators and downstream effectors of this mechanism. I have presented my ongoing work in multiple conferences, and I hope to submit these findings as a first-author publication within 1-2 years. I plan to expand these studies to other *TP53* mutations and publish the results as a second first-author paper during my graduate studies. My results will provide a platform to identify novel therapeutic targets in treating advanced ESCC, which can potentially be extrapolated to other squamous cell carcinomas (SCCs) with a similar genetic background. I have also contributed to two other projects: 1) We investigated mutant p53-mediated role of Survivin in lung metastasis from esophageal squamous cell carcinoma (ESCC) through Yes-Associated Protein (YAP) signaling, and 2) we studied downregulation of endocytic recycling factor Rab Coupling Protein (Rab11-FIP1) by mutant p53 that is critical for organoid formation, cell invasion and epithelial-mesenchymal transition (EMT). For my contributions, I am a co-author on two manuscripts, published earlier this year.

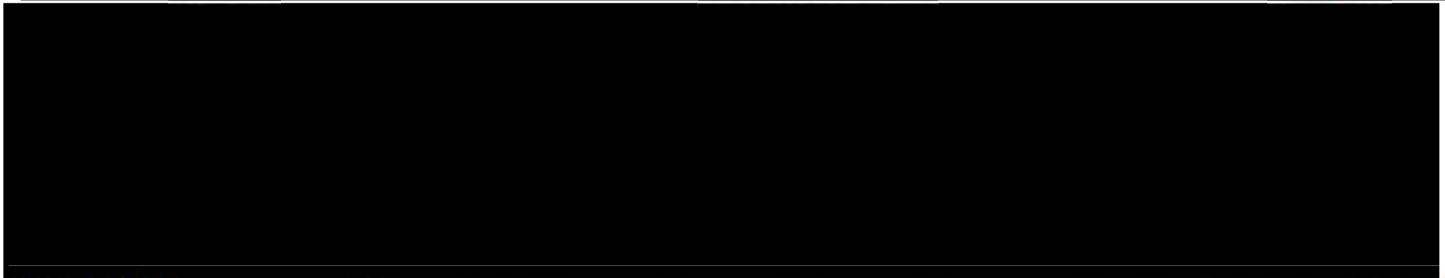
- f. Invited oral presentation: [REDACTED]
- g. Poster presentation: [REDACTED]
- h. Poster presentation: [REDACTED]
- i. Poster presentation: [REDACTED]
- j. Poster presentation. [REDACTED]
- k. Poster presentation. [REDACTED]


D. Scholastic Performance


YEAR	COURSE TITLE	GRADE
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[REDACTED]		
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YEAR	COURSE TITLE	GRADE
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GPA: 3.471.  ^{SO}
used. Grade of P (Pass) fulfills a graduation requirement of a zero-unit course and is not calculated in the GPA.

A large black rectangular redaction box covers the entire content of the table below the text.

GPA: 4.1633. Columbia University's Graduate School grading system is as following: A, excellent; B, good; C, fair; D, passing but poor; F, failure. Plus/Minus letter grades are also used. A few specific graduate-level courses are offered only on a Pass/Fail basis, such as Research and Seminar courses. In Spring 2020, courses typically on a letter-grade scale changed to Pass/Fail due to the difficulties posed by the SARS-CoV-2 pandemic.

[REDACTED]

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: [REDACTED] MD

eRA COMMONS USER NAME (credential, e.g., agency login): [REDACTED]

POSITION TITLE: [REDACTED] Professor [REDACTED]

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE <i>(if</i>	Completion Date MM/YYYY	FIELD OF STUDY
[REDACTED]			

A. Personal Statement

Vision: GI cancers are a major health issue in the US and worldwide. Mortality is related to metastasis, especially to the lungs and liver. The elucidation of underlying molecular and cellular mechanisms, development and characterization of model systems, and the bridging of mouse studies with human tissues/3D organoids all converge on translational applications. To that end, we are known nationally and internationally for our work in esophageal cancers (ESCC, EAC) as well as pancreatic and colon cancers. **Research:** Our group has been dedicated with passion to the elucidation of mechanisms underlying esophageal epithelial biology and metastasis, both cell autonomous and non-cell autonomous. As part of this effort, our group has developed innovative 3D organoid culture model systems, xenograft implantation (subcutaneous and orthotopic) and genetically engineered mouse models. We seek to translate these discoveries into novel and innovative diagnostics and therapeutics. We have published extensively in these topics in [REDACTED]

[REDACTED] We present our work at national meetings such as [REDACTED]. Additionally, our efforts in esophageal/GI cancer biology research have spawned our lab trainees obtaining independent faculty positions with their own federal and non-federal funding. **Commitment**

to Education/Training: I was the PI of an [REDACTED] prior to joining Columbia University. I have been fortunate to receive mentorship/teaching awards at [REDACTED]. Having trained [REDACTED] fellows and [REDACTED] students (at different stages), their career development is of paramount importance. Many have obtained [REDACTED] grants. Overall, I am committed unequivocally to the scientific and career development of [REDACTED] and will continue supporting her studies proposed for the HICCC TAM Pre-doctoral Student Pilot Award.

Ongoing and recently completed projects that I would like to highlight include:

[REDACTED]

[Redacted]

[Redacted]

**B. Positions, Scientific Appointments, and Honors
Positions and Scientific Appointments**

[Redacted]

[Redacted]

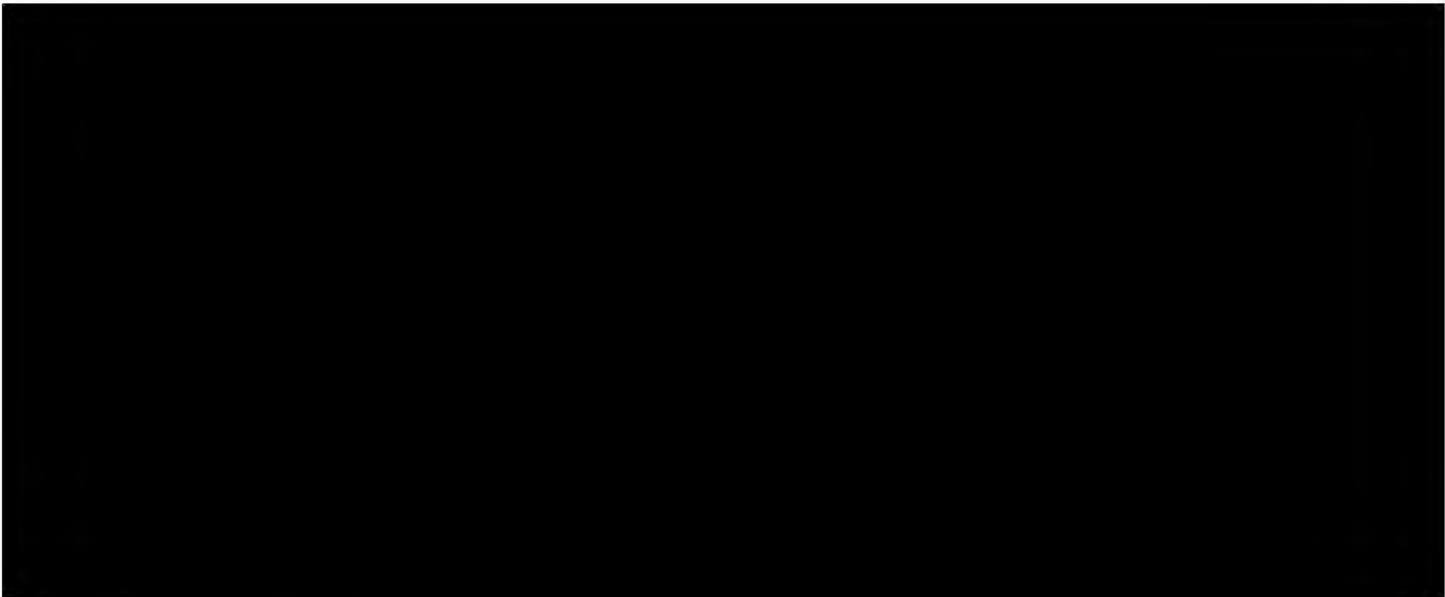
Other Experience and Professional Memberships

[Redacted]

Honors and Awards (selected)

[Redacted]

[Redacted]



C. Contributions to Science

1. **Pancreatic Ductal Development, Inflammation, and ADM:** Our group is the first to identify the intersection of pancreatic ductal development, acinar-ductal metaplasia (ADM) during acute pancreatitis, and ductal abnormalities (PanIN) during early pancreatic carcinogenesis. An RNA microarray study revealed that nearly 80 genes comprise this intersection, and the Prrx1 homeodomain transcriptional factor is a leading upregulated gene. Prrx1 is critical for ADM and PanIN. Lineage labeling reveals that Prrx1 is restricted to ductal cells and expands during chronic pancreatitis. These cells have stem cell like properties with evidence of self-renewal. Furthermore, Prrx1 transcriptionally induces Sox9, a master of ADM formation. We have also published on the importance of p120catenin in metastatic organotropism. This work was published in the following:



2. **Development of 3D Organoid Models:** Our long-standing passion for the development and characterization of 3D organotypic culture models of normal esophageal epithelial biology and esophageal cancer is illustrated by the first demonstration of its utility in the following publication:



We are able to recapitulate the key oncogenic drivers in esophageal squamous cell carcinoma through EGFR activation and p53 mutation with a dramatic invasion of tumor cells and activation of fibroblasts. The latter involves AKT activation, which could be attenuated through AKT inhibition or modifying the source of fibroblasts from other organs. This work is being used by a number of investigators across the country and world, and led to our publishing all the necessary technical aspects (isolation of mouse and human esophageal epithelial cells, overexpression/shRNA of genes using lentiviral and retroviral transduction, flow cytometry/sorting and functional assays in

I am the primary designer of this system and the senior author. This has resulted in an approved patent application through the

- [REDACTED]
3. **Identification of Esophageal Stem Cells:** Our group is the first to identify and characterize esophageal stem cells in the basal compartment in the epithelium, using a side-population approach. This population retains BrDU, is enriched for CD34, and is able to reconstitute damaged esophageal epithelium in an *in vivo* model:

- [REDACTED]
4. **Mouse Models of Esophageal Squamous Cell Cancer:** Our group has generated the first genetically engineered mouse models of esophageal squamous cell cancer (ESCC) using an oral-esophageal specific promoter (EBV-L2) and crossing with a p120catenin (p120ctn) conditional knockout with accompanying desmoplasia, cancer associated fibroblasts and recruitment of myeloid derived suppressor cells. This is the first demonstration that p120ctn, a critical component of the adherens junction, is a true tumor suppressor gene and underscores the importance of p120ctn in cancer initiation:

[REDACTED]

Other groups are using this mouse model to study signaling transduction pathways, as well as to determine the cooperation of p120ctn loss with oncogenic mutations. We have derived cell lines from this mouse model and for use by other investigators.

5. **Pathogenesis of colonic epithelial cell biology and cancer:** Three pathways are important in the pathogenesis of colon cancer: APC/Beta-catenin mediated chromosomal instability; mismatch repair gene mutations and microsatellite instability; and, Braf mutation and broad based promoter methylation. We have unraveled the critical role of Lin28b, an mRNA binding protein and ortholog of Lin28a (both Lin28a and Lin28b are critical in iPS cell formation), in promoting colon adenomatous polyp and cancer formation, without requiring Wnt signaling derangements. This would mean another pathway mediated by Lin28b overexpression, via downregulation of Let-7 microRNA, is important in the pathogenesis of colon cancer. This work was published as follows:

[REDACTED]

We are sharing this mouse model with investigators on a regular basis. Moreover, we have derived 3D enteroid cultures from the intestines/colons of these mice to continue to investigate underlying mechanisms but also to use on a translational basis for drug testing. We have published protocols to that end in Cold Spring Harbor Protocols.

[REDACTED]

October 25, 2022

Dear Selection Committee Members:

I am delighted to provide my wholehearted and unequivocal support for the candidacy and application of [REDACTED] PhD student ([REDACTED] and HICCC trainee associate member) for the TAM Pre-doctoral Student Pilot Award. I cannot emphasize how impeccable her credentials and qualifications have been throughout her training and in my lab. Of note, she has worked in some prominent labs and acquired interdisciplinary research experience.

I suspect she is amongst the top, if not at the top, of her PhD class in [REDACTED] and perhaps, in her entire PhD class at [REDACTED] HICCC. In our lab, she has exceeded my expectations through her native intelligence, tireless work ethic, dedication, collaborative spirit, unbridled enthusiasm and incredible productivity ([REDACTED] publications [REDACTED]). Additionally, [REDACTED] performance in coursework, seminars and group discussions has been stellar. In accordance, [REDACTED] has been an exceptional teacher and mentor of students at [REDACTED] and at Columbia, as she has served as teaching assistant in the latter in spite of a hectic course load and intensive research projects. Furthermore, she has done compassionate voluntary work to promote young girls in science. Her career to date has been replete with honors and awards, including [REDACTED] publications and [REDACTED] review articles. Since she joined to the lab, she has presented at [REDACTED]. She has [REDACTED] first-authored manuscripts to be submitted and first-author on an invited review article to [REDACTED]. Additionally, she received the [REDACTED] fellowship (on her first round of submission).

[REDACTED] has articulated a vision of comparing the key tumorigenic characteristics of distinct *TP53* mutations using elegant *in vitro* and *in vivo* approaches. To that end, she is focused on esophageal squamous cell cancer (ESCC) as it represents a common malignancy and is the 6th leading cause of cancer mortality worldwide. Additionally, our lab has published extensively on the common epigenetic/genomic/transcriptomic mechanisms across squamous cell cancers (pan-SCC) originating from the oral cavity, head and neck, esophagus and lung. Thus, [REDACTED] work in the long-term has potential mechanistic and therapeutic applications to other SCCs, and contribute significantly to the precision medicine efforts.

[REDACTED] research is representative of the basic-translational continuum. I could go on for an extended period of time (and space) extolling her virtues, but there is no doubt that she is stellar. Thank you for your time.

Sincerely

[REDACTED]

{ REPORT [REDACTED] COMPREHENSIVE CANCER CENTER

[REDACTED]

October 26, 2022

Dear 

I hereby confirm that I have been consulted and I am pleased to offer our full support for your efforts to elucidate mutant p53-mediated mechanisms contributing to metastasis from esophageal squamous cell carcinoma (ESCC). We offer multiple platforms for single-cell genomics including the 10x Genomic Chromium, a microfluidic system for large-scale single-cell RNA-seq (scrRNA-seq) and ATAC-seq (scATAC-seq); herein, we will help you conduct the sequencing and bioinformatic analysis and in so doing, contribute to your learning these approaches. We recently developed a production-scale pipeline for joint RNA-seq/ATAC-seq from the same individual cells using this platform. We also employ an automated, multi-well plate-based system for index sorting and lower-throughput scrRNA-seq. The research staff in the Core is highly experienced and processes ~1,000 samples per year for Columbia investigators. Our experimental services include quality control of single-cell suspensions, single-cell capture and library construction, and high-throughput sequencing on the Illumina NextSeq500 and NovaSeq6000 instruments. In addition, we offer comprehensive services for computational analysis data ranging from standard data processing, clustering, differential expression analysis, and visualization to more complex, custom analysis projects. Finally, before your experiments begin, we are happy to provide additional consulting on experimental planning and design.

I look forward to continuing to work with you on your exciting Trainee Associate Membership Program Predoctoral Pilot Award proposal.

Best regards,



Peter A. Sims, Ph.D.

Associate Professor, Columbia University
Faculty Director, Columbia Single Cell Analysis Core
Dept. of Systems Biology
Dept. of Biochemistry and Molecular Biophysics