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

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

U.S. Army Medical Research and Development Command
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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

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 $\leq$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

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

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INSERT THE STANDARD FORM 298 HERE: *Sample SF 298 is provided at http://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting. Choose corresponding Distribution Statement.*

An abstract shall be provided in Block 14 and shall state the purpose, scope, and major findings and be an up-to-date report of the progress in terms of results and significance. Abstracts will be submitted to the Defense Technical Information Center (DTIC) and shall not contain proprietary information. Subject terms are keywords that may have been previously assigned to the proposal abstract or are keywords that may be significant to the research.

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

Major 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
    - **Major Task 2:** Develop potent BKIs for CRPC while retaining minimal off Target Activity
    - **Major 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.

What was accomplished under these goals?

Subtask 1: Determine kinase profile affected by BKIs in CRPC cells; - Using the kinobead assay – profiles of inhibited kinases in LNCaP, LNCaP95 and VCaP cells were determined. Depending on BKI and SAR type 1 kinases inhibited varied between 1 and 10 kinases identified in a 250kinase screen.

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.

We first looked at the effects of 300 kinase inhibitors and their effects on proliferation in a range of prostate cancer cell lines that were AR positive and negative, requiring that they be active in the low uM to nM range in castration resistant AR- positive cells and have no effect in AR negative lines. We also developed a kinase-activity dead molecule with the same base structure (1817) to serve as a negative control. 70 of our 300 BKIs met the inclusion criteria.

The RNA seq data sown in Figures 1 and 2 of the supplementary data show that 4 different BKIs suppress the androgen receptor transcriptome as well as the CCP31 gene cancer cell proliferation profile. AR ChIP has been started using the unique ChIP PIXUL platform. In studies done so far we have not seen alteration in nuclear translocation of the AR when treated with BKIs. This was somewhat surprising given the decrease in ser81 phosphorylation noted on the AR. Additional studies will be done.

- In order to determine the phosphorylation targets of our BKIs we first developed a BKI probe with an aliphatic side chain to which a sepharose bead was attached. We demonstrated that this BKI was functional. We subsequently used this construct for pull-down of proteins from LNCaP 95 cell line. Pull -downs were the assayed for proteins by MS, or after selection of kinases using our kinobead assay, evaluated for change in phosphorylation status of kinases. These studies were done a 30 min, 2 hr and 4 hr after treatment with four BKIs. As shown in figure 3 in the appendices. There was no change in proteins after 4 hours but a marked alteration in phosphorylation status, **Figure 3**. As shown in the VENN diagram in **Figure 4** in the appendices at 30 min only 1 kinase was dephosphorylated, PRKAA2, the α2 catalytic subunit of AMPK. The de-phosphorylation occurred at ser486. This is a suppressive phosphorylation that when decreased activates AMPK. Indeed as shown in appendices **Figure 5**, our BKIs activate AMPK as determined by p-acetyl coenzyme A carboxylase increase.
- We have constructed an additional 250 BKIs using 2 scaffolds and are currently determining for aim 2 the most active molecule with few off-target effects.

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

This project has provided traing opportunities fortwo undergraduate students and one post-doctoral students. We have monthly combined lab meetings between the Plymate, Maly , and van Voorhis laboratories.

How were the results disseminated to communities of interest?

Presentations at Seattle Program in Prostate Cancer

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

Further develop MOA and testing of lead molecules in animal models in preparation for IND application.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Inhibition of androgen receptor signaling with current agents significantly prolongs life but all patients become resistant to these agents. Our findings provide a new unique way to further suppress AR activity by altering the cancer cell metabolism with minimal toxicity.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Patents granted:

United States Patent
Van Voorhis et al.

(10) **Patent No.: US 10,350,211 B2**
(45) **Date of Patent: Jul. 16, 2019**

BUMPED KINASE INHIBITOR COMPOSITIONS AND METHODS FOR TREATING CANCER

(58) **Field of Classification Search**
CPC ... A61K 31/519; A61K 31/4985; A61P 33/02; A61P 35/00; C07D 487/04

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*

- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

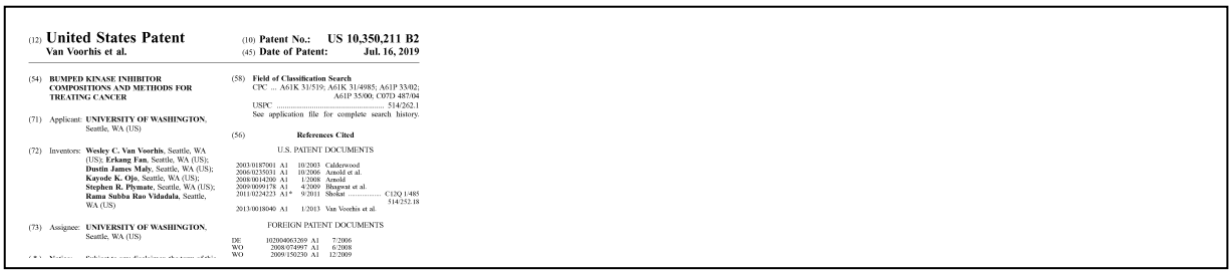
- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.



(12) **United States Patent**
Van Voorhis et al.

(10) **Patent No.: US 10,350,211 B2**
(45) **Date of Patent: Jul. 16, 2019**

(54) **BUMPED KINASE INHIBITOR COMPOSITIONS AND METHODS FOR TREATING CANCER**

(71) Applicant: **UNIVERSITY OF WASHINGTON**, Seattle, WA (US)

(72) Inventors: **Wesley C. Van Voorhis**, Seattle, WA (US); **Erkang Fan**, Seattle, WA (US); **Dustin James Maly**, Seattle, WA (US); **Kayode K. Ojo**, Seattle, WA (US); **Stephen R. Pfymate**, Seattle, WA (US); **Rama Subba Rao Vidadala**, Seattle, WA (US)

(73) Assignee: **UNIVERSITY OF WASHINGTON**, Seattle, WA (US)

(58) **Field of Classification Search**
CPC ... A61K 31/519; A61K 31/4985; A61P 33/02; A61P 35/00; C07D 487/04
USPC 514/262.1
See application file for complete search history.

(56) **References Cited**

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2013/0018040	A1	1/2013	Van Voorhis et al.	

FOREIGN PATENT DOCUMENTS

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WO	2008/074997	A1	6/2008
WO	2009/150230	A1	12/2009

- **Other Products**

- *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Stephen Plymate*
Project Role: *PI*
Nearest person month worked: *2*

Contribution to Project: *Overall project PI*
Funding Support: DOD, NCI, VAMC

Name: *Dustin Maly , PHD*
Project Role: *Co-PI*
Nearest person month worked: *2*

Contribution to Project: *Dr. Maly designed BKIs.*
Funding Support:NIH, DOD

Name: *Wes van Voorhis*
Project Role: *Co-PI*
Nearest person month worked: *1*

Contribution to Project: *Performed PK and Toxicity studies*
Funding Support: NIH, DOD, Gates Foundation

Name: *Takuma Uo*
Project Role: *Res Assistant Professor*
Nearest person month worked: *1*

Contribution to Project: *AMPK assay*
Funding Support: DOD, VA

Name: *Cynthia Sprenger*
Project Role: *res AssistantProffesor*
Nearest person month worked: *1*

Contribution to Project: *Designed animal work*
Funding Support:DOD, VA

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:.

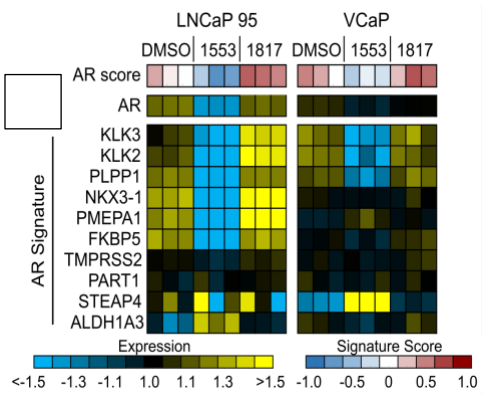

Figure 1. AR gene signature in LNCaP95 and VCaP cells is suppressed by 1553.

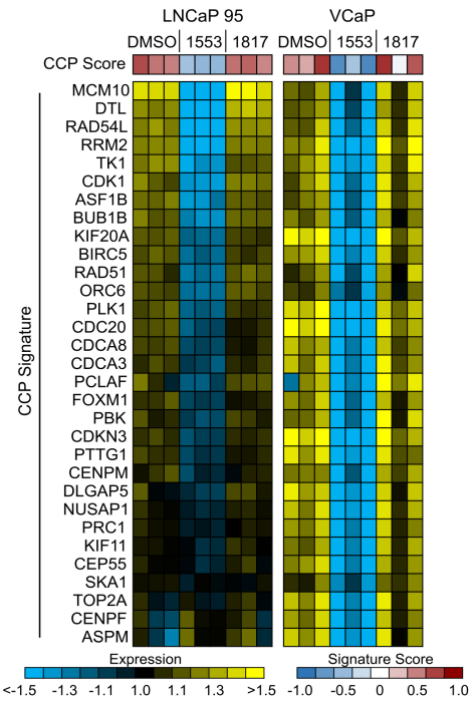

Figure 2. Cell Cycle Progression signature in LNCaP95 and VCaP cells is suppressed by 1553.

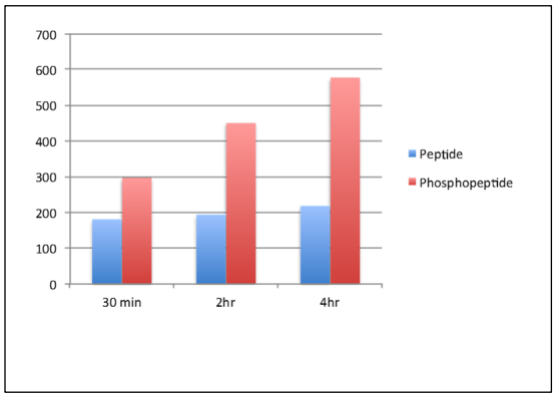

Figure 3. Results of phosphoprotein and phosphoproteome studies in LNCaP95 cells demonstrating no increase in changes in the diversity of peptides detected over the 4 h time period but significant increases in the diversity of phosphoproteome.

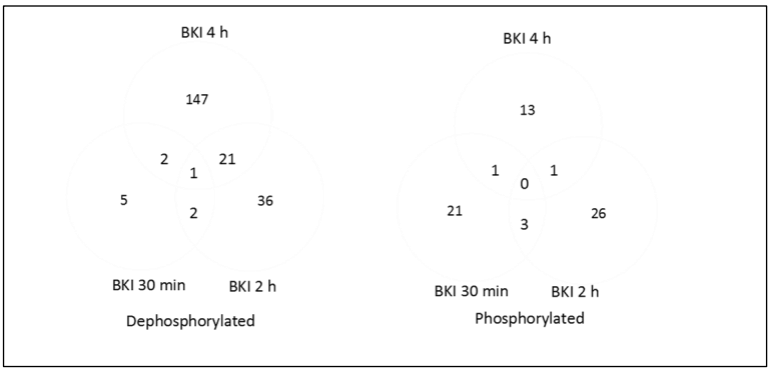

Figure 4. Results of phosphoproteome analysis showed only one protein differentially dephosphorylated at 30 min that maintained dephosphorylation at 2 and 4 hrs, PRKAA1 (Ser 486 of the $\alpha 1$ subunit of AMPK). No proteins were differentially phosphorylated at the 30 min time point and maintained phosphorylation at each subsequent time point. This suggests derepression of AMPK is a proximal event in the BKI effect on CRPC.