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TITLE: Design and Evaluation of Small Molecules That Target the Dimerization Interface Of Full-Length and Splice-Variant Forms of the Androgen Receptor.

PRINCIPAL INVESTIGATOR:
Artem Cherkasov

CONTRACTING ORGANIZATION: University of British Columbia

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The main factor required for all stages of prostate cancer (PCa) development and progression is the androgen receptor (AR), a molecular signaling protein resident within prostate cancer cells that is switched on by testosterone, which in turn interacts with DNA to orchestrate a genetic program that promotes the growth of PCa cells. The major problem for PCa patients is that these therapeutic approaches invariably fail because the testosterone binding site on the AR protein becomes mutated. These mutations often switch the AR protein on permanently without the normal signal from testosterone, enabling it to continue interacting with DNA and orchestrating the genetic program that promotes the growth of prostate cancer cells. We will develop a completely new series of novel drug-like small molecules that target another essential function that we recently elucidated for mutated AR proteins. This process is known as dimerization, whereby two mutated AR proteins must physically bind to each other to interact with DNA and orchestrate the genetic program that promotes growth of PCa cells. Here, we aim to interfere with this dimerization process by developing a drug-like small molecule that functions as an "anti-dimer" therapy. We hypothesize that anti-dimer drugs will be effective for treating PCas that contain mutant forms of AR that underlie resistance to conventional therapies.

15. SUBJECT TERMS

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A. INTRODUCTION

The androgen receptor (AR) is a hormone inducible transcription factor that drives expression of tumor promoting genes in prostate cancer (PCa). The AR is activated by steroid recruitment to its ligand binding domain (LBD) resulting in nuclear translocation and AR dimerization via the DNA binding domain (DBD) engaging with chromatin androgen response elements harbored in promoters and enhancers of downstream target genes. Current PCa therapies inhibit AR transcriptional activity through the LBD, but only temporarily prevent AR-driven tumor growth due to the emergence of mutations in the LBD or expression of constitutively active splice variants such as AR-V7 lacking an LBD. This stage of the disease where AR transcriptional activity resumes despite treatment with therapies designed to inhibit this activity is referred to as castration-resistant PCa (CRPC). CRPC is the lethal manifestation of the disease where new therapies are urgently needed. Here, computer-aided drug-design was used to develop small molecules that specifically block the interaction between two AR monomers via the DBD-dimerization interface. The potential importance of this mode of inhibition is supported by our work showing an absolute requirement of dimers for the chromatin binding and transcriptional activation of AR and AR variants. We hypothesize that an 'anti-dimer' compound will circumvent all known AR-dependent resistance mechanisms in CRPC. The major goals of this research are to perform hit-to-lead optimization of VPC-17005 to improve drug potency and bioavailability, investigate the drug binding site and molecular mechanism of action in cellular models of prostate cancer, and conduct pre-clinical evaluation of our most promising lead in animal models

B. KEYWORDS

Prostate cancer, castration-resistant prostate cancer, androgen receptor, *in silico* drug discovery, cryo-EM, x-ray crystallography, pre-clinical, chromatin binding.

C. ACCOMPLISHMENTS

C.1 Major Goals of the Project as Outlined in the Approved SOW.

The major goals for years 1-2 of the project are outline below.

Specific Aim 1: To improve the potency and stability of anti-dimer compounds using rational design and medicinal chemistry.

Major Task 1: Virtual screen and evaluation of additional chemical scaffolds.

Major Task 2: Determine the mechanism of action and potential clinical relevance of lead compounds.

Specific Aim 2: To employ, biophysical approaches including cryo-EM and X-ray crystallography to visualize the molecular interaction between the AR and anti-dimer compound.

Major Task 3: Protein production and development of biophysical assays to monitor compound interactions with the ARDDB.

Major Task 4: Structure determination of protein:compound complexes.

C.2 Accomplishments Under These Goals

University of Minnesota Site (Dehm, PI):

Aim 2, Major Task 2: Determine the downstream mechanism of action and potential clinical relevance of lead compounds.

In the previous reporting period, we evaluated three anti-dimer compounds, namely, VPC-17281, VPC-17160 and VPC-17166 developed by the Cherkasov lab. In this grant period, we evaluated an additional putative AR-targeted inhibitor (VPC-220010) developed by the Cherkasov lab. We determined the IC50 value of this compound for inhibition of cell growth across a panel of AR/AR variant-positive as well as AR negative prostate cancer cell lines. As shown in **Figure 1,** the AR NTD inhibitor VPC-220010 displayed lower IC50s in AR-positive prostate cancer cell lines, and higher IC50s in AR-negative prostate cancer cell lines. These results are consistent with an anti-AR mechanism of action.

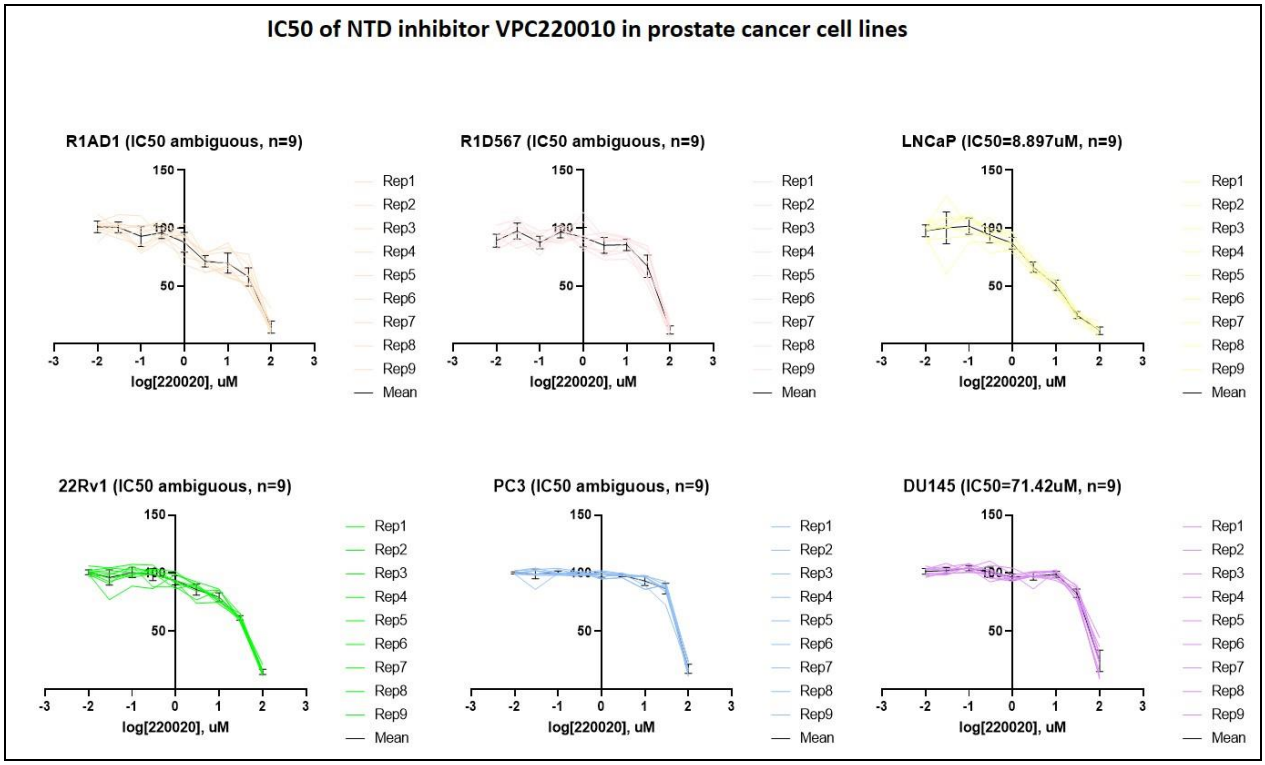

Figure 1. AR-positive (R1-AD1, LNCaP, R1-D567, 22Rv1) and AR-negative (PC3, DU-145) prostate cancer cell lines were tested for growth inhibition by VPC220010. Y-axes show % cell growth (6 days treatment) relative to DMSO (vehicle-treated) control (n=9).

To complement the *in silico* drug screening approach employed by the Cherkasov lab, we utilized gene expression data from the isogenic prostate cancer cell line model R1-AD1/R1-D567, to predict drugs that

may have greater potency in cells expressing AR variants (R1-D567) compared with cells expressing full-length AR (R1-AD1). This gene expression-based screen nominated nutlin-3a as a drug with potentially greater potency in R1-D567 compared with R1-AD1 cells. Noteworthy, nutlin-3a is not being developed in the clinic because of sub-optimal pharmacological properties. Therefore, we conducted subsequent validation and mechanistic studies using a newer second-generation MDM2 antagonist, idasanutlin.

We first evaluated the IC50 values of idasanutlin for inhibition of cell growth in R1-AD1 and R1-D567 cells. As shown in **Figure 2**, idasanutlin displayed lower IC50 in R1-D567 cells (IC50=0.02617uM) than in R1-AD1 cells (IC50=0.07583uM). These data demonstrated that idasanutlin has greater potency in cells expressing AR variants (R1D-567) compared with isogenic cells containing full length AR (R1-AD1).

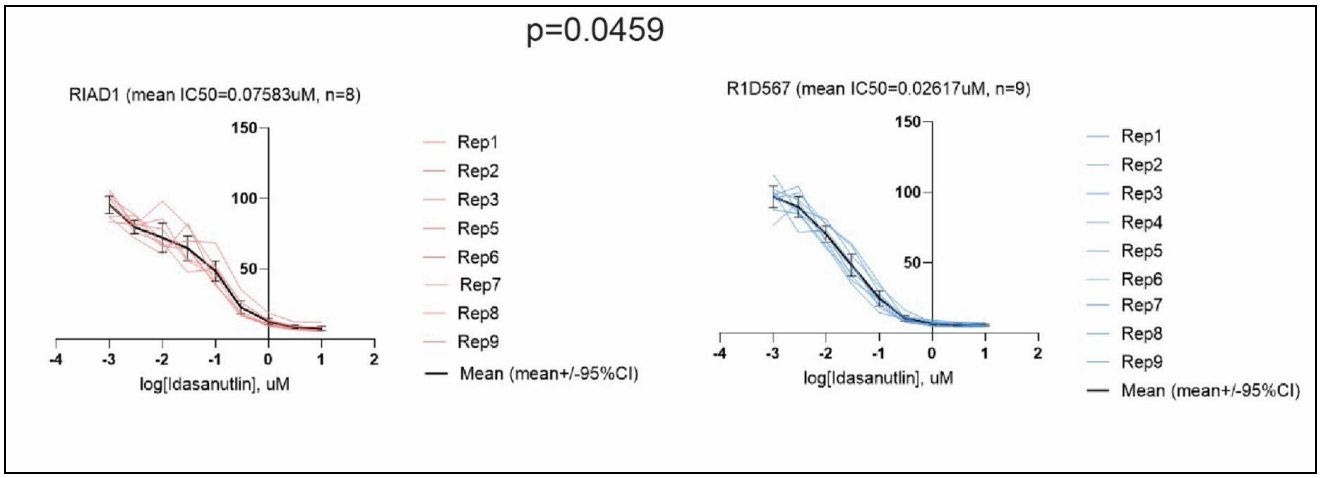

Figure 2. AR-FL expressing (R1-AD1) and AR-V expressing (R1-D567) prostate cancer cell lines were tested for growth inhibition by idasanutlin. Y-axes show % cell growth (6 days treatment) relative to DMSO (vehicle-treated) control (n=9). Statistical analysis was performed by *t*-test using Prism Graphpad software.

Next, we examined if idasanutlin exhibits differential effects on the protein expression levels of AR/AR-variants in R1-AD1 and R1-D567 cells. As shown in **Figure 3A**, idasanutlin did not change AR FL protein expression in R1-AD1 cells at all the time points examined. Interestingly, idasanutlin induced a significant decrease in AR-variant expression starting at 12h and persisting over 72h (**Figure 3B**). As shown in **Figure 3C&3D**, idasanutlin did not exert effects on mRNA expressions of AR/AR-variants in R1-AD1 or R1D-567 cells, indicating a post-transcriptional effect on AR/AR-variants. Treatment with different concentrations of idasanutlin did not affect AR protein expression but did reduce AR-variant protein expression as low as 30 nM **(Figure 3E &3F**). Real time PCR analysis did not show any effect of idasanutlin on mRNA expression of AR or AR variants, even at doses as high as 300 nM (**Figure 3G&3H**). Collectively, these data indicate that idasanutlin preferentially inhibits protein expression of AR variants vs. AR in an isogenic cell line model.

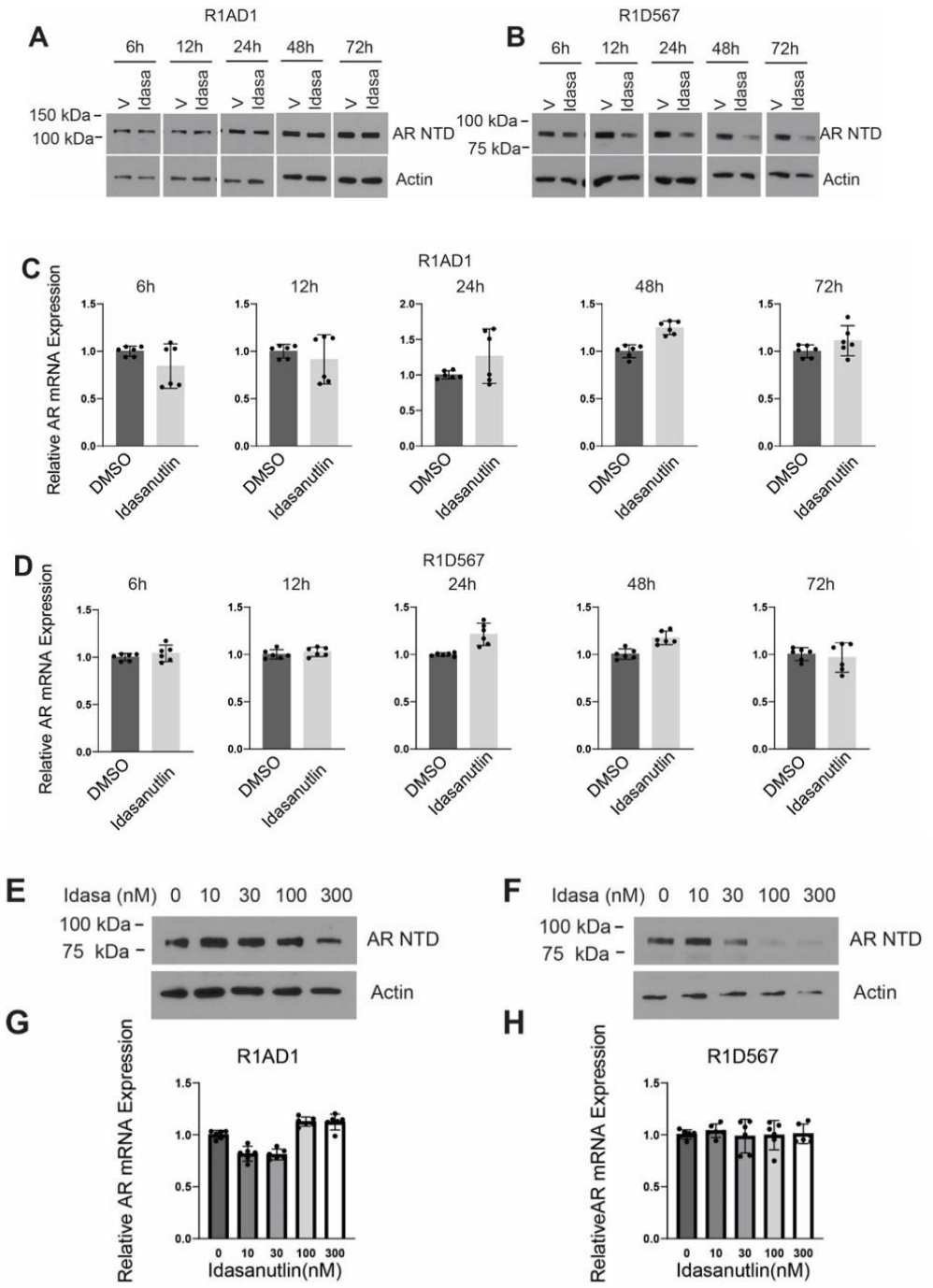

Figure 3. Idasanutlin preferentially inhibits protein expression of AR variants. **3A&3B.** AR-positive R1-AD1 and AR-V-positive R1-D567 cells were treated with 100nM idasanutlin or vehicle control (DMSO) for 6, 12, 24, 48 and 72hr and harvested for Western blot analysis of AR. Actin was used as the loading control (n=2). **3C&3D**, R1-AD1 and R1D-567 cells were treated with 100nM idasanutlin or vehicle control (DMSO) for 6, 12, 24, 48 and 72hr and harvested for real time-PCR analysis of AR/AR-variant. **3E&3F**, R1AD1 and R1D-567 cells were treated with different concentrations of idasanutlin for 72hr and harvested for Western blot analysis of AR. **3G&3H**, R1AD1 and R1D-567 cells were treated with different concentrations of idasanutlin for 72hr and harvested for real time-PCR analysis of AR/AR-variant.

Idasanutlin is a specific inhibitor of an E3 ubiquitin ligase protein, Mouse double minute 2 (Mdm2), which is a negative regulator of the p53 tumor suppressor. Idasanutlin disrupts the interaction between Mdm2 and p53 and thereby prevents Mdm2-dependent ubiquitination and degradation of p53. Mdm2 is also a downstream target of p53, wherein active p53 transcriptionally induces Mdm2 expression. To validate the molecular mechanism of idasanutlin, we examined the protein expression of p53 and Mdm2 in idasanutlin-treated R1-AD1and R1-D567 cells. As shown in **Figure 4A&4B**, idasanutlin induced a significant increase in both p53 and Mdm2 expression from 6h-72hr after treatment in both cell lines. As shown in **Figure 4C&4D,** idasanutlin induced a dramatic increase of p53 and Mdm2 expression dose-dependently. Collectively, these data validate the functional role of idasanutlin as a specific Mdm2 inhibitor. Furthermore, they indicate that the preferential decrease in AR-variants was not correlated with different mechanisms of action of idasanutlin on the Mdm2/p53 axis in R1-AD1 and R1D-567 cells.

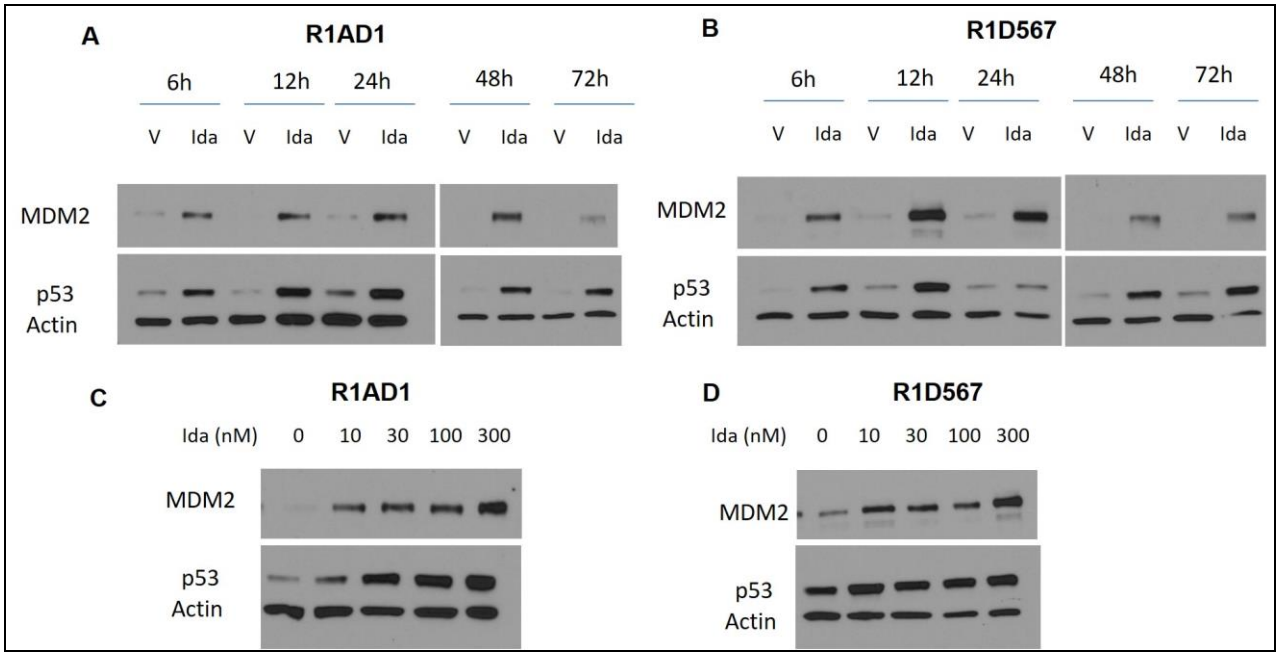

Figure 4. Idasanutlin increased p53 and Mdm2 expressions in both R1-AD1 and R1-D567 cells. **4A&4B.** R1-AD1 and R1D-567 cells were treated with 100nM idasanutlin or vehicle control (DMSO) for 6, 12, 24, 48 and 72hr and harvested for Western blot analysis of p53 and Mdm2. Actin was used as the loading control (n=2). **4C&4D**, R1AD1 and R1D-567 cells were treated with different concentrations of idasanutlin for 72hr and harvested for Western blot analysis of p53 and Mdm2.

Since Mdm2 is involved in ubiquitin-proteasome-dependent protein degradation, we next investigated if the idasanutlin-induced decrease of AR-variant protein expression was mediated by the ubiquitin-proteasome pathway (UPP). We treated R1D-567 cells with vehicle control or idasanutlin in the presence/absence of MG132, a proteasome inhibitor. As shown in **Figure 5,** inhibition of proteasome by MG132 prevented the idasanutlin-induced decrease in AR-variant expression as early as 6h and persisted until 24h after treatment. These data indicate that UPP system is involved in mediating AR-variant decrease by idasanutlin.

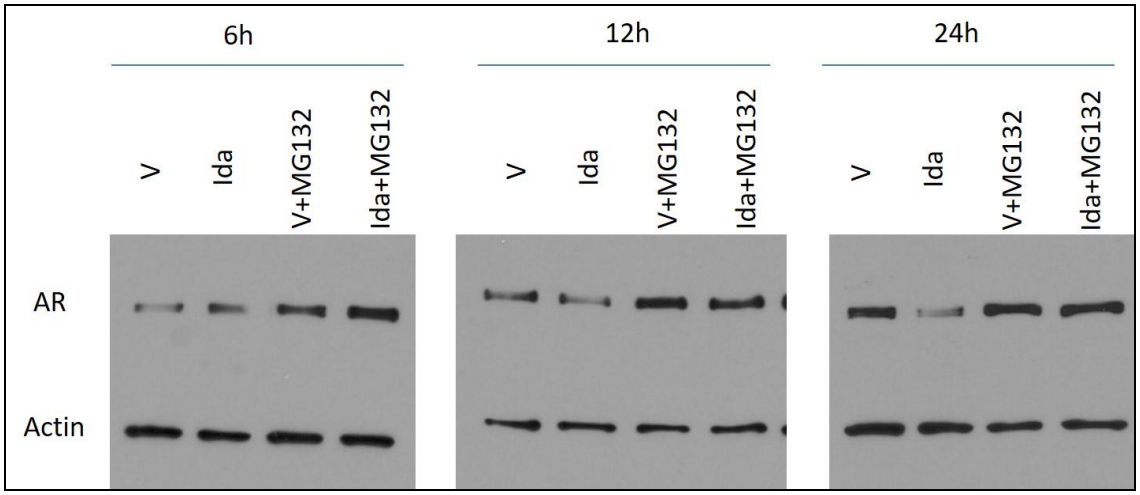

Figure 5. Ubiquitin-proteasome pathway is involved in idasanutlin-induced decrease in AR-variants in R1D-567 cells. R1D-567 cells were treated with vehicle control (DMSO) or 100nM idasanutlin in the presence/absence of MG132 for 6, 12, 24, 48 and 72hr and harvested for Western blot analysis of AR. Actin was used as the loading control.

Vancouver Prostate Centre Site (Cherkasov, PI):

Aim 1, Major Task 1: Virtual screen and evaluation of additional chemical scaffolds.

Two approaches were employed to identify new anti-dimer compounds. Since the AR-DBD dimerization interface consists of two AR protein interactions, a protein-protein interaction (PPI) small molecule database for screening was prepared. The previous "finger printing" enabled rapid screen of the 1.3 M compounds—but restricted/biased to identifying structures similar to VPC-17160 and VPC-17166. To address this processing restriction, the Cherkasov lab pioneered artificial intelligence (i.e., Deep Docking) to process the ZINC15 collection by virtual screening. They optimized deep-learning technology to rapidly screen 1.3 billion compounds using various independent processing parameters-thereby eliminating the original bias. The PPI and Deep Docking methods was first evaluated with the human estrogen receptor AF-2 domain and further refined with the SARS-Cov-2 3CL protease. Unfortunately, due to the pandemic curtailment of research at the Vancouver Prostate Centre, Deep Docking was not applied to the AR dimer interphase.

Aim 1, Major Task 2: Determine the mechanism of action and potential clinical relevance of lead compounds. Due to the pandemic curtailment of research, no significantly more potent compounds were identified nor evaluated.

Aim 2, Major Task 3: Protein production and development of biophysical assays to monitor compound interactions with ARDBD. The Cherkasov lab has previously expressed and purified the individual components for the formation protein:DNA:compound ternary complexes for the bio-layer interferometry (BLI) assay. Conditions were optimized for the generation of the complexes. However, no new compounds were ordered for evaluation.

University of British Columbia Site (Strynadka, PI):

Aim 2, Major Task 4: Structure determination of protein:compound complexes.

Subtask 1: Protein crystallization of ARDBD with or without DNA. The Strynadka lab has overexpressed ARDBD in recombinant pET-28 expression systems, isolated and purified the protein by FPLC Chromatographic including cobalt affinity chromatography, ion exchange and size exclusion chromatography with intervening thrombin-mediated removal of the tag of 6xHis localized at the N-terminus. Pure, tagless ARDBD was screened for crystallization by sitting drop vapor diffusion trials using 600 conditions spanning a range of precipitants, salts, additives and pH. Crystals were obtained for ARDBD without DNA or compound (confirmed by UV-Vis imaging) and the condition optimized in order to obtain crystals suitable for high resolution x-ray diffraction analysis. Data has been collected confirming apo crystals can be made.

Subtask 2: X-ray diffraction data collection of protein:DNA:compound or protein:compound crystals. Crystals from the various screened conditions were mounted under cryogenic stream and a direct photon detector at x-ray microfocus Beamline 5.1a at the Advanced Photon Source (Chicago, IL) or Advanced Light Source (U. Berkeley). Waiting for further compounds to screen.

Subtask 3: Cryo-EM imaging and structure determination. The Strynadka lab overexpressed, reconstituted and purified the ARDBD-Tri-ADR3 complex by FPLC size exclusion chromatography. Carbon coated copper grids were ionized using a Pelco ionization chamber. The samples were applied to these charged carbon coated copper grids and stained with uranyl acetate. The negatively stained grids were subsequently imaged using a 120V transmission Thermo Fisher Talos electron microscope. The resulting images showed promise in terms of homogeneity of particles and orientation but lacking in particle density. Sample preps have been further optimized to increase yield and concentration for cryo-EM imaging and data collection. There is concern that heterogeneity arising from conformational flexibility between the tandemly placed proteins may make high resolution data collection challenging, however new advances in particle picking of minor populations should aid in mitigating this issue which has been validated on the inhibitor-free structures. Reagents and methods are thus in place for structural analysis of identified future compounds in complex with small molecule inhibitors.

C.3. Opportunities for Training and Professional Development.

Nothing to report

C.4 Results Disseminated to Communities of Interest

Nothing to report.

C.5 Plans for Next Reporting Period

University of Minnesota Site (Dehm, PI):

In the next reporting period, the Dehm lab will continue to evaluate compounds with anti-AR and anti-AR-variant activities nominated by the Cherkasov lab as well as other *in silico* strategies. For compounds that are found to have the desired anti-AR and/or anti-AR-variant activities, we will continue to pursue the mechanism(s) of action.

Vancouver Prostate Centre Site (Cherkasov, PI):

The Cherkasov lab will deploy the refined Deep Docking technology to virtually screen 1.3 billion compound from the Enamine *REAL* Database—to identify novel chemical scaffolds (aka, "backbones") for evaluation.

University of British Columbia Site (Strynadka, PI): The Strynadka lab will characterize the atomic resolution structures of identified anti-AR small molecule compounds by now established xray crystallographic and cryoEM methods.

D. IMPACT

D.1 Impact on the Development of the Principle Disciplines(s) of the Project.

University of Minnesota Site (Dehm, PI): Nothing to report.

Vancouver Prostate Centre Site (Cherkasov, PI):
The Cherkasov lab initially screened 15M compounds to identify candidate AR-dimer inhibitors. 1.3B compounds—87 times more than before—will be screened to identify new scaffolds. The deployment of Deep Docking virtual screen will take approximately 3 weeks to perform—compared to the approximate 3 years it would take to use regular docking. This will provide a significant impact on attaining the milestones of this project.

University of British Columbia Site (Strynadka, PI): Nothing to report.

D.2 Impact on Other Disciplines.

University of Minnesota Site (Dehm, PI): Nothing to report.

Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to report.

University of British Columbia Site (Strynadka, PI): Nothing to report.

D.3 Impact on Technology Transfer.

University of Minnesota Site (Dehm, PI):

Vancouver Prostate Centre Site (Cherkasov, PI):

University of British Columbia Site (Strynadka, PI):

D.4 Impact on Society Beyond Science.

University of Minnesota Site (Dehm, PI): Nothing to report.

Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to report.

University of British Columbia Site (Strynadka, PI): Nothing to report.

E. CHANGES/PROBLEMS

E.1 Changes in Approach and Reasons for Change.

University of Minnesota Site (Dehm, PI):

Nothing to report

Vancouver Prostate Centre Site (Cherkasov, PI):

There were no acceptable candidates nor scaffolds identified for further preclinical development from screening 15M compounds. It was clear that a significantly larger compound data base needed to virtually screen. Therefore, development and refinement machine learning algorithms to virtually screen the Enamine *REAL* Database was needed. The initial half of Year-2 was focused on developing and benchmarking the Deep Docking technology.

In the 2nd half of Year-2 the COVID-19 pandemic disrupted research activities due to the closure of the Vancouver Prostate Centre and its research facilities starting March 20, 2020 through June 30 in response to the COVID-19 pandemic. The closure of the research facilities curtailed ongoing research on this project. In addition, VPC phased-in research resumption guidelines did not enable any work on this project through August 31, 2020.

University of British Columbia Site (Strynadka, PI): UBC research facilities were closed as well from March 20, 2020 through June 30 in response to the COVID-19 pandemic with resulting curtailment of research activities on this project.

E.2 Changes that Had a Significant Impact on Expenditures

University of Minnesota Site (Dehm, PI):

Laboratory experimentation at University of Minnesota has been halted and/or reduced from March 2020-present to address the COVID-19 pandemic. This reduced expenditures on reagents and supplies for this project in YR2.

Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to report.

University of British Columbia Site (Strynadka, PI): Nothing to report.

E.3 Significant Changes in Use or Care of Human Subjects, Vertebrate Animals, Biohazards, and/or Select Agents

University of Minnesota Site (Dehm, PI): Nothing to report.

Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to report.

University of British Columbia Site (Strynadka, PI): Nothing to report

F. PRODUCTS

University of Minnesota Site (Dehm, PI): Nothing to report.

Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to report.

University of British Columbia Site (Strynadka, PI): Nothing to report

G. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

G.1 Individuals Who Have Worked on the Project

University of Minnesota Site (Dehm, PI):

Name: Dr. Scott Dehm
Project Role: PI, responsible for all work at University of Minnesota site
Researcher Identifier:
Person months worked: 1.2
Contribution to Project: Dr Dehm
Funding Support:

Name: Dr. Yingming Li
Project Role: Research Associate
Researcher Identifier:
Person months worked: 1.2
Contribution to Project: Laboratory management and project supervision
Funding Support:

Name: Dr. Aashi Chaturvedi
Project Role: Postdoctoral Associate

Researcher Identifier:
Person months worked: 7.2
Contribution to Project: Laboratory experimentation to evaluate therapeutics with anti-AR and anti-AR-variant mechanisms of action
Funding Support:

Name: Dr. Yeung (Louisa) Ho
Project Role: Postdoctoral Associate
Researcher Identifier:
Person months worked: 4
Contribution to Project: Laboratory experimentation to evaluate therapeutics with anti-AR and anti-AR-variant mechanisms of action
Funding Support:

Vancouver Prostate Centre Site (Cherkasov, PI):

Name: Dr. Artem Cherkasov
Project Role: PI
Researcher Identifier:
Person months worked: 2
Contribution to Project: Dr Cherkasov oversees all aspects of computational drug design, molecular Modeling and bioinformatics, provides guidance and consultation to Dr. Kriti Singh, and assists with the analysis and interpretation of data.
Funding Support: Salary 100% covered by the University of British Columbia (no salary paid from this grant.

Name: Dr. Francesco Gentile
Project Role: Post-doctoral Fellow
Researcher Identifier:
Person months worked: 1
Contribution to Project: Dr. Gentile conducted computational drug design, molecular modeling and bioinformatics studies under the supervision of Dr. Cherkasov.
Funding Support: This project only

Name: Dr. Kriti Singh
Project Role: Post-doctoral Fellow
Researcher Identifier:
Person months worked: 1
Contribution to Project: Dr. Singh conducted computational drug design, molecular modeling and bioinformatics studies under the supervision of Dr. Cherkasov.
Funding Support: This project only

Name: Ana Karla Nunez
Project Role: Co-Op Student
Researcher Identifier:
Person months worked: 1

Contribution to Project: Ms. Nunez performed evaluation of compounds designed in Year 1, under the supervision of Dr. Cherkasov.
Funding Support: This project only

Name: Sarah Truong
Project Role: Co-Op Student
Researcher Identifier:
Person months worked: 3
Contribution to Project: Ms. Truong performed evaluation of compounds designed in Year 1, under the supervision of Dr. Cherkasov.
Funding Support: This project only

University of British Columbia Site (Strynadka, PI):

Name: Dr. Natalie Strynadka
Project Role: PI
Researcher Identifier:
Person months worked: 6
Contribution to Project: Supervision and oversight of all structure determination aspects of the project.
Funding Support: Salary 100% covered by the University of British Columbia (no salary paid from this grant.

Name: Dr. Liam Worrall
Project Role: Research Associate –
Researcher Identifier:
Person months worked: 6
Contribution to Project: Xray crystallographic and cryoEM data collection and analysis, processing and model building, refinement.
Funding Support: 50% of salary

Name: Dr. Craig Robb
Project Role: Research Associate
Researcher Identifier:
Person months worked: 6
Contribution to Project: – Ultra-pure, high yield protein production and crystallization screening, analysis
Funding Support: 50% of salary

G.2 Changes in Active Other Support of the PD/PI(s) or Senior/Key Personnel Since the Last Reporting Period.

University of Minnesota Site (Dehm, PI):

PC190269 (PI: Dehm) 07/01/2020-06/30/2023 1.8 calendar months DOD $245,381 direct/year
Targeting the CPSF Complex to Prevent Expression of AR Variants in Prostate Cancer
The goal of this study is to test whether the CPSF complex component CPSF1 represents a tractable therapeutic target for blocking expression and activity of AR variants in castration-resistant prostate cancer, and to advance the therapeutic development of an antisense morpholino targeting the AR:CPSF1 interaction. Aim 1: Evaluate the role and clinical relevance of CPSF1 in prostate cancer Aim 2: Test therapeutic targeting of CPSF1 to block expression of AR variants

R01CA244299-01A1 (PI: Dehm) 07/01/2020 – 06/30/2025 2.4 calendar months NIH/NCI $307,469 direct/year
mRNA Polyadenylation in Prostate Cancer
The proposed studies are focused on understanding how alterations in the expression and activity of the machinery governing mRNA polyadenylation impacts prostate cancer development and progression using models of AR-variant (AR-V) driven castration-resistant prostate cancer. Aim 1: Evaluate the spectrum of coordinate AR-V expression in prostate cancer. Aim 2: Study the mechanisms by which polyadenylation regulators coordinate expression of AR-Vs Aim 3: Determine the biological and therapeutic significance of coordinate AR-V expression

PC190189 (PI: Felices) 08/01/2020-7/31/2023 0.24 calendar months DOD $213,074 direct/year
Driving Natural Killer Cell Immunotherapy in the Castration Resistant Prostate Cancer Setting with Novel Tri-Specific Killer Engager Molecules
Objective/Goal: The goal of this proposal is to create two novel TriKEs targeting prostate cancer relevant tumor antigens, PSMA and Trop-2, in order to improve outcomes in men with mCRPC.
Specific Aims: SA1) Creation and Validation of Prostate Cancer specific TriKEs. TriKEs will be created using a mammalian expression platform and specific binding to PSMA or Trop-2 will be evaluated in a number of systems. The IL-15 moiety will also be tested in proliferation assays. Cytotoxic and inflammatory functions of the TriKE molecules will be tested against prostate cancer cell lines in a number of assay formats. SA2) Evaluating the ability of TriKEs to promote NK cell function on mCRPC patient samples using patient NK cells and patient derived xenograft models. We will also evaluate, using high content CyTOF analysis, what the best window for treatment is in mCRPC patients.
Role: Co-Investigator

R01CA256157 (Halabi/Armstrong/Dehm, MPI) 9/1/20 – 8/31/25 1.8 calendar months NCI/NIH $729,580
Clinical genomic predictive model of first line androgen receptor inhibitor therapy outcomes in men with mCRPC

The goal of this project is to develop prognostic models of clinical outcomes that will incorporate baseline AR and non-AR ctDNA aberrations in men with mCRPC (A031201). In addition, we identify potential predictive biomarkers for overall survival benefit with combined abiraterone acetate and enzalutamide versus enzalutamide treatment alone from men with mCRPC enrolled in a phase III trial on the Alliance Trial A031201.

Vancouver Prostate Centre Site (Cherkasov, PI):

Cherkasov (PI) 3/1/2020-2/28/2022
Canadian Institutes of Health Research (CIHR) CAD $999,000
Augmented discovery of potential inhibitors of SARS-CoV-2 3CL protease.
We are deploying a unique and robust approach to identify compounds that inhibit the SARS-CoV-2 $3CL^{pro}$ (the protease required for viral replication) and verify anti-COVID-19 activity by viral replication assays. In addition, we will use X-ray crystallography to generate new high-resolution 3D crystal structures of the protease to accelerate future QSAR modeling for therapeutic drug development.

Cherkasov (PI) 5/1/2020-2/28/2022
Canadian Institutes of Health Research (CIHR) CAD $50,000
Sex as a Biological Variable Supplement: Augmented discovery of potential inhibitors of SARS-CoV-2 3CL protease.
The central objective in our primary project is to identify SARS-CoV-2 3CL protease inhibitors by biochemical and viral replication assays in primate (Vero, kidney) and human pulmonary (A549) cells. This supplemental project will enable us to evaluate our top candidate inhibitors in human airway organoids—the assay that most closely recapitulates the lung tissue—while determining if sex differences can contribute to host-cell responses to the best antiviral compounds identified in our COVID-19 Rapid Response pipeline. This investigation will also enable identification of potential biological factors and cellular hubs that are determining the responses of M- and F-organoids to SARS-CoV-2 infection and to prospective treatments.

Cherkasov (PI) 6/1/2020-5/31/2021
Canadian Institutes of Health Research (CIHR) CAD $2,109,120
Computer-aided discovery of synergistic drug combinations with remdesivir for COVID-19 through mechanism-based drug repurposing and combinatorial organoid screening.
We are building state-of-the-art small drug modeling and screening virology facilities. Herein, we propose to use these facilities to identify inhibitors for most prominent SARS-CoV-2 target proteins including $3CL^{pro}$, PL^{Pro}, Spike/ACE2 interface, RNA polymerase and Nsp15. Our efforts will be focused on existing drugs or natural products to either rapidly find stand-alone repurposing options for COVID19 treatment, and/or synergetic partners for remdesivir. In parallel, we will exercise substantial 'plan B' development of potent and selective novel anti-coronaviral agents that can be used in a long-term prospective.

University of British Columbia Site (Strynadka, PI):

Strynadka (PI) 3/1/2015-2/28/2022
Canadian Institutes of Health Research (CIHR) CAD $4,300,000
Structure based drug discovery on the bacterial membrane Use of xray crystallography and single particle cryoEM to determine atomic resolution structures of large membrane spanning complexes essential to bacterial pathogenicity and drug resistance.

H. SPECIFIC REPORTING REQUIREMENTS

This report is for a collaborative award (partnering PI option), and was prepared jointly by the three PIs. The tasks are clearly articulated for each responsible PI and project performance sites are clearly marked.

I. APPENDICES

None.