

AWARD NUMBER: W81XWH-15-1-0288

TITLE: High fidelity drug repurposing, molecular profiling and cell reprogramming

PRINCIPAL INVESTIGATOR: Dr. Chris Albanese

CONTRACTING ORGANIZATION: Georgetown University Medical Center

REPORT DATE: March 2020

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE March 2020		2. REPORT TYPE Final(Final pending Extension Approval)		3. DATES COVERED 09/01/2015 - 01/31/2020	
4. TITLE AND SUBTITLE High fidelity drug repurposing, molecular profiling and cell reprogramming			5a. CONTRACT NUMBER W81XWH-15-1-0288		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Dr Chris Albanese E-Mail:albanese@georgetown.edu			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Georgetown University 37 th & O Streets, NW Washington, DC 20057-0001			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel 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 To provide successful immediate-term treatment of PCa, and to prolong or prevent the need for androgen deprivation therapy and its lethal corollary, castrate resistant prostate cancer. We will integrate two paradigm-shifting Georgetown-Lombardi technologies (TMFS/network pharmacology and CRCs) to discover and test repurposed drugs that target PCa on an individual patient basis. Objective 1: We will enrich the FDA-approved drug database to include world-wide approved and experimental drugs and add new target structures as needed. Objective 2: TMFS will be applied to the molecular profiles derived from a series of <i>pten</i> mutant tumors from engineered mice and predicted drugs will be tested on conditionally reprogrammed cultures of these cells <i>in vitro</i> as well as <i>in vivo</i> as allografts. Objective 3: We will also complete the characterization of 10 human normal and prostate cancer CRC lines by Illumina bead array and RNAseq. Objective 4: These datasets will be interrogated for potential targets and repurposable drugs identified using TMFS. The drugs will be tested on prostate cells growth <i>in vitro</i> to calculate the LD50 in preparation for future studies <i>in vivo</i> .					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
Unclassified	Unclassified	Unclassified	Unclassified		Jennifer Shankle (301) 619-2193

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

	<u>Page</u>
1. Introduction.....	2
2. Keywords.....	2
3. Accomplishments.....	2
4. Impact.....	3
5. Changes/Problems.....	4
6. Products.....	4-5
7. Participants & Other Collaborating Organizations.....	5
8. Special Reporting Requirements.....	NA
9. Appendices.....	NA

Introduction

In 2012, we published three landmark papers. First, we used a novel computational modeling method of “Train, Match, Fit, Streamline” (TMFS) to predict alternative targets, i.e. “drug repurposing”, for all FDA-approved and experimental drugs. We have recently integrated our proprietary TMFS with network pharmacology, which will help to further define and refine the drug-disease inferences generated by our modeling system. We also described a powerful new epithelial cell culture technique, yielding Conditionally Reprogrammed Cells, or CRCs, for the rapid and prolonged culturing of both normal and malignant prostate epithelium. Finally, we previously established that CRC approach could be rapidly (14 days) used for identifying a “repurposed” drug, vorinostat, for the successful treatment of a lethal case of recurrent respiratory papillomatosis. With support from the DOD we now have 7 matched sets of normal and malignant prostate cells growing as CRCs.

Our goal is to affect a paradigm shift in the way prostate cancer is treated. Indeed, despite radical primary therapy for curative intent, 30-35% of patients experience disease recurrence and few options exist for effective interdiction. To accomplish this goal we need better ways of identifying approved drugs, of validating the predicted target interactions and finally for applying these data to patient samples.

In this proposal we integrate two paradigm-shifting Georgetown-Lombardi technologies (TMFS/network pharmacology and CRCs) to discover and test repurposed drugs that target PCa on an individual patient basis. We will, for the first time, synergistically link our in silico approved-drug repurposing software and databases with our breakthrough CRC technology. This proposal promises to provide significant short- and long-term benefits to the research community and, ultimately, directly to patients. For example, while family history, PSA profile and an abnormal digital rectal exam are associated with more lethal cancers, there are no predictive biomarkers to guide optimal selection of a management strategy for an individual patient (e.g. active surveillance, radical prostatectomy, radiation or hormonal/chemo therapies).

CRCs are perfect for medium and high throughput drug screening, similar to what has been done for decades using transformed cell lines, only bringing the data to the bedside through the use of patient derived samples. Additionally, our novel TMFS system also brings an unparalleled level of accuracy and specificity to in silico identification of approved drugs and new targets.

