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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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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 EC50's of 8uM. However, a more ideal candidate to take to the clinic will have an EC50 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 EC50 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						
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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	3
2. Keywords	3
3. Accomplishments	3-7
4. Impact	8
5. Changes/Problems	9-10
6. Products	10-11
7. Participants & Other Collaborating Organizations	11
8. Special Reporting Requirements	16
9. Appendices	16

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 AR Ser81 phosphorylation, which is necessary to activate AR to stimulate transcription. Develop new BKI's for treatment of AR- driven castrate resistant prostate cancer.

2.

KEYWORDS:

Bumped kinase inhibitor (BKI) Androgen receptor (AR), Castration resistant prostate cancer (CRPC), phospho-proteome.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Task 1: Establish Mechanism (s) of action of BKIs

Subtask 1: Determine kinase profile affected by BKIs in CRPC cells

Subtask 2: Determine role that serine phosphorylation plays in the pathway of suppression of tumors growth

- Treat cell lines with lead BKI candidates and determine knockdown effects of CDKs on cell proliferation
- Determine effects of serine phosphorylation on nuclear translocation, chromatin interaction (CHiP), and AR transcriptome
- Determine phosphorylation targets of BKIs

Task 2: Develop potent BKIs for CRPC while retaining minimal off Target Activity

Task 3: Analyze leads for potency, efficacy, pharmacokinetics, and safety to progress the optimal leads to a pre-clinical candidate and a back-up molecule. A Target Candidate Profile (TCP) and work flow to evaluate BKIs will direct us to optimize BKIs to efficiently choose a pre-clinical candidate for an IND.

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Task 1 was complete in 2019 and is in last year's report.

Task 2. In Figure 1 we show in the right hand panel those BKIs that have specificity for our target LNCaP 95 cell line leave the PC-3 line un-affected from this group of compounds. From this group of compounds we have selected 1553, 1676, and GP 228 to bring forward for clinical consideration based on potency. For a negative control compound we are using BKI-1817 since it has no activity on either PC-3 or LNCaP95 cells.

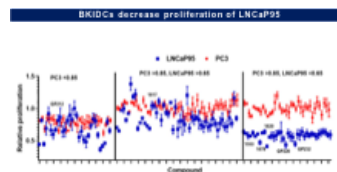


Figure 1.

In Figure 2 we see in the principle component analyses of 1553, 1676, and GP228 that their transcriptomes are similar. This finding was in spite of 1676 having no obvious kinase type 1 activity on our kinobead assay, Figure 3

