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TITLE: Bumped-Kinase Inhibitors as Castrate-Resistant Prostate Cancer Drugs

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CONTRACTING ORGANIZATION: University of Washington, Seattle, WA

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Background: Kinase inhibitors present exciting therapies for cancer including prostate cancer. We have developed a class of kinase inhibitors, bumped kinase inhibitors (BKIs), that have narrow kinase specificity due to their unique binding of the ATP-binding site and activity against androgen receptor (AR) positive prostate cancer cells. Despite of the life extending therapies of the newest drugs targeting the AR e.g. abiraterone and enzalutamide, tumors almost universally acquire resistance, and survival is extended by only four months.
Hypotheses 1: BKIs are specific candidates for treatment of AR-driven CRPC. **2:** BKIs act directly or indirectly by inhibition of AR Ser81 phosphorylation necessary to activate AR to stimulate transcription.

Study Design: In aim1, we will use BKI-kinome screening of prostate cancer cells to discover kinase targets of our BKI's, BKI induced changes in phosphoproteome, and BKI effects on pSer81 to determine the targets and pathways effected by BKIs. We currently have a good lead BKIs with EC_{50}'s of 8uM. However, a more ideal candidate to take to the clinic will have an EC_{50} of <3uM. A structure–activity relationship model (SAR) has been developed. Therefore, in Aim 2 additional BKIs will be synthesized using this SAR and screened for CRPC activity with a goal for an EC_{50} of ≤ 3 μM. Selected BKIs will be screened against enzalutamide resistant PDX models. A Target Candidate Profile (TCP) and work flow to evaluate BKIs for efficacy, pharmacokinetic and safety properties will efficiently direct us to choose a pre-clinical candidate for an IND

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

Subject: Recurrence through continued androgen receptor (AR) signaling remains the driver in > 90% of men who become resistant to therapy. New treatments are urgently needed for this progressive disease. *Therapies that inhibit factors important in activating AR may be the most successful against these constitutively active variants and prevent further progression of CRPC.* Kinase inhibitors have the potential to inhibit androgen receptor signaling and function. **Purpose and Scope of Research:** Establish that BKIs are specific candidates for treatment of AR-driven. BKIs work as PK inhibitors and act directly or indirectly by inhibition of kinase activity, which is necessary to activate AR to stimulate transcription. Develop new BKI's for treatment of AR- driven castrate resistant prostate cancer.

2. KEYWORDS:

Prostate, Cancer, Kinase, Inhibitor Androgen Receptor Glycolysis ATP Hexokinase Glut1acetyl coenzyme A carboxylase

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1: Establish Mechanism (s) of action of BKIs – The original completion date for this Major task was 30 Sept 2020. This Task was completed 30 August 2021.
Major Task 2: Develop potent BKIs for CRPC while retaining minimal Off Target Activity – 250 BKI type molecules were synthesized and tested on prostate cancer cell models as well as non-prostate cancer cell lines. Four PDX human prostate cancer xenografts were tested. This task was scheduled to be completed September 2020 and was completed July 2020
Major Task 3: Analyze lead compounds for potency, efficacy, and safety. This task was scheduled to be completed September 2020 and was completed August 2021.

Tasks one and 2 were delayed due to a slowdown in laboratory and animal work due to the COVID pandemic. However, all tasks were completed 9/30/2021 with the 1 year no cost extension.

What was accomplished under these goals?

In this final reporting period we demonstrated that our lead compound 1553, which is orally bioavailable, suppressed growth of LuCaP lines in which the target of the MOA was expressed but had no effects in those lines where the target was not expressed. Importantly as we have shown the primary target for the mechanism of action is hexokinase 2. This is the initial glycolytic enzyme after glucose enters the cell and fixes it for glycolysis. We demonstrated that this was the target for our lead clinical candidate and mechanism of action was demonstrated by three methods (1) Radiolabeled uptake. Uptake of 3H-3-O-methylglucose and 3H-2-deoxyglucose was measured as carried out previously {Sweet, I. R., Cook, D. L., Lernmark, A., Greenbaum, C. J., Wallen, A. R., Marcum, E. S., Stekhova, S. A. and Krohn, K. A. (2004) Systematic screening of potential beta-cell imaging agents. Biochem Biophys Res Commun. **314**, 976-983}.

(2) In vivo cross-linking with strept-avidin pull-down using a modification we made of 1553 in which we added a lipid linker with an attached biotin bead.