By using repurposed drugs to delay, or perhaps even negate, the need for androgen deprivation therapy (ADT) success with treating localized disease can be viewed as could be viewed as chemoprevention, as it is ADT that typically leads to castrate resistant PCa, and while we anticipate finding repurposed drugs for all stages of PCa, it is advantageous to avoid ADT as long as is possible for many biological and health reasons.

We anticipate that this combination of our TMFS and CRC technologies represent the future of a truly personalized approach to PCa research and patient treatment

Keywords

Drug repurposing, primary cell, conditionally reprogrammed cells. localized prostate cancer, androgen independent prostate cancer, network pharmacology

Final report - *(please note: a no-cost extension request is currently under review with this award's DoD contracting officer; hence, we anticipate one more year of work on this project, and some tasks below accordingly refer to work to be done in the coming year)*

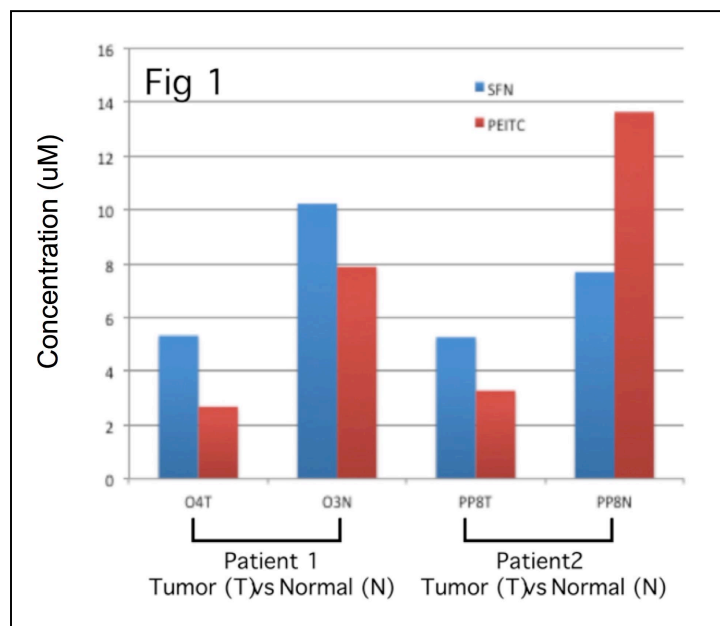
Accomplishments

Major Task 1: Aim 1: Broaden FDA-approved drug database to include all approved and experimental drugs world-wide; add new target structures as needed.		Albanese	Dakshanamurthy Byers
--	--	----------	----------------------

Major Task 1 is now complete, as detailed below. The original Aim 1 was to broaden the FDA-approved drug database to include all approved and experimental drugs world-wide; add new target structures as needed.

Subtasks 1, 2, and 3 have been completed, and we have achieved Milestones 1 and 2 as proposed.

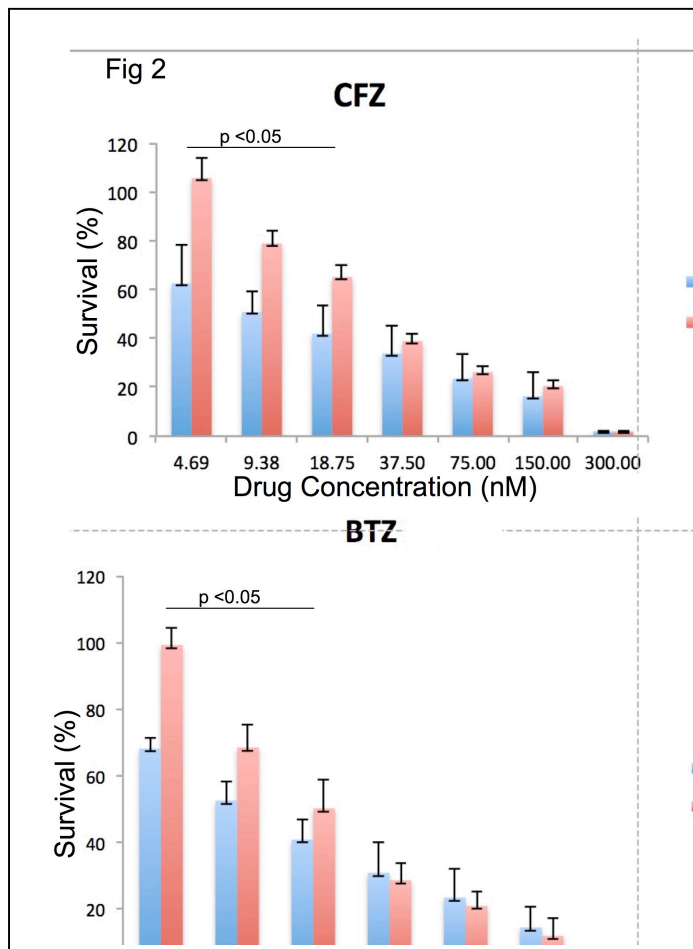
Milestone(s) Achieved:



Milestone 1: Using the combined efforts of the requested computational chemist and programmer we will be able to incorporate world-wide approved drugs into the database and screen them during the first year of this award.

Milestone 2: Addition of new targets will be linked to pathways and networks identified in the other aims and will proceed for both years of the proposal.

Major Task 2 is complete. We completed the work outlined in Major Task 2, Subtask 1, to establish CRC lines from mouse models (see below). Significant unforeseen difficulties were encountered deriving continuous cultures of prostate cancer cells the mouse prostate. The next step, is to publish with the patient-derived data



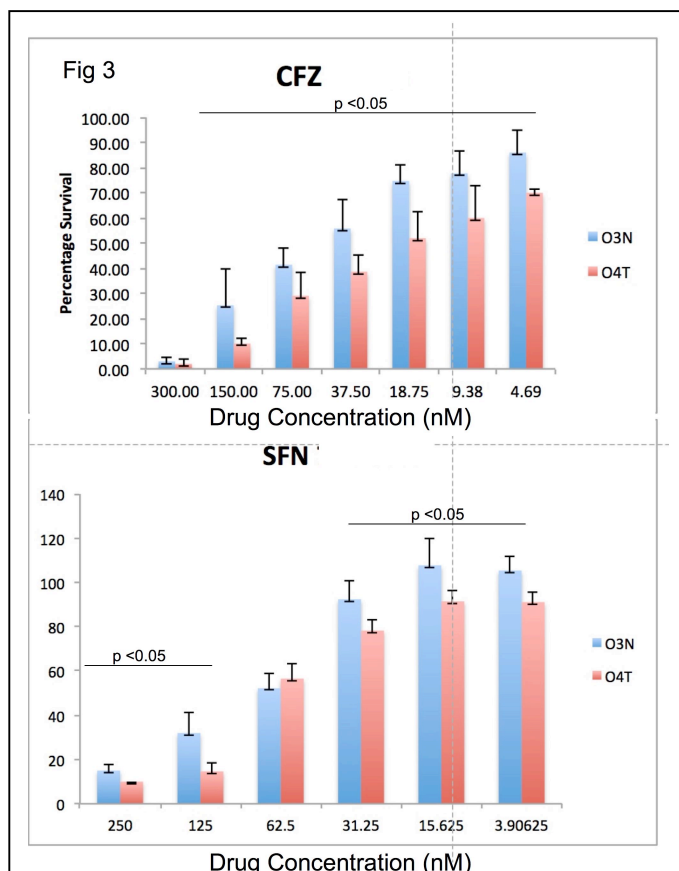
<p>Major Task 3: In parallel we have a growing collection of human prostate cells that have been prepared through conditional reprogramming with linked access to original formalin fixed cancer tissue, clinical data and, in a subset, frozen cancer tissue. We will complete molecular characterization of PCa CRCs by Illumina Human HT-12 v4 bead chip arrays to search for potential targets and develop them for testing of predicted drugs.</p>		Albanese	Dakshanamurthy Byers
--	--	----------	-------------------------

Major Task 3, Milestone 1 is complete. See below for details of completed Illumina transcription profiling and expression analyses.

To date in addition to the molecular profiling by Illumina bead array of 2 matched pairs of normal human prostate and prostate cancer CRCs used in the study, we performed proteomic arrays and have published these data (Tricoli et al, 2017). The proteomic and the transcriptomic data were used to verify the differential targeting of the tumor vs normal prostate cells by the repurposed drugs we have identified.

In the year 2 update, the microarray data were processed by TMFS and over a dozen FDA approved and experimental drugs were identified. Some of the tops scoring “hits” were already in clinical trials, and these were excluded for the time being.

Hits that were similar between the 2 patients and these hits included Sulforathane (SFN) and Phenethyl Isothiocyanate (PEITC). The previous dose response curves and the EC 50’s for normal and tumor lines were established are shown in Fig 1, in both patients 1 and 2, the tumor cells were significantly more sensitive to the compounds than the matched normal cells, as predicted by TMFS.



Additional drugs were recently identified and tested using these 2 lines including Carfilzomib (CFZ) and Bortezomib (BTZ).

As seen in Fig 2, both BTZ and CFZ were highly effective at low nanomolar (nM) concentration in reducing survival of the tumor cells (blue bars, PP8T) from the patient with Gleason 8 cancer. The tumor cells were significantly more sensitive ($p < 0.05$) at the lower, more clinically relevant concentrations of both drugs.

Similarly, the tumor cells (Fig 3, red bars, O3T) cells from the patient with Gleason 7 cancer were more sensitive to these drugs, vs the matched normal cells at all concentrations of drug with the exception of 62.5nM SFN, which appears to be an outlier.

RNAseq analyses were performed on normal and tumor cells treated with CFZ, BTA and SFN and at the submission of this report, we are analyzing these complex data to validate that the pathways identified are indeed targeted and to better understand the mechanism of action. These complex data will round out the requirements for the second paper on using the combination of TDCM and the primary cells to provide personalized cancer therapy via drug repurposing. We performed the complicated biostatistical analyses and used IPA and other pathways analysis software packages to comprehensively define the pathways that differentiate the effects of the repurposed drugs in the tumor cells.

For the **mouse studies**, we were unable to get the cells to grow *ex vivo*

Impact

Collectively, our new findings strongly suggest that the CRC and TMFS innovations synergize to give broad applicability in both rapidly identifying approved drugs and testing their efficacy in a personalized approach to patient treatment. We have identified repurposed drugs that target primary human prostate tumor cells at **nanomolar** concentrations, and do so with **more efficacy than with normal cells** from the same patient.

We firmly believe that the CRC technology in combination with our TMFS computational modeling software and database represents the future of personalized/precision prostate cancer research and treatment.

Changes/Problems

None

Products

Manuscript. Oncotarget, 2017,

Characterization of the Effects of Defined, Multidimensional Culture Conditions on Conditionally Reprogrammed Primary Human Prostate Cells.

Lucas Tricoli^{1,4}, Aisha Naeem¹, Erika Parasido¹, John P. Mikhael¹, Muhammad Umer Choudhry¹, Deborah L. Berry¹, Iman A. Abdelgawad², Richard J. Lee³, Adam S. Feldman³, Chukwuemeka Ihemelandu¹, Maria Avantaggiati¹, Deepak Kumar⁴, Stephen Byers¹, Rosa Gallagher⁵, Julia Wulfkuhle⁵, Emanuel Petricoin⁵, Olga Rodriguez^{1,6}, Chris Albanese^{1,6}.

¹Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC

²National Cancer Institute of Egypt, Cairo, Egypt

³Massachusetts General Hospital Cancer Center, Boston, MA

⁴Julius L. Chambers Biomedical/Biotechnology Research Institute, North Carolina Central University, Durham, NC.

⁵Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA.

⁶Preclinical Imaging Research Laboratory, Georgetown University Medical Center, Washington, DC

Manuscript : submitted under review

PROS-20-031: Predicting new drug indications for prostate cancer: The integration of an *in-silico* proteochemometric network pharmacology platform with patient-derived primary prostate cells

Aisha Naeem^{1,2*}, Sivanesan Dakshanamurthy^{1*}, Henry Walthieu¹, Erika Parasido¹, Maria Avantaggiati¹, Lucas Tricoli³, Deepak Kumar³, Richard J. Lee⁴, Adam Feldman⁴, Muhammad Saad Noon⁵, Stephen Byers¹, Olga Rodriguez^{1,6}, Chris Albanese^{1,6#}

¹Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC

²College of Health And Life Sciences, Hamad Bin Khalifa University, Doha, Qatar

³North Carolina Central University Julius L. Chambers Biomedical/Biotechnology Research Institute, Durham, NC.

⁴Massachusetts General Hospital Cancer Center, Boston, MA

⁵Data Science Institute, University of Arizona

⁶Center for Translational Imaging, Georgetown University Medical Center, Washington, DC.

Participants & Other Collaborating Organizations

Dr. Chris Albanese

Dr. Stephen Byers

Dr. Siva Daksanamurthy

Dr. Olga Rodriguez

Dr. Lucas Tricoli

Dr. Aisha Naeem

All at Georgetown University Medical Center, Washington DC

Special Reporting Requirements

None

Appendices

None