AWARD NUMBER: W81XWH-21-1-0697

TITLE: Mechanisms of Adstiladrin Sensitivity and Resistance in Bladder Cancer

PRINCIPAL INVESTIGATOR: David J. McConkey

CONTRACTING ORGANIZATION: Johns Hopkins University, Baltimore, MD

REPORT DATE: August 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2022	Annual	01Aug2021-31Jul2022

4. TITLE AND SUBTITLE

Mechanisms of Adstiladrin Sensitivity and Resistance in Bladder Cancer

5a. CONTRACT NUMBER
W81XWH-21-1-0697

5b. GRANT NUMBER
CA201117

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

David McConkey

E-Mail: dmcconk1@jhmi.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins University
Department of Urology
600 North Wolfe Street
Baltimore, MD 21287-2101

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our hypothesis is that Adstiladrin works by novel mechanisms of action that involve both the innate and adaptive arms of the immune system, and that acquired resistance involves metabolic plasticity resulting in downregulation of fatty acid biosynthesis. Our objective is to use genomic and metabolomic approaches to identify these mechanisms in order to develop biomarkers for patient selection and novel targets for future combination therapies. Our Specific Aims are: (1) Perform genomic and metabolomic analyses on tumors and urine from the Phase 3 clinical trial; (2) Comprehensively characterize the effects of Ad-IFN on the tumor immune microenvironment; and (3) Define the role of fatty acid metabolism in Ad-IFN-induced cell death.

15. SUBJECT TERMS
Bladder cancer, gene therapy, immunotherapy, biomarkers

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:**

Our project involves preclinical experiments in mouse models and biomarker analyses of primary human tissues (tissue and urine) collected within the context of a completed Phase-3 clinical trial. The Johns Hopkins team is responsible for performing genomic analyses on the tissue and urine to identify biomarkers associated with response and/or resistance. Our work involves whole transcriptome RNAseq on RNA extracted from FFPE unstained slides and urine sediment and DNA panel exome sequencing on DNA extracted from the same unstained slides.

2. **KEYWORDS:**

Interferon, gene therapy, immune signatures, liquid biopsies

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Following is a summary of the tasks in the approved SOW that are being performed at Johns Hopkins. Progress on the approved tasks being performed at MD Anderson are summarized in a separate Annual Report.

Major Task 1: Submission and approval of IRB and HRPO documents (months 0-6) **(completed)**

Specific Aim 1: Perform genomic and metabolomic analyses on tumors and urine from the Phase 3 clinical trial.

Major Task 3: Prepare RNA and DNA from FFPE unstained slides (n= 151 tumors) **(in progress)**

Major Task 4: Perform whole transcriptome RNAseq on RNA from FFPE unstained slides (n = 151 tumors) **(in progress)**

Major Task 5: Perform bladder cancer panel DNA sequencing on DNA from FFPE tumors **(in progress)**

What was accomplished under these goals?

Our progress was hampered as a consequence of the COVID-19 pandemic. Specifically, our research assistant (Jack Mountain) resigned from the laboratory to continue his Master's degree in Engineering, and pandemic-associated delays prevented us from hiring his replacement (Sam Jin) until June, 2022. In an effort to speed up progress, we also hired a new postdoctoral fellow (Yujiro Hayashi, MD, PhD), who will also work on the project.

1) Major activities: We obtained IRB and HRPO approval for all of the work outlined in Specific Aim 1. We created a final inventory of the available FFPE tissue sections and arranged for them to be transported by auto to Johns Hopkins, where they are now stored at -20^{o} C. Using DNA isolated using the same workflow outlined in our grant and an independent cohort of tumors from Johns Hopkins (and supported by an independent source of funds), we have performed panel DNA sequencing and downstream bioinformatics analyses on n = 57 tumors using the clinical-grade Oncomine Comprehensive Assay v3, and we have prepared libraries from n = 16 of the same DNA isolates using our bladder cancer-specific panel. We will sequence the latter this week and compare the sequencing quality metrics and mutation calls to those generated by the clinical panel in order to validate our custom assay.

Dr. Hayashi's doctoral thesis work focused in part on measuring *TERT* promoter mutations in urothelial cancers and cell-free DNA in urine. Because of this, he joins the project with good previous experience with primary tumor and urine nucleic acid extraction and next generation sequencing. He is currently working with Kai Aragaki on the custom DNA sequencing panel described above. In preparation for the work described in the SOW for Year 2, he has also been learning how to extract RNA from urine sediment, perform quality control on the RNA, and prepare Ampliseq RNA sequencing libraries (using a separate source of funds). He plans to use the corresponding urine supernatants from the same voided urine samples to isolate exosomes (using Qiagen's miRCURY kit) and cell-free DNA (using QIAmp Circulating Nucleic Acid kits). Dr. Hayashi used the QIAmp kit in characterizing *TERT* and *FGFR3* mutations in urine from patients with upper tract urothelial cancers (PMID: 30887605).

2) Specific Objectives: Because the Phase-3 clinical trial tissues are irreplaceable, our overall objective is to ensure that we are using the tissues in the best possible manner. Specifically, we need to (1) ensure that the personnel tasked with sample preparation and sequencing are well-trained and consistently produce high-quality results, (2) confirm that our methods are the most up-to-date and optimal for measuring the biomarkers in question, and (3) ensure that the tissue supply is adequate for the methods proposed.

3) Significant results or key outcomes. We have restored and enlarged the research team, which will enable us to meet the Year 2 targets outlined in the approved SOW. We have successfully transported the Phase-3 primary tumor samples to Johns Hopkins.

4) Other achievements. Not applicable.

What opportunities for training and professional development has the project provided?

In preparing for the project, Kai Aragaki (a graduate student) and Yujiro Hayashi (a surgeon-scientist and postdoctoral fellow) have expanded their bladder cancer knowledge base and have learned how to make RNA and DNA sequencing libraries.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to complete the sequential extraction of RNA and DNA from the pretreatment clinical trial tumor tissues. We plan to select a urine RNA isolation platform (i.e., urine sediment or exosomes) and apply it to the longitudinal urine samples collected in the clinical trial. We plan to choose either to use our own assay (Ion Torrent's Ampliseq Cell-Free Assay) or a commercial assay (from either Convergent or Predicine) to measure urine cell-free DNA in the same urine isolates.

4. **IMPACT: What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to report.

  Books or other non-periodical, one-time publications.

  Nothing to report.

  Other publications, conference papers and presentations.

  Nothing to report.

- **Website(s) or other Internet site(s)**

  Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Yujiro Hayashi*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Hayashi has learned how to isolate RNA from urine sediment and use it to produce Ampliseq whole transcriptome libraries.*
Name:	*Samuel Jin*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Sam will help Dr. Hayashi perform RNA and DNA extractions and sequencing. He has learned PCR and some other basic laboratory techniques.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No grant identifying number
Title: Targeting FGFR3 in bladder cancer
Time commitment: 10% effort (1.2 calendar months)
Supporting agency: Commonwealth Foundation
Performance period: July 1, 2022 – June 30, 2025
Level of funding: direct/year
Project goal: The overall goal of this project is to identify the molecular mechanisms linking FGFR3 to suppression of interferon pathway activation in human bladder cancer cells.
Specific Aims: (1) Identify the molecular mechanisms underlying IFN pathway activation and TRAIL upregulation in response to FGFR3 inhibition. (2) Define the role of IGF-1R in FGFR inhibitor-induced cell death. (3) Identify gene expression profiles associated with response in patients treated with pemigatinib as part of a Phase 2 window of opportunity clinical trial.
Role: PI
Project overlap: None.

What other organizations were involved as partners?

Organization Name: The University of Texas MD Anderson Cancer Center
Location of Organization: (if foreign location list country) Houston, Texas
Partner's contribution to the project (identify one or more)

- *Financial support;* Provided a subcontract that supports the urine liquid biopsy feasibility studies.
- *Facilities (e.g., project staff use the partner's facilities for project activities):* The MD Anderson group maintained the primary tumor tissue samples at -20^{o} C prior to their being transferred to Johns Hopkins.
- *Collaboration (e.g., partner's staff work with project staff on the project);* The MD Anderson group members are partners in the research being performed at Johns Hopkins. All project personnel participate in weekly Zoom meetings to discuss project progress and plans.
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site);* Two MD Anderson urologic oncology fellows drove the Phase-3 clinical trial specimens from Houston to Baltimore (to ensure that they would not be damaged or lost in transit).

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

The MD Anderson team has submitted an independent annual report for this performance period.

9. APPENDICES:

Nothing to report.