Developing a novel mRNA-LNP therapy for treating HPV+ head and neck cancers

AIMS/HYPOTHESES: The incidence of human papillomavirus-positive (HPV+) Head and neck squamous cell carcinoma (HNSCC) has risen significantly in recent years and has surpassed cervical cancer as the most common HPV-related malignancy. The standard therapy for HPV+ HNSCCs includes high dose radiation plus cisplatin, which destroy tumor cells via oxidative damage to their DNA, proteins, and lipids. However, this treatment often leaves severe and long-lasting morbidities and disabilities, highlighting the need to reduce radiation doses and avoid cytotoxic chemotherapy. In addition, a significant subset of HPV+ HNSCCs recur after standard therapy, and the mechanisms underlying therapy resistance remain poorly understood. Currently available anti-PD-L1 drugs and other immunotherapies also had limited impact on treatment resistance and survival after recurrence. Obstacles to more effective and less toxic therapy for HPV+ HNSCCs include immune suppression in the tumor microenvironment (TME) and resistance to DNA-damaging agents in some cases. Activation of Stimulator of Interferon Genes (STING) has shown remarkable potential to invigorate the immunologically “cold” TME as well as to potentiate radiotherapy responses. However, we recently discovered that the STING pathway is repressed in many human cancers, including those induced by HPV (Liu et al., PNAS, 2020). In addition, HPV encoded E2 and E7 proteins have been shown to suppress the STING signaling pathway. Furthermore, most of the HNSCC cell lines we examined express a lower level of STING compared to normal cells. Together, the dampened STING signaling in HPV+ HNSCCs could contribute to tumor resistance to radiation and immune therapies. To overcome the suppressed STING signaling in cancer cells, we have developed an mRNA-lipid nanoparticle (LNP) that can introduce naturally occurring constitutively active “hot” STING mutants into STING-silenced tumor cells (US Provisional Patent No. 63/385,053). We discovered that “hot” STING mRNA-LNPs applied to cancer cells not only reinvigorate antitumor immune response but also induce cancer cell death. Together, our findings support the hypothesis that targeted reactivation of STING in HPV+ HNSCCs using the permanently active “hot” STING mutants may counteract HPV-mediated suppression of the STING signaling pathway and overcome the tumor resistance to radiation and immune therapies. “Hot” STING mRNA-LNP-induced cancer cell death could also bolster tumor immunogenicity and reactivate antitumor immune cytotoxicity to amplify tumorcidal activity. The major challenge in optimizing the efficacy of LNP cancer therapy is to achieve tumor-specific delivery of the therapeutic molecules. In this synergy grant, we will apply the most advanced LNP technology to generate mRNA-LNPs coated with HNSCC-targeting molecules that can selectively deliver “hot” STING mutants into HPV+ HNSCCs (Aim 1)(Fig. 1). We will examine these mRNA-LNPs for their abilities to selectively deliver “hot” STING into HNSCC cells and to reignite antitumor immune cytotoxicity both in vitro and in vivo (Aim 2)(Fig. 1). This proposal is built on our strong data demonstrating a new technology to restore STING antitumor immunity in cancer cells. To complete this study, we have assembled a collaborative team with matching expertise in HPV oncogenesis and STING reactivation mRNA-LNP technology (J You, Dept of Microbiology), and HNSCC clinical studies and xenotransplantation (D. Basu, Dept of Otorhinolaryngology).

SIGNIFICANCE: As the sixth most common cancer worldwide, HNSCCs are responsible for more than 450,000 deaths annually and account for about 5% of malignancies in the United States. HPV+ HNSCC has one of the most rapidly increasing incidences of any cancer in high-income countries and its incidence is anticipated to increase to 1.08 million new cases annually by 2030. FDA-approved HPV vaccines do not treat established cancer. Current standard treatment for HPV+ HNSCCs, including surgical excision, primary radiotherapy, or chemoradiotherapy, often carry debilitating, long-lasting side effects that can even become life-threatening in the years and decades after treatment. In addition, more than half of patients with HNSCC eventually experience disease recurrence and/or metastasis despite surgery and first-line, standard therapy, and the treatment strategies for recurrent disease also carry a high risk of toxic side effects. Many HNSCC patients also do not respond to PD-1/PD-L1 immune checkpoint blockade therapies, posing additional clinical challenges. Therefore, there is a significant need for novel therapies with less toxicity and more efficacy for HNSCCs. This synergy grant aims to address this significant clinical problem and develop a novel and effective therapeutic strategy for HPV+ HNSCCs.
**APPRAOH:**

