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

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CONTRACTING ORGANIZATION:
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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.

15. SUBJECT TERMS

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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 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

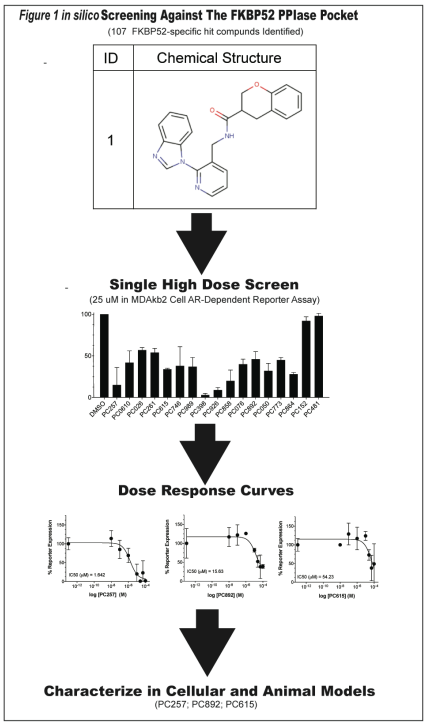
Major Task 6: Perform PK/PD on selected candidate compounds

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

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

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. Thus, we are currently working with *Maia Biotechnology Inc.* on the preclinical chemistry needed to improve metabolic stability. In addition to the GMC1 analogues, we also successfully identified a new chemotype, termed PC257, that can be pursued independently of GMC1 as a novel drug molecule targeting FKBP52 for the treatment of prostate cancer (**Fig. 1**). This molecule is independent of any agreements with Maia, and, 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 of our focus in Y3 has been to characterize PC257 for the treatment of prostate cancer. While the current COVID19 pandemic significantly affected our ability to progress on PC257, we were still able to make significant progress towards the stated goals in Y3. That progress is summarized below.



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

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)

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

Dr. Chaudhary (Partnering PI) was able to generate the mechanistic data demonstrating that GMC1 inhibits AR nuclear translocation (see below). This was the final data needed to publish the initial GMC1 manuscript. We currently have a full working draft of the manuscript and plan on submitting in September or October. In addition, we have a completed manuscript detailing the novel GMC1 formulation ready for submission as soon as the initial GMC1 manuscript is accepted. Finally we have a full draft of a manuscript detailing the broader *in silico* screen that led to the identification of PC257. We anticipate two manuscript resulting from the work on PC257 in the next year including one detailing the *in silico* screen as well as one detailing the characterization of PC257 in cellular and animal models.

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.

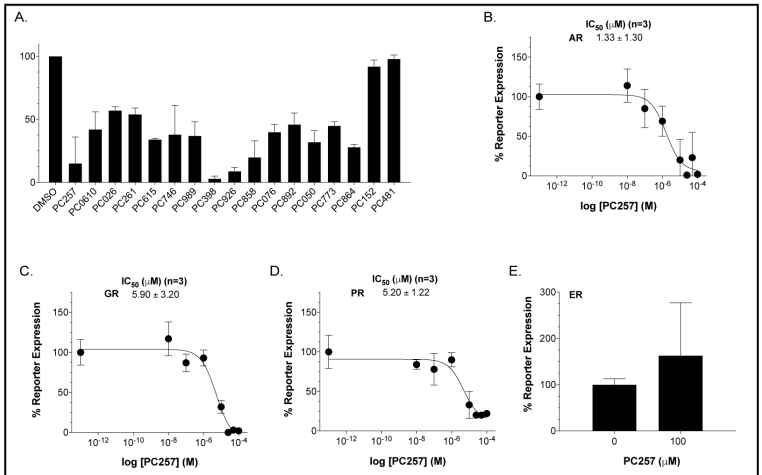


Figure 2: PC257 specifically inhibits AR, GR, and PR activity in hormone-dependant reporter assays. **A.** Novel chemotypes were assessed in AR-dependent reporter assays in MDA-kb2 cells at a single high dose. Those that inhibited by AR by 50% or more were then screened in full dose response assays. 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.

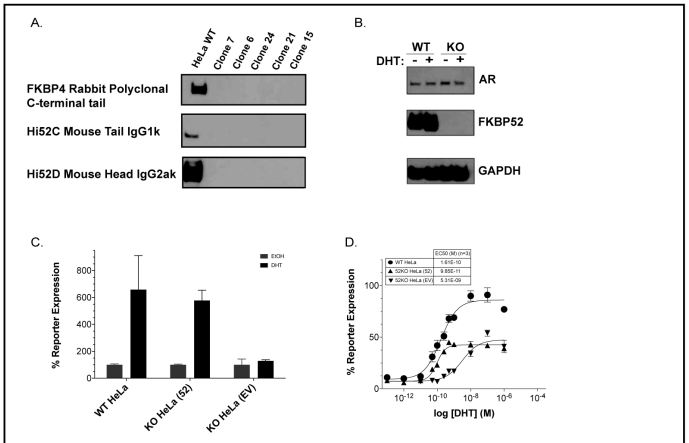


Figure 3: CRISPR/Cas9 FKBP52 KO human cell model. A. Western blot from 52KO HeLa cell clones using FKBP52 antibodies targeting a variety of epitopes on FKBP52. B. Clone 15 was selected and a western blot for AR and FKBP52 were performed in the presence and absence of hormone. C. AR-dependent reporter 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. We have now 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. 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**). Finally, PC257 was shown to abrogate endogenous DHT-dependent FKBP51 and/or PSA gene expression in 22Rv1 and LNCaP 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.

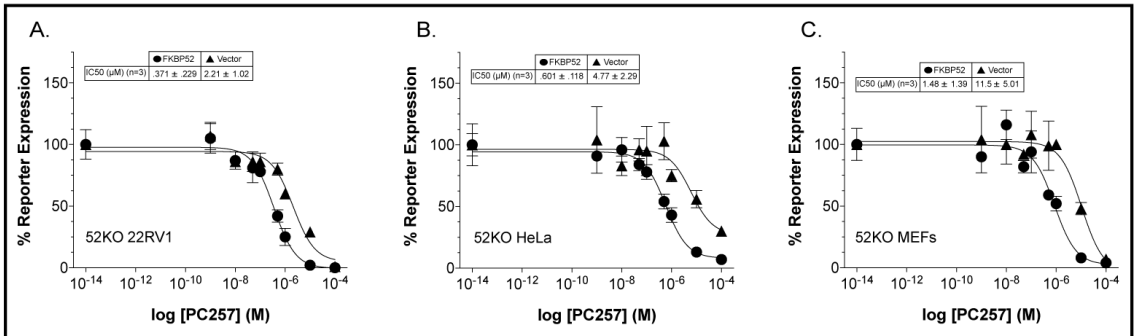


Figure 4: PC257 preferentially targets FKBP52-regulated AR activity. AR-mediated reporter assays in the presence of a range of PC257 concentrations with or without exogenous FKBP52 expression in 52KO 22Rv1 prostate cancer cells (**A**), 52KO HeLa cells (**B**), and 52KO MEFs (**C**).

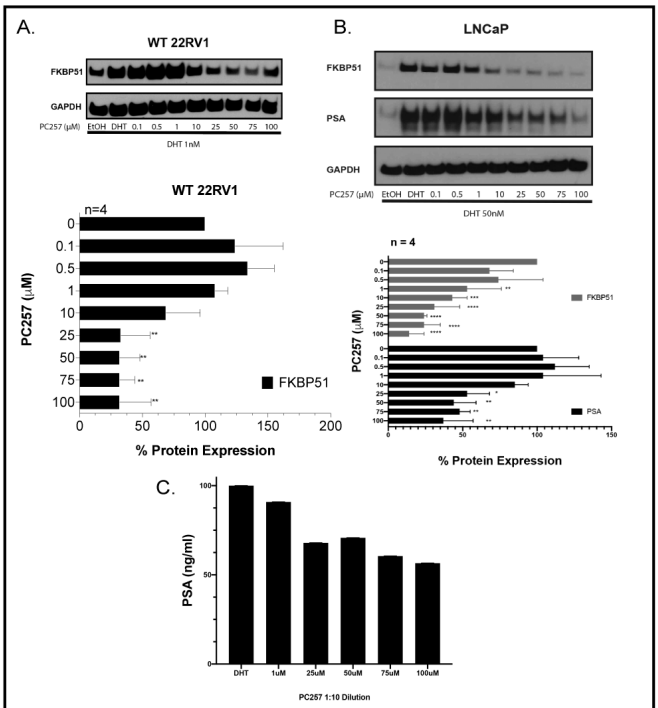


Figure 5: PC257 effectively inhibits endogenous AR-dependent gene expression in 22Rv1 prostate cancer cells. **A.** A range of PC257 concentrations were assessed for the ability to inhibit DHT-induced FKBP51 expression in 22Rv1 cells by western blot flollowed by quatitation by densitometry. **B.** A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

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

We have chosen 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. We are currently working on assessing the drug solubility and stability properties in preparation for formulation for optimal *in vivo* delivery. The current COVID19 pandemic situation delayed our progress towards completing formulation and initial animal evaluations as our respective labs were shutdown from March through June of this year. 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. We will be working in the no cost extension period to finalize the characterization of PC257 in cellular and animal models.

Vancouver Prostate Centre Site (Cherkasov, PI):

In the year 2 reporting period, 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.

Aim 1, Major Task 1: In Y3, we aimed to continue 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 aimed to define 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 current COVID-19 situation delayed this.

Aim 3, Major Task 5: Assess *in vitro* efficacy of lead molecules. We planned to genetically engineer, express, and purify human recombinant FKBP52 protein to subsequently conduct FKBP52/compound binding studies to determine dissociation constant (Kd). Unfortunately, the COVID-19 pandemic resulted in the curtailment of these activities for the last 4 months of Y3.

Clark Atlanta University Site (Chaudhary, PI):

Year 3 Report. The final report will be submitted 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)

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.

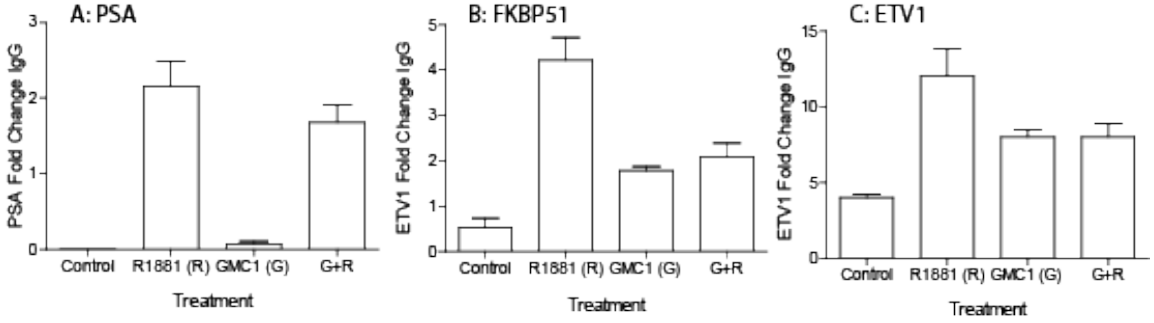


Fig. 6: Effect of GMC1 on the binding of androgen receptor on the respective Androgen Response Elements in the prompter of PSA (A), FKBP51 (B) and ETV1 (C). The data is mean±SEM of three separate experiments and is represented as fold change with IgG. Abbreviations: R: R1881, G: GMC1

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.

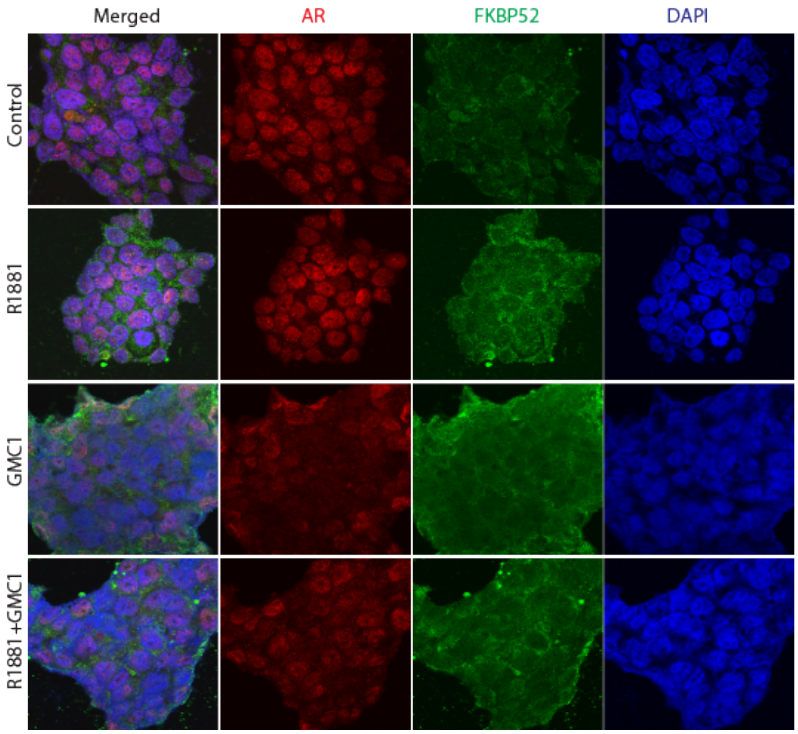


Fig. 7: Confocal immune-fluorescence analysis to confirm that GMC1 alters AR signaling and nuclear translocation in 22Rv1 cells. ICC we performed to study the AR (Red) and FKBP52 (Green) interaction inside the cell before and after treating 22Rv1 cells with R1881 (1nm) and GMC1 (30um). The merged panel 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.

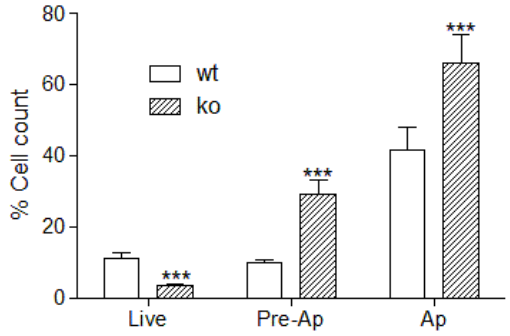


Fig. 8: Fraction of Live vs. apoptotic cells as determined by flow cytometry. The cells were stained with propidium iodide (live) and Annexin V (apoptotic marker). The data expressed as mean+SEM is from 3 different experiments. ***: p.0.001 – all data is compared within each group.

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 (ongoing).

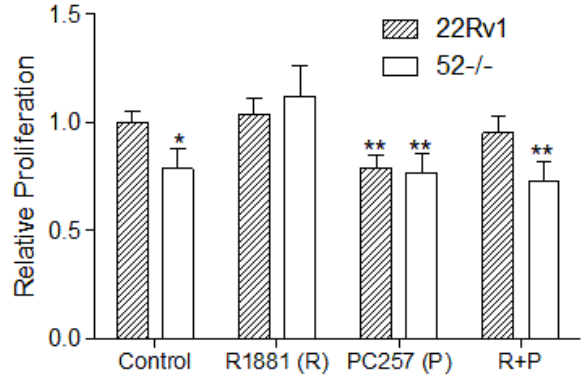


Fig. 9: Effect of PC257 on the proliferation 22Rv1 and rrRv1 cells lacking FKBP52 (52-/-) in the presence or R1881 (R) or PC257 (P) or both (R+P). The data, represented as mean±SEM of three different experiments in triplicate. The data is normalized to the proliferation of 22Rv1 cells without any treatment (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. We will continue our studies on PC257 in terms of its effect on migration and apoptosis.

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 of the xenografts and expanded studies could not be performed due to the closure of our university in response to COVID-19.

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 Texas at El Paso Site (Cox, PI):

As detailed above, we were able to make initial progress on completion of the analog screens and cellular characterization of PC257 before the shutdown including submission of the provisional patent. We anticipate converting the provisional to a full patent in January, 2021. In the no cost extension period we will also be working to finalize the cellular characterization of PC257 including assessing PC257 effects on endogenous PSA expression and secretion and proliferation in a variety of prostate cancer cell lines. We will also be working to complete the PC257 stability, solubility and formulation studies in preparation for Dr. Chaudhary to complete the initial animal evaluations by the end of the no cost extension period.

Vancouver Prostate Centre Site (Cherkasov, PI):

During the no-cost extension, we will genetically engineer, express, purify and quality control recombinant human FKBP52 protein for candidate compound (PC257) binding studies. We plan to employ biolayer interferometry, surface plasmon resonance, isothermal titration calorimetry, microscale thermophoresis for these studies.

Clark Atlanta University Site (Chaudhary, PI):

We will start the proposed animal studies to investigate the *in vivo* efficacy of GMC1 and PC257. In addition, we will develop toxicity profiles of PC257 *in vitro* before performing additional *in vitro* studies including MTT/ Annexin (PI), Matrigel transwell migration assay and anchorage-independent growth in a soft agar assay in a variety of androgen-sensitive and castration-resistant cell lines.

D. IMPACT

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

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.2 Impact on Other Disciplines

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.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. We are continuing to work under these agreements to try and move analogues of MJC13 and GMC1 towards IND. 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). It is anticipated that this provisional will be converted to a full patent application by January, 2021. It is also important to note that this IP is independent of the agreements with *Maia Biotechnology Inc*. and will be pursued independently of those agreements.

Vancouver Prostate Centre Site (Cherkasov, PI):

Dr. Cherkasov (Partnering PI) is Co-Inventor on the PC257 provisional patent 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

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

E. CHANGES/PROBLEMS

E.1 Changes in Approach and Reasons for Change

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 July. We have applied for and been approved for a no cost extension for another year and as detailed in section C.5, all three PIs have begun to restart their labs and have plans to complete all Y3 studies in the extension period.

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 will be working to identify institutional sources of salary support for the personnel required to complete the project. Dr. Cox had some academic salary funds remaining that will be used to support one or two graduate students in the Fall and/or Spring semesters.

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

Singh, J., Tait, B., Hutt, D.H., Brown, S., Guy, N.C., Sivils, J.C., Dickey, C., Chadli, A., Finley, D., **Cox, M.B.**, Dyson, J., Gestwicki, J., Balch, W.E. Management of Hsp90-dependent protein folding by small molecule targeting the Aha1 co-chaperone. *Cell Chemical Biology.* (in press).

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.

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.

Vancouver Prostate Centre Site (Cherkasov, PI): Nothing to Report
Clark Atlanta University Site (Chaudhary, PI): Nothing to report

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: This project only

Name: Ashley Payan
Project Role: Graduate Student
Researcher Identifier: N/A
Person months worked: 12
Contribution to Project: Ashley conducted all experiments (reporter assays for screening derivatives), and collected and analyzed data.
Funding Support: This project only

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. 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

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

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

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

Name: Dr. Shravan Kumar Komaragiri
Project Role: Post-doctoral Fellow
Researcher Identifier: https://orcid.org/0000-0003-0889-9906
Person months worked: 12
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

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:

Cox (PI) 6/1/2018-5/31/2019
Lizanell and Colbert Coldwell Foundation $70,000
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.

1R13CA236020-01 Cox (PI) 11/1/2018-10/31/2019
NIH/NCI $5,000
This supported travel awards for trainees to attend the 2018 Annual Meeting of the Society for Basic Urologic Research (SBUR)

Cox (PI) 11/15/2018-11/14/2019
Maia Biotechnology Inc. $46,000
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.

Cox (PI) 1/1/2019-12/31/2019

Maia Biotechnology Inc. $46,000
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.

2U54MD007592-26
Kirken (PI) 4/1/2019-3/31/2024
NIH/NIMHHD $19,198,789
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) 6/1/2019-5/31/2021
Lizanell and Colbert Coldwell Foundation $170,000
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.

3 linked awards (2RL5GM118969-06, 2TL4GM118971-06, and 2UL1GM118970-06)
Echegoyen (PI) 7/1/19-6/31/2024
NIH/NIGMS $15,200,000
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.

Vancouver Prostate Centre Site (Cherkasov, PI):

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

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.

Cherkasov (PI) 9/1/2018 – 8/31/2021
US Department of Defense USD $464,659
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 $450,000
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 $9,000,000
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 $1,000,000
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