Top candidate was HK2 as shown in the **table (below).** (3) CRISPr/Cas9 knock-out demonstrate a loss of 1553 activity in LNCaP cell line, **Figure (below)**.

Importantly, HK2 has low expression in normal prostate tissue but at high levels in high grade prostate cancer.

In our human prostate PDX mouse study , we have previously shown that LuCaP 35 growth was significantly suppressed by 1553; in contrast, the LuCaP 77 model growth was not affected by 1553. LuCaP35 expresses high levels of HK2 but LuCaP 77 has undetectable levels. Thus Major Tasks 1 and 2 are complete.

We subsequently had moved to Major Task 3. As we have shown in mice there was no toxicity seen at therapeutic levels of 1553 with oral administration after 9 weeks treatment. To further evaluate toxicity went on to study the rat model with daily doses of 1553 per OS of 30, 60, and 100 mgs per kg daily. Our therapeutic dose is 20 mgm/kg 3 x a week in mice. At the 60 and 100 mgs/kg doses mice lost weight and had to be terminated at 1 week or less. Histopathology showed no specific organ damage; however, chemistry showed marked elevation of glucose to 350-400mg/dl. We measured serum insulin levels and they were 5x normal compared to control mice. These data are consistent with on target effects of inhibition of HK2. At 30 mgs /kg daily there was minimal elevation of glucose and no wt loss. Trough levels of drug in the 60 and 100 mg/kg dose were 20-30 fold above the therapeutic levels. This these studies demonstrate on target effects of 1553 and no off target toxicity. On target toxicity occurred at blood levels significantly higher than therapeutic levels. We conclude, that based on this data our lead compound can move forward toward pre-GLP and GLP work.

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

Table. Top candidates from pull-down assay.

-Log Student's T-test p-value X_C	Student's T-test Difference X_C	Protein names	Gene names
1.562061	2.783851	Hexokinase;Hexokinase-2	HK2
2.57524	2.36061	Prefoldin subunit 2	PFDN2
4.136963	2.153111	Protein ERGIC-53	LMAN1
3.120451	1.629074	Tumor protein D54	TPD52L2

