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| <b>14. ABSTRACT</b><br>Despite significant advances in the treatment, prostate cancer (PCa), remains a leading cause of cancer death among men. Androgen deprivation therapy (ADT) constitutes the main therapeutic option for patients with advanced PCa. However, the major cause of death in men with metastatic prostate cancer involves progression to castration-resistant prostate cancer (CRPC). Characterizing mechanisms of resistance to ADT could enable the development of more effective therapeutic strategies. We performed a systematic genome-wide suppressor RNAi screen in the androgen-sensitive LNCaP cells, and identified genes whose silencing drives resistance to ADT in androgen-sensitive LNCaP cells, using nextgen sequencing. Performing cell viability assays, we validated the ability of shRNAs against ~20 top-tier genes to increase proliferation under androgen-deprived conditions, and also tested their effect on cell proliferation in the presence of the direct AR antagonist MDV3100. This study has identified several genes whose silencing may modulate resistance to ADT in prostate cancer. |                                        |                                         |                                                       |                                                          |                                                   |
| <b>15. SUBJECT TERMS</b> none listed                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                        |                                         |                                                       |                                                          |                                                   |
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## 1. INTRODUCTION

Prostate cancer is the second most common cause of cancer death among men in the United States. Prostate cancer malignant cells require androgen receptor (AR) signaling and the presence of androgens for their growth and survival [1]. Therefore, androgen deprivation therapy (ADT, castration) has been implemented as a first-line therapy for patients with metastatic disease [2]. Unfortunately, however, although almost all men with advanced prostate cancer initially respond to castration, the major cause of death in men with metastatic prostate cancer involves progression to castration-resistant prostate cancer (CRPC) [3].

Mechanisms underlying resistance to castration-based therapies in PCa have been intensely studied over the past years [3]. It is well established that the AR pathway plays a central role in the progression of PCa [4]. Indeed, CRPC cells maintain active AR signaling, despite castrated androgen levels [5, 6].

Recent therapeutic advances and clinical benefits for CRPC have been achieved by treatment with abiraterone, which inhibits the enzyme (CYP17) that catalyzes the formation of testosterone precursors [7, 8], or with antiandrogens, such as the second-generation AR antagonists MDV3100, approved for the treatment of CRPC in august 2012 [9, 10]. Other antiandrogens are in late-stage development, such as ARN-509, an anti-androgen with similar *in vitro* activity to MDV3100 but with greater *in vivo* activity in CRPC xenograft models [10, 11].

However, resistance/relapse of castration-resistant prostate cancer inevitably occurs even after treatment with these agents, and CRPC remains a formidable medical challenge.

## 2. KEYWORDS

|         |   |                                                          |
|---------|---|----------------------------------------------------------|
| ADT     | – | Androgen Deprivation Therapy                             |
| PCa     | – | Prostate Cancer                                          |
| AR      | – | Androgen Receptor                                        |
| CRPC    | – | Castration Resistant Prostate Cancer                     |
| CSS     | – | Charcoal-Stripped Serum                                  |
| FBS     | – | Fetal Bovine Serum                                       |
| GFP     | – | Green Fluorescent Protein                                |
| LNCaP   | – | Lymph Node Carcinoma of the Prostate                     |
| MDV3100 | – | Enzalutamide                                             |
| MOI     | – | Multiplicity Of Infection                                |
| mRNA    | – | Messenger RNA                                            |
| PSA     | – | Prostate-Specific Antigen                                |
| qRT-PCR | – | Quantitative Real-Time PCR                               |
| R1881   | – | Methyltrienolone                                         |
| RIGER   | – | RNAi gene enrichment ranking                             |
| RNAi    | – | RNA interfering                                          |
| RPMI    | – | Roswell Park Memorial Institute (culture <b>medium</b> ) |
| shRNA   | – | short hairpin RNA                                        |

## 3. OVERALL PROJECT SUMMARY

Because our mechanistic understanding of prostate cancer resistance to castration-based therapies still remains highly incomplete, the overarching goal of this project is to apply systematic loss-of-function

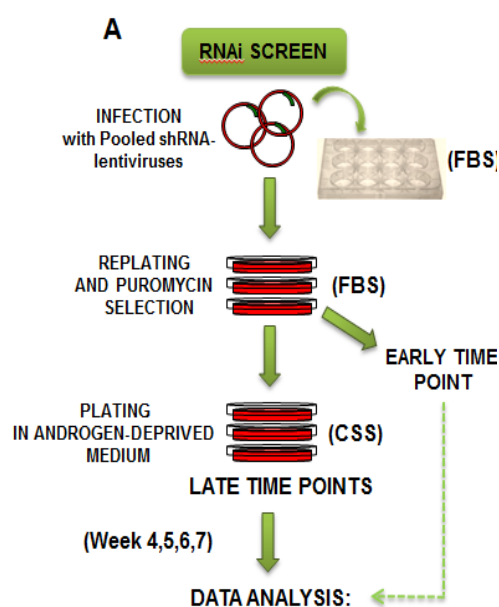
screens to discover genes whose altered expression mediates resistance to ADT. Using an androgen-dependent prostate cancer cell model (LNCaP), we performed a genome-scale smallhairpin RNA (shRNA) screens, to identify genes whose silencing (loss-of-function) allow these cells to proliferate in the absence of androgen. Applying computational approaches, we identified several candidate genes, among which phosphatase INPP5A was the top hit. Silencing of this gene results in LNCaP cells proliferation in the absence of androgens. Interestingly, while INP55A silencing results in re-activation of the androgen receptor signaling in these cells, we found that the AR inhibitor enzalutamide did not completely prevent the growth of LNCaP cells lacking INPP5A in androgen deprived conditions, thus suggesting a combined AR-dependent and AR-independent mechanism of resistance.

#### 4. KEY RESEARCH ACCOMPLISHMENTS

As stated in the approved SOW, our goals were:

**SPECIFIC AIM 1. To perform a systematic RNAi screen to identify loss of function mechanisms of resistance to androgen deprivation therapy.**

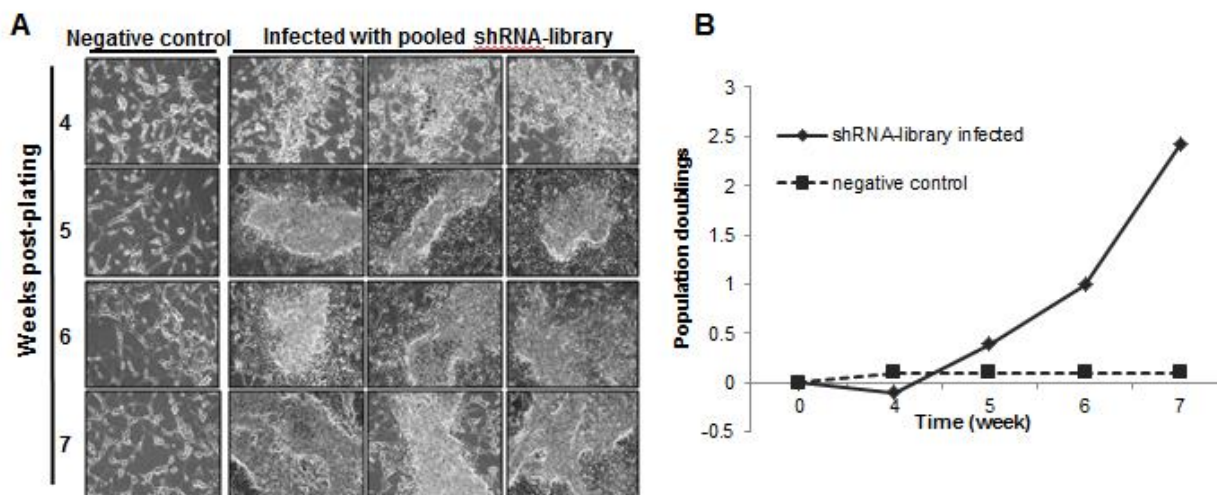
**Major task 1: Perform a systematic RNAi suppressor screen.** To identify genes whose silencing confer resistance to androgen deprivation *in vitro*, we performed a pooled genome-scale RNAi suppressor screen using the androgen-dependent LNCaP cell line cultured in charcoal-stripped serum (CSS), as schematized in **Figure 1**. After optimizing the screening conditions as shown in the approved award (preliminary data, 3), LNCaP cells cultured in FBS-RPMI were plated in eight 12-well plates ( $3 \times 10^6$  cells/well) (2 independent plates per time point; 4 late time points total). Cells were spin-infected with the pooled genome-scale shRNA library including 98,000 shRNAs targeting >16,000 genes, at 0.4 MOI in presence of polybrene (8  $\mu$ g/ml). Twentyfour hours after infection, cells from each 12-well plate were pooled and plated in 100 mm plates in FBS-RPMI. Forty-eight hours later, puromycin (1  $\mu$ g/ml) was added to select for infected cells. Once



selected (4 days), cells were pooled and resuspended in RPMI medium without serum. Half of the suspension was harvested and cell pellets frozen to determine the levels of shRNAs at the EARLY time point (**Fig. 1**). The remaining cells were replated and cultured in 5% charcoal-stripped (CSS)-RPMI medium. Cells were passaged as required and CSS-RPMI medium was replaced twice a week. The screen procedure described above was repeated in two biological replicates. Outgrowth of resistant cells was assessed by monitoring the emergence of colonies, indicative of acquired ability of individual clones to proliferate in androgen-deprived medium. Emergence of colonies was clear at 4-weeks post-plating (**Fig. 2A**), though they were already evident at 2-weeks (data not shown). Cells were harvested at EARLY time point (zero), and at LATE time points (week 1-7), and counted. Cell population doublings were calculated over the seven weeks. After 4 weeks, cells infected with

**Figure 1. Systematic RNAi suppressor screen in androgen-sensitive LNCaP cells.**

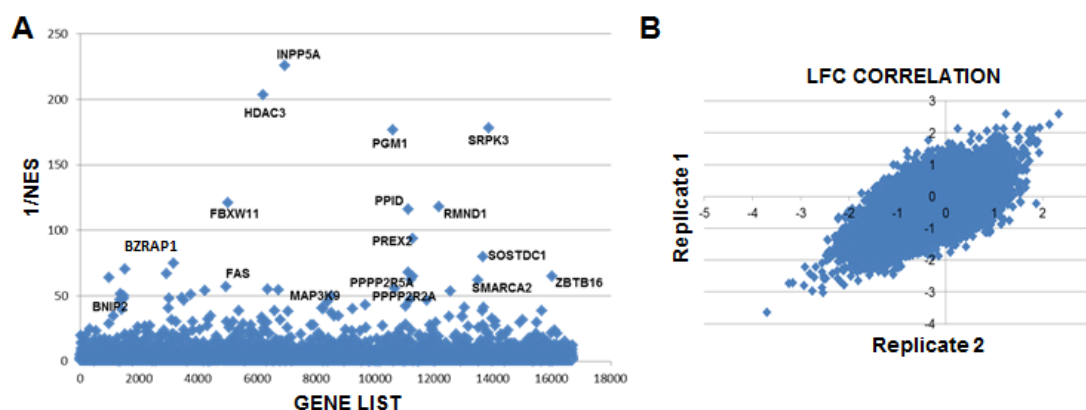
the lentiviral library showed the ability to proliferate in androgen-deprived condition (**Fig. 2B**). Cells were



**Figure 2. RNAi suppressor screen data analysis identifies top-tier genes driving resistance to androgen deprivation.**

(A). Emergence of resistant colonies in LNCaP cells infected with the pool library and not in LNCaP cells infected with control-shRNAs. (B). Population doublings of shRNA-library infected compared to control-infected LNCaP cells over seven weeks in CSS.

harvested at 4, 5, 6 and 7 weeks after plating, and genomic DNA was isolated from all harvested cells, including the EARLY time point harvested immediately following infection. The lentiviral vector component was isolated by PCR, barcoded Illumina sequencing libraries were generated, and the amplified lentiviral DNA was pooled and characterized by massively parallel sequencing using standardized Illumina HiSeq protocols. Using RNAi Gene Enrichment Ranking (RIGER) analysis [12, 13], the fold gain or loss for each shRNA was calculated relative to the EARLY time point, a rank of top-tier genes for which at least 2 shRNAs/gene were enriched >4 fold was established (second best shRNA). Normalized Enriched Score (NES) was calculated for each gene to show this level of enrichment [12]. Results of this analysis are shown in **Figure 3A**, where the inverse of normalized enriched score is depicted. Log fold Change (LFC) correlation between the two replicates of the experiments is shown in **Figure 3B**. The two experimental replicates showed a significant correlation.

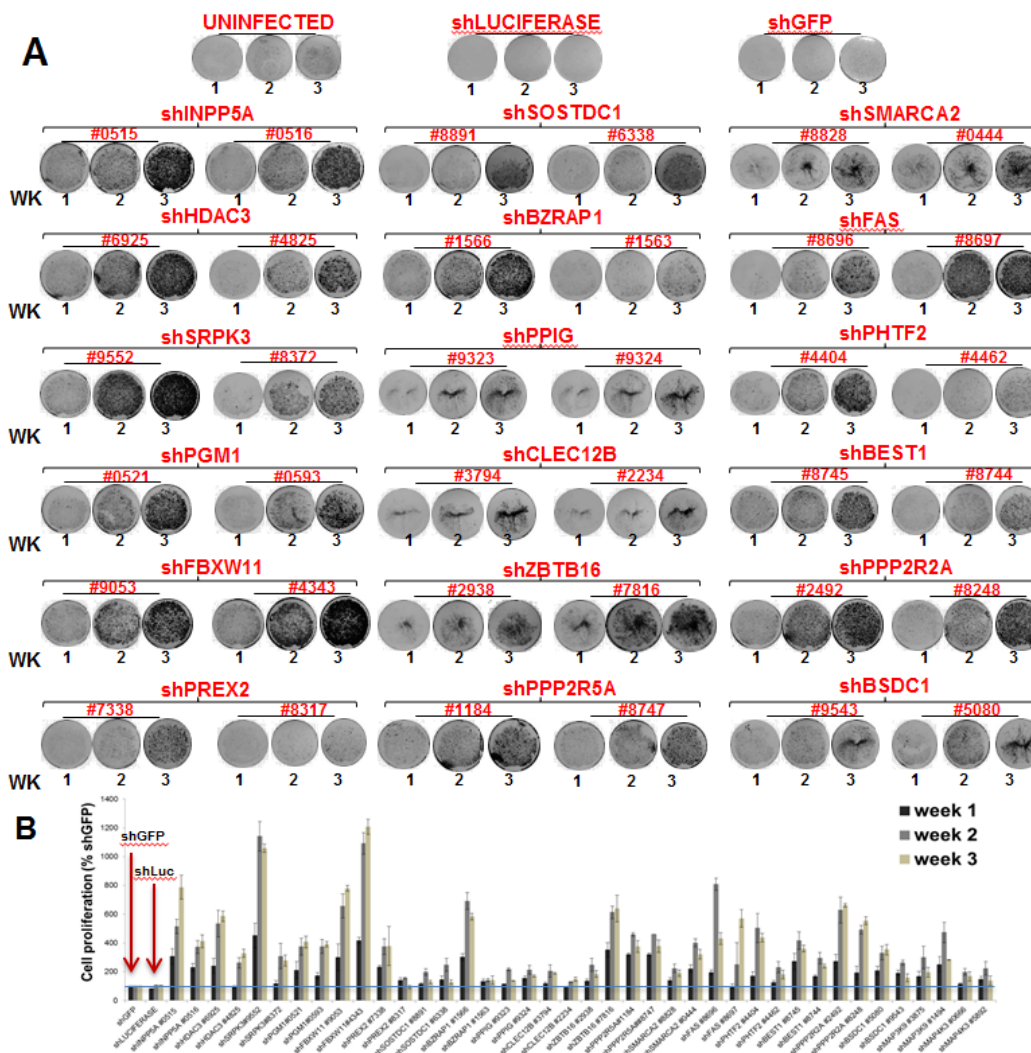


**Figure 3. Top-tier genes identified in the genome-scale shRNA screen with LNCaP cells cultured in androgen-deprived medium.** (A). Top hits identified by Second best hairpin ranking (RIGER). (B). Results from screen duplicates showed high correlation.

## Major Task 2: Validation of candidate genes *in vitro*.

In the aim 1b of the approved award, we stated that we would validate the top screen hits by quantifying the growth of cells in CSS-RPMI medium using viability assays (Cell Titer Glo) performed in 96-well format. However, the top screen hits did not show a growth as robust as the one achieved in the primary screen, when infected in 96-well format and culture in CSS-RPMI medium. This was probably due to the short timing of this experiment (one week). To address this issue, LNCaP cells were infected in 10 cm plates, and clones stably expressing 2 shRNAs against each of the 20 top hits were generated, as well as shGFP and shLuciferase controls. After selection in puromycin (1.5 µg/ml), cells were counted and plated in 12-well plates CSS-RPMI medium. One, two and three weeks after plating, cells were stained with crystal violet. **Figure 4A** shows the crystal violet staining, indicative of cell proliferation achieved 1, 2 and 3 weeks after plating. Knock-down of most top hits did result in increase of proliferation in androgen-deprived conditions. To quantify proliferation levels relative to shGFP-expressing cells, crystal violet of week 1-2-3 was dissolved in 10% acetic acid and viability assessed by measuring absorbance at 595 nm. (**Fig. 4B**).

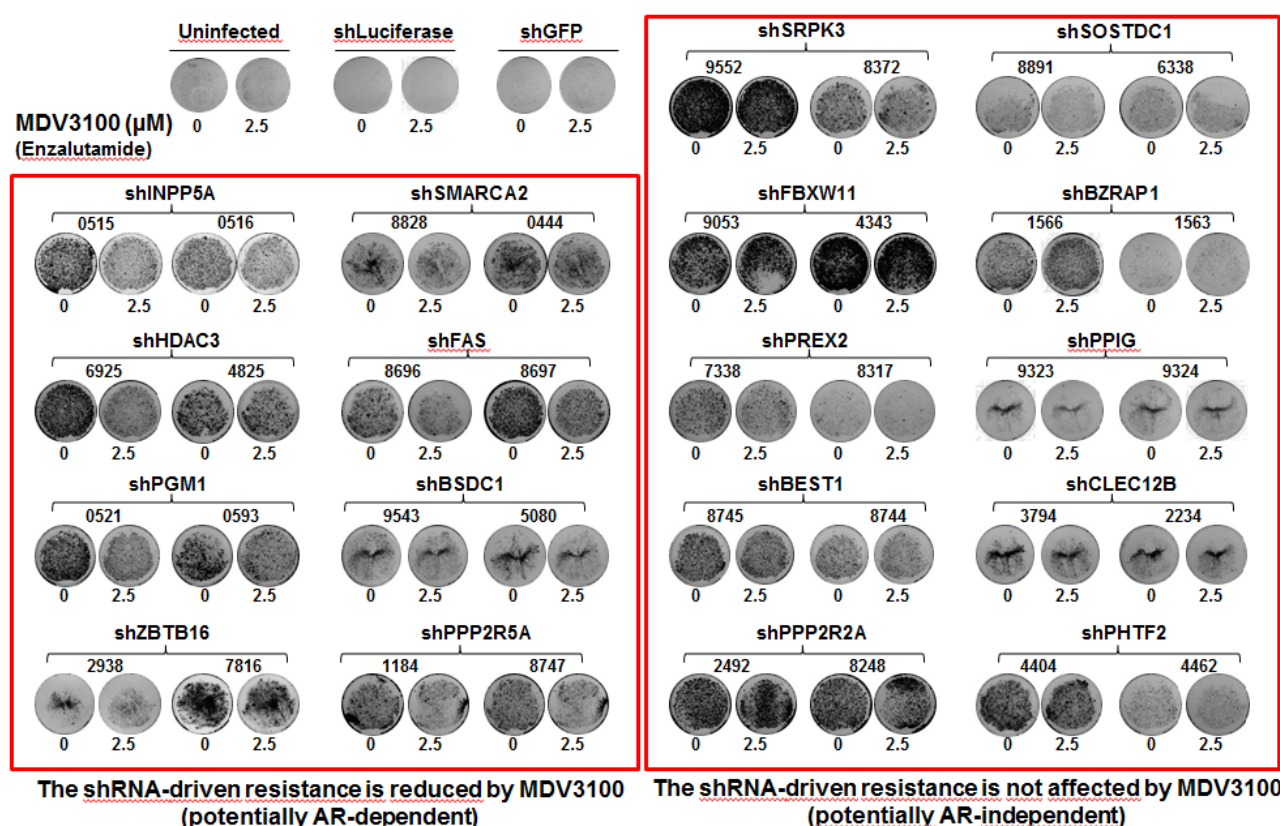
In the original award, we stated that we would also determine the effect of the top candidate shRNAs in cells cultured in FBS, in order to exclude from our analysis shRNAs that promote cell proliferation even in presence of androgens. However, using the 12-well plate format, cells reached 100% confluency too rapidly, without the possibility to determine any difference among the clones. Although this stated goal has not been met, we determined the growth in FBS of the top hit that we chose as focus of our research, calculating long-term population doublings in either FBS- and



**Figure 4. Validation of candidate genes *in vitro*.** (A) Crystal violet colony assays performed with LNCaP cells stably expressing shRNAs against the genes indicated over the course of 3 weeks. (B) Cell proliferation results of validation experiments calculated relative to the control shGFP by measuring absorbance of dissolved crystal violet at 595 nm.

CSS-RPMI medium (showed later in the text).

Validated shRNAs were also tested for their ability to confer resistance to treatment with the AR antagonist MDV3100. To this aim, we performed colony formation assays of stable clones cultured in CSS-RPMI media, in the presence of enzalutamide (MDV3100, 2.5  $\mu$ M), an inhibitor of AR nuclear translocation, for 3 weeks. MDV3100 concentration was determined following drug optimization experiments (data not shown). As can be seen in **Figure 5**, while the shRNA-driven resistance of some genes was greatly reduced upon AR inactivation (**Fig. 5 left panel**), knock-down of several genes still allowed growth of LNCaP cells in CSS in the presence of enzalutamide (**Fig. 5 right panel**), thus suggesting a potential mechanism of resistance independent of AR-signaling. These experiments have revealed a series of potential candidates whose silencing may drive proliferation in an AR-independent manner. These findings will give us novel opportunities to acquire a more comprehensive understanding of the molecular basis of castration resistant prostate cancer, since, to date, most of the mechanisms known to drive resistance to castration result in re-activation of the AR pathway.



**Figure 5. Sensitivity of validated shRNAs to the AR antagonist MDV3100.** Crystal violet colony assays performed with LNCaP cells stably expressing shRNAs against the genes indicated, cultured in androgen-deprived medium in the presence or absence of enzalutamide, over 3 weeks.

### Major Task 3: Validation of candidate genes *in vivo*.

Validation of candidate genes *in vivo* has not been completed yet. However, the castration procedure has been added and approved in the animal protocol of Dr. Garraway's (mentor) laboratory, and optimization experiments aiming to learn the procedure have just started.

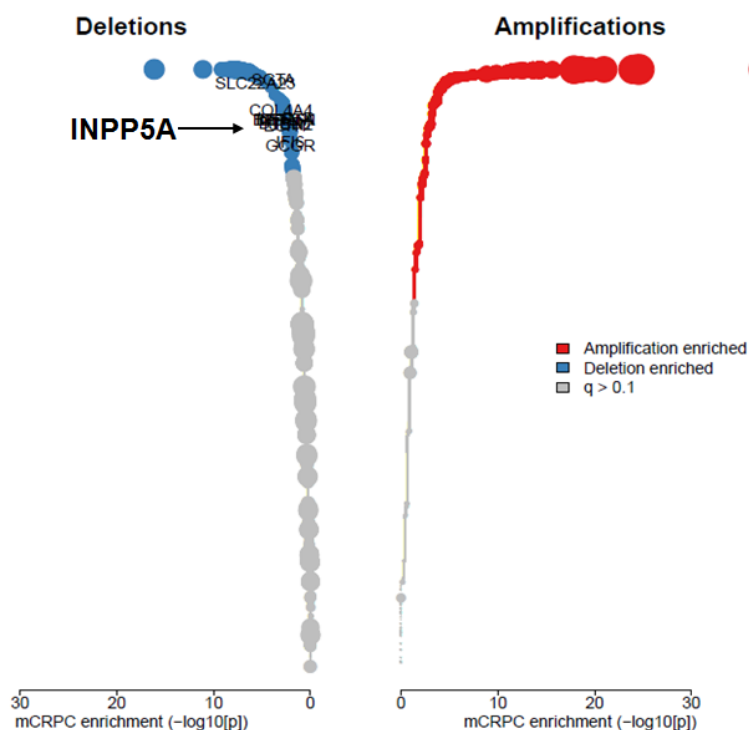
#### Major Task 4: Analysis of gene expression of the validated resistance genes in human tumors.

To explore the gene expression of validated genes identified from the loss-of-function screen in human PCa, we performed whole exome sequencing on biopsies deriving from castration-resistant prostate tumors (mCRPC n=104, bone/ lymph node/ soft tissue metastasis), and assessed the copy number of the top 30 screen hits. Indeed, Dr. Levi Garraway has access to a tissue collection of patients from DFCI and BWH, together with samples derived from the Stand Up 2 Cancer Prostate Cancer Dream Team (SUC2), led by Drs. Arul Chinnayian and Charles Sawyers. The tissue collection was accompanied with a patient demographics and information about previous therapy. The whole exome sequencing was performed at the Broad Institute. As shown in **Figure 6**, a number of top 30 screen hits were significantly deleted in human

mCRPC, while no screen hit was found significantly amplified.

These results suggested that some of the genes identified in our *in vitro* screen might play a role resistance to castration in human tumors, and identified candidate pathways underlying *de novo* or acquired resistance to ADT.

In future studies, we will assess the “druggability” of regulators and/or downstream effectors of the identified candidates *in vitro* and in *in vivo* models.



**Figure 6. Analysis of copy number variation of the top 30 screen hits in metastatic CRPC.**

#### MILESTONE ACHIEVED:

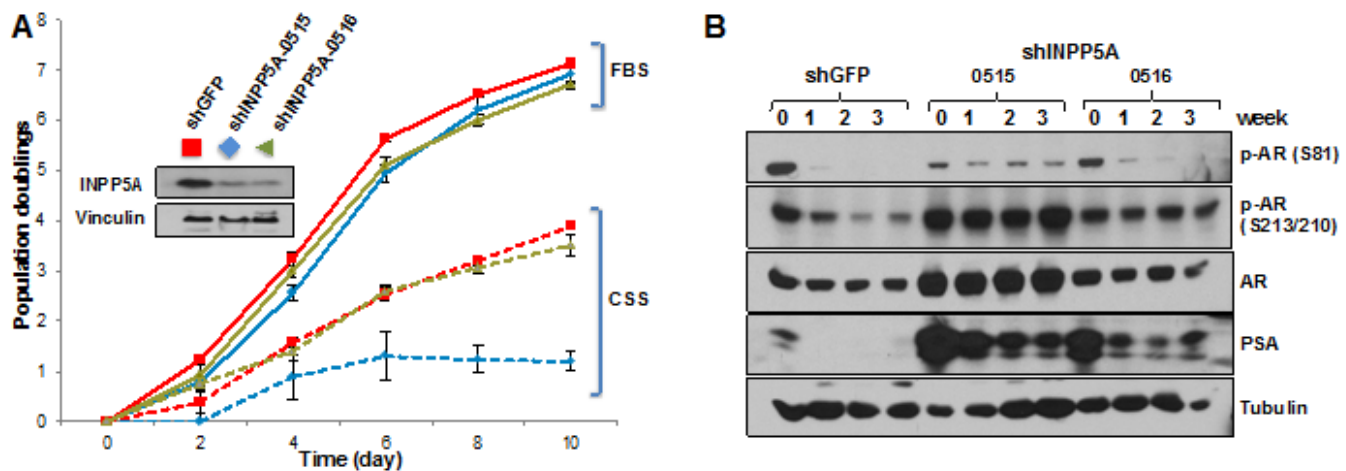
1. Identification of a panel of candidate genes whose silencing exerts the most robust effect on the proliferation of LNCaP cells upon androgen deprivation.
2. Validation of “hits” obtained from the screen in LNCaP cell lines.
3. Analysis of copy number alterations of candidate genes in human metastatic PCa tumors, and identification of validated candidate genes that are deleted in the clinic.

**SPECIFIC AIM 2. To determine whether shRNAs identified by suppressor screen drive castration resistance through AR-dependent or -independent mechanisms.**

**Major task 1: Analysis of AR pathway activation in cells stably-expressing validated shRNAs**

The top and strongest hit of our shRNA screen was the membrane-associated type I inositol-1,4,5-trisphosphate (InsP3) 5-phosphatase. Because the analysis of copy number variation performed on metastatic CRPC revealed INPP5A as a gene also significantly deleted in human tumors (**Fig. 6**), we decided to focus our studies on follow-up of this gene. INPP5A hydrolyzes Ins(1,4,5)P3 and Ins(1,3,4,5)P4, involved in release of ER and extracellular Ca<sup>2+</sup>, which acts as a signaling second messenger [14, 15]. Loss of INPP5A, therefore, results in an increase of cytoplasmic Ca<sup>2+</sup>. Several studies suggest that INPP5A might have a suppressor role in different cancer types, including in skin cancer and in brain tumors and leukemia [16-19]. Validation assays subsequent to those shown in Fig. 4B confirmed the initial finding that silencing of INPP5A can sustain the growth of LNCaP cells in androgen-deprived medium (**Fig. 7A**). As shown in **Fig. 7A**, while no significant changes in population doublings of shINPP5A-LNCaP compared to shGFP-LNCaP cells were found when cells were cultured in complete FBS-RPMI medium, shINPP5A-LNCaP cells showed the ability to double in CSS-RPMI, which was lost in control shGFP-LNCaP cells. INPP5A protein levels in LNCaP cells expressing control shGFP or two shRNAs against INPP5A are shown in **Fig. 7A** (inset).

The colony formation assay in the presence of enzalutamide (**Fig. 5**) showed a partial inhibition of shINPP5A-mediated resistance in CSS-RPMI in presence of the AR antagonist MDV3100, suggesting that silencing of INPP5A allowed LNCaP cells to proliferate in androgen-deprivation conditions by re-activating the AR signaling pathway. To directly address AR pathway activation in LNCaP cells upon silencing of INPP5A, LNCaP clones stably expressing shGFP or the INPP5A-specific shRNAs were grown in CSS media and collected at 1, 2 and 3 weeks after plating. Protein lysates were generated, and immunoblot analyses were performed to determine levels of the AR-driven PSA gene, as well as of total and phospho-AR. As shown in **Figure 7B**, both total and p-AR levels, as well as PSA levels, were significantly higher in shINPP5A expressing cells in comparison to those expressing shGFP, thus confirming that silencing of INPP5A re-activates the AR pathway.



**Figure 7. INPP5A silencing in LNCaP cells and AR signaling.** (A) LNCaP cells stably expressing the shRNAs indicated were grown in complete FBS medium or androgen-deprived CSS-medium, and population doublings were counted every 2 days for 10 days. Level of INPP5A knock-down shown in inset. (B). Effect of INPP5A silencing in LNCaP cells grown in CSS medium on total and phospho-AR, and PSA levels.

To better investigate the involvement of the AR pathway re-activation in shINPP5A-driven resistance to ADT, we will further characterize the response of shINPP5A-LNCaP clones to the AR antagonist MDV3100, together with the analysis of AR localization by immunofluorescence.

**Major task 2: To investigate whether activation of the AR pathway is necessary for shRNA-driven resistance.**

To address this question, the ability of INPP5A shRNAs to promote cell proliferation in CSS medium will be tested in presence of an AR-specific shRNA. Because cells will be concomitantly infected with two different shRNAs (against INPP5A and AR), we are currently testing different IPTG-inducible AR shRNAs for their ability to knock-down AR protein level upon stimulation with IPTG (data not shown).

**MILESTONE ACHIEVED:**

1. Classification of validated genes into genes whose silencing drives resistance to castration through AR-dependent or -independent mechanisms (based on sensitivity to MDV3100).
2. Identification of loss of INPP5A as a candidate of resistance to androgen-deprivation.
3. Analysis of AR pathway re-activation in shINPP5A-LNCaP cells.

**5. CONCLUSIONS:**

In conclusion, during the first year reporting period, we used a prostate cancer cell model (LNCaP) that requires androgen for survival, and performed a genome-scale small-hairpin RNA (shRNA) screen to identify a rank of genes whose silencing (loss-of-function) allow these cells to proliferate in the absence of androgens. The validated hits showed different sensitivity to enzalutamide (MDV3100), a drug currently used in the clinic, that interferes with the function of the androgen receptor, indicating different dependencies on the AR pathway. To date, among the validated genes, our work has identified the inositol phosphatase INPP5A as one of the strongest hit, whose silencing sustains the growth of LNCaP cells and an active AR pathway, despite the lack of androgens. INPP5A regulates phospho-inositols and calcium signaling pathways, implicating that some effectors of these pathways could potentially be targetable with existing drugs, to ultimately revert the resistance phenotype.

**ACCOMPLISHMENTS TO ACHIEVE FOR THE NEXT REPORTING PERIOD:**

1. Validate candidate genes on additional androgen-sensitive PCa cell lines. VCaP cells are much more challenging to culture in androgen-deprived RPMI medium, and thus compromised our ability to validate hits identified with LNCaP cells. However, we will obtain an additional cell line, LAPC-4, which contains a wild type AR and is an androgen dependent cell line, from our colleague Philip W. Kantoff of the Dana-Farber Cancer Institute [20].
2. Validate candidate genes *in vivo*.
3. Characterize of dependencies on AR functions in shINPP5A-LNCaP cells.
4. Generate LNCaP cells whose *INPP5A* genomic locus has been modified using targeted CRISPR genomic editing technology, thus resulting in CRISPR-driven INPP5A knock-out.
5. Undertake detailed mechanistic studies to identify mechanism of resistance driven by INPP5A silencing.

## **OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT:**

As stated in the approved SOW, our goals were:

### **Major Task 1: Training and educational development in prostate cancer research**

To execute the loss-of-function screening, I exploited the unique resources of DFCI and the Broad Institute, (Dr. Garraway is a Senior Associate Member of the Broad Institute), and I deeply collaborated with expert mentors of the Broad Institute genomic perturbation platform. To set up both the RNAi screening optimization and execution accordingly to the platform guidelines, I was constantly mentored by experts of the platform. In addition, the Broad institute gave us the opportunity to leverage constant collaborations with experts in cancer genomics, molecular oncology, prostate oncology, computational biology and statistics.

Part of my training in cancer biology has included the attendance of regular seminars and conferences. Indeed, each Tuesday I have attended the meeting of the Cancer Program of the Broad Institute, and on Tuesday afternoon the institute-wide series of Seminars in Oncology at DFCI, where invited speakers present their latest work. Moreover, at the Broad Institute I have attended a monthly “Resistance meeting”, where postdoctoral fellows present their work focused on the study of resistance to anticancer treatment in different cancer types, and a monthly “Genomic Perturbation Platform meeting”, where gain- or loss-of-function screening-based projects, executed on different cancer models, are presented.

Dr. Garraway and Dr. Hahn have consolidated several platforms at DFCI/Broad Institute to create an integrated approach for the study of resistance mechanisms to anticancer agents in specific cancers, including prostate cancer. In a monthly meeting under the direct supervision of both Dr. Garraway and Dr. Hahn, post-docs and graduate students from the two labs had the opportunity to discuss on the progress of projects exclusively focusing on prostate cancer.

Finally, in Dr. Garraway’s laboratory, we have a weekly meeting on Wednesday morning dedicate to critically discuss each ongoing project in the lab. In addition to these meetings, I have met formally with Dr. Garraway at least twice per month, to discuss new results and plans in detail, and to receive both scientific and technical advices. Importantly, I also had the opportunity to review and criticize papers from high impact journals, focusing on prostate cancer research, under the direct Dr. Garraway’s supervision.

The attendance to all the mentioned meetings has guaranteed a continued collaborations and mentorship, significantly improving my development in the area of prostate cancer molecular oncology.

Besides the attendance of meetings, I have attended the international conference “American Association for Cancer Research” (AACR) annual meeting (april 5-9, 2014, San Diego), the “Ninth Annual Broad Institute Scientific Retreat” (November 12-13, 2013, Boston), and the “STARR Cancer Consortium Retreat” (September 23-24, 2013, Cold Spring Harbor Laboratory).

## **DISSEMINATION OF RESULTS TO COMMUNITIES OF INTEREST:**

To achieve the stated goals, I have presented my work at several seminars at DFCI/Broad institute department meetings:

- Cancer Program of the Broad Institute
- Genomic Perturbation Platform monthly meeting (twice)
- Garraway lab weekly meeting (every three months)

In addition, I have presented a poster at the following meetings:

- “American Association for Cancer Research” (AACR) annual meeting
- “STARR Cancer Consortium Retreat”

During the STARR Cancer Consortium retreat, I had the opportunity to disseminate our results to five biomedical research institutions — Memorial Sloan Kettering Cancer Center, the Broad Institute of MIT and Harvard, Cold Spring Harbor Laboratory, The Rockefeller University, and Weill Cornell Medical College. During the AACR annual meeting, I could interact and exchange among scientists from all over the world.

#### **MILESTONE ACHIEVED:**

1. Presented research at the monthly department group meetings
2. Attended conferences and retreats
3. Presentation of project data at a national meeting

### **IMPACT**

#### **IMPACT ON THE DEVELOPMENT OF THE PRINCIPAL DISCIPLINE(S) OF THE PROJECT:**

The specific project developed in the first year reporting period leveraged some technical skills critical in the field of resistance of prostate cancer to castration-based therapy. These skills have included lentiviral shRNA-based systematic functional studies, sequencing methods, along with analysis of survival shift of prostate cancer cells in castration conditions. Importantly, having access to patients tissue collection will make an incredible impact on the knowledge of genetic basis of resistance to anti-androgen-based therapies. Indeed, the evaluation of clinical alterations of the identified genes in human metastatic CRPC, together with knowledge of recurrent CaP alterations, may provide the framework for improved diagnosis and, in the future, application of durable therapeutic approaches for CaP.

Thus, as the project is progressing toward completion, the identified loss-of-function alterations in prostate cancer may lead to significant advances in the knowledge of dysregulation of mechanisms driving resistance, opening new prospects of combination studies.

**IMPACT ON OTHER DISCIPLINES:** Nothing to Report

**IMPACT ON TECHNOLOGY TRANSFER:** Nothing to Report

**IMPACT ON SOCIETY BEYOND SCIENCE AND TECHNOLOGY:** Nothing to Report

### **CHANGES/PROBLEMS**

Nothing to report

### **6. PRODUCTS**

**PUBLICATIONS, CONFERENCE PAPERS, AND PRESENTATIONS:** Nothing to report

**JOURNAL PUBLICATIONS:** Nothing to report

BOOKS OR OTHER NON-PERIODICAL, ONE-TIME PUBLICATIONS: Nothing to report

OTHER PUBLICATIONS, CONFERENCE PAPERS, AND PRESENTATIONS:

Poster presentations made in the last year are listed above (section “dissemination of results to communities of interest”).

WEBSITE OR OTHER INTERNET SITE(S): Nothing to report

TECHNOLOGIES OR TECHNIQUES: Nothing to report

7. INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES: Nothing to report

OTHER PRODUCTS:

Research material: generation of LNCaP cells stably expressing screen top hits shRNAs.

### **PARTECIPANTS AND OTHER COLLABORATING ORGANIZATIONS:**

INDIVIDUALS THAT HAVE WORKED ON THE PROJECT: Ginevra Botta; NO CHANGE

CHANGE IN THE ACTIVE OTHER SUPPORT OF THE PD/PI, OR SENIOR/KEY PERSONNEL SINCE THE LAST REPORTING PERIOD: Nothing to report

OTHER ORGANIZATIONS INVOLVED AS PARTNERS: Nothing to report

### **SPECIAL REPORTING REQUIREMENTS:**

COLLABORATIVE AWARDS: Nothing to report

QUAD CHARTS: Nothing to report

### **APPENDICES**

Nothing to report

## **10. REFERENCES**

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