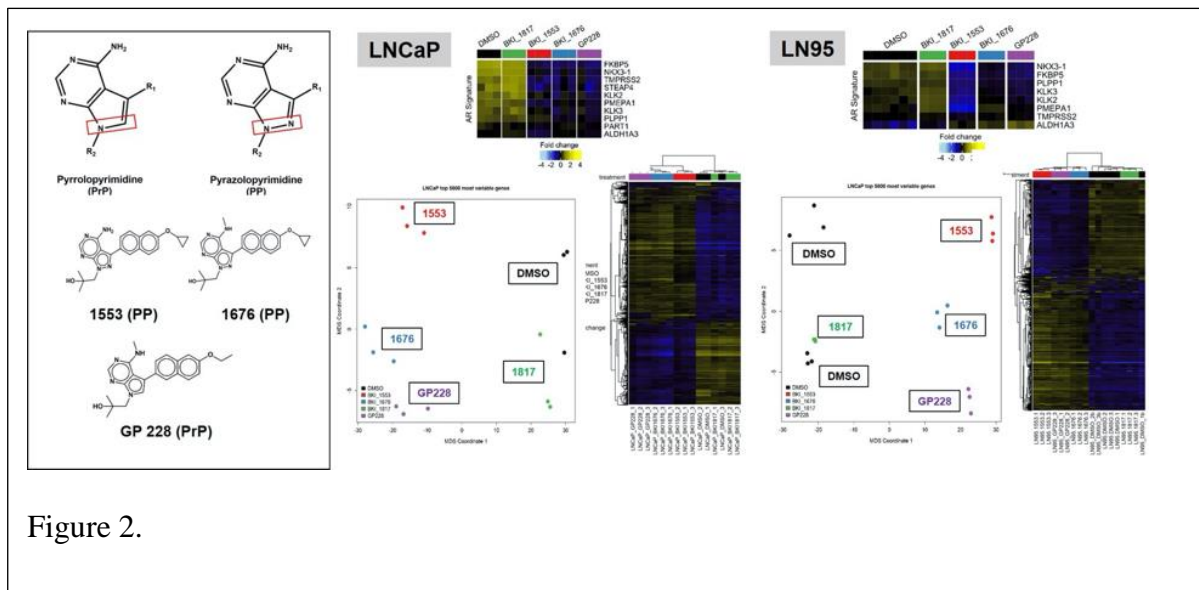
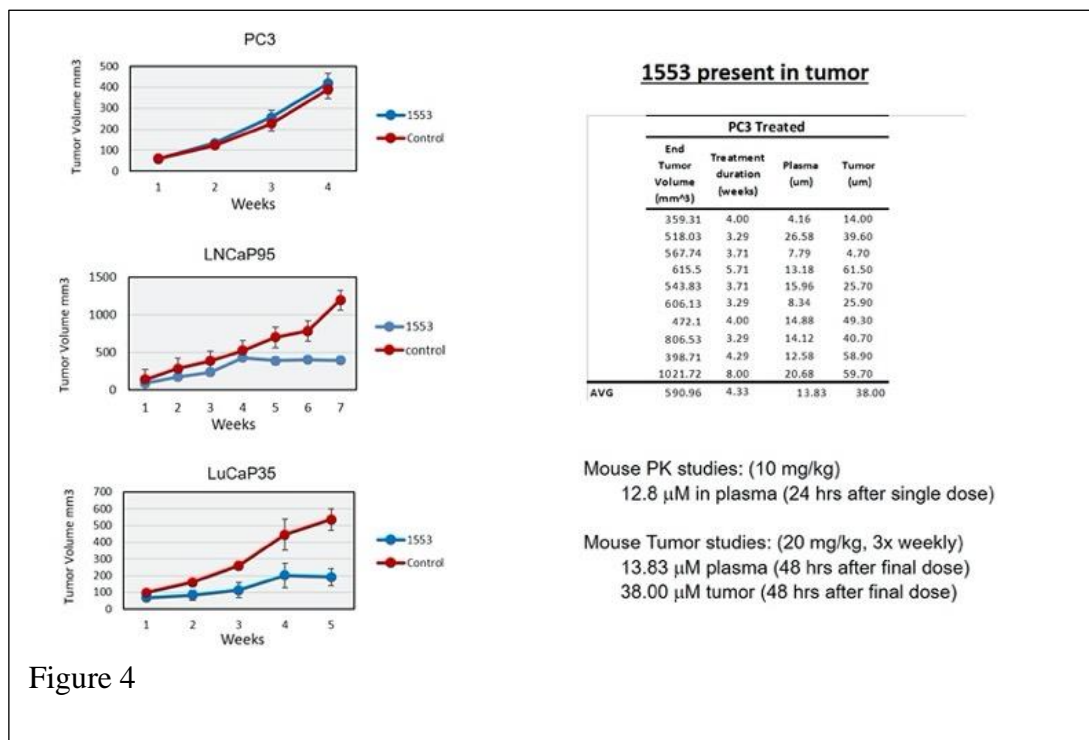
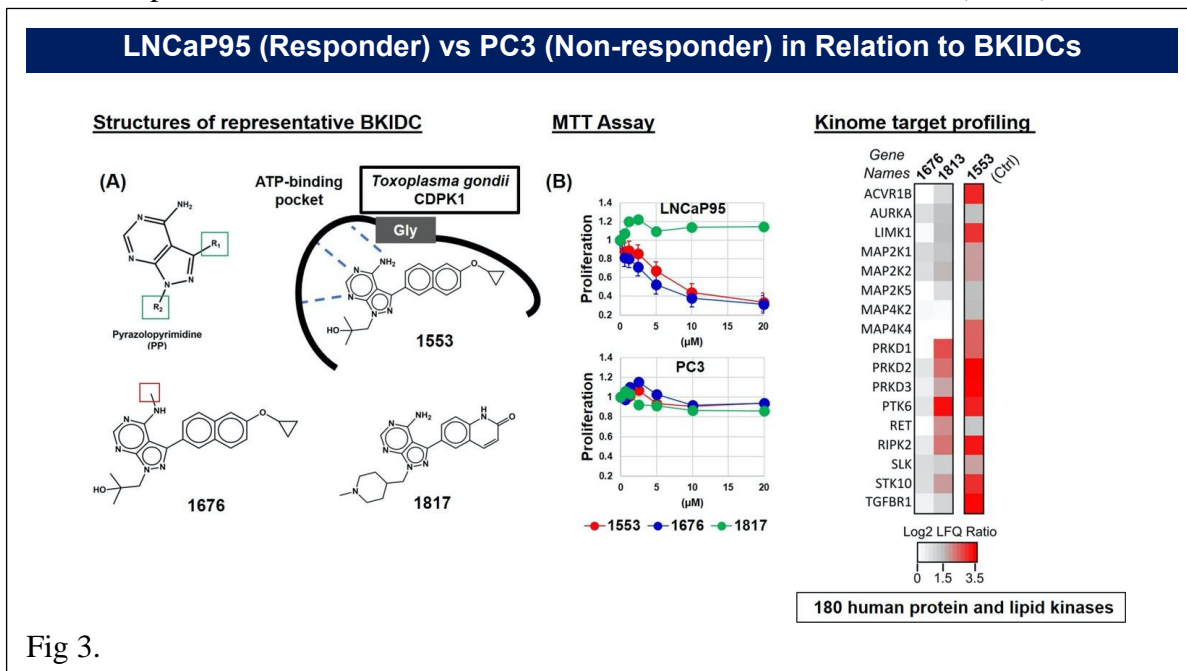


