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TITLE: Direct Targeting of the FKBP52 Cochaperone for the Treatment of Castration-Resistant Prostate Cancer

PRINCIPAL INVESTIGATOR: Dr. Jaideep Chaudhary

CONTRACTING ORGANIZATION: Clark Atlanta University

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14. ABSTRACT

Prostate cancer affects one in seven men in the United States and is a major leading cause of cancer death among men. Current treatment strategies exploit the dependence of AR for hormone activation and current therapies are ineffective in castration resistant prostate cancer (CRPC). Based on this rationale, we are pursuing a unique non-AR based strategy. The folding, activation, and nuclear translocation of steroid hormone receptors involves no less than twelve proteins and at least four distinct complexes. At least one of these proteins, the FKBP52 cochaperone, is a highly promising therapeutic target for the disruption of a number of mechanisms important in prostate cancer. The proposed research is focused on the preclinical development of GMC1, a drug-like small molecule that targets FKBP52 regulation of steroid hormone receptor activity. During the first year of this award we have made progress in the hit-to-lead optimization process and have identified a number of novel derivatives with activity. We have also established protocols and assays for assessing lead drug effects in cellular and animal models.

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A. INTRODUCTION

Prostate cancer affects one in seven men in the United States and is a major leading cause of cancer death among men. Current treatment strategies exploit the dependence of AR for hormone activation and current therapies are ineffective in castration resistant prostate cancer (CRPC). Based on this rationale, we are pursuing a unique non-AR based strategy. The folding, activation, and nuclear translocation of steroid hormone receptors involves no less than twelve proteins and at least four distinct complexes. At least one of these proteins, the FKBP52 cochaperone, is a highly promising therapeutic target for the disruption of a number of mechanisms important in prostate cancer. The proposed research is focused on the preclinical development of GMC1, a drug-like small molecule that targets FKBP52 regulation of steroid hormone receptor activity. The major goals of this research are to perform hit-to-lead optimization of GMC1 to improve drug solubility and potency, 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 compounds in animal models of prostate cancer.

B. KEYWORDS

Prostate cancer, castration-resistant prostate cancer, androgen receptor, glucocorticoid receptor, progesterone receptor, testosterone, FKBP52, FKBP4, FKBP51, FKBP5, immunophilin, cochaperone, beta-catenin, anti-androgen, pre-clinical, FKBP inhibitor

C. ACCOMPLISHMENTS

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

The major goals for years 1-3 including the year 4 extension of the project are outlined below.

Specific Aim 1: Use structure-based drug design methodology and in silico library screening to identify small molecules targeting the FKBP52 PPIase pocket.

Major Task 1: Conduct large-scale in silico screen against the FKBP52 PPIase pocket

Specific Aim 2: Perform a detailed evaluation of all candidate drug compounds in multiple cellular models of prostate cancer.

Major Task 2: Functional screening of hit molecules and molecule modifications

Major Task 3: Verify drug-binding site for the most promising lead molecules.

Milestone 1: Seek patent protection for the 10 most promising lead molecules

Major Task 4: Characterize drug effects in cellular models of prostate cancer and characterize mechanism of action

Specific Aim 3: Perform preclinical evaluations in murine prostate cancer models.

Major Task 5: Assess in vitro efficacy of lead molecules

Milestone 2: Publication on novel drugs and their in vitro characterization

Major Task 6: Perform PK/PD on selected candidate compounds

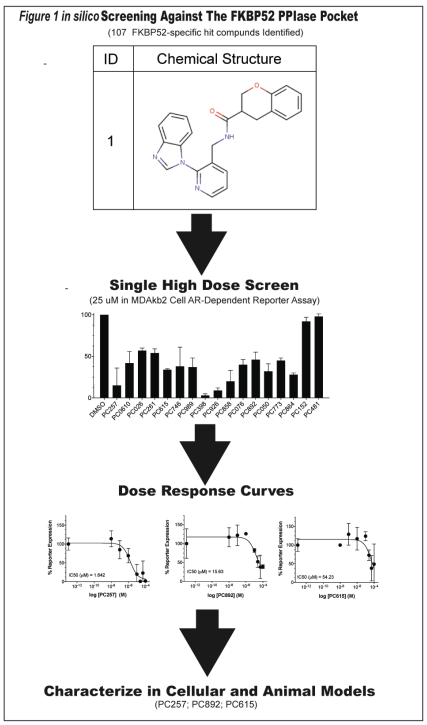
Major Task 7: Assess the efficacy of at least 3 selected candidate molecules in *in vivo* mouse xenograft models

C.2 Accomplishments Under These Goals

It is important to note that the Y3 annual report (2020) represented a report of the progress on all aims and major tasks related to the entire 3-year project period that was originally proposed. Due to delays related to the pandemic, we requested and were granted a 1-year extension. Thus, the report below represents the information submitted in Y3 with updates related to the scope of work proposed in the extension request, progress made in Y4, as well as more details on the project overall. In short, the Cherkasov group successfully performed a large, broad scale in silico screen for novel FKBP52 targeting drugs that represent novel chemotypes independent of GMC1; the drug we previously identified from a more limited preliminary screen. The Cox group screened these molecules in cellular assays to identify PC257, a molecule that specifically inhibits FKBP52-regulated AR, GR and PR activity in reporter assays. In addition, work by both the Cox and Chaudhary groups demonstrated that PC257 effectively inhibits endogenous AR-dependent gene expression and proliferation in prostate cancer cells. The Chaudhary group has completed preliminary assessments of PC257 in soft agar colony formation assay. Thus, we have identified and characterized a novel drug candidate that displays more consistency and potency than our previous leads, secured intellectual property protection of this novel drug, and the necessary data to attract commercial interest. As reported in Y2, we secured sponsored research agreements with *Maia Biotechnology Inc.* to add our previously developed drug candidates, MJC13 and GMC1, to their pipeline, and to continue to optimize the chemistry of the drugs to improve metabolic stability. That work is still ongoing. In addition, we are now currently negotiating the potential licensing of PC257 with *Maia Biotechnology Inc.* We still have future work to understand the PC257 targets and/or binding sites on FKBP52, and to optimize PC257 for further development. Ultimately, extensive evaluation of all of our drugs in animals did not make sense given that we are working to optimize the chemistry. The future goals for this work after this grant ends are to identify analogues of MJC13, GMC1 and PC257 that display favorable PK/PD and metabolic stability, and it will be these new lead molecules that will need to be extensively evaluated for toxicity and therapeutic efficacy in animal models.

University of Texas at El Paso Site (Cox, PI):

Aim 2, Major Task 2: Functional screening of hit molecules and molecule modifications



As reported in Y1, we began the screening of molecules that came out of the *in silico* screens described below. In Y2 we completed the screening process to identify new leads based on GMC1 in addition to identifying completely new chemotypes independent of GMC1 to pursue as possible drug candidates. At the time of the Y2 report, we were working with *Maia Biotechnology Inc.* to complete the ADME studies on the more potent GMC1 analogues identified in our screens. Those ADME studies were completed, and it was found that while two of the five analogues showed better PK/PD profiles than GMC1, their metabolic stability was still not where it needs to be to move the molecules forward towards IND enabling studies. We are still working with *Maia Biotechnology Inc.* on the preclinical chemistry needed to improve metabolic stability. At the current time Maia is in the process of issuing an IPO, which will likely generate the funds needed to continue our future work with Maia. In addition to the GMC1 analogues, we also successfully identified a new chemotype, termed PC257, that is being pursued independently of GMC1 as a novel drug molecule targeting FKBP52 for the treatment of prostate cancer and it is this molecule that was the primary focus of this DOD-funded project (**Fig. 1**). While this molecule was independent of any agreements with Maia, we are currently in the process of negotiating licensing to add PC257 to the Maia pipeline. Based on the data we were able to generate, PC257 is a more potent inhibitor of FKBP52-regulated AR activity than any other drug molecule we have identified and characterized

previously. Thus, all our focus in Y3 and Y4 was to characterize PC257 for the treatment of prostate cancer.

Milestones 1: Seek patent protection for the 10 most promising lead molecules:

The provisional patent covering PC257 and derivatives was filed in January, 2020 (Claims Priority to U.S. Provisional Patent Application No. 62/963,873, filed January 21, 2020). In addition, the full conversion was submitted in January 2021 and is currently pending.

Milestone 2: Publication on novel drugs and their in vitro characterization:

The publications on the novel drugs GMC1 and PC257 are still pending. Given that these drugs have been the subject of our ongoing work with *Maia Biotechnology Inc.* to identify more metabolically stable derivatives that we can also pursue composition of matter on, we have held off on publishing these drugs for now. We still have a full draft of a manuscript detailing the broader *in silico* screen that led to the identification of PC257 in addition to a draft manuscript detailing the in vitro and in vivo characterization of GMC1. We will get these manuscripts out when the timing is right and will likely expand them to include the new lead molecules that result from our work with *Maia Biotechnology Inc*.

Aim 2, Major Task 3: Verify drug-binding site for the most promising lead molecules.

As reported in Y1, we performed functional mutagenesis to verify the GMC1 target site on FKBP52. We were able to demonstrate that a few mutations in the PPIase pocket (proposed GMC1 target site) that did not affect FKBP52 regulation of AR reduced GMC1 inhibition ability. Given that our focus ultimately shifted to the new chemotype PC257, we also worked with the Cherkasov group to perform more detailed *in vitro* drug binding studies for our new chemotype, PC257 in Y4 (see below). Our group developed the PET28 expression plasmids that were used by the Cherkasov group for the bacterial expression and purification.

Aim 2, Major Task 4: Characterize drug effects in cellular models of prostate cancer and characterize mechanism of action:

Given that PC257 is a new chemotype for which we can pursue composition of matter, we also must characterize PC257 for effects on hormone receptor signaling and verify its mechanism of action in cellular models in addition to performing efficacy studies in animal models (see below). We have demonstrated that PC257 specifically inhibits AR, GR and PR-dependent luciferase reporter gene expression in the 1-5 uM range but has no effect of ER-mediated reporter expression (**Fig. 2**). The fact that it inhibits the three receptors known to be regulated by FKBP52 strongly indicates that the molecule is targeting FKBP52.

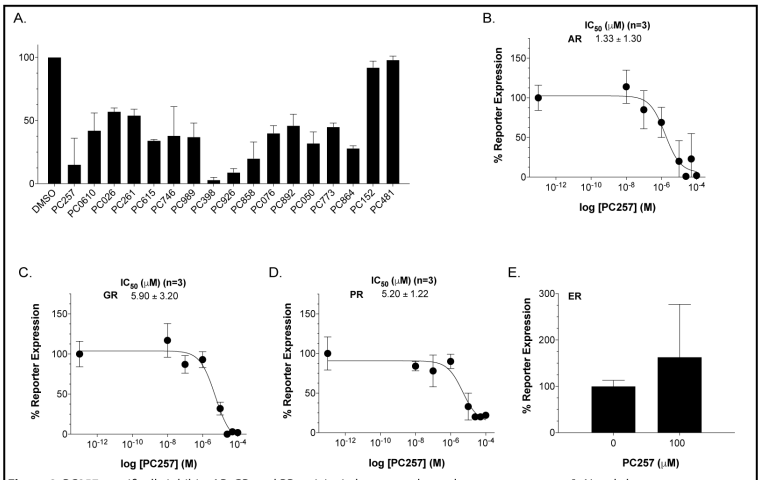


Figure 2: PC257 specifically inhibits AR, GR, and PR activity in hormone-dependant reporter assays. **A**. Novel chemotypes were assessed in AR-dependent reporter assays in MDA-kb2 cells at a single high dose. Those that inhibited by AR by 50% or more were then screened in full dose response assays. PC257 was selected as the most potent FKBP52 inhibitor out 107 screened. Hormone-dependent AR- (**B**), GR- (**C**), PR- (**D**), and ER- (**E**) dependent reporter assays were performed in the presence or absence of a range of PC257 concentrations.

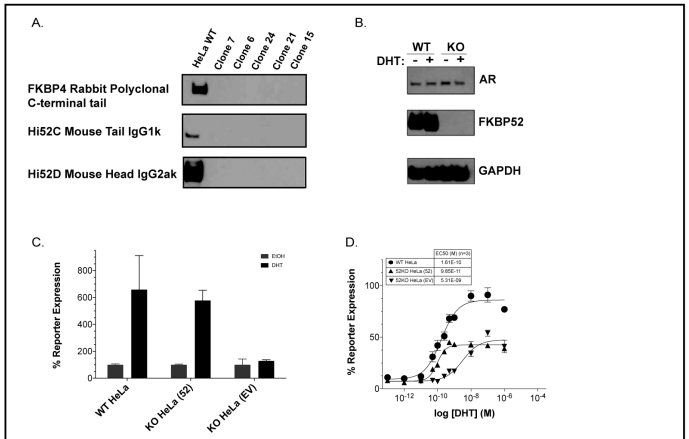


Figure 3: CRISPR/Cas9 FKBP52 KO human cell model. A. Western blot from 52KO HeLa cell clones using FKBP52 antibodies targeting a variety of epitopes on FKBP52. B. Clone 15 was selected and a western blot for AR and FKBP52 were performed in the presence and absence of hormone. C. AR-dependent reporter assays in the presence or absence of hormone and FKBP52 expression plasmid. D. DHT-dependet AR-mediated reporter assays in the presence of a range of DHT concentrations demonstrating a loss of hormone potentcy in the 52KO that can be restored by exogenous expression of FKBP52.

Previously, all assays to assess the preferential targeting of FKBP52 were performed in 52KO mouse embryonic fibroblasts as this was the only cell model with complete deletion of FKBP52. Thus, we generated 52KO human cell models in HeLa (**Fig. 3**) and 22Rv1 (data not shown) using CRISPR/Cas9. In both of these 52KO cellular models, AR activity is significantly reduced and can be restored upon exogenous expression of FKBP52 (**Fig. 4**). These models will provide a variety of more relevant cellular models to assess PC257 effects on AR in the presence or absence of FKBP52. In addition, the 52KO 22Rv1 cells will be used to validate FKBP52 as a target by demonstrating the effect of 52KO in xenografts. Since we are targeting the FKBP52 PPIase pocket, which is highly conserved among family members, we fully expect that PC257 targets a variety of the FKBPs and may confound our ability to show FKBP52-specific targeting. That being said, our data demonstrate that AR activity in reporter assays in 52KO MEFs, 52KO HeLa, and 52KO 22Rv1 cells is preferentially targeted by PC257 with IC50s ranging from 371 nM to 1.48 uM in the presence of FKBP52 and ranging from 2.21 uM to 11.5 uM in the absence of FKBP52 (**Fig. 4**). In Y4 we completed our dataset demonstrating that PC257 abrogates endogenous DHT-dependent FKBP51 and PSA gene expression in a wide variety of prostate cancer cells at concentrations between 10 and 25 uM (**Fig. 5**), which is more potent in this assay than any previously characterized drug molecules including MJC13 and GMC1. Finally, in Y4 we conducted studies to assess the effects of PC257 on 22Rv1 prostate cancer cell proliferation. As shown in Figure 6, PC257 effectively blocks R1881-dependent 22RV1 cell proliferation starting at concentrations between 10-30 uM, which is consistent with PC257 concentrations that are effective in the hormone-dependent gene expression studies in **Figure 5**.

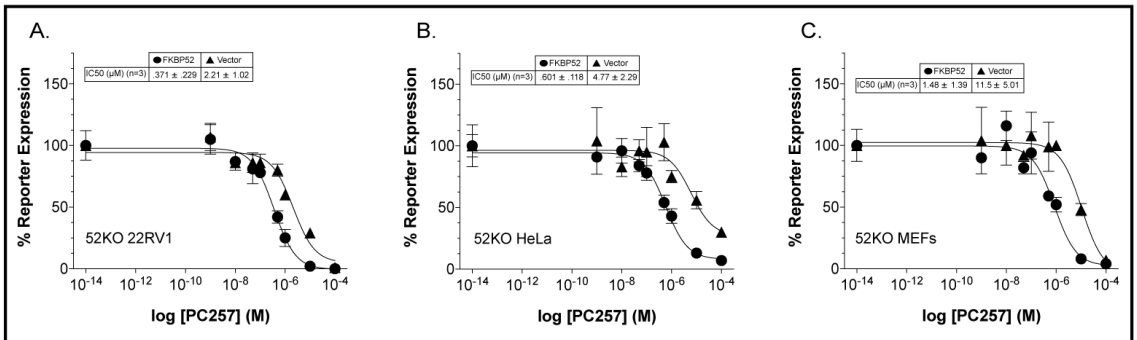


Figure 4: PC257 preferentially targets FKBP52-regulated AR activity. AR-mediated reporter assays in the presence of a range of PC257 concentrations with or without exogenous FKBP52 expression in 52KO 22Rv1 prostate cancer cells (**A**), 52KO HeLa cells (**B**), and 52KO MEFs (**C**).

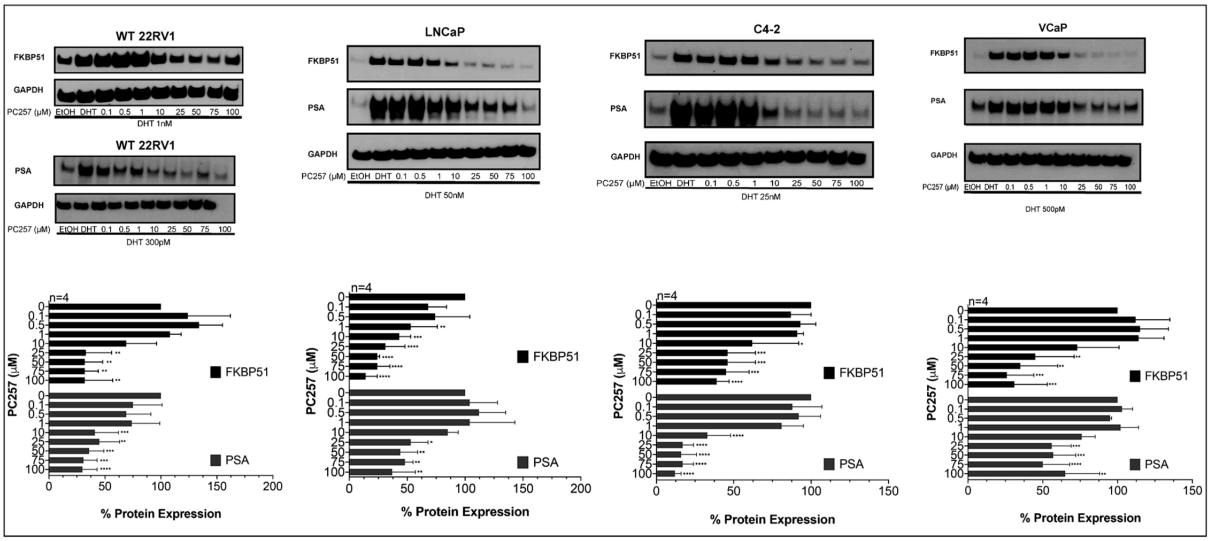


Figure 5: PC257 effectively inhibits endogenous AR-dependent gene expression in 22Rv1 prostate cancer cells. A range of PC257 concentrations were assessed for the ability to inhibit DHT-induced FKBP51 and PSA expression in 22Rv1, LNCaP, C4-2, and VCaP cells by western blot flollowed by quatitation by densitometry.

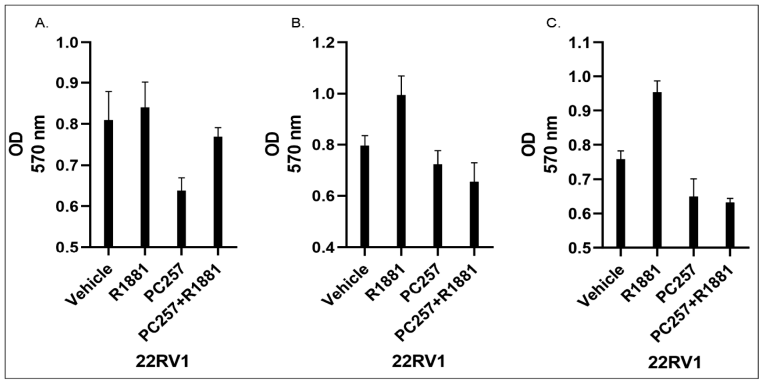


Figure 6: PC257 Inhibits AR-dependent Proliferation in WT 22RV1 Cell Lines. PC257 effectively abrogates prostate cancer cell proliferation. **A.** The cells were treated with 10μM PC257, incubated, followed by 1nM R1881. **B.** The cells were treated with 30μM PC257, incubated, followed by 1nM R1881. PC257 (30μM) alone and in combination with R1881 inhibits proliferation when compared to vehicle control. **C.** Cells were treated with 75μM PC257, incubated, followed by 1nM R1881, the same effect is apparent with PC257 (75μM) alone and in combination with R1881. The absorbance was measured at 570nM using a 96-well plate. The data is a representation of three independent experiments.

Aim 3, Major Task 7: Assess the efficacy of at least 3 selected candidate molecules in *in vivo* mouse xenograft models.

We chose PC257 as the most promising lead molecule to move forward to animal studies based on its potency as well as its preferential targeting of FKBP52. The COVID19 pandemic delayed our progress towards completing formulation and initial animal evaluations in Y3 as our respective labs were shutdown from March through June of 2020. In addition, once we were back in the lab, our respective animal facilities were some of the last facilities to get back up and running given the number of personnel required to maintain these facilities with active research. In addition, it is important to note that the cost to have PC257 custom synthesized is cheap suggesting that the molecule is relatively simple to synthesize, which has positive implications for scale-up later in the development process. In Y4 we completed the PC257 solubility assessment and formulation for *in vivo* administration as proposed last year. PC257 was formulated with a PLGA (poly(lactic-co-glycolic acid) 50:50). PLGA has been approved by U.S. FDA in 1989 as a drug

delivery vehicle and since then 19 different drugs have been approved by U.S. FDA formulated with PLGA. The PLGA is a co-polymer that has both hydrophilic and hydrophobic moieties and thereby if facilitate loading most of the hydrophobic drug molecules and enhance their solubility/dispersibility in aqueous solution. The PC257 and PLGA formulation was prepared by W/O emulsion method where the PLGA (100 mg) and PC257 (10/20/40 mg) were dissolved in dichloromethane, individually. PVA (1%) solution was added slowly dropwise at high steering speeds. The agitation was continued for overnight at room temperature. The mixture was then kept in -20 ^{o}C for 1 hour followed centrifuged for 30 min at 3900 RPM to separate the precipitated phase from the supernatant phase. Then, the supernatant was removed, followed by lyophilizing the formulation by freeze dryer under high vacuum for 24-48 hr. The formulations were characterized by measuring drug loading content, solubility in PBS, particle size and zeta potential values. These formulations will be used for all future *in vivo* evaluations of PC257 in animal models. See below for the work performed in the Chaudhary lab during to further characterize both GMC1 and PC257 in cellular and animal models during the project period.

Vancouver Prostate Centre Site (Cherkasov, PI):

As reported in the Y1-2 reporting periods, the Cherkasov group at VPC performed a large-scale virtual screening of 138M compounds, and completed the hit selection for wet lab evaluation, which led to the identification of a new lead drug molecule termed PC257 that was characterized by Drs. Cox and Chaudhary.

Aim 1, Major Task 1: In Y3, we continued to expand the repertoire of available FKBP52 inhibitors through the development of broader screens based on the idea that the more candidates in the pipeline, the better chance that one will move forward towards commercialization. Thus, we defined a large-scale *in silico* screen against the FKBP52 PPIase pocket. We initially screened 138M compounds *in silico* in Y2. While the Y2 compounds were being evaluated, we began working on deploying a machine learning approach to *in silico* screening for this project. To identify more novel scaffolds and corresponding analogues we need to expand the chemical library screening from 138M to 1.4B molecules or greater—which in turn should increase the number of novel candidates by one order of magnitude. Other members of Dr. Cherkasov's *in silico* team demonstrated that they could screen a 1.3B-compound data base in two weeks with the Machine Learning approach (aka "Deep Docking")—compared to the 3 years of CPU time it would take to complete via regular docking. We were considering deploying DD once the *in vitro/in vivo* studies were completed. However, the COVID-19 situation delayed this, and we instead focused on the studies to assess PC257 binding to the proposed drug target FKBP52 instead (see below).

Aim 2, Major Task 3: Verify drug-binding site for the most promising lead molecules.
In Y4 we proposed to genetically engineer, express, and purify human recombinant FKBP52 protein to subsequently conduct FKBP52/compound binding studies to determine dissociation constant (Kd). Below details those studies that were completed in Y4.

Protein expression: The plasmid pET28 encoding full length FKBP52 with a C-terminus $(His)_6$-tag was transformed with pRARE into E.coli BL21(DE3) for protein expression. The expression was induced with 0.5 mM IPTG and cells were incubated at 30°C for 5 hours and then collected by centrifugation at 3000 x g for 10 min. The cell pellet was resuspended in lysis buffer (20 mM Tris pH 7.5, 200 mM NaCl, 8 mM bME, 1 mM PMSF, protease inhibitor cocktail (cOmplete™, Sigma-Aldrich). The cells were lysed by sonication and the sample was cleared by centrifugation at 20,000 x g for 30 min. The supernatant was incubated with Ni-NTA Agarose beads (Qiagen) for 1 h at 4°C. His-tagged protein was eluted from the resin in elution buffer (20 mM Tris pH 7.5, 200 mM NaCl, 300 mM Imidazole, 5 mM bME, 0.1 mM PMSF). After an overnight dialysis against dialysis buffer (20 mM Tris pH 7.5, 50 mM NaCl, 5 mM bME, 0.1 mM PMSF) at 4°C, the protein was applied to ion exchange chromatography (HiTrap Capto Q 5/50, Cytiva Life Science) using dialysis buffer with 0.1-1M NaCl gradient. Further purification was accomplished by size exclusion chromatography (Superdex 75 Increase 10/300GL, GE Healthcare) in buffer containing 20 mM Tris pH 7.5, 150 mM NaCl, 0.2 mM TCEP, 0.1 mM PMSF. Fractions containing purified protein were pooled the concentration determined by the Bradford assay.

Protein Labeling: A sample of the protein was used for protein labeling with the red fluorescent dye NT647 via Monolith Protein Labeling Kit RED-NHS (Amine Reactive) from Nanotemper Technologies (Munich, Germany). The labeling process was done according to the manufacturer's protocol. The dye carries a reactive NHS-ester group that reacts with primary amines to form a covalent bond. The protein concentration and degree of labeling (DOL) were then determined via absorbance at 280nm and 650nm recorded using NanoDrop™ (https://nanotempertech.com/dol-calculator).

MST: MST assay was performed to determine the binding affinity of selected molecules using reagents, consumables, and Monolith NT.115Pico from Nanotemper Technologies. A serial dilution of the molecules was prepared with 100% DMSO and subsequently mixed with the labeled fluorescent protein in the assay buffer (20 mM Tris pH 8.0, 100 mM NaCl, 0.1% NP40, 0.2 mM TCEP and 0.1 mM PMSF). The final concentration of protein is 5nM with various concentration of small molecules in MST buffer containing 5% DMSO. The labeled protein and small molecule mix were allowed to incubate in the dark at RT for 5 minutes, prior to filling the capillaries. MST assays were performed at a final concentration of 5 nM labeled protein, with 5-10% LED/excitation power and medium MST power using premium capillaries for Monolith NT.115. The data for the estimation of Kd were analyzed using MO Affinity Analysis software from Nanotemper.

Results:

In this study, we expressed and purified FKBP52-His protein for binding studies using MST technology. Fractions containing pure FKBP52 were collected after size exclusion chromatography (Figure 1) for binding studies.

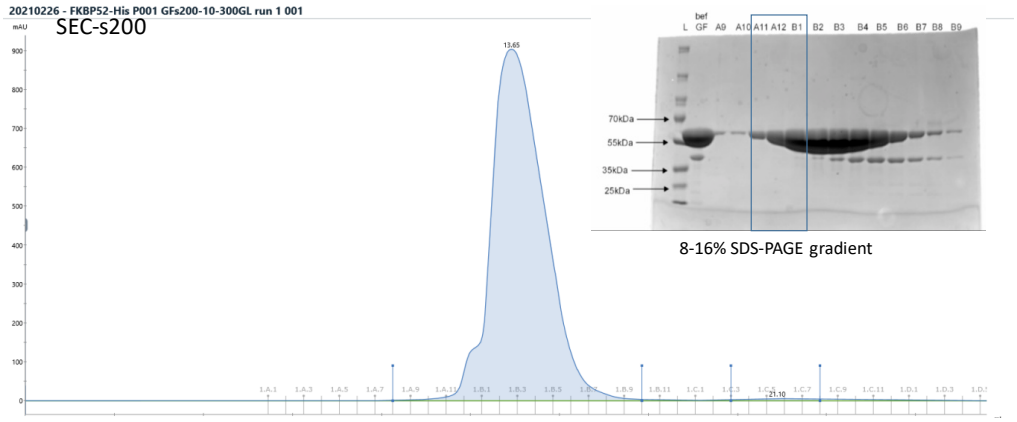


Figure 7: Purity of FKBP52 protein after size exclusion chromatography (SEC) as shown by SDS-PAGE and Coomassie staining.

In order to evaluate the direct binding of GMC1 and PC257 to FKBP52 protein, we used Microscale Thermophoresis (MST) to measure the directed movement of fluorescently labelled protein in a temperature gradient alone or when bound to the studied small molecules. We first confirmed that the protein alone is not aggregated in the experimental conditions of the assay and then studied the binding in presence of various concentrations of the small molecule inhibitors (Figure 2). Both inhibitors didn't show any direct binding to FKBP52 in this assay. Due to precipitation seen in the tubes, we were not able to test concentrations above 500 µM of these molecules.

Unfortunately, no direct binding of PC257 up to 500uM was observed in these studies. We still have confidence that PC257 targets FKBP52 given that PC257 specifically inhibits only the FKBP52-regulated receptors. It is likely that, like our previous drug MJC13 that only targeted AR with FKBP52 bound, PC257 targets an FKBP52 conformation that exists only in complex with the receptors. Thus, future binding studies will include Hsp90-receptor heterocomplex formation to assess drug binding in the context of the receptor heterocomplex.

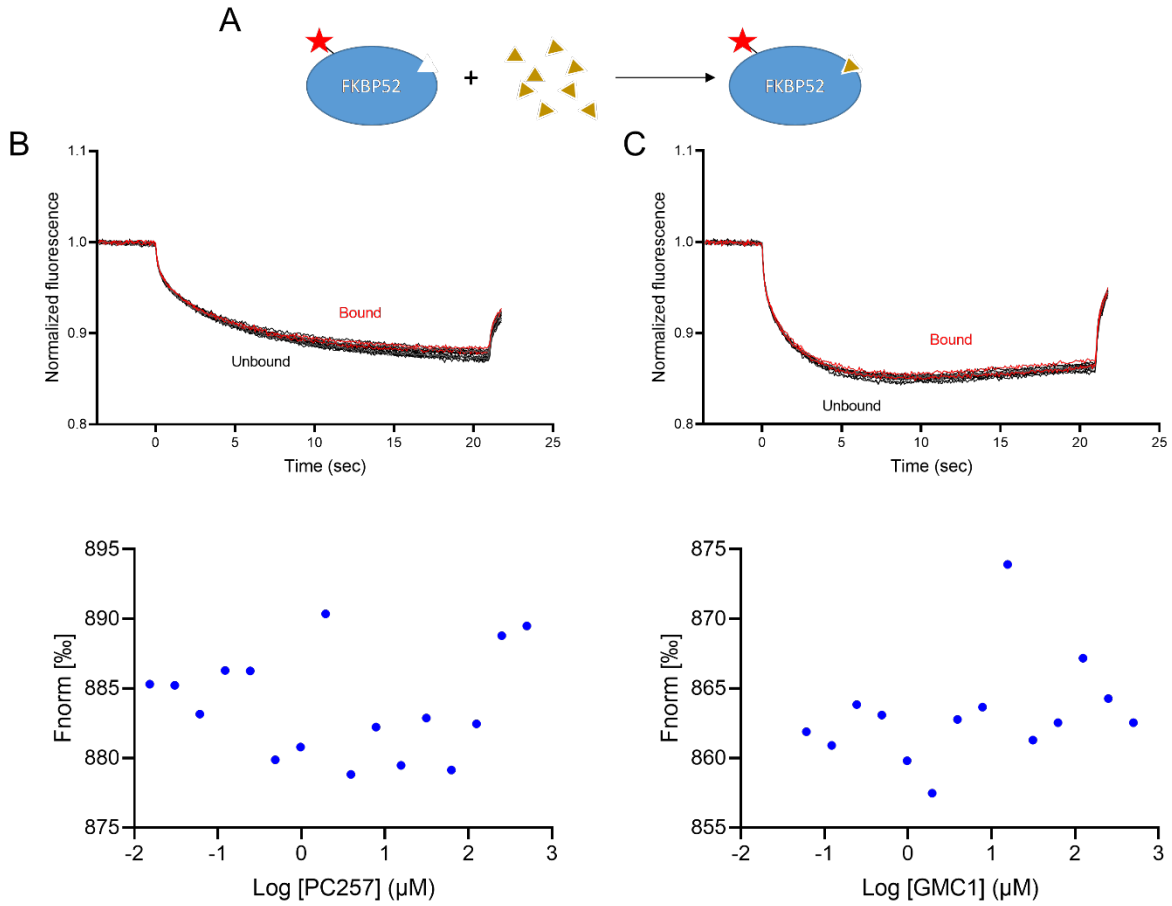


Figure 8: A- Schematic representation of the studied interaction using Microscale Thermophoresis (MST, NanoTemper). The direct binding between fluorescently tagged FKBP52 protein and the small molecule inhibitors PC257 (B) and GMC1 (C) was evaluated. A representative plotting of the change in thermophoresis (upper panel) and of the corresponding fitting of the data is represented (n=3).

Clark Atlanta University Site (Chaudhary, PI):

Year 4 Report. Final report at the end of No Cost Extension Period
In year 3 the PI proposed the following specific aims, major and sub tasks:
Specific Aim 3: Perform preclinical evaluations in murine prostate cancer models.

Major Task 5: Assess *in vitro* efficacy of lead molecules
Subtask 1: Assess effects on proliferation (MTT assay), apoptosis (AnnexinV/ PI assay followed by flow cytometery), Matrigel transwell migration assay and anchorage-independent growth in a soft agar assay in a variety of androgen sensitive and castration-resistant cell lines.
Milestone 2: Publication on novel drugs and their in vitro characterization

Major Task 6: Perform PK/PD on selected candidate compounds
Subtask 2: We will test our current lead compounds *in vivo* in mice along with preliminary pharmacokinetic evaluations in a nude mouse xenograft model.

Major Task 7: Assess the efficacy of at least 3 selected candidate molecules in *in vivo* mouse xenograft models
Below is the detailed outcome for each of the proposed tasks in the Statement of Work (SOW):

1. **Specific Aim 3:** Perform preclinical evaluations in murine prostate cancer models.

The lab received a new potentially active compound PC257 identified by Dr. Cherkasov and validated by Dr. Cox. In the meantime, our lab continued to investigate the molecular mechanism of action of GMC1, the lead compound and against which the efficacy of all newly developed compounds will be measured. We continued to replicate our previous results for statistical validation.

- Major Task 5: Assess *in vitro* efficacy of lead molecules
    - *Subtask 1*: Assess effects on proliferation (MTT assay), apoptosis (AnnexinV/ PI assay followed by flow cytometery), Matrigel transwell migration assay and anchorage-independent growth in a soft agar assay in a variety of androgen sensitive and castration-resistant cell lines.

Specific Aim 3/ Major task 5 was addressed by the following three experiments:
EXPERIMENT 1: Effect of GMC1 on Androgen receptor function.
EXPERIMENT 2: Investigate the Apoptosis in 22Rv1 and 22Rv152-/- cells
EXPERIMENT 3: Effect of PC257 on 22Rv1 and 22Rv1 FKBP52 knockout (52-/-) cells (MTT assay). This section also includes data generated in the nocost extension period: Final apoptosis data (continuation from "Experiment 2", transwell migration assay and soft agar colony formation assay

In year 3 we focused on establishing a clear molecular mechanism of action of GMC1 in addition to addressing the effect of GMC1 on cancer phenotype (proliferation, apoptosis etc). Dr. Cox lab generated 22Rv1 cells that lacked FKBP52 (52-/-), a great resource that will be used to investigate and compare the effect of small molecule inhibitors of FKBP52.
EXPERIMENT 1: Effect of GMC1 on Androgen receptor function.
The overall objective was to investigate whether GMC1 attenuates Androgen Receptor function, a key target for the treatment of castration resistent prostate cancer (CRPC) and the focus of this proposa. The following experiments wre performed to investigate whether GMC1 alters:
a) The stability of AR (Ongoing)
b) Binding of AR to the Androgen Respnse Element (ARE) on gene promoters – Experiment 1a
c) Expression of AR – Experiment 1b
d) Translocation of AR – Experiment 1c
Experiment 1a: Effect of GMC1 on the binding of androgen receptor to the respective Androgen Response Element on androgen respnsive genes
We expected that GMC1 may alter the androgen receptor activity thus altering the growth of CRPC cells. The direct evidence to support this mechanism was established by investigating the effect GMC1 on androgen receptor binding to the androgen receptor response element (ARE), in the promoters of known androgen receptor regulated genes such as PSA(**Fig. 6A**), FKBP51 (**Fig. 6B**) and ETV1 (**Fig. 6C**). We had access to the resources needed to perform these experiments (chromatin immuno-precipitation) from our previous study. The cells were treated with either R1881 (synthetic androgen analog), GMC1 or a combination of R1881+GMC1. The DNA was isolated. Cross-linked and immune-precipitated with androgen receptor antibody. This was followed by reverse cross-linking and performing PCR with primers spanning ~200 bp around the known ARE. The results shown in Fig. 1 clearly demonstrated that GMC1 decreased the binding of AR to its respective ARE on PSA, FKBP51 and ETV1. This is a major observation and established the molecular mechanism of action of GMC1 i.e. GMC1 may act by blocking the binding of AR to its respective response element. It is to be noted that although GMC1 also decreases but not abolishes AR expression as shown in our earlier reports and shown below in Fig. 2.

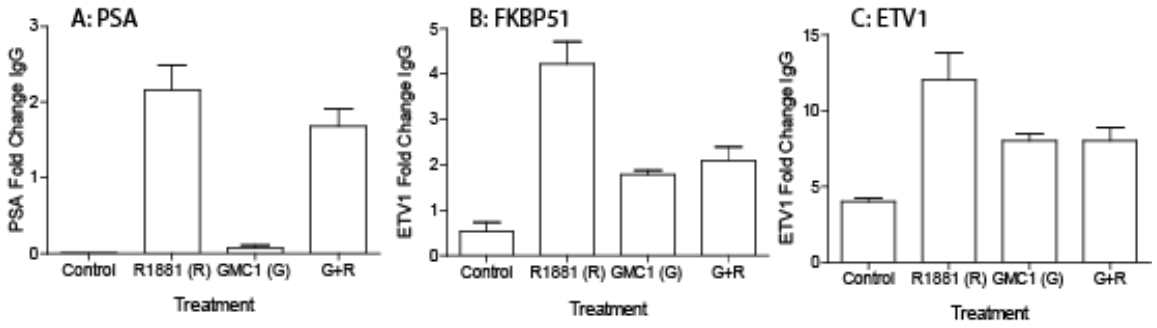


Fig. 6: Effect of GMC1 on the binding of androgen receptor on the respective Androgen Response Elements in the prompter of PSA (A), FKBP51 (B) and ETV1 (C). The data is mean±SEM of three separate experiments and is represented as fold change with IgG. Abbreviations: R: R1881, G: GMC1

Experiment 1b and 1c: Immunolocalization of AR and FKBP52 in 22Rv1 Cells (these results were mentioned in last years rport but not shown because of lack of replicates).

Based on the results from experiment 1, Here we report that GMC1 does not appear to significantly alter the stability of AR (**Fig. 7**) or reduce overall AR expression but a decrease is clearly observed in the nuclear compartment, possibly reflected accurately in our ChIP assays (**Fig. 6**). These results are similar to LNCaP cells reported in last year's report.

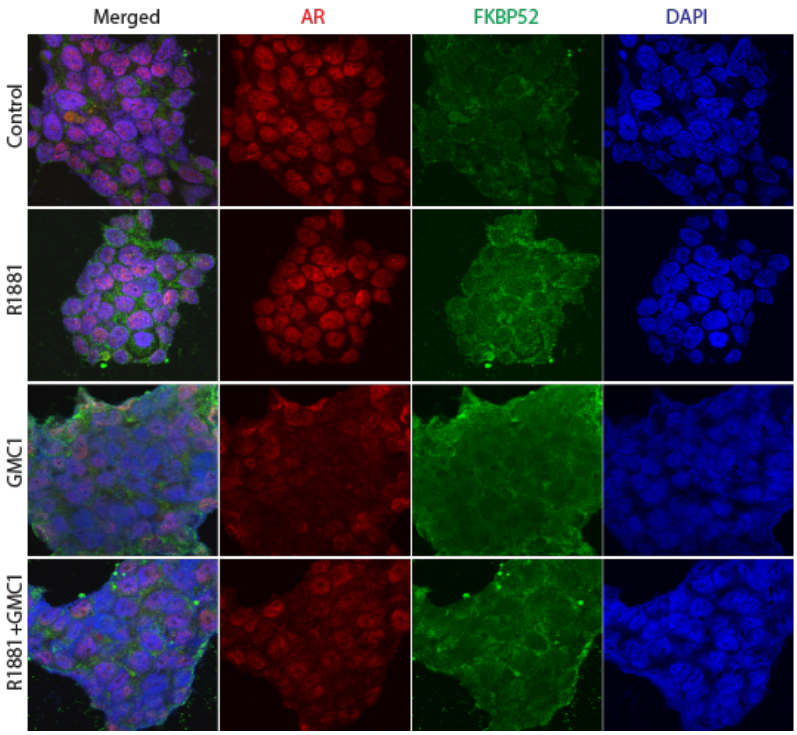


Fig. 7: Confocal immune-fluorescence analysis to confirm that GMC1 alters AR signaling and nuclear translocation in 22Rv1 cells. ICC we performed to study the AR (Red) and FKBP52 (Green) interaction inside the cell before and after treating 22Rv1 cells with R1881 (1nm) and GMC1 (30um). The merged panel is a merge between blue (DAPI, nucleus) AR and FKBP52. The data is representative of 3 experiments.

EXPERIMENT 2: Investigate the Apoptosis in 22Rv1 and 22Rv152-/- cells

The effect of FKBP52 gene knockout in 22Rv1 cells as a mimic for small molecule inhibitor was used toe measure apoptosis. As shown in **Fig. 8**, loss of FKBP52 significantly increased apoptosis (Flow Cytometry: Live - Propidium iodide, apoptotic -Annexin V). These data sets will be use in the future to investigate the efficacy of FKBP52 small molecule inhibitors.

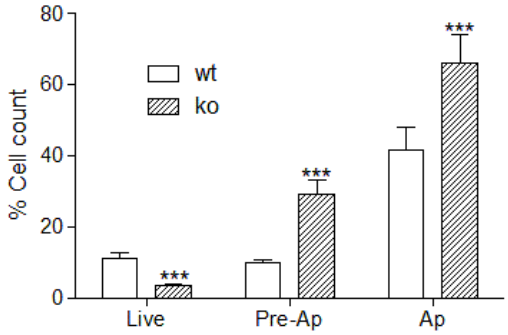


Fig. 8: Fraction of Live vs. apoptotic cells as determined by flow cytometry. The cells were stained with propidium iodide (live) and Annexin V (apoptotic marker). The data expressed as mean+SEM is from 3 different experiments. ***: p.0.001 – all data is compared within each group.

EXPERIMENT 3: Effect of PC257 on 22Rv1 and 22Rv1 FKBP52 knockout (52-/-) cells (MTT assay)
In addition to the above, we also initiated investigations on the efficacy and molcualr mechanism of action of PC257. This section also includes data generated in the nocost extension period: Final apoptosis data (continuation from "Experiment 2", transwell migration assay and soft agar colony formation assay

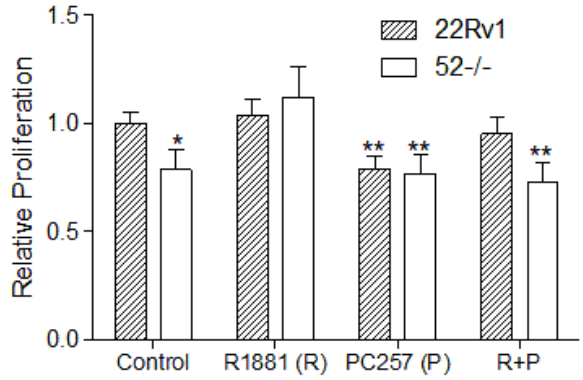


Fig. 9: Effect of PC257 on the proliferation 22Rv1 and 22rRv1 cells lacking FKBP52 (52-/-) in the presence or R1881 (R) or PC257 (P) or both (R+P). The data, represented as mean±SEM of three different experiments in triplicate. The data is normalized to the proliferation of 22Rv1 cells without any treatment (control)

The effect of PC257, the new candidate molecule, on proliferation was performed. The study was performed with different concentrations of PC257 (10um, 30um and 75um, 10um data shown in **Fig. 9** and discussed below) in the presence or absence of 1nm R1881. The results demonstrated that PC257 alone significantly decreased the proliferation of 22Rv1 cells at 10um. At this concentration however, PC257 had no effect on proliferation in the presence of R1881 suggesting that increased androgen concentration was able to overcome inhibitory effect of PC257. The effect of R1881 on 22Rv1 proliferation was not statistically significant suggesting that these cells are castration resistant. The basal proliferation rate (control) of 52/- cells was significantly lower that 22Rv1 cells suggesting FKBP52 is required to maintain the proliferative potential of these cells. Overall, the results suggest that PC257 inhibits proliferation. Similar effects PC257 on 52-/- cells suggests that PC257 may have off target effects on possibly other immunophilins which are structurally similar to FKBP52.

In the no cost extension period, we were able to investigate the effect of PC257 on the rate of apoptosis in 22Rv1, 52-/-, C42B and LnCAP cells. As shown in Fig. 10, treatment with R1881 significantly decreased the rate of apoptosis in 22Rv1 and LnCAP cells, whereas PC257 significantly increased apoptosis in al cell lines tested. Treatment of cells with R188+PC257 protected the cells against apoptosis induced by PC257 except 52-/- cells. The results suggested that PC257 promotes apoptotic death whereas R1881 protect cells from apoptosis in the prostate cancer cell lines used in this study

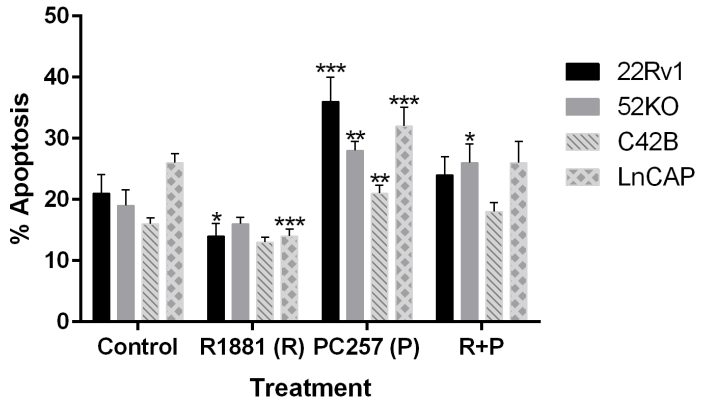


Fig. 10: Effect of PC257 (30uM) on apoptosis (Pre-apoptotic+Apoptotic) on 22Rv1, 22Rv1 (52-/-: 52KO), C4-2B and LNCaP cells. The protocol was as described in Fug. 9 legend). The cells were either untreated (Cintrol) or treated with R1881 ®, PC257 (P, 30uM) or a combination of R+P. The data is expressed % cells (Mean ± SEM) undergoing apoptosis from 3 different experiments in triplicate. *: P<0.05, **: P<0.01, ***: P<0.001.

Major Task 6: Perform PK/PD on selected candidate compounds
Subtask 2: We will test our current lead compounds *in vivo* in mice along with preliminary pharmacokinetic evaluations in a nude mouse xenograft model.

Major Task 7: Assess the efficacy of at least 3 selected candidate molecules in *in vivo* mouse xenograft models
We are excited to report the IACUC protocol for the use of GMC1 was approved and that we have also received MRMC ACURO approvals. This will now allow us to start the proposed in vivo efficacy studies for GMC1 and PC257.

We have a performed a small pilot study on 20 SCID mice (gift from Taconic) to determine the toxicity and effective concentrations of GMC1 on inhibiting 22Rv1 xenografts. GMC1 was not toxic at 5mg/ kg body weight.

However, further analysis on the effect of PC257 in xenografts and expanded studies as proposed could not be performed due to the closure of our university in response to COVID-19. Furthermore, in the no cost extension period, our funds could only support the post-doctoral fellow till December 2020. The lack of personnel and Covid-19 restrictions severely limited our ability to perform any large-scale xenograft studies. However, with limited resources we were able to investigate the effect of PC257 in the migration (transwell migration assay) and soft agar colony formation assay (the pre-requisite and justification for performing xenograft studies) on the cell lines.

PC257 significantly attenuated the migration potential of 22Rv1 and C4-2B prostate cancer cell lines but had no effect on the 22Rv1 FKBP52-/- knockout cells (Fig. 11). These results clearly suggest that FKBP52 is required for the migration of at least 22Rv1 cells. PC3 cells which lack AR were resistant to PC257, suggesting that a fully functional AR is also required for the inhibitory effect of PC257.

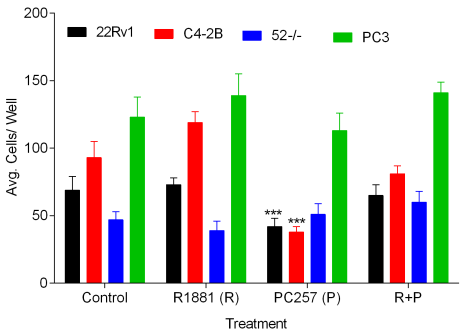


Fig. 11: Effect of PC257 (30uM) on cell migration and invasion using Trans well migration assay (Millipore QCM). PC257 (P) significantly reduced the migratory/ invasive potential of 22Rv1 and C4-2B cells as compared to R1881 (R), untreated controls (Control) and a combination of R+P. No significant effect of PC257 was observed in 22Rv1 52-/- and PC3 cells which lack AR. The data is expressed as Mean±SEM from 3 different experiments in triplicate. ***: P<0.001.

Given the inherent difficulties in investigating the mechanisms of tumor progression *in vivo*, cell-based assays such as the soft agar colony formation assay (soft agar assay), which measures the ability of cells to proliferate in semi-solid matrices, remain a hallmark of cancer research. A key advantage of this technique over conventional 2D monolayer or 3D spheroid cell culture assays is the close mimicry of the 3D cellular environment to that seen *in vivo.* Importantly, the soft agar assay also provides an ideal tool to rigorously test the effects of novel compounds (such as PC257) on cell proliferation, migration, and potential tumor formation in vivo. The results of these studies are required for proposing any study using mice xenograft models. In this context, and to prepare for the IACUC approval for mouse xenograft studies, we investigated the efficacy of PC257 on the soft agar colony formation assay. The results shown in Fig. 12, demonstrated that PC257 (P) significantly reduced the colony forming ability of 22Rv1 and C4-2B in a soft agar assay. Moreover, R1881 was able to rescue the colony forming ability (broadly, tumorgenicity) C4-2B cells but not 22Rv1 cells. These results are very significant and suggest that PC257 may inhibit the tumor forming ability of 22Rv1 and C4-2b in the mouse xenograft study.

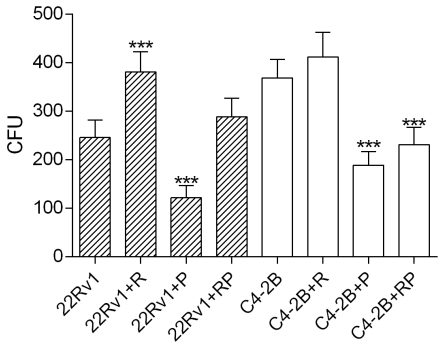


Fig. 12: Effect of PC257 on the colony formation (invasion, migration and colony formation) of 22Rv1 and C4-2B cells. The 22Rv1 52-/- , LNCaP and PC3 cells were not used in this study because of lack of an effect of PC257 in invasion/ migration assay (see Fig. 11). In contrast to R1881, PC257 significantly decreased the colony formatting potential of 22Rv1 cells that was rescued by R1881. PC257 also reduced the colony forming ability of C4-2B cells that was not rescued by R1881. The data is expressed as Mean±SEM from 3 different experiments in triplicate. ***: P<0.001.

C.3 Opportunities for Training and Professional Development

This project provided training opportunities for a number of graduate students and post-doctoral fellows (see section G.1 below). Importantly, this project in the Cox lab served as the primary dissertation project for Ashley Payan. Ashley successfully defended he dissertation and graduated in May, 2021 despite the challenges and delays caused by the pandemic. In total, four graduate students and four post-doctoral fellows were trained on this project across all three sites.

C.4 Results Disseminated to Communities of Interest

Nothing to report

C.5 Plans for Next Reporting Period

University of Texas at El Paso Site (Cox, PI): Nothing to report

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

Clark Atlanta University Site (Chaudhary, PI): Nothing to report

D. IMPACT

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

The drugs we are developing as part of this project are at the forefront of an emerging concept to target novel AR co-regulators for the treatment of prostate cancer, as well as other diseases. The drugs developed here are first-

in-class for the direct targeting of an androgen receptor-associated co-chaperone for the treatment of castration-resistant prostate cancer (CRPC), which will help pave the way for the development of similar technologies. In fact, investigators around the world are now developing co-chaperone targeting drugs targeting a wide variety of cochaperones for a wide variety of disease settings. This is evidenced by the collaborative work the Cox and Chasudhary groups have done with Dr. Balch at Scripps to characterize Aha1 targeting drugs for the treatment of prostate cancer (see section F below). While no single drug is likely to represent a "magic bullet", preliminary findings suggest that our strategy will lead to more potent and effective drugs that are likely to show efficacy towards the treatment of CRPC, either alone or in combination with existing therapeutics, thereby addressing a major unmet need in the fight against prostate cancer. Thus, we are confident that, in the long-term, our work will directly and/or indirectly add new weapons to the clinicians' arsenal of treatments that will provide greater options for the design of individualized and/or combination therapies, and significantly contribute to the reduction of death from prostate cancer.

University of Texas at El Paso Site (Cox, PI): See above

Vancouver Prostate Centre Site (Cherkasov, PI): See above

Clark Atlanta University Site (Chaudhary, PI): See above

D.2 Impact on Other Disciplines

Refer to section D.1 for context. We believe our work, in the long-term, will help inform the targeting of Hsp90-associated cochaperones in a wide variety of disease settings in addition to prostate cancer. These could include breast cancer, obesity, chronic pain management, and neurodegenerative diseases to name a few.

University of Texas at El Paso Site (Cox, PI): See above
Vancouver Prostate Centre Site (Cherkasov, PI): See above
Clark Atlanta University Site (Chaudhary, PI): See above

D.3 Impact on Technology Transfer

University of Texas at El Paso Site (Cox, PI):

We discussed the sponsored research agreements with *Maia Biotechnology Inc.* in the Y2 report and provided updates in Y3. We are continuing to work under these agreements to try and move analogues of MJC13 and GMC1 towards IND. *Maia Biotechnology Inc.* is currently working to issue an IPO this coming year so work is currently on hold as they work on this. It is anticipated that a successful IPO will provide them with the necessary capital to push their FKBP52 targeting program forward. As mentioned above, the provisional patent covering PC257 and derivatives was filed in January, 2020 and is in full effect (Claims Priority to U.S. Provisional Patent Application No. 62/963,873, filed January 21, 2020). In addition, this provisional was converted to a full patent application in January, 2021. It is also important to note that this IP is currently independent of the agreements with *Maia Biotechnology Inc*. We are currently negotiating with *Maia Biotechnology Inc.* to secure licensing on PC257 as well.

Vancouver Prostate Centre Site (Cherkasov, PI):

Dr. Cherkasov (Partnering PI) is Co-Inventor on the PC257 provisional and full patent applications and an inter-institutional sharing agreement is in place.

Clark Atlanta University Site (Chaudhary, PI): Nothing to report

D.4 Impact on Society Beyond Science and Technology

Given the nature of the drug development and commercialization process, including the bench-to-bedside timeframe, significant future economic impacts associated with these technologies must first be discussed in relation to long-term benefits and impacts. Novel technologies and new goods are at the heart of economic progress and the major long-term economic benefits of novel drug technologies include increased longevity of patients, reduced limitations on patient activities including working, and reduced medical expenditures. Given the novel therapeutic strategies we are developing, and the impressive effects observed in preclinical animal studies we strongly believe that these technologies will ultimately have a major impact in all of these areas. In addition, these impacts will be both direct and indirect given that our novel approaches will not only be used directly to benefit patients, but also will, and already are influencing the direction of prostate cancer drug development worldwide leading to additional novel technologies. Although the realization of these major impacts are in the future, there are also more immediate, short-term economic impacts associated with these technologies. These novel drug technologies and the development towards commercialization attract funding which benefits our respective universities and our regional economies through increased research expenditures and the creation of jobs. As these technologies move further through the development process, we anticipate future R&D funding to increase exponentially. Finally, the future impacts that high quality education and training of students have on the economy cannot be overlooked. Many students and post-doctoral fellows, some of whom are underrepresented minorities in science, have been trained in association with the research projects leading to the development of these technologies. Many of these trainees will go on to make their own contributions to society in the future.

University of Texas at El Paso Site (Cox, PI): See above
Vancouver Prostate Centre Site (Cherkasov, PI): See above
Clark Atlanta University Site (Chaudhary, PI): See above

E. CHANGES/PROBLEMS

E.1 Changes in Approach and Reasons for Change

As reported in Y3, the COVID-19 pandemic disrupted research activities due to the closure of the University of Texas at El Paso, the Vancouver Prostate Centre, and Clark Atlanta University from March through the end of June in response to the COVID-19 pandemic. The closure of the research facilities at all three institutions forced us to stop all our ongoing research. In addition, phased-in research resumption guidelines did not enable any work on this project through the end of August 2020. Thus, we applied for a no cost extension for another year and as detailed above. While all three PIs restarted their labs in the Fall of 2020, work remained slow for some time, especially work involving vertebrate animals. Given the challenges with facility access amidst the testing and vaccination efforts, these were some of the last facilities to get back up and running fully. This significantly delayed animal studies and we instead shifted to focus on the characterization of PC257 including assessing drug binding to FKBP52. Despite these challenges we were able to generate at least some preliminary animal data that is sufficient to attract commercial interest.

University of Texas at El Paso Site (Cox, PI): See above
Vancouver Prostate Centre Site (Cherkasov, PI): See above
Clark Atlanta University Site (Chaudhary, PI): See above

E.2 Changes that Had a Significant Impact on Expenditures

As a result of the COVID-19 pandemic and shutdown, all three PIs had few expenditures between the months of March through July. Any expenditures recorded during this time were minor expenditures required to maintain the labs (e.g. liquid nitrogen to maintain cell storage). While the labs were shut down the lab personnel continued on their grant supported salaries and remained engaged remotely by working on draft manuscripts and making

progress on the written dissertations in the case of the students. In the no cost extension period, all three PIs had to work to identify institutional sources of salary support for the personnel required to complete the project. Dr. Cox had some academic salary funds remaining that he be used to support one or two graduate students in the Fall and/or Spring semesters to work on these projects. Dr. Chaudhary could not support his post-doctoral fellow beyond Dec. 2020 which significantly impacted the proposed animal studies.

University of Texas at El Paso Site (Cox, PI): See Above
Vancouver Prostate Centre Site (Cherkasov, PI): See Above
Clark Atlanta University Site (Chaudhary, PI): See Above

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

University of Texas at El Paso Site (Cox, PI): Nothing to report
Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to report
Clark Atlanta University Site (Chaudhary, PI): Nothing to report

F. PRODUCTS

University of Texas at El Paso Site (Cox, PI):

The following publications reference support from this award:

Research Articles

Ekpenyong, O., Cooper, C., Ma, J., Guy, N.C., Payan, A.N., Ban, F., **Cherkasov, A.**, **Cox, M.B.**, Liang, D., Xie, H. (2020) Bioanalytical Assay Development and Validation for the Pharmacokinetic Study of GMC1, a Novel FKBP52 Co-chaperone Inhibitor for Castration Resistant Prostate Cancer. *Pharmaceuticals*. 13(11):386. PMCID: PMC7698315

Singh, J., Hutt, D.H., Tait, B., Guy, N.C., Sivils, J.C., Ortiz, N., Payan, A.N., Komaragiri, S.K., Owens, J.J., Culbertson, D., Blair, L.J., Dickey, C., Kuo, S.Y., Dyson, H.J., **Cox, M.B.**, **Chaudhary, J.**, Gestwicki, J.E., Balch, W.E. (2020) Management of Hsp90-dependent protein folding by small molecule targeting the Aha1 co-chaperone. *Cell Chemical Biology.* 27(3): 292-305.e6. PMCID: PMC7144688

Harris, D.C., Garcia, Y.A., Storer Samaniego, C., Rowlett, V.W., Ortiz, N.R., Payan, A.N., Maehigashi,T., and **Cox, M.B.** (2019) Functional comparison of human and zebra fish FKBP52 confirms the importance of the proline-rich loop in the regulation of steroid hormone receptor activity. *International Journal of Molecular Sciences*. **20**(21): pii: E5346. PMCID: PMC6862696

Review Articles

Mazaira, G.I., Zgajnar, N.R., Lotufo, C.M., Daneri-Becerra, C., Sivils, J.C., Soto, O.B., **Cox, M.B.**, and Galigniana, M.D. (2018) The Nuclear Receptor Field: A Historical Overview and Future Challenges. Nuclear Receptor Research. 5: Article ID 101320, 21 pages.

Book Chapters

Mazaira, G.I., Zgajnar, N.R., Lotufo, C.M., Daneri-Becerra, C., Sivils, J.C., Soto, O.B., **Cox, M.B.,** and Galigniana, M.D. (2019) Nuclear Receptors: A Historical Perspective. *Methods in Molecular Biology.* **1966**: 1-5.

Inventions and Patent Applications

Cox, Marc B., Ban, Fuqiang, and Cherkasov, A. "Next Generation FKBP52 Targeting Drugs for the Treatment of Prostate and Breast Cancer"; Claims Priority to U.S. Provisional Patent Application No. 62/963,873, filed January 21, 2020. Converted to non-provisional in January, 2021, US patent number 17/153,340.

Vancouver Prostate Centre Site (Cherkasov, PI): See collaborative publication in the list above.

Clark Atlanta University Site (Chaudhary, PI): See collaborative publication in the list above.

G. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

G.1 Individuals Who Have Worked on the Project

University of Texas at El Paso Site (Cox, PI):

Name:	Dr. Marc B. Cox
Project Role:	PI
Researcher Identifier:	https://orcid.org/0000-0001-7854-2676
Person months worked:	2.8
Contribution to Project:	Dr. Cox provided oversight of the project, provided guidance and consultation to Ashley Payan, and assisted with the analysis and interpretation of data.
Funding Support:	In addition to the academic salary and summer salary funded by this grant, The remainder of Dr. Cox's Salary is funded by the institution and other grants in Y1-4.

Name:	Ashley Payan
Project Role:	Graduate Student
Researcher Identifier:	N/A
Person months worked:	12
Contribution to Project:	Ashley conducted all experiments to identify and characterize PC257, and collected and analyzed data. This project served as Ashley's dissertation project, and she successfully defended her dissertation in May, 2021.
Funding Support:	This project only in Y1-4.

Name:	Nina Ortiz
Project Role:	Graduate Student
Researcher Identifier:	N/A
Person months worked:	3
Contribution to Project:	Nina helped develop the 52KO cell lines described above and helped complete the dataset described in Figure 5 while Ashley was writing her dissertation and preparing for her defense.
Funding Support:	This project only in Y4

Name:	Olga Soto
Project Role:	Graduate Student
Researcher Identifier:	N/A
Person months worked:	9
Contribution to Project:	Olgo provided overall support for continuance of the project while Ashley focused on graduating. This included prepping the bacterial expression plasmids used by the Cherkasov group in Y4 and finalizing some of the datasets in Figures 5 and 6 above.
Funding Support:	This project only in Y4

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) in Y1-4.

Name: Dr. Fuqiang Ban
Project Role: Post-doctoral Fellow
Researcher Identifier:
Person months worked: 2
Contribution to Project: Dr. Ban conducted and/or supervised Dr. Kriti and the Masters student in computational drug design, molecular modeling and bioinformatics studies to generate novel derivatives to be tested. He also assisted with the analysis and interpretation of data.
Funding Support: salary fully covered by other sources (no salaried paid from this grant) in Y1

Name: Dr. Kriti Singh
Project Role: Post-doctoral Fellow
Researcher Identifier:
Person months worked: 12
Contribution to Project: Dr. Kriti conducted computational drug design, molecular modeling and bioinformatics studies under the supervision of Dr. Cherkasov. She also initiated plans to clone, express, and purify FKBP52 protein to conduct compound binding studies.
Funding Support: This project only in Y1-3

Name: Godwin Woo
Project Role: Master's Student
Researcher Identifier:
Person months worked: 3
Contribution to Project: Mr. Woo worked alongside Dr. Kriti on computational drug design, molecular modeling and bioinformatics studies under the supervision of Dr. Cherkasov.
Funding Support: This project only in Y1-3

Name: Christophe Sanchez
Project Role: Co-Op
Researcher Identifier:
Person months worked: 1
Contribution to Project: Mr. Sanchez performed planning of FKBP52 expression, under the supervision of Dr. Singh.
Funding Support: This project only in Y2-3

Name: Michael Llamosa
Project Role: Post-doctoral Fellow
Researcher Identifier:
Person months worked: 5

Contribution to Project: Dr. Llamosa worked alongside Dr. Kriti on computational drug design, molecular modeling and bioinformatics studies under the supervision of Dr. Cherkasov.
Funding Support: This project only in Y2-3

Name: Eric LeBlanc
Project Role: Research Associate
Researcher Identifier:
Person months worked: 5
Contribution to Project: Dr. LeBlanc performed evaluation of compounds designed in Year 1, under the supervision of Dr. Cherkasov.
Funding Support: This project only in Y2

Clark Atlanta University Site (Chaudhary, PI):

Name: Dr. Jaideep Chaudharry
Project Role: PI
Researcher Identifier: https://orcid.org/0000-0002-4440-6585
Person months worked: 1
Contribution to Project: Dr. Chaudhary provided oversight of the project, provided guidance and consultation to Dr. Komaragiri, and assisted with the analysis and interpretation of data.
Funding Support: This project only in Y1-4

Name: Dr. Shravan Kumar Komaragiri
Project Role: Post-doctoral Fellow
Researcher Identifier: https://orcid.org/0000-0003-0889-9906
Person months worked: 12 (Dr. Kumar was supported only for 5 months (August 20-Dec. 2020) in the cost extension period
Contribution to Project: Dr. Kumar established experimental protocols (cell culture, immune-histochemistry etc.), collected and analyzed data and managed the supply chain.
Funding Support: This project only in Y1-4

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

University of Texas at El Paso Site (Cox, PI):

The following funding has been activated since negotiation and setup of this award:

RP210153
Cox (PI) 8/31/21-8/30/2026
Cancer Prevention and Research Institute of Texas
UTEP/UTMDACC Partnership for Hispanic Cancer Disparities Research
This grant aims to incentive junior faculty to pursue research relevant to our local population by funding junior faculty research projects, hiring new faculty with expertise in this area, and, in partnership with UTMDACC, providing mentoring and professional development opportunities to enhance their success. Ultimately, our goal is to build capacity in Hispanic cancer disparities research in the El Paso region.

3 linked awards (2RL5GM118969-06, 2TL4GM118971-06, and 2UL1GM118970-06)
Echegoyen (PI) 7/1/19-6/31/2024
NIH/NIGMS
Phase II of BUILDing SCHOLARS

This supports the second 5 year cycle of our BUILDing SCHOLARS undergraduate training program. I serve as PI on the Administrative core and as Deputy Director of the Program.

Cox (PI) 6/1/2019-2/31/2022
Lizanell and Colbert Coldwell Foundation
Proof-of-Concept Study of Surface-Directed AR Inhibitors for the Treatment of Prostate Cancer
This project supports the continued characterization of the mechanisms by which FKBP52 and beta-catenin regulate unique androgen-regulated, genome-wide transcriptional programs and defines how targeting these factors through targeting AR BF3 with MJC13 affects these unique transcriptional programs.

2U54MD007592-26
Kirken (PI) 4/1/2019-3/31/2024
NIH/NIMHHD
Border Biomedical Research Center
This supports the next 5 year cycle of our Research Centers in Minority Institutions (RCMI) center. I serve as PI of the Investigator Development Core of the Center.

Cox (PI) 1/1/2019-12/31/2019
Maia Biotechnology Inc.
This Sponsored Research Agreement (SRA) with Maia Biotechnology supports the optimization of our AR BF3 targeting drug, MJC13, to improve potency and solubility, and to support studies aimed at securing IND status.

Cox (PI) 11/15/2018-11/14/2019
Maia Biotechnology Inc.
This Sponsored Research Agreement (SRA) with Maia Biotechnology supports the structure activity relationship analysis of our first-in-class FKBP52 targeting drug, GMC1, by providing medicinal chemistry support.

1R13CA236020-01 Cox (PI) 11/1/2018-10/31/2019
NIH/NCI
This supported travel awards for trainees to attend the 2018 Annual Meeting of the Society for Basic Urologic Research (SBUR)

Cox (PI) 6/1/2018-5/31/2019
Lizanell and Colbert Coldwell Foundation
A Novel Approach to Treating Castration Resistant Prostate Cancer
The overall goal of this project is to further our understanding of the mechanisms by which FKBP52 and beta-catenin regulate unique androgen-regulated, genome-wide transcriptional programs and define how targeting this mechanism affects those transcriptional programs.

Vancouver Prostate Centre Site (Cherkasov, PI):

The following funding has been activated since negotiation and setup of this award:

Cherkasov 3/1/2021-2/28/2022
Canadian Institutes of Health Research (CIHR) CAD
COVID-19 Variant Network - Computer-aided discovery of synergistic drug combinations with remdesivir for COVID-19 through mechanism-based drug repurposing and combinatorial organoid screening.
A SARS-CoV-2 variant network will be established to rapidly characterize the biology, infectivity, growth characteristics, and antiviral drug susceptibly of emerging SARS-CoV-2 Variants of Concern (VOCs) in BC and across Canada. This information will be used to inform decision makers about potential increased transmissibility and resistance to potential therapeutics. The Network will address VOCs SARS-CoV-2 England (VOC202012/01), South

Africa [SARS-CoV-2 South Africa (501Y.V2)], and Nigeria [SARS-CoV-2 Nigeria (484)]. This work will form the foundation of future work evaluating the effectiveness of antiviral therapeutics and targets against emerging VOCs.

Cherkasov (PI) 3/1/2021-2/28/2022
Canadian Institutes of Health Research (CIHR) CAD
COVID-19 Variant Supplement - Computer-aided discovery of synergistic drug combinations with remdesivir for COVID-19 through mechanism-based drug repurposing and combinatorial organoid screening.
We will map known and emerging variants of SARS-CoV-2 Spike and 3CLpro proteins and apply Molecular Dynamics (MD) simulation to explore how these mutations affect the drug binding sites or the overall stability of the proteins. We will then employ the machine learning Deep Docking platform (DD) developed by Dr Cherkasov's team to screen 40 billion compounds predicted to bind with high affinity to these mutated target sites of the two SARS-CoV-2 proteins. We plan to refine our list of identified compounds using variety of docking programs with DD and implement more computationally extensive and accurate scoring functions that will be applied to further refine and narrow the list of potential ligands.

Cherkasov (PI) 8/1/2021-8/31/2022
Michael Smith Foundation for Health Research CAD
Artificial intelligence based discovery of estrogen receptor activation function 2 (AF2) inhibitors as the first-in-class therapies for drug resistant breast cancers.
We will employ the machine learning Deep Docking platform (DD) developed by Dr Cherkasov's team to screen 40 billion compounds predicted to bind with high affinity to the estrogen receptor activation function 2 (ER-AF2) binding domain. We plan to refine our list of identified compounds using variety of docking programs with DD and implement more computationally extensive and accurate scoring functions that will be applied to further refine and narrow the list of potential ligands.

Cherkasov (PI) 8/1/2020-8/31/2021
Canada's Digital Technology Supercluster CAD
Raven2: AI platform for novel drug discovery
Raven2 is a national effort with participants in both Quebec and British Columbia that unleashes the full power of Variational AI's generative artificial intelligence algorithm to generate novel and optimized compounds in months versus years against human coronavirus targets, specifically the SARS-CoV-2 3CLpro main protease, other potential human viruses (for future rapid pandemic response), as well as being generalizable to multiple diseases. Training data set (structures of compounds that bind to 3CLpro catalytic site) will be provided by Dr. Cherkasov's team deploying their machine learning Deep Docking platform (DD).

Cherkasov (PI) 4/1/2020-3/31/2026
Canadian Foundation for Innovation CAD
CFI Infrastructure Operating Fund
Operating fund for "Accelerated Drug Discovery Using Clinical Translation (ADDUCT) grant. Fund not CFI authorized until all equipment was installed and operational.

Cherkasov (PI) 3/1/2020-2/28/2022
Canadian Institutes of Health Research (CIHR) CAD
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
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
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.

Cherkasov (PI) 9/1/2018 – 8/31/2021
US Department of Defense USD
Design and evaluation of small molecules that target the dimerization interface of full-length and splice variant forms of the androgen receptor.
The overall goal of this project to evaluate that breaking or preventing human androgen receptor dimerization will bypass all drug-resistance mechanisms whereby antagonists such as enzalutamide are rendered ineffective by ligand binding domain (LBD mutants or when variants lacking the AR-LBD are expressed. Small drug inhibitors will be designed by computer assisted drug design (CADD) and evaluated via cell-based assays to inhibit full length and LBD-deleted androgen receptor mediated transcriptions of reporter molecules.

Cherkasov (PI) 4/1/2018-3/31/2021
Canadian Institutes of Health Research (CIHR) CAD
Design and evaluation of small molecules that target the dimerization interface of full-length and splice-variant forms of the androgen receptor as a potential treatment for advanced prostate cancer.
The goal of this project is to improve the potency and specificity of low molecular weight compounds to target the human androgen receptor dimerization interface using rational design.
We will employ biophysical approaches and cryogenic electron microscopy (cryo-EM) to investigate the molecular interaction between the AR and anti-dimer compounds.

Cherkasov (PI) 7/1/2018-30/6/2022
Canadian Foundation for Innovation CAD
Accelerated Drug Discovery Using Clinical Translation (ADDUCT).
ADDUCT expands upon existing CFI infrastructure grants to the VPC and to UBC's Advanced Structural Biology of Re-emerging Infectious Diseases (ASTRID) initiative, and also brings in expertise from the Centre of Drug Research and Development and the BC Cancer Agency, for targeted drug development to generate new drugs and treatment options for prostate, bladder and renal cancer patients. It will fund expansion in many areas of the bench-to-bedside pipeline, primarily focussed on targeted drug discovery and increasing the capacity for protein production, protein structural determination, and computer aided drug design.

Cherkasov (Co-PI) 4/1/2018-31/3/2021
Canadian Cancer Society CAD

Development of anti-estrogens with a novel mechanism of action for treatment of hormone resistant breast cancer.
From this study, we anticipate that our novel human estrogen receptor inhibitors will be further improved and will lead to new therapeutic strategies that can be used alternatively, complementarily, or synergistically with the current breast cancer treatments. The potential impact of the proposed research will be to create an entirely new class of drugs to treat breast cancer even in its most deadly, hormone-resistant forms. There is a great need for novel therapeutic strategies in breast cancer that can overcome tamoxifen resistance and improve patient survival.

Clark Atlanta University Site (Chaudhary, PI): Nothing to report

H. SPECIAL 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