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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 *pten* mutant tumors from engineered mice and predicted drugs will be tested on conditionally reprogrammed cultures of these cells in vitro as well as in vivo 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 *in vitro* to calculate the LD50 in preparation for future studies in vivo.

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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 depravation 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

Year 4 Report - *(please note: a no-cost extension request was approved for this project, and the previously submitted Final Report covers most of the material included in this year 4 report)*

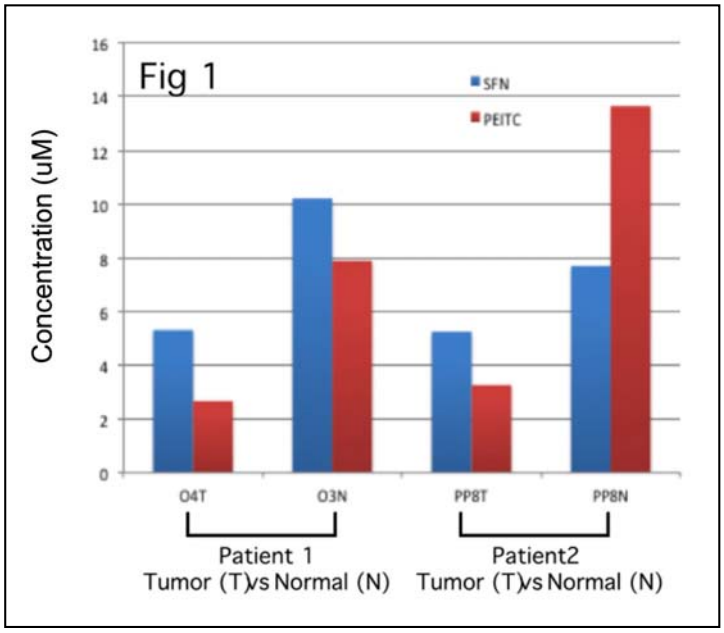
Accomplishments

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

<u>Major Task 1 was completed in Year 3; *no work for this Major Task was conducted during Year 4*</u>.

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:

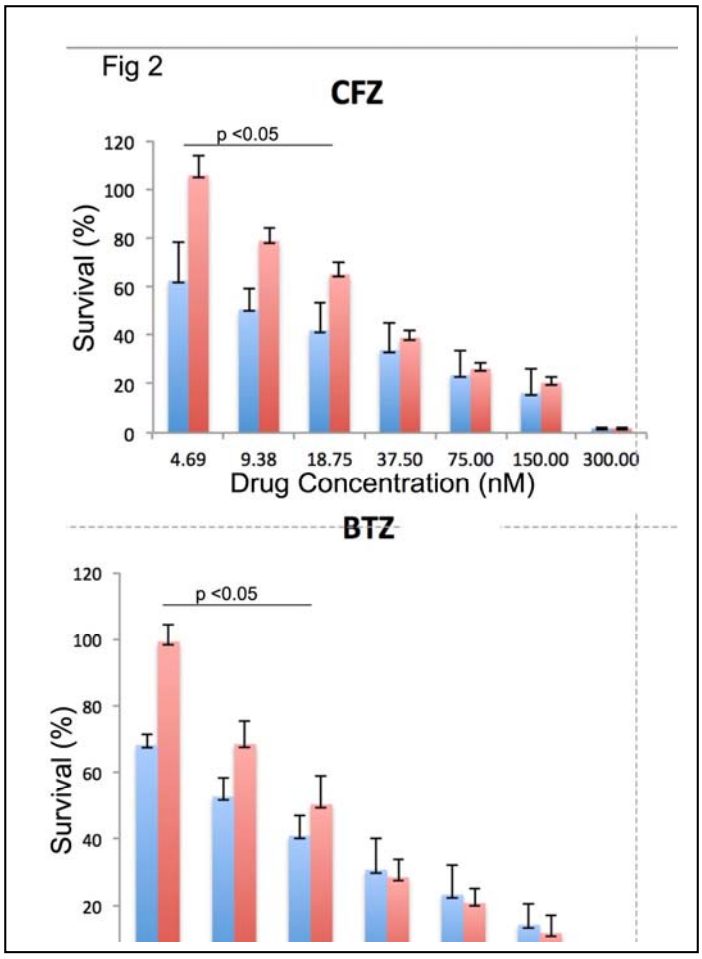


<u>Milestone 1</u>: Using the combined efforts of the requested computational chemist and programmer we will be able to incorporate world-wide approved drugs into the database and screen them during the first year of this award.

<u>Milestone 2</u>: 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: Use genetically engineered mouse model test systems to assess pharmacological response of prostate cancer cells with altered pten status.		Albanese	Dakshanamurthy Byers

<u>Major Task 2 is complete.</u> *During Year 4, 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.

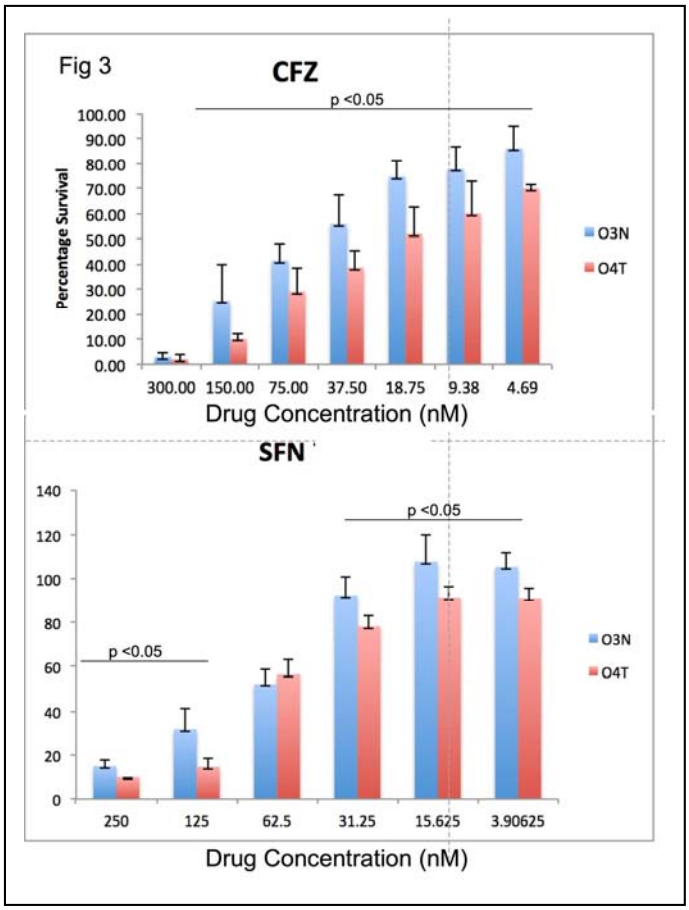


Major Task 3: In parallel we have a growing collection of human prostate cells that have been prepared through conditional reprogramming with linked access to original formalin fixed cancer tissue, clinical data and, in a subset, frozen cancer tissue. We will complete molecular characterization of PCa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

Major Task 3, Milestone 1 was completed during Year 4. 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, 03T) 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 biostatical 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**, using MRI we identified mice with prostate tumors and successfully developed continuous cultures.

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 Naeem[1], Erika Parasido[1], John P. Mikhaiel[1], Muhammad Umer Choudhry[1], Deborah L. Berry[1], Iman A. Abdelgawad[2], Richard J. Lee[3], Adam S. Feldman[3], Chukwuemeka Ihemelandu[1], Maria Avantaggiati[1], Deepak Kumar[4], Stephen Byers[1], Rosa Gallagher[5], Julia Wulfkuhle[5], Emanuel Petricoin[5], Olga Rodriguez[1,6], Chris Albanese[1,6].

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***Manuscript: completed and submitted during Year 4* - Accepted, July 22, 2020**
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
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Special Reporting Requirements

None

Appendices

None