Figure 2.

Since we have shown that BKI 1553 has good oral bioavailability and at a PO dose of 20mg/kg three times a week has plasma and tumor levels that are at or above our in vitro EC50 (Table), we chose it as our lead



compound to take forward for in vivo studies. In figure 4. We see that as expected it has no activity against PC3 xenografts in SCID mice but markedly suppressed growth of LNCaP95 xenografts and LuCap35 human PDX model, Figure 4. These studies treated the mice with BKI1553 up to 7 weeks and no obvious toxicity was noted. In vitro toxicity confirmed lack of toxicity.

Task 3. Two back-up molecules that are orally bioavailable with similar PK characteristics have been developed but we will continue to move forward with 1553. A second human PDX model, LuCap77 will be tested. Additionally, for Task 3, we have received We-Reach funding from the University of Washington/NIH. This funding will allow us to perform a pre-GLP rat toxicity study by our selected CRO. In addition, polymorph studies will be performed to develop a stable oral tablet. Once the rat toxicity study has been completed, it should permit searching for a Pharma partner to perform the more expensive GLP rat and dog toxicity studies. On the chance that a pharma partner is not found we will apply for a phase 2 STTR grant from NCI to complete these studies. Finally, a pre-IND meeting will be arranged with FDA once rat pre-GLP studies are complete.

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

Nothing to Report

How were the results disseminated to communities of interest?

Pacific Northwest SPORE teleconference – Oct 15 2020

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

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan additional animal safety studies and a FDA pre-IND meeting

4. IMPACT:

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

We have developed the plan to move towards an IND and phase 1 clinical trial and IND with 1553

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:

Due to COVID-19 progress on animal studies was stopped from March to Sept 2020.

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

COVID-19 will continue to delay progress , exactly how cannot be determined at this time.

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 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: Wesley Van Voorhis

Project Role: PI

Research Identifier:

Nearest person month worked: 0.7

Contribution to Project:

Funding Support:

Name: K.K. Ojo

Project Role: Co-Investigator

Research Identifier:

Nearest person month worked: 2.4

Contribution to Project:

Funding Support:

Name: Lynn Barrett

Project Role: Research Manager

Research Identifier:

Nearest person month worked: 1

Contribution to Project:

Funding Support:

Name: Ryan Choi

Project Role: Research Scientist

Research Identifier:

Nearest Person Month Worked: 1.1

Name: Matt Hulverson

Project Role: Research Scientist

Research Identifier:

Nearest person month worked: 0.2

Contribution to Project:

Funding Support:

Name: Claire Colson

Project Role: Grant and Fiscal Specialist

Research Identifier:

Nearest person month worked: 1

Contribution to Project: coordination of the project grant and fiscal activities

Funding Support:

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

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: