

AWARD NUMBER: W81XWH-12-1-0401

TITLE:

Development of Novel Drugs That Target Coactivation Sites of the Androgen Receptor for Treatment of Antiandrogen-Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR:

Artem Cherkasov

CONTRACTING ORGANIZATION:

University of British Columbia

Prostate Centre at the VGH

2660 Oak Street

Vancouver, BC, Canada

V6H 3Z6

REPORT DATE:

December 2015

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 December 2015			2. REPORT TYPE Final		3. DATES COVERED 30 Sep 2012 - 29 Sep 2015	
4. TITLE AND SUBTITLE Development of Novel Drugs That Target Coactivation Sites of the Androgen Receptor for Treatment of Antiandrogen-Resistant Prostate Cancer					5a. CONTRACT NUMBER	
					5b. GRANT NUMBER W81XWH-12-1-0401	
					5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Artem Cherkasov; Paul Rennie E-Mail: acherkasov@prostatecentre.com					5d. PROJECT NUMBER	
					5e. TASK NUMBER	
					5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of British Columbia Prostate Centre at the VGH 2660 Oak Street Vancouver, BC, Canada V6H 3Z6					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 Interest in developing androgen receptor (AR) inhibitors with novel mechanism of action is slowly increasing since commercial anti-androgens (Bicalutamide, Flutamide, Nilutamide and Enzalutamide) face therapeutic limitations. Current therapies fail over a period of time because they all target hormone binding pocket on AR to which the receptor has already developed effective resistance mechanisms. One of the promising strategies to combat drug resistance is to develop the inhibitors that target an alternative binding pocket of the AR, called Binding Function 3 (BF3). In the current study, we report indole chemical series, identified through systematic <i>in silico</i> screen, as leading AR BF3 inhibitors based on parental compound VPC-13566, which demonstrated excellent anti-androgen potency, anti-PSA activity and abrogates androgen-induced proliferation of LNCaP and Enzalutamide-resistant prostate cancer cell lines. These studies resulted with a lead inhibitor that was similar to 13566 in activity while being almost 10-fold more stable in microsomes experiment. This lead compound thus possess drug-like properties that could have great potential for alternative treatment of prostate cancer.						
15. SUBJECT TERMS Prostate cancer, small molecule drugs, androgen receptor, chemical genomics, drug resistance, hormone resistance, computer-aided drug design						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC	
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)	
Unclassified	Unclassified	Unclassified	Unclassified			

Table of Contents

	<u>Page</u>
1. Introduction.....	3
2. Keywords.....	3
3. Overall Project Summary.....	3
4. Key Research Accomplishments.....	9
5. Conclusion.....	9
6. Publications, Abstracts, and Presentations.....	9
7. Inventions, Patents and Licenses.....	10
8. Reportable Outcomes.....	10
9. Other Achievements.....	10
10. References.....	11

- 1. INTRODUCTION:** Androgen receptor (AR), a member of the nuclear hormone receptor (NHRs) is a ligand-dependent transcription factor (1) with significant therapeutic relevance in prostate cancer (PCa) (2). Conventional AR-based therapeutics have mainly focused on targeting the traditional hormone binding pocket of the receptor (3;4). However, over a period of time, therapeutic efficacy of these drugs suffered from the problem of drug resistance (5) due to 1) mutations at the ligand binding pocket causing structural and functional changes of the receptor and 2) conversion of antagonist into an agonist in a physiological context. Antiandrogen-based therapy including second generation androgen receptor (AR) inhibitors, Enzalutamide represents a typical case, where drug resistance is inevitable and its onset is associated with poor prognosis and high mortality (6). This highlights the urgency to develop entirely new types of anti-AR therapeutics with novel mode of action *i.e.* rather than blocking ligand interaction with AR the novel class of drugs should ideally bind to alternative sites on the receptor thereby effectively disrupting the association of coactivators that bind to these sites. Recently, a co-regulatory surface site called the Binding Function 3 (BF3) has been identified by Fletterick *et al* (7). The specific function of this pocket has not yet been fully characterized, although recent reports provide some evidence of its possible involvement in the AR association with FKBP52 - an important positive regulator of this receptor (8) and possible cross-talk with the AF2 site (9). In addition, BF3 is conserved among other members of NHR family, thereby offering a better opportunity to understand the molecular mechanism of co-factor recruitment and subsequently its inhibition. Since AF2 and BF3 surface pockets play pivotal role in mediating AR function, therapeutic targeting of these sites offers a rich vein for the discovery of novel drugs for PCa with alternative mechanism of action and thereby circumvents treatment resistance seen with conventional anti-androgens. These drugs would provide an additional line of therapy for patients combating castration-resistant PCa thereby considerably expanding their life span.
- 2. KEYWORDS:** Prostate cancer, small molecule drugs, androgen receptor, chemical genomics, drug resistance, hormone resistance, computer-aided drug design
- 3. OVERALL PROJECT SUMMARY:**

- a. What were the major goals of the project?**

Specific Aim 1: to use the generated structure-activity data and the resolved crystal structure to design and synthesize AR AF2 and AR BF3 binders with enhanced target-affinity and 'drug-like' properties.

Specific Aim 2: to experimentally evaluate the developed synthetic derivatives.

Specific Aim 3: to select several lead compounds for pharmacological development.

ACCOMPLISHMENTS:

Key outcome 1: Molecular modeling and synthesis of derivatives of our lead BF3 binders VPC-0098 and a closely related VPC-4035.

During the first year of the project we identified 2 derivatives of VPC-0098 with excellent activity in the low nanomolar range (VPC-13562 and 13566). In the following two years, we made multiple attempts to enhance the activity profile of these new BF3 leads via structure based lead optimization and importantly to improve their stability and their formulation so that ultimately these lead derivatives will be compatible for oral dosing in future phase I clinical studies. Each subsequent chemical series was identified based on the scaffold of active compounds VPC-13566 from the previous series. Based on these findings, a total of 250 small molecule compounds were synthesized in year 2 and 3 either at Enamine

(<http://www.enamine.net/>) or at our collaborator Robert Young's laboratory and send to the VPC for further biological testing and characterization.

Key outcome 2: Identification of a new lead compound that combine excellent activity against the AR while being stable metabolically.

eGFP Cellular AR Transcription Assay.

All the 250 compounds were screened for their ability to inhibit AR transcriptional activity using a nondestructive, cell-based enhanced green fluorescent protein (eGFP) AR transcriptional assay (10). To ensure these values are true positive hits in the AR transcriptional eGFP assay, we validated their activity by a second transcription assay, based on light detection instead of fluorescence, by quantifying their effect on the production of the prostate specific antigen (PSA) in prostate cancer cell lines (11). PSA is AR-regulated serine protease and is widely used as a biomarker for PCa. As expected, hit compounds induced an equivalent decrease in PSA levels in LNCaP (12) cells as with the IC₅₀ values found with the eGFP assay. Table 1 below summarize compounds that were found to have an IC₅₀ below 100 nM. For comparison purposes, in this assay, gold standards Enzalutamide and Bicalutamide shows IC₅₀ of 100 nM and 600 nM respectively. Importantly, eleven compounds exhibited exceptional activity under 50 nM.

Table 1: Compounds that exhibited an IC₅₀ under 0.1 μM in transcription assays (eGFP and PSA)

Internal number	eGFP (μM)	PSA (μM)	Half-Life (min.)
13591	0.012	0.027	23
13786	0.013	0.021	n/a
13621	0.020	0.008	13
13697	0.029	0.029	n/a
13642	0.032	0.050	11
13610	0.033	0.013	27
13785	0.036	0.045	n/a
13713	0.037	0.091	36
13698	0.041	0.041	n/a
13696	0.042	0.042	n/a
13766	0.045	0.020	32
13582	0.051	0.058	32
13676	0.053	0.034	13
13585	0.057	0.028	37
13566	0.060	0.090	29
13760	0.062	0.052	35
13793	0.065	0.139	n/a
13807	0.065	0.135	n/a
13716	0.067	0.058	14
13688	0.069	0.068	30
13695	0.069	0.087	41
13694	0.070	0.067	46
13699	0.073	0.073	n/a
13674	0.075	0.051	43
13737	0.080	0.037	12
13738	0.083	0.062	37
13703	0.087	0.097	16

13584	0.088	0.050	47
13770	0.093	0.017	14
13754	0.094	0.051	n/a
13579	0.100	0.042	41
13622	0.100	0.075	17
13692	0.100	0.082	58
13764	0.100	0.082	19

Determination of metabolic stability

Metabolic stability refers to the susceptibility of compounds to biotransformation in the context of selecting and/or designing drugs with favorable pharmacokinetic properties. Metabolic stability results are usually reported as measures of intrinsic clearance, from which secondary pharmacokinetic parameters such as bioavailability and half-life can be calculated when other data on volume of distribution and fraction absorbed are available. These parameters are very important in defining the pharmacological and toxicological profile of drugs as well as patient compliance. Preliminary data using microsomes studies have shown that many of our best compounds (under 0.1 μM) did not show a great half-life in metabolic stability (Table 1) with numbers under 40 minutes. However, based on this information we have been capable of identifying weak points in the structure of the compounds and improve this aspect in year 3. Based on these information we managed to create a compound 13789 that retained excellent activity while being stable for more than 200 minutes in microsomes experiments (Table 2).

Table 2: Half life of the most stable compounds in microsomes studies

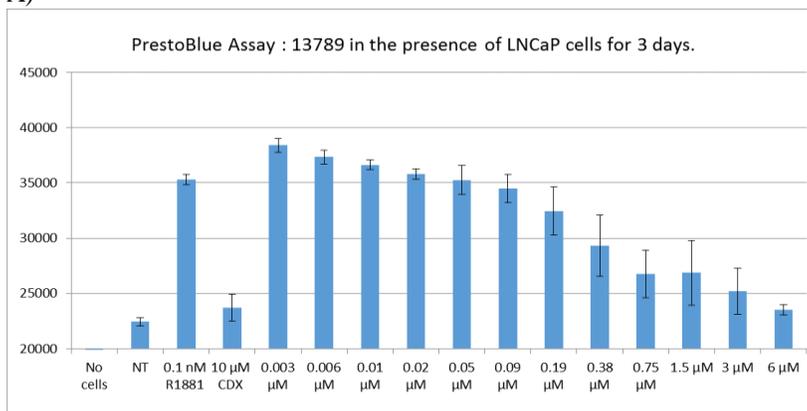
Internal number	eGFP (μm)	PSA (μm)	Half-Life (min.)
13789	0.190	0.087	230
13732	0.420	0.350	199
13665	0.270	0.210	186
13730	0.149	0.198	158
13627	1.517	1.160	154
13677	0.156	0.110	144
13717	0.370	0.330	120
13762	0.470	0.420	119
13773	0.327	0.303	116
13731	0.304	0.255	114

Cell growth assay.

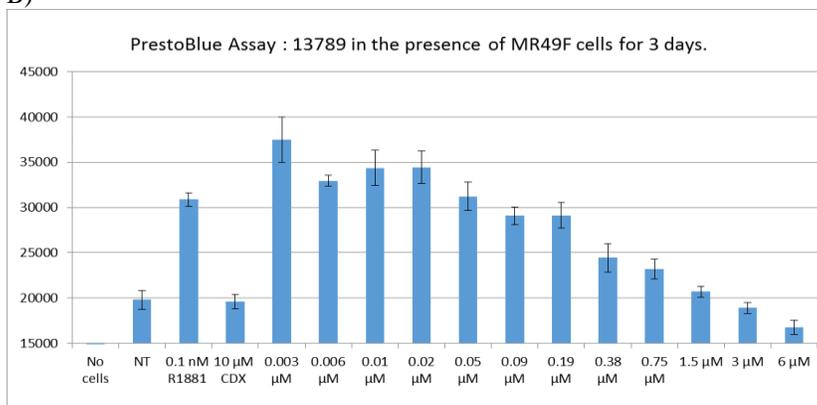
To determine the translational potential of the most potent BF3 inhibitors listed in Table 1, we evaluated their ability to reduce growth of PCa models stimulated by the androgen R1881 *i.e.* LNCaP, and androgen-independent PC3 cell line. The cell viability was assessed after 3 days of incubation with the test compound in a concentration dependent manner. Figure 1 shows a typical experiment with our lead compound VPC-13789 where this compound is very effective to inhibit the growth of LNCaP cells (Figure 1A). This compound was also very effective in

inhibiting the growth of MR49F cells resistant to Enzalutamide (Figure 1B). Moreover, compound 13688 did not show any effect on AR independent PC3 cell lines (figure 1C), confirming its AR-specific activity. Cell growth experiments were performed on all our compounds that are under 1 μM in eGFP activity.

A)



B)



C)

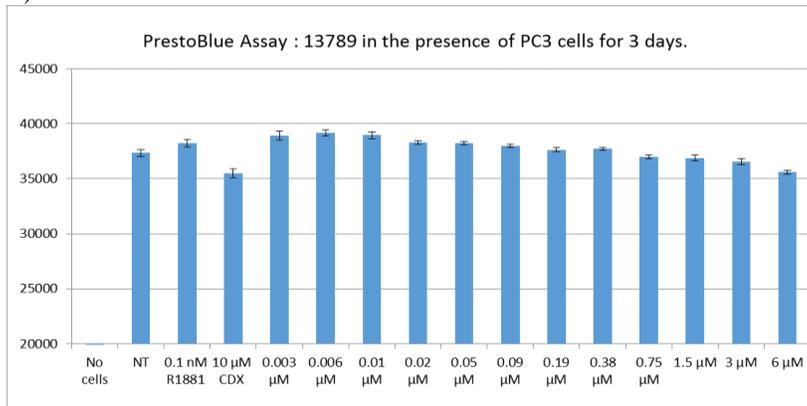


Figure 1: Cell growth assay using PrestoBlue reagent with A) LNCaP cells B) Enzalutamide-resistant MR49F cells and C) AR-independent PC3 cells.

Determination of binding to the BF3 site

Biolayer interferometry (BLI) studies demonstrate a direct reversible interaction between these compounds and a purified AR ligand binding domain in a dose dependent manner. When a compound binds to the AR, there is a shift in wavelength that is detectable and measurable using the instrument. However BLI data for the compound VPC-13789 was not that great. So we also developed an assay that test the ability of a BF3 compound to displace a peptide that

bind to the BF3 site and it was positive for 13566 and 13789, confirming the mode of binding of these compounds.

Key outcome 3: Compound 13566 can effectively inhibit the growth of prostate cancer cell lines in mice xenograft tumour model

Efficacy studies in human xenograft tumour bearing mice.

In year 2 we proceeded to test compound VPC-13566 identified in Year 1 for its ability to reduce the growth of tumor cell lines in xenograft models. We completed these studies in year 3.

The *in vivo* effect of VPC-13566 was evaluated with a xenograft model of castration-resistant prostate cancer. The pharmacokinetic analysis of VPC-13566 and its metabolites following intravenous (IV) or intraperitoneal (IP) administration demonstrated moderate metabolic stability of VPC-13566, with three glucuronides established as primary biotransformation products. The hydroxylation products were observed in lesser amounts. The IV and IP serum profiles of VPC-13566 suggest that it could be administered IP with substantial retention up to 24h. For IV and IP, C_{max} was established at approximately 14 and 1 μ M and half-lives were estimated as 2.6 and 2.8 hours respectively. Based on the initial *in vitro* data (AR eGFP IC₅₀ = 0.05 μ M) it was to be expected that the plasma concentration of the drug should remain within the predicted therapeutic window for at least 24 hours when delivered IP. Initial toxicity experiments demonstrated doses of 100 mg/kg twice a day (single dose of 200 mg/kg once per day for 2 days per week) for 4 weeks could be tolerated by the mice with no visible signs of toxicity, decrease in body weight, or histological abnormalities in major organs (data not shown). A dose of 100 mg/kg administered IP twice a day (single dose of 200 mg/kg once per day for 2 days per week) was chosen based on these preliminary studies.

The *in vivo* screening for tumor growth was performed using the castration-resistant LNCaP xenograft model. Mice were implanted with LNCaP tumor cells and castrated upon serum PSA reaching 25 ng/ml. When tumor regrowth was observed and serum PSA returned to pre-castration levels, the mice were treated with VPC-13566, vehicle control, or the AR inhibitor enzalutamide. Tumor growth was effectively suppressed by VPC-13566 ($p < 0.05$) and enzalutamide ($P < 0.01$) (Figure 2A). Moreover, VPC-13566 significantly decreased serum PSA to a comparable degree as enzalutamide ($p < 0.01$, Figure 2B). Together, these results indicate that this class of AR inhibitor has the potential to yield an AR targeting drug that could have clinical utility in the treatment of patients with castration-resistant tumors.

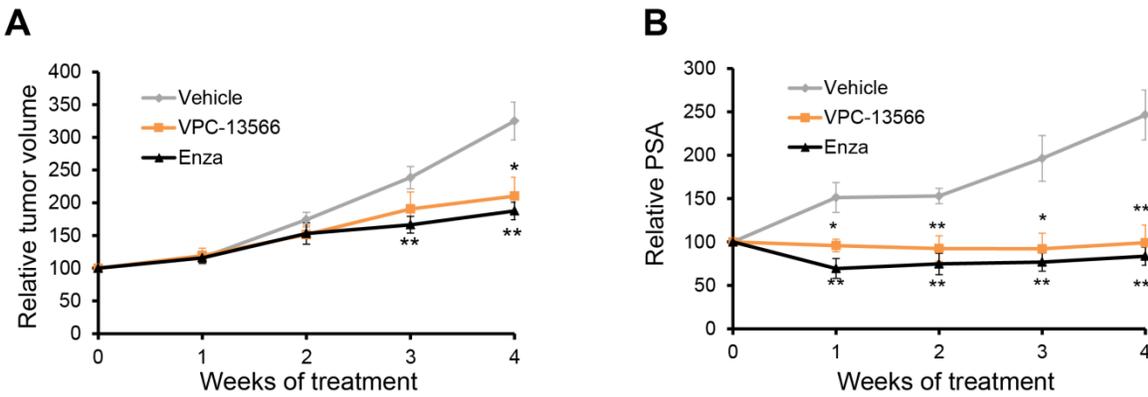


Figure 2: In vivo effect of VPC-13566 in LNCaP castration resistant xenograft model. **A-** The in vivo effect of VPC-13566 on the tumor volume of LNCaP mice xenografts. Data are presented as mean \pm SEM of % tumor volume at time of treatment initiation (each group, n=5). A p value < 0.05 was considered significant (*) and < 0.01 very significant (**) compared to vehicle control (two-tailed T-Test). **B-** The in vivo effect of VPC-13566 on PSA level of LNCaP xenograft bearing mice. Data are presented as mean \pm SEM of % PSA level at time of treatment initiation (each group, n=5). A p value < 0.05 was considered significant (*), and < 0.01 very significant (**) compared to vehicle control.

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

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

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

Nothing to report

IMPACT

Nothing to report

CHANGES/Problems

Nothing to report

List of Personnel

- Ph. D student: Ravi Munuganti
- PDF: Mohamed Hessein
- PDF: Christophe André

4. KEY RESEARCH ACCOMPLISHMENTS:

- Using our in-silico model we identified and characterized 33 small molecule compounds inhibitor of the AR with an IC₅₀ under 100 nM in transcription assay
- Eleven of these compounds exhibit exceptional activity under 50 nM which is twice more potent than gold standard Enzalutamide
- These compounds are effective to reduce the growth of Enzalutamide-resistant cell lines *in vitro*.
- Parental compound VPC-13566 identified in year 1 of the project is very effective in reducing tumor growth of LNCaP cell lines.
- The half-life of new compounds from year 3 in metabolic conditions have been much improved, and we have identified a new lead compound #13789 that is very active (150-200 nM IC₅₀) and with a half-life in microsome studies over 200 minutes.

5. CONCLUSION:

Over the last 3 years, there has been considerable progress in our project to develop small molecules inhibitors that target the BF3 site of the AR for the treatment of prostate cancer. Based on the chemical scaffold VPC13566 previously reported in year 1 of the project, we conducted a systematic *in silico* screen and identified approximately 250 indole based compounds for biological testing. These compounds were evaluated successfully using a series of *in vitro* assays confirming their potency via AR guided mechanism of action in various prostate cancer cell lines. Several compounds demonstrated inhibition of AR in low nanomolar range. It should be noted that these derivatives show two to five fold increase in their potency when compared to gold standard Enzalutamide. This class of inhibitors is promising and is currently under further investigation. Importantly, VPC-13566 exhibited a strong anti-proliferative effect on LNCaP cell line in xenograft mouse models, confirming that these compounds can be truly effective anti-tumor agents.

Very importantly, the results from year 3 of the project have identified a lead compound that is almost drug-like in its properties (compound 13789), with an IC₅₀ around 150-200 nM while having an excellent half-life of over 200 minutes in microsome experiments. It is our hope that this compound will attract further interest and eventually be developed as a drug to act as complementary therapeutics to treat castration-resistant prostate cancer.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

1) Discovery of 1H-indole-2-carboxamides as novel inhibitors of the androgen receptor binding function 3 (BF3). Ban F, Leblanc E, Li H, Munuganti RS, Frewin K, Rennie PS, Cherkasov A. J Med Chem. 2014 Aug 14;57(15):6867-72. doi: 10.1021/jm500684r. Epub 2014 Jul 25. PMID:25025737

2) Identification of a Potent Antiandrogen that Targets the BF3 Site of the Androgen Receptor and Inhibits Enzalutamide-Resistant Prostate Cancer Ravi S.N. Munuganti, Mohamed D.H. Hassona, Eric Leblanc, Kate Frewin, Kriti Singh, Dennis Ma, Fuqiang Ban, Michael Hsing, Hans Adomat, Nada Lallous, Christophe Andre, Jon Paul Selvam Jonadass, Amina Zoubeidi, Robert N. Young, Emma Tomlinson Guns, Paul S. Rennie, and Artem Cherkasov, Chemistry and Biology, 2014 Nov 20;21(11):1476-85

3) The development of anti-androgens with a new mechanism of action for treatment of castration resistant prostate cancer. Ravi SN Munuganti, Mohamed H Hassona, Eric Leblanc, Fuqiang Ban, Emma T. Guns, Paul S. Rennie, Artem Cherkasov. Poster presented at the AACR meeting, April 05-09, 2014 in San Diego, USA:

4) Targeting binding function-3 site on the androgen receptor to treat Enzalutamide-resistant prostate cancer. Ravi Shashi Nayana Munuganti, Mohamed DH Hassona, Eric Leblanc, Emma T. Guns, Paul S. Rennie, Artem Cherkasov. Poster presented at the AACR meeting, April 21, 2015 in Philadelphia, USA.

5) Functional analysis of androgen receptor mutations that confer anti-androgen resistance identified in circulating cell-free DNA from prostate cancer patients. Nada Lallous, pH.D; Stanislav V. Volik, Ph.D.; Shannon Awrey; Eric Leblanc, Ph.D.; Ronnie Tse; Josef Murillo; Kriti Singh; Arun A. Azad; Alexander W. Wyatt, Ph.D.; Stephane LeBihan; Kim N. Chi, M.D.; Martin E. Gleave, M.D.; Paul S. Rennie, Ph.D.; Colin C. Collins, Ph.D.; Artem Cherkasov, Ph.D. *Accepted for publication in Genome Biology*.

6) Targeting Binding Function-3 of the Androgen Receptor Blocks its Co-Chaperone Interactions, Nuclear Translocation, and Activation. Nada Lallous, Eric Leblanc, Ravi S.N. Munuganti, Mohamed D.H. Hassona, Shannon Awrey, Nader Al Nakouzi, Helene Morin, Mani Roshan-Moniri, Kriti Singh, Sam Lawn, Takeshi Yamazaki, Hans Adomat, Christophe Andre, Mads Daugaard, Robert N. Young, Emma Tomlinson Guns, Paul S. Rennie, Artem Cherkasov. Submitted to PNAS.

7. INVENTIONS, PATENTS AND LICENSES:

Nothing to report.

8. REPORTABLE OUTCOMES:

a) We have employed these AR BF3-directed small molecules to demonstrate that inhibition of AR activity through the BF3 functionality can block translocation of the receptor into the nucleus. Furthermore, using this AR BF3 binder as a chemical probe, we have identified a co-chaperone, small glutamine-rich tetratricopeptide repeat (TPR)-containing alpha (SGTA), as an important AR-BF3 interacting partner. This is to our knowledge the first time such a role was found for SGTA. This data has been submitted for publication to PNAS.

b) We have tested 13566 against a panel of clinically relevant AR mutations that confer resistance to the usual anti-androgens. 13566 has effectively inhibited all AR variants, including those that confer resistance to enzalutamide, and to emerging drug candidates such as ARN509. Thus, VPC-13566 could represent a viable treatment option for CRPC patients.

9. OTHER ACHIEVEMENTS:

Nothing to report.

10. REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in Science, Military Medicine, etc.)

1. Lu NZ, Wardell SE, Burnstein KL, Defranco D, Fuller PJ, Giguere V, et al. International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: Glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacological Reviews*. 2006;58:782-97.
2. Denmeade SR, Isaacs JT. A history of prostate cancer treatment. *Nature Reviews Cancer*. 2002;2:389-96.
3. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a Second-Generation Antiandrogen for Treatment of Advanced Prostate Cancer. *Science*. 2009;324:787-90.
4. Clegg NJ, Wongvipat J, Joseph JD, Tran C, Ouk S, Dilhas A, et al. ARN-509: A Novel Antiandrogen for Prostate Cancer Treatment. *Cancer Research*. 2012;72:1494-503.
5. Lassi K, Dawson NA. Emerging therapies in castrate-resistant prostate cancer. *Current Opinion in Oncology*. 2009;21:260-5.
6. Adamo V, Noto L, Franchina T, Chiofalo G, Picciotto M, Toscano G, et al. Emerging targeted therapies for castration-resistant prostate cancer. *Frontiers in endocrinology*. 2012;3:73-.
7. Estebanez-Perpina E, Arnold AA, Nguyen P, Rodrigues ED, Mar E, Bateman R, et al. A surface on the androgen receptor that allosterically regulates coactivator binding. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104:16074-9.
8. De Leon JT, Iwai A, Feau C, Garcia Y, Balsiger HA, Storer CL, et al. Targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108:11878-83.
9. Grosdidier S, Carbo LR, Buzon V, Brooke G, Nguyen P, Baxter JD, et al. Allosteric Conversation in the Androgen Receptor Ligand-Binding Domain Surfaces. *Molecular Endocrinology*. 2012;26:1078-90.
10. Tavassoli P, Snoek R, Ray M, Rao LG, Rennie PS. Rapid, non-destructive, cell-based screening assays for agents that modulate growth, death, and androgen receptor activation in prostate cancer cells. *Prostate*. 2007;67:416-26.
11. Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. *Journal of Clinical Oncology*. 2003;21:383-91.
12. Horoszewicz JS, Leong SS, Chu TM, Wajsman ZL, Friedman M, Papsidero L, et al. The LNCaP cell line--a new model for studies on human prostatic carcinoma. *Progress in clinical and biological research*. 1980;37:115-32.