**Aim 1. Engineer tumor-specific “hot” STING mRNA-LNPs for targeted delivery in HNSCC.** We will generate “hot” STING\(^{R284S}\) mutant mRNA-LNPs conjugated with antibodies for HNSCC-specific markers. Several cell surface growth factor receptors are frequently amplified or overexpressed in HNSCCs. For example, EGFR is highly overexpressed on the cell surface of ~90% of HNSCCs\(^{11,15-18}\) and is often associated with an aggressive phenotype\(^{11,19,20}\) HER2, MET, and FGFR are also amplified and overexpressed in a significant subset of HNSCCs\(^{11,21-26}\). These receptors therefore presented attractive targets for therapeutic mRNA-LNP delivery. We will encapsulate STING\(^{R284S}\) mRNA in LNPs using the NanoAssemblr® microfluidic mixing system (Precision Nanosystems Inc) as we described\(^ {9}\). Using a selective mRNA expression method named ASSET (Anchored Secondary scFv Enabling Targeting)\(^ {27}\), we will coat STING\(^{R284S}\) mRNA-LNPs with antibodies recognizing EGFR, HER2, MET, and FGFR\(^ {18,28}\) to promote selective LNP binding of the HNSCC cell surface receptors and specific delivery of STING\(^{R284S}\) into HNSCCs. Luciferase mRNA will be encapsulated and modified using the same method and used as a negative control. For the tumor targeted mRNA-LNPs (mRNA-tLNPs) produced in this study, we will quantify mRNA encapsulation efficiency, LNPs size distribution, ultrastructure, ASSET incorporation, and coating of targeting antibodies on LNPs as described previously\(^ {27,29}\).

**Aim 2. Target tumor-specific “hot” STING mRNA-LNPs to HNSCCs.**

2A. Deliver “hot” STING mRNA-LNPs into tumor cells. We will test the delivery efficiency of HNSCC-targeted STING\(^{R284S}\) mRNA-tLNPs including anti-EGFR-STING\(^{R284S}\), anti-HER2-STING\(^{R284S}\), anti-MET-STING\(^{R284S}\), and anti-FGFR-STING\(^{R284S}\) mRNA-tLNPs. Normal human keratinocytes, fibroblasts, and HPV+ HNSCC cells (collected by D. Basu) will be treated with untargeted STING\(^{R284S}\) mRNA-LNPs, STING\(^{R284S}\) mRNA-tLNPs, or Luciferase mRNA-tLNPs. We will perform IF staining, RNAscope, and RT-qPCR to validate HNSCC-specific expression of “hot” STING delivered by the mRNA-tLNPs. We will perform cytokine and proliferation assays\(^ {6,9}\) to determine if, compared to Luciferase control, STING\(^{R284S}\) mRNA-tLNPs stimulate antitumor cytokine production and preferentially induce the death of HNSCC cells.

2B. Tumor targeting in vivo. We showed that expression of STING\(^{R284S}\) stimulates robust cancer cell death\(^ {6,9}\). This is an exciting finding because, in the in vivo setting, tumor antigens released by dying cancer cells could be engulfed by antigen-presenting cells to activate T cells, thus amplifying the tumoricidal effect. Using a murine model of HPV+ HNSCC\(^ {30,31}\), we will determine if “hot” STING mRNA-tLNPs can achieve tumor-specific STING reactivation, stimulate antitumor cytotoxicity, and repress HNSCC tumor growth in vivo. Using the methods described in **Aim 1**, we will generate the mouse version of human STING\(^{R284S}\) mRNA-tLNPs, mSTING\(^{R283S}\) mRNA-tLNPs. After establishing the HPV+ HNSCC tumor growth in C57BL/6 mice\(^ {30,31}\), the mice will be intratumorally injected with 5ug\(^ {32}\) Luciferase mRNA-tLNPs, or each of the mSTING\(^{R283S}\) mRNA-tLNPs. We will monitor tumor growth kinetics to determine whether, compared to the luciferase control, treatment with mSTING\(^{R283S}\) mRNA-tLNPs could induce tumor regression in mice. When the tumor size is 20 mm in its greatest dimension, the mice will be sacrificed and the tumors will be harvested to assess treatment response. We will perform IF staining, IHC, RT-qPCR, and RNAscope\(^ {33}\) to 1) confirm the specific mSTING\(^{R283S}\) expression in HNSCC cells, 2) assess the reduction or disappearance of HNSCC tumor cells, 3) test if STING downstream T cell chemoattractants, such as CCL5 and CXCL10, are specifically induced in tumors injected with mSTING\(^{R283S}\) mRNA-tLNPs compared to the control, and 4) determine if mSTING\(^{R283S}\) expression induces intratumoral T cell infiltration, T cell killing, and tumor regression. Finally, the tumor sections will be co-stained using STING and Cleaved caspase-3 antibodies to determine if expression of mSTING\(^{R283S}\) induces tumor cell death. We will also use the Dead-End Colorimetric Apoptosis Detection assay\(^ {34}\) to determine if the percentage of apoptotic cells increases in tumors treated with mSTING\(^{R283S}\) mRNA-tLNPs.

**LIKELY IMPACT/FUTURE GOALS:** Successful completion of this study will generate tumor-specific mRNA-LNPs that can selectively deliver “hot” STING into HNSCCs to reactivate tumoricidal activity, while minimizing off-target toxicity towards healthy tissues. As demonstrated by the success of COVID-19 vaccines\(^ {35-37}\), the highly potent mRNA-LNPs have shown great promise for therapeutic applications and therefore will allow us to rapidly translate our findings into clinical setting. Our future goals are to test these mRNA-LNPs in clinical trials to determine if targeted delivery of “hot” STING mRNA-LNPs can enhance cancer cell susceptibility to ionizing radiation, reignite antitumor immune cytotoxicity, and repress tumor growth. Together, our studies will open new avenues for therapeutic combinations of “hot” STING mRNA-LNPs, DNA-damaging agents, virus-targeted therapies, and/or PD-L1 antibody treatment to improve therapeutic outcomes for HPV+ HNSCCs. This study will also serve as a platform to expand the technology across a diverse array of STING-deficient cancers\(^ {4}\) that are refractory to current therapies. We anticipate the studies proposed in this synergy grant will generate sufficient data for applying new external funding and ultimately translation into the clinics.
References
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: You, Jianxin

eRA COMMONS USER NAME (credential, e.g., agency login): JIANYOU

POSITION TITLE: Associate Professor

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.)

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<td>Instructor</td>
<td>12/2007</td>
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A. Personal Statement. The goal of the proposed project is to develop a novel mRNA-LNP based immunotherapy to improve the treatment outcome for human papillomavirus (HPV)+ head and neck squamous cell carcinoma (HNSCC). My role in this project is to work closely with members of our collaborative team to provide guidance on experiment design, project execution, data analysis, and summarization of research results.

Research in my lab studies how DNA tumor viruses modulate host cellular function to achieve persistent infection and cellular transformation. We also investigate the function of STING (Stimulator of Interferon Genes) in eliciting antiviral and antitumor innate immunity. Our overall research goal is to develop innovative therapeutic strategies to block pathogenic viral infection and cancer. Over the last 20 years, I have investigated two oncogenic DNA tumor viruses: human papillomavirus (HPV) and Merkel cell polyomavirus (MCPyV). We examined how the molecular interplay between the tumorigenic viruses and their host targets leads to persistent infection and cancer. I have an excellent track record in viral oncology research, with specific training and expertise in key research areas for these studies. I received my Ph.D. degree from Johns Hopkins University under the supervision of the late Professor Cecile Pickart and identified a novel ubiquitin E3 ligase in the Ubiquitin-proteasome pathway. I completed my postdoctoral training in Dr. Peter Howley’s laboratory at Harvard Medical School where my work identified BRD4 as the long sought-after receptor for papillomavirus to gain a foothold on mitotic chromosomes in order to establish persistent infection (Cell 2006;117:349-360). Since joining Penn faculty in 2008, my laboratory has successfully employed a number of innovative techniques to further investigate the virus-host interaction in HPV-induced oncogenesis. In the last 10 years, we have also applied our viral oncology expertise to study MCPyV, the most recently discovered tumor virus that can cause a highly aggressive skin cancer, MCC. Our research provided important insights into MCPyV life cycle and oncogenic mechanism. In our recent study, we combined cell culture with ex vivo approaches and identified human dermal fibroblasts as the target cells that are productively infected by MCPyV in the human skin (Cell Host Microbe 2019;19:775-87). This study established the first cell culture model for studying MCPyV infection, host response, and viral oncogenic mechanism. Recently, we discovered that STING is silenced in MCC and several other types of human cancer (Proc Natl Acad Sci U S A. 2020 117:13730-13739). In this application, we propose to apply the most advanced LNP technology to generate mRNA-LNPs coated with HNSCC-targeting molecules that can selectively deliver constitutively active “hot” STING mutants into STING-repressed HPV+ HNSCCs. We will examine these mRNA-LNPs for their abilities to selectively deliver “hot” STING into HNSCC cells and to reignite antitumor immune cytotoxicity both in vitro and in vivo. Our published data presented in the proposal demonstrate feasibility and our expertise for the proposed studies. To ensure...
success, we have assembled a collaborative team with matching expertise in STING reactivation mRNA-LNP technology (J You, Dept of Microbiology) and HNSCC clinical studies and xenotransplantation (D. Basu, Dept of Otorhinolaryngology). As a principle investigator, I have successfully directed multiple NIH funded projects, collaborated with many other scientists, and published articles in leading peer-reviewed journals for each project. In summary, I have demonstrated a record of productive research in areas of high relevance for our proposed research, and my expertise and experience have prepared me to successfully lead the proposed work.

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

1R21AI149761-01A1
You (PI)
03/01/2020-02/28/2022
Overcoming the immune evasion mechanism of Merkel cell polyomavirus-associated Merkel cell carcinoma

2R01-CA187718-07
You (PI)
04/01/2015-11/30/2026
Merkel cell polyomavirus infection, host response, and viral oncogenic mechanism

Citations:

B. Positions, Scientific Appointments, and Honors

Positions
2014-present Associate Professor of Microbiology, University of Pennsylvania Perelman School of Medicine
2008-2014 Assistant Professor of Microbiology, University of Pennsylvania Perelman School of Medicine
2006-2007 Instructor in Pathology, Harvard Medical School
2001-2006 Post-doctoral Fellow, Harvard Medical School
1996-2001 Graduate Research Assistant, Johns Hopkins University
1993-1996 Graduate Research and Teaching Assistant, University of Maine

Scientific Appointments
2021-present Standing member of NIH Virology A (VIRA) study section
2021-present Topic Editor, Viruses
2021-present Editorial Board Member, Tumour Virus Research
2021-present Editorial Board Member, Frontiers in Microbiology
2019 NIH VIRA study section (June 27-28, 2019) Ad hoc Reviewer
2018 NIH VIRA study section (June 21-22, 2018) Ad hoc Reviewer
2018 NIH Study Section ZRG1 OBT-H (02) M Cancer Biology Ad hoc Reviewer
2017 NIH VIRA study section (October 26-27, 2017) Ad hoc Reviewer
2017 NIDCR DSR Scientific Grants Review study section Ad hoc Reviewer
C. Contribution to Science

1. **MCPyV infection and Merkel cell carcinoma.** MCPyV is a novel human polyomavirus discovered in MCC, a highly aggressive form of skin cancer. Epidemiological surveys and sequencing analyses have shown that MCPyV is also an abundant virus frequently shed from healthy human skin. However, many aspects of the MCPyV life cycle remain poorly understood and it is not clear how MCPyV infection causes MCC. We were the first group to report the mechanistic details of MCPyV replication (PLoS Pathogens 2012;8:e1003021). Our additional studies revealed the host factors required for MCPyV replication as well as the impact of the host DNA damage response (DDR) on MCPyV replication and cellular transformation. We found that, during MCPyV infection, the virus activates and recruits host DDR factors to support viral DNA replication. In our recent study, we discovered the human skin cell type that is productively infected by MCPyV and revealed its host cellular tropism (Cell Host Microbe 19:775-87). Our study established the first cell culture model for MCPyV infection. Our results illustrated how the major MCC risk factors, such as UV radiation, wounding, and aging, may boost viral infection to induce tumorigenesis. During the last few years, we published a total of nineteen manuscripts on MCPyV and MCC, providing novel insights to elucidate the life cycle and oncogenic mechanism of this newly discovered DNA tumor virus. Building on these discoveries, our ongoing studies investigate how the interplay between MCPyV and the host immune defense system contributes to MCC oncogenesis. The ultimate goal of our study is to develop novel cancer therapeutics for treating MCPyV-induced MCCs.


2. Selective reactivation of STING signaling to target “cold” tumors. Tumor immune suppression represents a major obstacle to achieving effective cancer immunotherapy. Poor intratumoral T cell infiltration and activation are the major barriers that prevent MCC eradication by the immune system. However, the mechanisms that drive the immunologically “cold” tumor microenvironment remain poorly understood. We recently discovered that STING silencing causes the immunologically “cold” MCC tumor microenvironment by blocking cytokine production, and consequently impeding cytotoxic T cell infiltration, activation and killing of tumor cells (Proc Natl Acad Sci U S A. 2020 117:13730-13739). Reactivating STING in MCC cells stimulates their antitumor inflammatory cytokine/chemokine production. More importantly, stimulation of STING causes robust cell death in MCCs as well as several other STING-silenced cancers. Our findings therefore suggest that targeted activation of STING could be a viable strategy for bolstering antitumor adaptive immunity in many STING-silenced cancers. This approach could synergize with existing immune-checkpoint therapies to improve treatment for MCC and many other STING-silenced cancers. Our ongoing studies focus on developing novel therapeutic strategies to reactivate STING for overcoming the immunosuppressive microenvironment in STING-deficient “cold” tumors that are often refractory to current therapies. STING is also a key mediator of antiviral immunity. We recently discovered that activation of STING signaling could be an effective strategy for conquering coronavirus immune evasion mechanism and blocking viral infection. Our studies thus provide new insights for developing innovative broad-spectrum therapeutics against multiple coronavirus strains to face the challenge of future coronavirus outbreaks.


3. HPV-host interaction during viral latent infection and host malignant progression. High-risk type HPVs such as HPV-16 and -18 are associated with cervical, anogenital, and head and neck cancers. Currently available HPV vaccines protect against up to ten major types of cancer-causing strains. However, the vaccines do not treat established cancer and are not useful for those people who are already infected. Alternative approaches are therefore needed for curing ongoing HPV infections. This is particularly important because high-risk HPVs need to persistently infect host cells for decades in order to accumulate substantial cytogenetic changes for developing invasive tumors. HPVs establish persistent infection by maintaining their genomes as episomes in infected cells. Our previous work identified BRD4 as an important host receptor, which tethers the viral E2 protein/episome complex to mitotic chromosomes in latently infected cells, thereby ensuring faithful partitioning of viral episomes to daughter cells during mitosis (Cell 117:349-360). This study provided the first insight into the cellular machinery essential for papillomavirus retention in host cells. Our follow-up studies established BRD4’s role in the HPV life cycle and associated oncogenesis, uncovering the E2-BRD4 interaction as an important antiviral target. We also demonstrated that BRD4 could be a common target for other episomal viruses, such as Kaposi’s sarcoma-associated herpesvirus.


4. **BRD4 function in cancers.** The epigenetic reader BRD4 plays a vital role in transcriptional regulation, cellular growth control, and cell-cycle progression. Dysregulation of BRD4 function has been implicated in the pathogenesis of a wide range of cancers. We were the first to clone and express the human BRD4 gene (Cell 117:349-360). Recently, studies by others identified BRD4 as a critical therapeutic target in acute myeloid leukemia, breast cancer, colon cancer, and many other types of cancers. BRD4 is also the target of a genetic translocation t(15;19), which results in the formation of a novel fusion oncogene BRD4-NUT that defines the highly malignant NUT midline carcinoma (NMC). In the last few years, we have elucidated the mechanism by which BRD4-NUT oncogene alters BRD4 function to cause the highly malignant NMC (Cancer Research 74:3332-43). We also investigated the normal BRD4 function in stem cells and mouse embryos, and demonstrated that BRD4 preferentially occupies pluripotency genes in embryonic and cancer stem cells to regulate their transcription (Cell Death Differ. 21:1950-60). We discovered that BRD4 is hyperphosphorylated in NMC and uncovered BRD4 hyperphosphorylation as a mechanism that activates downstream oncogenes to drive cellular transformation in NMC (Proc Natl Acad Sci U S A 114:E5352-E5361). This study revealed a mechanism that regulates BRD4 biological function through phosphorylation, which, when dysregulated, could lead to oncogenesis. Our studies made major contributions to the field by providing novel insights into the biological function of BRD4 and the mechanisms that contribute to oncogenesis when BRD4 function is altered.