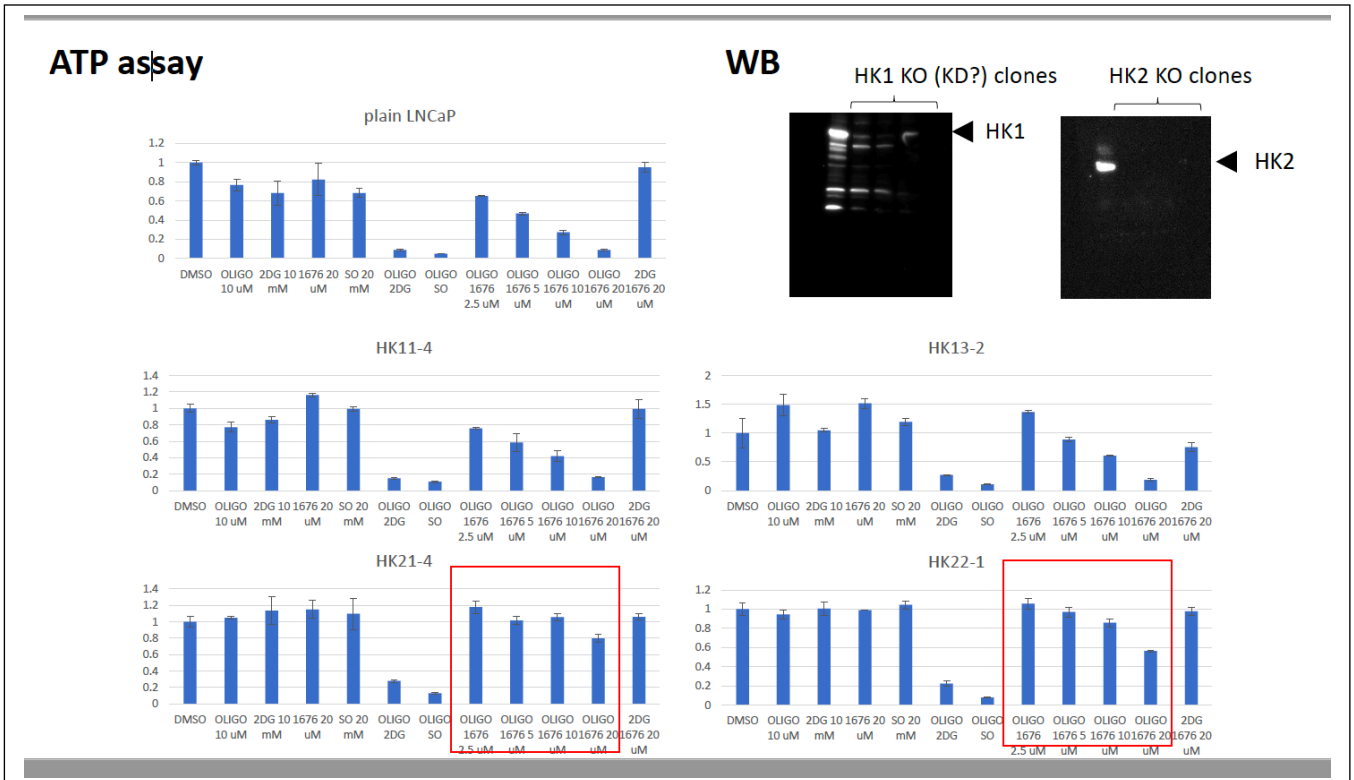

Figure. Western Blot **(WB)** demonstrating CRISPr knock -out of hexokinase 1 and 2. ATP assay showing resistance to SGI 1676 based on ATP suppression in combination with oligomycin. HK2 KO blocks effects of 1676 HK21-4 and HK22-1 but no effects of HK-1 KO.

Three undergraduate students worked 1:1 with Dr. Uo and Dr. Plymate for the academic year on this project. They became proficient with protein and RNA analysis used in this project.

How were the results disseminated to communities of interest?

Results presented at AACR. Publication for *Cancer Discovery* in progress.

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

This is the Final Report on this grant. We now have a DOD Translational grant and NIH WE REACH grant to move the lead compound to an IND and clinical trial

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

This project led to development of a first in class glycolysis (glucose) inhibitor that can be translated to a drug for prostate cancer. The results of this study also led to the acquisition of a WE-Reach grant. This is a combined NIH/University of Washington sponsored grant that is designed to lead towards approval of a drug or device for patient use. In this case pre-GLP studies including toxicity studies in two species and pre-IND submission.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Led to development of a small company, ProsTech, for drug development.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to Report

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

None

Changes that had a significant impact on expenditures

None

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.

  Using biochemistry and biophysics to extinguish androgen receptor signaling in prostate cancer.

Asangani I, Blair IA, Van Duyne G, Hilser VJ, Moiseenkova-Bell V, **Plymate S**, Sprenger C, Wand AJ, Penning TM. J Biol Chem. 2021 Jan-Jun;296:100240. doi: 10.1074/jbc.REV120.012411. Epub 2021 Jan 9. PMID: 33384381 Free PMC article.

Androgen Receptor Signaling and Metabolic and Cellular Plasticity During Progression to Castration Resistant Prostate Cancer.
Uo T, Sprenger CC, **Plymate SR.** Front Oncol. 2020 Oct 9;10:580617. doi: 10.3389/fonc.2020.580617. eCollection 2020. PMID: 33163409

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**

Small molecule synthesis

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

Patent application number, issued patent number, trademark registration number, copyright number, etc.	Title: Status and date	For a patent/patent application the major types of claims
Provisional Patent filed by UW on 7Jan2020, UW 48731	MODIFIED PYRROLO- AND PYRAZOLO- PYRIMIDINES FOR PROSTATE CANCER THERAPY	The claims of this patent cover derivatives of pyrazolo-pyrimidines (PP, BKI-1553 is a PP) and pyrrolo-pyrimidines that are active on prostate cancer, but not covered by previous patents, protecting 1553 and providing further diversity of backups

Other Products

Led to development of a small company, ProsTech, for drug development.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Wesley C. Van Voorhis
Project Role: PI
Research Identifier: ORCID ID: 0000-0001-6141-2015
Nearest person month worked: **0.1**
Contribution to Project: Planning of experiments, discussion with other Co-PIs/Co-Invs, interpretation of data, communication with all groups
Funding Support: NA

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

Not Relevant, note FINAL REPORT

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Reports will be reported separately, as required.

QUAD CHARTS:

9. APPENDICES: