

AWARD NUMBER: W81XWH-14-1-0429

TITLE: Understanding the Role of MDSCs in Castration-Resistant Prostate Cancer and Metastasis

PRINCIPAL INVESTIGATOR: Dr. Prasenjit Dey

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center  
Houston, TX 77030

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

*Form Approved*  
*OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE</b> October 2015			<b>2. REPORT TYPE</b> Annual Progress Report			<b>3. DATES COVERED</b> 15 Sep 2014 - 14 Sep 2015			
<b>4. TITLE AND SUBTITLE</b>  Understanding the Role of MDSCs in Castration-Resistant Prostate Cancer and Metastasis						<b>5a. CONTRACT NUMBER</b>			
						<b>5b. GRANT NUMBER</b> W81XWH-14-1-0429			
						<b>5c. PROGRAM ELEMENT NUMBER</b>			
<b>6. AUTHOR(S)</b>  Prasenjit Dey, Ph.D.  E-Mail: pdey@mdanderson.org						<b>5d. PROJECT NUMBER</b>			
						<b>5e. TASK NUMBER</b>			
						<b>5f. WORK UNIT NUMBER</b>			
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  The University of Texas MD Anderson Cancer Center 1515 Holcombe Boulevard Houston, TX 77030-4009						<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>			
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012						<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>			
						<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>			
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited									
<b>13. SUPPLEMENTARY NOTES</b>									
<b>14. ABSTRACT</b> The androgen receptor is a primary target of prostate cancer treatment and prevention. For metastatic prostate cancer (androgen responsive), androgen deprivation therapy (ADT) has been the standard mode of treatment. The tumors initially respond with the inhibition of growth, but most tumors invariably relapse leading to a lethal castration resistant prostate cancer (CRPC), indicating a shift in tumor responsiveness. The proliferating primary tumor recruits a variety of stromal cells in its surrounding microenvironment, which facilitates its growth and ultimately invasion and metastasis to distant organs. The myeloid derived suppressor cells (MDSC) and regulatory T cells (Treg) are important components of the immune suppressive network of the tumor microenvironment (TMEN). MDSCs play a pivotal role in suppression of both innate and adaptive immunity and its presence is documented both in preclinical model and cancer patients. The role of MDSCs in prostate cancer CRPC and metastasis is poorly understood. The unanswered questions include how MDSCs facilitate the tumor cells to evade the host immune surveillance, and what pathway regulates MDSC recruitment, accumulation, proliferation and differentiation in prostate cancer. Using the PB-Cre4/PTEN <sup>L/L</sup> /SMAD4 <sup>L/L</sup> and PB-Cre4/PTEN <sup>L/L</sup> /Trp53 <sup>L/L</sup> / SMAD4 <sup>L/L</sup> mouse model this project will first characterize MDSC population in both primary tumors as well as castration resistant tumors. Then we will identify novel prostate cancer specific MDSC markers using genomic and proteomic technology and finally, we will propose pre-clinical model to validate our newly discovered targets to deplete MDSCs to prevent/treat CRPC.									
<b>15. SUBJECT TERMS</b>									
<b>16. SECURITY CLASSIFICATION OF:</b>						<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC	
<b>a. REPORT</b>		<b>b. ABSTRACT</b>		<b>c. THIS PAGE</b>		Unclassified		<b>19b. TELEPHONE NUMBER</b> (include area code)	
Unclassified		Unclassified		Unclassified					

## Table of Contents

	Page
1. Introduction.....	3
2. Keywords.....	4
3. Accomplishments.....	5
4. Impact.....	10
5. Changes/Problems.....	10
6. Products.....	11
7. Participants & Other Collaborating Organizations.....	12
8. Special Reporting Requirements.....	12
9. Appendices.....	12
• References	

## 1. INTRODUCTION:

The androgen receptor is a primary target of prostate cancer (PCa) treatment and prevention (Heinlein and Chang 2004). For metastatic prostate cancer, androgen deprivation therapy (ADT) has been the standard mode of treatment (Karantanos et al. 2013). The tumors initially respond with the inhibition of growth, but most tumors invariably relapse leading to a lethal castration resistant prostate cancer (CRPC). The primary tumors recruit a variety of stromal cells in its surrounding microenvironment, which facilitates its growth and ultimately invasion and metastasis to distant organs (Sluka and Davis 2013). The myeloid derived suppressor cells (MDSC) and regulatory T (Treg) cells are important components of the immunosuppressive network of the tumor microenvironment (TMEN) (Solito et al. 2012; Lindau et al. 2013; Sluka and Davis 2013). MDSCs are a heterogeneous subset of activated but immature myeloid cells (IMC) (Gabrilovich and Nagaraj 2009). In mice, MDSCs co-express myeloid cell lineage marker Gr-1<sup>mid</sup> and CD11b<sup>hi</sup> (Joyce and Pollard 2009) and comprise two main subsets-- monocytic (Ly6C<sup>hi</sup>) and granulocytic (Ly6G<sup>hi</sup>). It is well established that MDSCs suppress both innate and adaptive immunity and their presence in tumors is documented in both preclinical models and cancer patients (Sawant and Ponnazhagan 2013). However, the role of MDSCs in prostate cancer development and castration resistance is poorly understood. Recent data suggests that ADT promotes immune cells infiltration, which influences tumor relapse and resistance to castration (Ammirante et al. 2010; Nelson 2010). These immune cells cause inflammatory response leading to further infiltration and expansion of MDSCs into the tumor microenvironment making the tumor increasingly resistant to conventional chemotherapy and radiation-therapy (Shojaei et al. 2007; Finke et al. 2011). MDSCs populate TMEN during early stage of tumor development. Given castration causes increase in the immune cell infiltration, we hypothesize that inflammation signal post castration may lead to activation and further expansion of MDSCs within TMEN which may contribute to the development of CRPC. Given our preliminary data indicating the presence of large number of MDSCs in primary prostate tumors and depletion of MDSCs by neutralization antibodies against Gr-1 leads to regression of primary tumor in our animal model, we further hypothesized that depletion of MDSCs would have therapeutic benefit for prostate cancer patients. We have characterized MDSC population in both primary tumors as well as castration resistant tumors as described in major goal 1. Then we delve deep to identify novel prostate cancer specific MDSC markers using genomic and proteomic technology in major goal 2 (ongoing). Finally, in major goal 3 (ongoing) we will propose pre-clinical model to validate our newly discovered targets to deplete MDSCs to prevent/treat CRPC.

2. **KEYWORDS:** Castration resistant prostate cancer (CRPC), tumor microenvironment (TMEN), androgen deprivation therapy (ADT), myeloid cells, immune cells.

## 3. ACCOMPLISHMENTS:

### ○ What were the major goals of the project?

Major Goal 1. Characterization of the prostate cancer mouse model to study MDSCs in CRPC and metastasis.

Major Goal 2. Identification of the novel genetic pathways in prostate cancer specific MDSCs by transcriptomic and proteomic analysis.

Major Goal 3. Functional validation of the role of MDSC in tumor progression and metastasis.

### ○ What was accomplished under these goals?

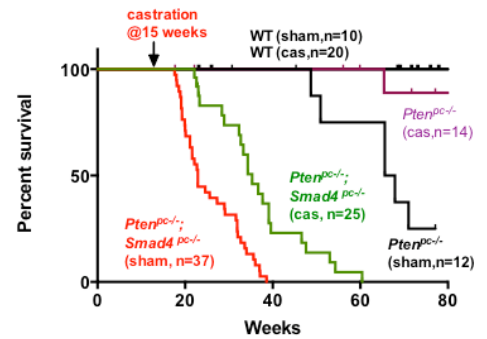
**Major Goal 1.** Characterization of the prostate cancer mouse model to study MDSCs in CRPC and metastasis.

**1) Major activity.** We have generated a mouse cohort for characterization of CRPC and MDSC. We conducted MRI scan of these mice to assess the tumor size and metastasis. We also harvested the tumor

and conducted immunohistochemistry to further characterize the tumor. We then assessed the immune landscape using flow cytometry and cyTOF mass cytometry analysis of primary and metastatic tumor samples.

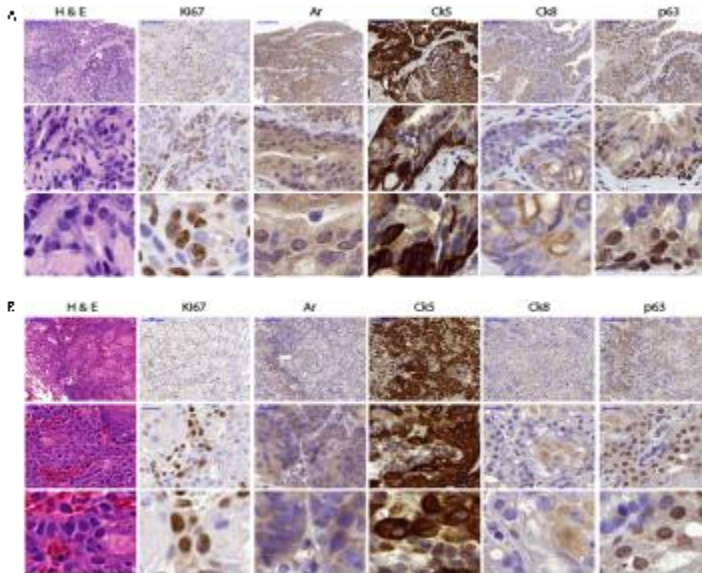
**2) Specific objective.** The specific objective of the Major Goal 1 is to further characterize the novel Luc/mTmG/PTEN<sup>pc-/-</sup> and Luc/mTmG/PTEN<sup>pc-/-</sup>/SMAD4<sup>pc-/-</sup> mouse model that was generated in the laboratory and to establish a cohort bearing primary, metastatic and castration resistant tumors for MDSC characterization.

**3) Significant results. (i) Characterization of mice cohort to study MDSC in CRPC.** We have performed castration in a larger cohort of mice and generated cumulative survival curve for this cohort (Figure 1). The castration was done at 15 weeks of age and we compared the survival of Luc/mTmG/PTEN<sup>pc-/-</sup> sham (n=12) vs. castrated (n=14). Also, we compared the survival of Luc/mTmG/PTEN<sup>pc-/-</sup>/SMAD4<sup>pc-/-</sup> sham (n=37) vs. castrated (n=25) mice. We observed an increase in overall survival in both the above tumor model upon castration compared to the sham control. Although the Luc/mTmG/PTEN<sup>pc-/-</sup>/SMAD4<sup>pc-/-</sup> tumors are comparatively more resistant to surgical castration compared to the Luc/mTmG/PTEN<sup>pc-/-</sup> mouse model (Figure 1).



**Fig 1.** Kaplan Meier survival analysis of PTEN<sup>-/-</sup> and PTEN<sup>-/-</sup>;SMAD4<sup>pc-/-</sup> castrated and sham castrated.

We have also collected prostate tissues as well as bone, lung, and lymph nodes from these mice. In addition, lung and bone tissues from Pten<sup>pc-/-</sup>/Smad4<sup>pc-/-</sup> mutant mice with lethal CRPC were examined for increased lung and bone metastasis. We also did marker studies with immunohistochemistry (IHC) to verify prostatic epithelial origin using luminal cell markers (CK8, CK18), basal cell markers (CK14, CK5, p63) and androgen receptor (Ar) and proliferation by ki67 (Figure 2). Consistent with human CRPCs, Ar is reactivated in Pten<sup>pc-/-</sup>;



**Fig 2.** H & E staining and Ki67, Ar, CK5, CK8, p63 IHC staining in CRPC primary tumors (A) and lung metastasis (B)

;Smad4<sup>pc-/-</sup> tumors as shown by the nuclear staining of Ar (Figure 2). Interestingly, in the treatment naïve prostate Pten<sup>pc-/-</sup>/Smad4<sup>pc-/-</sup> tumors, Ar is expressed in the majority of the cells (data not shown). However, some Ar+ tumor cells also expressed strong basal cell markers Ck5 (data not shown), suggesting that such basal type Ar+ cells down-regulate Ar expression in the CRPC tumors.

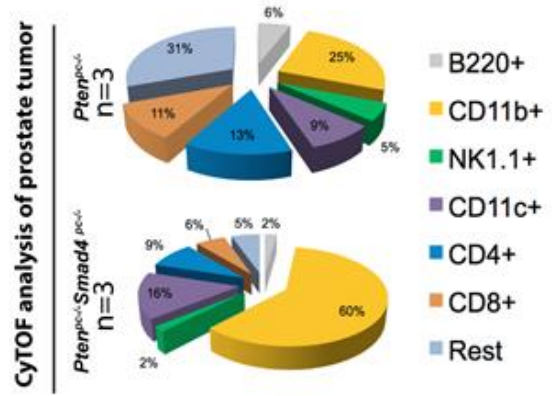
Further, a significant amount of normal epithelium was identified in castrated Pten<sup>pc-/-</sup> mice at 1 and 2 months post-castration, suggesting a clear response to castration. Interestingly, the stroma in the castrated Pten<sup>pc-/-</sup> mice is significantly expanded, which may account for the emergence of CRPC in Pten<sup>pc-/-</sup> mice 3, months post-castration (Lunardi et al. 2013; Wang et al. 2013). On the contrary, Pten<sup>pc-/-</sup>;

Smad4<sup>pc-/-</sup> mice are resistant to castration as compared to Pten<sup>pc-/-</sup> as shown by histopathological analysis by H & E staining. Although castration did slightly prolong the survival of Pten<sup>pc-/-</sup>;

succumbed to lethal CRPC (Figure 1), suggesting that this unique model may allow the identification of novel pathways that may play a role in the development of CRPC. Further characterization is ongoing and the data will be reported in the final report due next year.

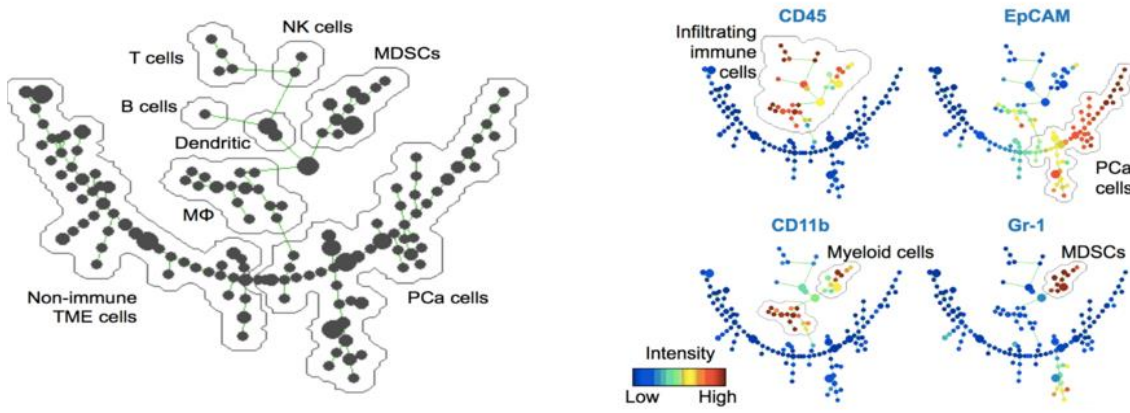
**(ii) Characterization of MDSCs and other immune cell populations in naïve vs. castrated tumor.**

We characterized the MDSC in a large cohort of mice using well-established markers such as CD11b<sup>+</sup>Gr-1<sup>+</sup>-MDSCs in stroma. To extensively audit the infiltrating immune cells in tumors, we performed mass cytometry (CyTOF) immunophenotyping (Bendall et al. 2011) to catalog tumor cell type constituents from 16-week old *Pten*<sup>pc/-</sup> and *Pten*<sup>pc/-</sup>;*Smad4*<sup>pc/-</sup> mice. Employing a 17-marker antibody panel, CyTOF confirmed a significant increase of CD45<sup>+</sup> infiltrating leukocytes in *Pten*<sup>pc/-</sup>;*Smad4*<sup>pc/-</sup> as compared to *Pten*<sup>pc/-</sup> tumors. Within the CD45<sup>+</sup> infiltrating cells, CD11b<sup>+</sup> myeloid cells represent a remarkably increased population in *Pten*<sup>pc/-</sup>;*Smad4*<sup>pc/-</sup> as compared to *Pten*<sup>pc/-</sup> tumors (Figure 3).



**Figure 3.** Percent distribution of immune cells in prostate tumor analyzed by cyTOF

The detailed immunophenotyping profiles enabled construction of the SPADE derived tree (Bjornson et al. 2013). SPADE (spanning-tree progression analysis of density-normalized events) is a computational approach to facilitate the identification and analysis of heterogeneous cell types. SPADE of the *Pten*<sup>pc/-</sup>;*Smad4*<sup>pc/-</sup> model displays the complexity of the TME which is composed of epithelial tumor cells (EpCAM<sup>+</sup> CD45<sup>-</sup>), non-immune TME cells (EpCAM<sup>-</sup> CD45<sup>-</sup>), and infiltrating



**Figure 4** SPADE tree derived from CyTOF (17-marker) analysis of whole tumor cell population from *Pten*<sup>pc/-</sup>;*Smad4*<sup>pc/-</sup> mice at 5-week, 8-week, and 14-week old (n=3). Live single cells were used to construct the tree. Cell populations were identified as PCa cells (EpCAM<sup>+</sup> CD45<sup>-</sup>), non-immune TME cells (EpCAM<sup>-</sup> CD45<sup>-</sup>), T cells (CD45<sup>+</sup> CD3<sup>+</sup> TCRβ<sup>+</sup>), B cells (CD45<sup>+</sup> B220<sup>+</sup> CD19<sup>+</sup>), NK cells (CD45<sup>+</sup> NK1.1<sup>+</sup>), dendritic cells (CD45<sup>+</sup> CD11c<sup>+</sup>), putative MDSCs (CD45<sup>+</sup> CD11b<sup>+</sup> Gr-1<sup>+</sup>), and macrophages (CD45<sup>+</sup> CD11b<sup>+</sup> Gr-1<sup>-</sup>). On the right panels, the tree is colored by the median intensity of individual markers shown on 33 the top to highlight infiltrating immune cells (EpCAM<sup>-</sup> CD45<sup>+</sup>), epithelial PCa cells (EpCAM<sup>+</sup> CD45<sup>-</sup>), total myeloid cells (CD45<sup>+</sup> CD11b<sup>+</sup>), and putative MDSCs (CD45<sup>+</sup> CD11b<sup>+</sup> Gr-1<sup>+</sup>).

immune cells (EpCAM<sup>-</sup> CD45<sup>+</sup>) that can be further grouped into various immune cell sub-populations (Figure 4). Among the infiltrating immune cells, there was a striking age-dependent increase of CD11b<sup>+</sup> Gr-1<sup>+</sup> cells in tumors (Figure 5). We are now in the process of conducting the CyTOF analysis of castrated and sham control tumor of *Pten*<sup>pc/-</sup> and *Pten*<sup>pc/-</sup>;*Smad4*<sup>pc/-</sup> mice.

## Methodology:

Wild type,  $Pten^{pc/-}$  and  $Pten^{pc/-};Smad4^{pc/-}$  mice are subjected to surgical castration. We collected tumors, DLN and lungs after animals developed castration resistance and from animals bearing castration naïve tumors. We performed luciferase imaging at regular intervals to monitor tumor progression. At least five samples per group of spontaneous prostate tumors and those regrown after castration as well as regional lymph nodes and distant organs, were collected at 15 and 90 days post castration for detailed histopathological analyses, including cell proliferation, apoptosis, senescence, angiogenesis, lymphangiogenesis, invasiveness and metastasis. Histological analyses were done to monitor features such as changes in cytoarchitecture and differentiation of epithelial and stromal components.

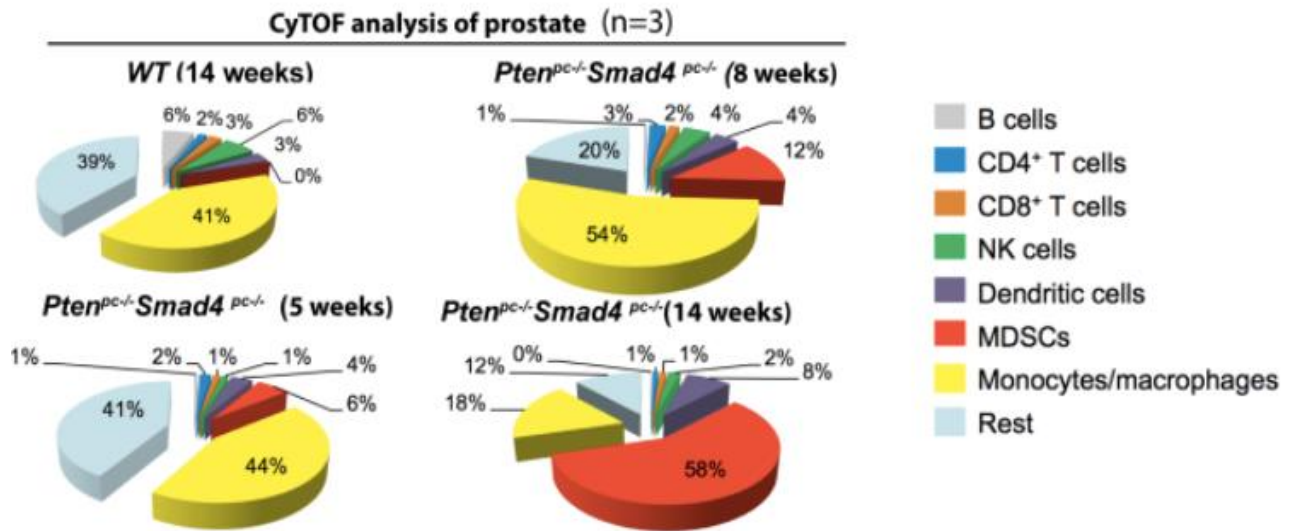


Figure 5. CyTOF analysis of tumors from 5, 8, and 14 weeks old  $Pten^{pc/-};Smad4^{pc/-}$  mice revealed an age-dependent increase in the MDSCs infiltration. Prostate from wild type (WT) mice at 16-week old was used as control (n=3 for each genotype).

Other selected methodology most pertinent to the study is detailed below.

CytoTOF. Metal-labeled antibodies against cell surface markers were purchased from DVS Sciences. Prostate tumor single cells were isolated using the Mouse Tumor Dissociation kit (cat# 130-096-730, Miltenyl Biotec). Cells from spleen and lymph node were isolated by mincing with a 5 mL syringe plunger against a 40 $\mu$ m cells strainer into a 60mm dish with RPMI medium containing 10% fetal bovine serum (FBS). The cells were depleted of erythrocytes by hypotonic lysis. Peripheral blood (100 $\mu$ L) was drawn using retroorbital bleeding and depleted of erythrocytes by hypotonic lysis. Next, tumor, spleen, lymph node, or blood cells were incubated with CD16/CD32 antibody (clone 2.4G2, BD Biosciences) to block Fc $\gamma$ R binding for 10 minutes then with antibody mix for 30 minutes at room temperature. Cells were washed once and incubated with MAXPAR<sup>®</sup>Nucleic Acid Intercalator-103Rh (Cat# 201103A, DVS Sciences) for 20 minutes for viability staining. Cells were fixed with 1.6% formaldehyde for 1 hour and incubated with MAXPAR<sup>®</sup>Nucleic Acid Intercalator-Ir (Cat# 201192A, DVS Sciences) at 4 $^{\circ}$ C overnight to stain the nuclei. The samples were analyzed with CyTOF instrument (DVS Sciences) in the Flow Cytometry and Cellular Imaging Core Facility at M.D. Anderson Cancer Center. The following markers were used to define different immune populations: T cells (CD3<sup>+</sup>TCR $\beta$ <sup>+</sup>, further classified as CD4<sup>+</sup> and CD8<sup>+</sup>), B cells (B220<sup>+</sup>CD19<sup>+</sup>), NK cells (NK1.1<sup>+</sup>), dendritic cells (CD11c<sup>+</sup>), MDSCs (CD11b<sup>+</sup> Gr-1<sup>+</sup>), and macrophages (CD11b<sup>+</sup> Gr-1).

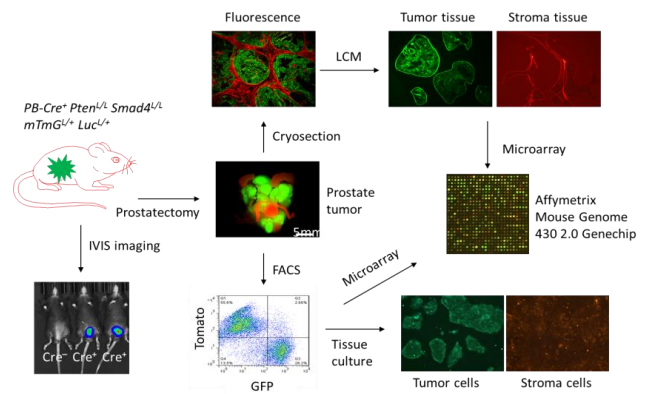
Flow cytometry. Fluorochrome-conjugated antibodies against CD45 (clone 30-F11), CD11b (M1/70), Gr-1 (RB6-8C5), Ly-6C (HK1.4) were purchased from eBiosciences. Antibody against Ly-6G (1A8) was purchased from BioLegend. Antibody against Cxcr2 (242216) was purchased from R&D Systems. Primary cells from mice were isolated with the same methods as in CyTOF. To assess cell viability, cells were incubated with SYTOX Blue (Life Technologies) prior to FACS analysis. All samples were acquired with the LSRFortessa analyzer (Becton Dickinson) and analyzed with FlowJo software (Tree Star).

**Major Goal 2.** Identification of the novel genetic pathways in prostate cancer specific MDSCs by transcriptomic and proteomic analysis.

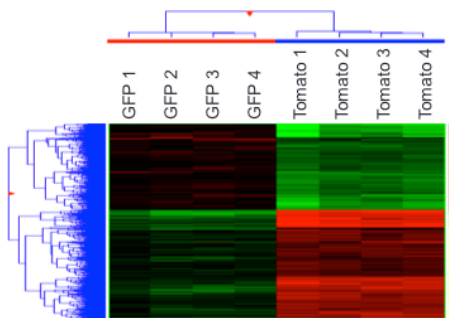
**1) Major activity.** We have isolated tumor, MDSC and metastatic samples by FACS sorting of primary and metastatic tumor samples. We have completed the processing of the above samples for RNA-seq and initiated the bioinformatic analysis.

**2) Specific objective.** RNA-seq of cancer cells and MDSCs from treatment naïve and castrated group. The specific objective of Major Goal 2 is to utilize powerful genomic profiling technologies to identify novel genes and pathways that drive the development of CRPC.

**3) Significant results.** Transcriptomic profiling of GFP+ tumor cells and Tomato+ stromal cells from  $Pten^{pc-/-}$   $Smad4^{pc-/-}$  shows a distinct expression pattern for these two populations. For this study we generated the novel prostate GEM model with fluorescence and luciferase reporters for non-invasive tracking of the tumor growth. Also, the fluorescence reporter enables FACS sorting of the cancer and non-cancer cells for downstream molecular profiling. We isolated GFP+ cancer cells and Tomato+ stromal cells, from which RNAs were isolated and subjected to microarray analysis (Figure 6). As expected, tumor and stroma



**Figure 6.** A novel metastatic prostate GEM model with fluorescence and luciferase reporters for noninvasive tracking of tumor growth as well as phenotypic and molecular dissection of tumor and stroma.



**Fig 7.** Hierarchical Clustering Analysis of GFP+ tumor cells and Tomato+ stromal cells.

showed distinct expression pattern by Hierarchical Clustering Analysis (Figure 7), and Ingenuity pathway analysis (IPA) indicated that, while tumor cells are enriched for pathways involving cell adhesion molecules and tight junction (consistent with their epithelial nature), stromal cells display activation of more diverse signaling pathways involved in chronic inflammation, such as cytokine/cytokine receptor interaction, chemokine signaling pathway, Jak-STAT pathway, TCR pathway, and BCR pathway ( $p < 0.01$ , data not shown). We are waiting for further bioinformatics analysis and validation experiments. The data from this analysis will be reported in the final report.

Methodology: To enable unambiguous distinction between tumor and stroma, we incorporated a dual fluorescent reporter allele, Rosa26-Lox-tdTomato-Lox-EGFP (a.k.a. mTmG)26, into the PB-Cre+ Pten<sup>pc/-</sup>;Smad4<sup>pc/-</sup> model. This allele allows Cre-dependent GFP expression in prostate epithelial cells as well as ubiquitous tdTomato expression in all other cells (Figure 6). We now can (1) visualize tumor and stroma distinctly by fluorescence imaging, (2) visually quantify metastases in lymph node and lung without laborious histological inspection, and (3) easily isolate tumor and stromal cells using flow cytometry or Laser Capture Microdissection. Furthermore, to enable noninvasive bioluminescence imaging (BLI) of tumor and metastasis development, we have further incorporated Rosa26-Lox-STOP-Lox-Luciferase allele and have confirmed the prostate-specific luciferase signal (Figure 6). Thus, we now have established a robust metastatic PCa model: PB-Cre<sup>+</sup>;Pten<sup>pc/-</sup>;Smad4<sup>pc/-</sup>;mTmG<sup>L/+</sup>;Luc<sup>L/+</sup>, which will facilitate monitoring of tumor development and tumor-stroma interactions through dual fluorescence and bioluminescence imaging. In parallel, we have also established a comparable model with only Pten loss: PB-Cre<sup>+</sup>;Pten<sup>pc/-</sup>;mTmG<sup>L/+</sup>;Luc<sup>L/+</sup>. It is important to note that we have now backcrossed these strains to C57BL/6 congenic background, which enables syngeneic transplantation.

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

- 1) Training from intra- and inter-laboratory interactions: DePinho lab provides the best possible research environment. At MDACC, we have weekly lab meeting and journal club with post-doctoral fellow and graduate students from Ronald DePinho, Raghu Kalluri and Giulio Draetta's labs, which provide great opportunities to present my work in a critical academic setting and to learn about emerging discoveries from other talented post-docs and graduate students. At MDACC, I am exposed to a whole array of facilities and cores, whose core teams are willing to assist me in different aspects of my research. In addition, I have maintained a good relationship with bioinformaticians and biostatisticians led by Dr. Zhang and the scientists from the Institute of Applied Cancer Science led by Dr. Draetta. By collaborating and exchanging ideas with these scientists and physicians, I not only benefit from their great science, but will also build up a strong network for my future independent career.
- 2) Grant writing: With the help of Dr. DePinho, I successfully got the Department of Defense Prostate Cancer Research Program Postdoctoral Training Award. In addition, I'm helping Dr. DePinho to put together a CIPRIT MIRA grant and a RO1 grant on the role of MDSC using the novel prostate cancer mouse model generated in our lab. These experiences are invaluable, as Dr. DePinho will work closely with me to make sure I learn and understand the craft of grant writing. I also attended one workshops conducted by MDACC named "Write Winning Grants". These training activities will help me obtain funding for my independent research laboratory.
- 3) Scientific communication in writing: I'm working closely with Dr. DePinho in writing my research article on the novel molecular mechanisms for CRPC in the Pten/Smad4 mouse model along with Drs. Guocan Wang and Xin Lu. I expect to write another co-first-author papers on several novel mouse PCa models in collaboration projects with Dr. Xin Lu in our lab before I finish my postdoctoral training here. Furthermore, I attended the "Writing and Publishing Scientific Articles Workshop" in MDACC. These training experiences will help me to write and publish high-profile papers of my own laboratory.
- 4) Scientific communication in speaking: I routinely present my work in the DePinho lab meeting every other month in MDACC. I also regularly present my work in the joined weekly lab meeting among Draetta lab/Kalluri lab/DePinho lab/IACS and in the weekly Cancer Biology Department Seminar. In addition, I present regularly in the weekly Department of Cancer Biology Journal club. Moreover, I took the workshops provided by MDACC such as "Giving Presentations: Learn Presentation Skills and Tips That Will Help You Keep Your Audience Enthralled" and on similar topics. I believe these training

activities will make presentation skill one of my strengths and help me interview for and attain a faculty position.

- 5) Lab management/Mentoring training: The DePinho lab is huge, so all of the post-docs participate heavily in ensuring that various aspects of the lab are running smoothly. I have trained 1 undergraduate intern students, one medical intern student. These experiences have provided me the great opportunity to practice my mentoring and management skills. In terms of education for myself, I attended the “Faculty Mentoring Academy Series” in MDACC. Finally, I will continue to learn from Dr. DePinho, who is a master of mentoring himself, to improve a wide range of skills on mentoring and management.
- 6) Seminar series/Conferences/Workshops: MDACC offer a wide range of top-notch seminars/series/conferences, where I keep abreast of current research findings around the world. I also plan to attend various international conferences including but not limited to AACR Annual conference 2016 and SITC 2016. I have also attended the CRI-CIMT-EATI-AACR - The Inaugural International Cancer Immunotherapy Conference: Translating Science into Survival at New York in Sep 2015 and AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics at Boston in Nov 2015. In addition to the workshops mentioned in other sections, I will attend “Faculty Development Workshop and Seminar Series” of MDACC regularly to help me prepare the transition to independent PI.
- **How were the results disseminated to communities of interest?**

The primary goal of this project is to elucidate the role played by castration in inflammation induced MDSC infiltration into tumor microenvironment. I have accomplished all the goals proposed for the 1st year of the project as stated in statement of works. I have generated all the cohorts necessary for the study and isolate tissues for further downstream analysis. I have also completed immunophenotyping by flow cytometry and CyTOF and are in the process of analyzing the data. I also have completed collection of samples for genomic/transcriptomic analysis by microarray and RNAseq and the data are now been analyzed by bioinformaticians.

Major goals achieved in 1st year-

- a. Completed the Kaplan-meier survival analysis of Pten/Smad4 mice with a large cohort.
  - b. Comprehensive histopathological analysis of CRPC in the Pten/Smad4 mice suggests an expansion of the prostate tumor cells that express basal cell markers, such as Ck5 and p63. These basal type CRPC cells are highly proliferative, suggesting it may contain the cancer stem cell population.
  - c. Transcriptome profiling of GFP+ tumor cells and Tomato+ cells from my Pten/Smad4/mTmG model identified tumor- and stroma-specific genes and pathways
- **What do you plan to do during the next reporting period to accomplish the goals?**

I will continue to characterize the CRPC model and conduct unbiased analysis of the transcriptomic data and identify pathways that can be used as target in CRPC. Also, I am currently analyzing the CyTOF and flow cytometry data to identify the pro-tumorigenic immune cells in the TMEN. In the 2nd year of my project, I will continue the cytokine and proteomic profiling of the CRPC tumor. This will further give us impetus to new knowledge about immune cells mediated castration resistance and will help me identify new players in this already complex phenomena. Perform RPPA for the tumor and metastatic samples collected in Major Goal 1. I will also undertake the expression validation of the targets identified in Major Goal 1 using NanoString.

#### 4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**

Although a large number of studies have demonstrated a direct relationship between MDSC frequency and tumor burden [17], our understanding of the role of MDSCs in tumor progression, particularly castration resistant prostate cancer, remains largely speculative. Here, using a highly invasive Pten/Smad4 deficient prostate cancer model, we intend to establish the signaling circuits involved in the recruitment of MDSCs to the TME and demonstrate a critical role of these cells in facilitating tumor progression. Using the state-of-the-art CyTOF technology, we reveal that progression in the Ptenpc<sup>-/-</sup>/Smad4pc<sup>-/-</sup> model is associated with abundant immune cell infiltration characterized by prominent representation of CD11b<sup>+</sup> Gr-1<sup>+</sup> MDSCs. The basis for the increased frequency of MDSCs in the TME and, specifically in Ptenpc<sup>-/-</sup>/Smad4pc<sup>-/-</sup> model was not known and presumably could derive from either active chemoattraction or passive nonspecific responses to tissue stress associated with expanding tumor burden. Taking an unbiased approach to identify pathways that may recruit MDSCs, we seek to identify unique immune regulatory molecules that are activated predominately in castrated Ptenpc<sup>-/-</sup> and Ptenpc<sup>-/-</sup>/Smad4pc<sup>-/-</sup> models of prostate cancer.

- **What was the impact on other disciplines?**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology?**

Nothing to report

#### 5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Nothing to Report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to Report.

- **Changes that had a significant impact on expenditures**

No changes.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

No changes.

- **Significant changes in use or care of human subjects**

No changes.

- **Significant changes in use or care of vertebrate animals.**

No changes.

- **Significant changes in use of biohazards and/or select agents**

No changes.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**  
Nothing to Report.
- **Website(s) or other Internet site(s)**  
Nothing to Report.
- **Technologies or techniques**  
Nothing to Report.
- **Inventions, patent applications, and/or licenses**  
Nothing to Report.
- **Other Products**  
Nothing to Report.

**PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	Prasenjit Dey, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-4812-2248
Nearest person month worked:	12
Contribution to Project:	Dr. Dey is the principal investigator on the project.
Funding Support:	None

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**  
Nothing to Report.
- **What other organizations were involved as partners?**  
Nothing to Report.

**7. SPECIAL REPORTING REQUIREMENTS**

Nothing to Report

**8. APPENDICES:**

**References**

Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M. 2010. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* **464**: 302-305.

- Bendall SC, Simonds EF, Qiu P, Amir el AD, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI et al. 2011. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* **332**: 687-696.
- Bjornson ZB, Nolan GP, Fantl WJ. 2013. Single-cell mass cytometry for analysis of immune system functional states. *Curr Opin Immunol* **25**: 484-494.
- Finke J, Ko J, Rini B, Rayman P, Ireland J, Cohen P. 2011. MDSC as a mechanism of tumor escape from sunitinib mediated anti-angiogenic therapy. *Int Immunopharmacol* **11**: 856-861.
- Gabrilovich DI, Nagaraj S. 2009. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* **9**: 162-174.
- Heinlein CA, Chang C. 2004. Androgen receptor in prostate cancer. *Endocr Rev* **25**: 276-308.
- Joyce JA, Pollard JW. 2009. Microenvironmental regulation of metastasis. *Nat Rev Cancer* **9**: 239-252.
- Karantanos T, Corn PG, Thompson TC. 2013. Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. *Oncogene* **32**: 5501-5511.
- Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ. 2013. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. *Immunology* **138**: 105-115.
- Lunardi A, Ala U, Epping MT, Salmena L, Clohessy JG, Webster KA, Wang G, Mazzucchelli R, Bianconi M, Stack EC et al. 2013. A co-clinical approach identifies mechanisms and potential therapies for androgen deprivation resistance in prostate cancer. *Nature genetics* **45**: 747-755.
- Nelson BH. 2010. CD20+ B cells: the other tumor-infiltrating lymphocytes. *J Immunol* **185**: 4977-4982.
- Sawant A, Ponnazhagan S. 2013. Myeloid-derived suppressor cells as osteoclast progenitors: a novel target for controlling osteolytic bone metastasis. *Cancer Res* **73**: 4606-4610.
- Shojaei F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, Fuh G, Gerber HP, Ferrara N. 2007. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat Biotechnol* **25**: 911-920.
- Sluka P, Davis ID. 2013. Cell mates: paracrine and stromal targets for prostate cancer therapy. *Nat Rev Urol* **10**: 441-451.
- Solito S, Pinton L, Damuzzo V, Mandruzzato S. 2012. Highlights on molecular mechanisms of MDSC-mediated immune suppression: paving the way for new working hypotheses. *Immunol Invest* **41**: 722-737.
- Wang G, Lunardi A, Zhang J, Chen Z, Ala U, Webster KA, Tay Y, Gonzalez-Billalabeitia E, Egia A, Shaffer DR et al. 2013. Zbtb7a suppresses prostate cancer through repression of a Sox9-dependent pathway for cellular senescence bypass and tumor invasion. *Nature genetics* **45**: 739-746.