Complete List of Published Work in MyBibliography:
BIOGRAPHICAL SKETCH

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**NAME:** Basu, Devraj

**eRA COMMONS USER NAME** (credential, e.g., agency login): DEVBASU

**POSITION TITLE:** Associate Professor of Otorhinolaryngology-Head and Neck Surgery

**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.)*

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<td>Resident, Department of Otolaryngology, University, St. Louis, MO</td>
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<td>Washington University, St. Louis, MO</td>
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<tr>
<td>Clinical Fellow, Dept. of Otorhinolaryngology, University of Pennsylvania, Philadelphia, PA</td>
<td></td>
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**A. Personal Statement**

The goal of the proposed project is to develop a novel mRNA-lipid nanoparticle (LNP) based therapy to improve the treatment outcome for human papilloma virus-positive (HPV+) Head and neck squamous cell carcinoma (HNSCC). My role in this project is to provide clinical expertise in HNSCC as well as related cell lines, and to work with Dr. You to plan and direct the studies proposed in the application. As a surgeon-scientist, my work in treating head and neck cancer patients and leading a research lab focused on personalizing therapy for this disease leaves me ideally qualified to serve this role for this proposal. My group pursues novel approaches to identify the most treatment-refractory cases prospectively and address their mechanisms of therapy resistance. At the same time, we are working to develop biomarkers that allow de-escalation of therapy and abatement of treatment toxicity for treatment-sensitive patients. My lab’s efforts span both the generally more treatment-sensitive HPV-related subtype of oropharyngeal cancer, which is rapidly growing in incidence, and the more aggressive HPV-negative cancers, which are often tobacco-related. Our recent work to create experimental cancer models that serve this proposal have emphasized the HPV+ cancer subtype. We have partly overcome the poor growth potential of HPV+ HNSCCs outside patients to establish a unique panel of patient derived xenografts (PDXs) as well as multiple organoid and primary culture models. Our studies with these tools emphasize targeting mechanisms of tumor progression and treatment resistance that depend upon cooperativity among multiple tumor cell states. In the process, we have developed the expertise for analyzing the heterogeneity and phenotypic plasticity within and among HPV+ HNSCC cell lines, organoids, PDXs, and primary human cancer samples based on molecular and functional criteria. Using such models, my group seeks precise molecular definition of the tumor cell state dynamics that sustain malignant potential of an aggressive subset of these cancers in the face of therapy.
Ongoing and pending research support that I would like to highlight include:

NIH/NCI UH2 CA267502 Basu (PI) 1/09/2022 – 7/31/2024
Pursuing molecular biomarkers to guide adjuvant therapy for HPV+ head and neck cancers after transoral robotic surgery
The goal of this project is to identify viral and host molecular features that distinguish HPV-related head and neck cancers at high vs. low risk of lethal recurrence after transoral robotic surgery.

NIH/NIDCR R01 DE027185 Basu (PI) 7/01/2018 - 06/30/2022
JARID1B-mediated epigenetic regulation of oncogenic signals in oral cancer
The goal of this project is to determine the interplay between PI3-kinase signaling and JARID1B-mediated epigenetic regulation in the progression and therapy responses of oral cancer.

Four studies highlighting expertise and resources directly linked to this proposal:


B. Positions, Scientific Appointments, and Honors

Positions and Employment
2018-present Associate Professor, Dept. Otorhinolaryngology, University of Pennsylvania, Philadelphia, PA
2013-present Full Member, Abramson Cancer Center of the University of Pennsylvania, Philadelphia, PA
2009-present Adjunct Professor and Member, The Wistar Institute Cancer Center, Philadelphia, PA
2009-2018 Assistant Professor, Dept. Otorhinolaryngology, University of Pennsylvania, Philadelphia, PA
2007-2018 Staff Physician, Philadelphia Veterans Administration Medical Center, Philadelphia, PA
2007-2009 Visiting Scientist, The Wistar Institute, Philadelphia, PA

Other Experience and Professional Memberships
2018-present Member, American Society of Clinical Oncology
2017-present Fellow, The Triological Society
2009-present Member, American Association for Cancer Research
2008-present Member, American Head and Neck Society
2008-present Fellow, American College of Surgeons
2007-present Diplomate, American Board of Otolaryngology

Honors
C. Contributions to Science

1. HPV+ oropharyngeal cancer biology, translational therapeutics, biomarker development, and trials. Over the last four years, I have gradually shifted my lab’s focus toward pursuing mechanisms of progression and therapy resistance in HPV-related cancer, for which we developed unique experimental models during the past decade. Our studies presently focus on testing a working model for how variation in HPV oncoprotein levels and function among tumors diversifies their mitochondrial mass, progression, and therapy responses. These efforts include preclinical evaluation of emerging targets designed to deplete mitochondrial antioxidant capacity in certain tumors resist standard therapy. This work is integrated with an expansive project I initiated to compare genomic, transcriptomic, histomorphometric, and radiomic features of HPV+ oropharyngeal cancers that failed robotic therapy to those of carefully matched controls. This project is particularly innovative in collaborating with the NCI HPV Genomes Project to analyze genetic variants of oncoproteins that likely impact tumor phenotype. By integrating the multiple data sets through machine learning approaches, we are ultimately seeking to guide clinical trial design for HPV+ oropharyngeal cancer by identifying easily curable patients for safe reduction in adjuvant therapy and patients at high risk of treatment failure for testing of novel therapies. In addition to the four references above most directly related to this proposal, we list four other studies below highlighting our rapidly growing expertise related to preclinical models, retrospective analysis of patient cohorts, and clinical trials related to HPV+ cancers.


2. The role of tumor cell state heterogeneity and plasticity in determining therapy responses. My lab has pursued a novel conceptual framework for head and neck cancer therapy. Our overall approach has been to more accurately define head and neck cancer stem cells (CSCs) together with mechanisms for restoring them after targeted depletion. We initially isolated a drug-resistant subset of slow-cycling CSC subset based on high expression of the H3K4me3 demethylase JARID1B. An observed hyper-activation of the PI3-kinase (PI3K) pathway in these JARID1B\textsuperscript{high} cells renders them sensitive to depletion by PI3K-targeted drugs. We subsequently discovered that a JARID1B\textsuperscript{high} CSC pool depleted by PI3K targeting can be replenished by a distinct population of quiescent “G\textsubscript{0}-like” cells, which have both high growth potential and drug resistance. While lacking conventional stem cell markers and PI3K activation, G\textsubscript{0}-like cells showed the prominent Notch pathway activation previously linked to CSC population expansion and increased PI3K activation. We are
evaluating the hypothesis that Notch-mediated entry of proliferative cancer cells into a quiescent, \(G_0\)-like state creates a reservoir to prevent permanent CSC depletion by providing a direct precursor to \(\text{JARID1B}^\text{high}\) cells. In the process, we have developed new model systems to dissect mechanisms underlying the cell state transitions that prevent durable oral CSC depletion during treatment. In doing so, our work is also facilitating translational studies to jointly target two distinct tumor cell phenotypes that cooperatively sustain head and neck cancer growth via Notch and PI3K pathways.


3. The contribution of epithelial to mesenchymal transition to head and neck cancer progression.

After completing post-graduate medical training, my early mentored work in cancer biology helped to define mechanisms by which epithelial to mesenchymal transition (EMT) drives intrinsic resistance to EGFR inhibitors and other standard head and neck cancer drugs. Our work showed that EMT produces cellular heterogeneity within head and neck cancers by creating a subset of malignant cells that acquire mesenchymal-like gene expression and subsequently are enriched by cetuximab therapy. Such cells were represented as a discrete subpopulation within head and neck squamous cell carcinomas, where they arose from epithelial phenotype tumor cells and possessed a dynamic, reversible phenotype. The expanded mesenchymal-like subset drove tumor progression and expressed a distinct profile of secreted factors that enhanced cetuximab resistance by both tumor autonomous mechanisms and ones mediated by crosstalk with fibroblasts. Overall, this work dissected the impact of mesenchymal-like carcinoma cells on the tumor microenvironment during EGFR inhibition and in doing so provided conceptual foundation for new combination therapies with cetuximab.


4. **Regulation of T cell receptor activation by self peptides presented by MHC class II.**

Prior to my clinical training, my work as an immunology Ph.D. student helped characterize the spectrum of functionally significant interactions that occur between mature peripheral T cells and endogenous self-peptides. These studies provided the first clear *in vivo* evidence of T cell receptor (TCR) functional antagonism by a MHC class II-restricted self-peptide, resulting in down-regulation of responses to structurally related non-self-antigen. Further studies examined the structural recognition features of activating self-ligands in a spontaneous transgenic model of rheumatoid arthritis. In this context, my work precisely localized functionally relevant differences between the surfaces of two self-restricted MHC molecules, an exogenous peptide and an autoantigen, recognized by a single TCR. These studies highlighted how subtle structural distinctions attributable to single amino acids can stand at the interface between foreign antigen responsiveness and pathogenic auto-reactivity.


**Complete List of Published Work in MyBibliography:**
Budget and Justification

Personnel

Jianxin You, Ph.D. (Principal Investigator; 0% effort, 0 calendar months): $0
Dr. You is the Principal Investigator on this study. She is an Associate Professor in the Department of Microbiology at the University of Pennsylvania Perelman School of Medicine. Her lab recently developed the STING reactivation technology, which provides the basis for this study. Dr. You will set the overall goals of the project and oversee all aspects of the proposed work. She will work with members of the collaborative team to provide guidance on experiment design, project execution, data analysis, and summarization of research results. Dr. You is supported by other grants so no salary support is requested.

Devraj Basu, M.D., Ph.D. (Principal Investigator; 0% effort, 0 calendar months): $0
Dr. Basu is currently an Associate Professor in the Department of Otorhinolaryngology-Head and Neck Surgery at the University of Pennsylvania. As a surgeon-scientist, Dr. Basu treats head and neck cancer patients and also leads a research lab focusing on developing personalized therapy for this disease. He will provide clinical expertise in HNSCC as well as related cell lines and work with Dr. You to plan and direct the studies proposed in the application. Dr. Basu is supported by other grants so no salary support is requested.

Ranran Wang, PhD (Research Associate; 50% effort, 6 calendar months): $45,000
Dr. Wang is a Research Associate in Jianxin You’s laboratory. She will generate the tumor-targeted mRNA-LNPs for STING reactivation in HNSCC and perform in vitro and in vivo characterization of these LNPs as described in the proposal. Using the tumor tissues collected from the mice, she will also conduct functional analyses to determine the impact of STING reactivation on killing HNSCC cells and repressing tumor growth.

Malay Sannigrahi, PhD (Postdoctoral scientist; 17% effort, 2 calendar months) $10,755
Dr. Sannigrahi is a senior postdoctoral in the laboratory of Dr. Basu. His doctoral work studied the role of host microRNAs in regulating viral genome transcription in HPV+ cancer. He has extensive experience using the multiple experimental models of HPV+ cancer in Dr. Basu’s laboratory. He will provide HNSCC cell lines for Aim 2 of the proposal and aid in experimental design and data interpretation using these resources.

Supplies

mRNA-LNPs production and purification: $7,320
Molecular biology reagents will be needed for producing and purifying STING and control mRNAs. This category also covers the cost for encapsulating HNSCC-targeted mRNA-LNPs.

Tissue culture supplies: $10,000
Tissue cultures dishes, flasks, serum, media, antibiotics, plasticware and consumables will be needed for culturing cancer cells.

Molecular biology supplies and reagents: $3060
Reagents for molecular biology will be needed for analysis of DNA by qPCR, RNA by quantitative reverse transcriptase-PCR (qRT-PCR) and RNA scope, and protein by Western immunoblotting.

Antibodies: $2372
Primary antibodies, secondary fluorescently labeled antibodies, and enzymes will be needed for immunofluorescent staining, flow cytometry, Immuno-FISH, Western blotting, and LNP modification.

Animals: $17,993
Reagents are needed for the mouse xenograft experiments proposed in Aim 2. Total cost includes the expense for animal purchases, maintenance, as well as the supplies needed to grow and characterize the HNSPC xenografts, media, serum, animal housing, sacrificing and collecting samples.

Immunohistochemistry: $1,500
To assess treatment efficacy and immune response in the experiments proposed in Aim 2.

Microscopy Core: $2,000
Fluorescent microscopy analysis will be carried out to characterize the immuno-stained cancer cells as well as animal tissue sections. We anticipate carrying out 40 hours @ average of $50/hour of analysis.

Total costs: $100,000