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TITLE: Dissecting the Role of Cancer Cell Chromatin Remodeling on the Landscape of Tumor Immune Microenvironment

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14. ABSTRACT
We recently demonstrated that the chromatin remodeler Nsd2 is required for PCa metastasis, is associated with tumor progression, and that its silencing markedly reduced the metastatic burden and increased survival (Nature Communications, 2018). Our preliminary data indicates that Nsd2 antagonizes the SWI/SNF complex deregulating global chromatin accessibility and changes the landscape of the immune infiltrating microenvironment. Further, the remodeling of the immune infiltrate by epigenetic dysregulation is dependent on recurrently altered tumor suppressor genes in mCRPC. This data suggests that epigenetic remodeling altering chromatin accessibility in cancer cells impacts on the tumor immune microenvironment. We hence hypothesize that chromatin accessibility in treatment refractory mCRPC may act as a surrogate marker for epigenetic remodeling that can be correlated to distinct tumor immune infiltration phenotypes with implications for prostate cancer immune checkpoint inhibitors.

15. SUBJECT TERMS
None listed.

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1. Introduction

Resistance to Androgen Receptor inhibition is arguably the principal hallmark of lethal prostate cancer. Antiandrogens have greatly improved the outcomes of prostate cancer patients, particularly those with metastatic disease. However, the approval of these treatments at earlier stages has left those with progressive disease with limited therapeutic options. Immune check point inhibitors have significantly improved outcomes in various solid tumors, unfortunately, evidence from clinical trials has shown that prostate cancer remains largely primary refractory to immunotherapy, other than a small subset of patients with mismatch repair deficiency or microsatellite instability. Rational combination strategies may help improve immunotherapy response for a greater number of patients. There remains a scarcity of preclinical studies exploring novel immunotherapy combinations, in part due to a lack of appropriate preclinical models to explore the tumor immune microenvironment in prostate cancer and metastatic lesions, particularly to the bone, hindering the development of rational approaches to overcome primary immune-resistance.

Over the last decade, we have developed a series of genetically-engineered mouse prostate cancer models and human derived organoids allowing us to elucidate cancer cell-intrinsic mechanisms of progression and metastasis as well as treatment response and resistance. Of particular relevance is our contribution to uncover how a dysregulated epigenetic master regulators impinge on treatment resistance and neuroendocrine transdifferentiation. These preclinical models, together with our unique access to clinical specimens and our ability to perform cross-species multimodal single cell analysis makes us well poised to make breakthroughs in the relationship between epigenetic remodeling, transdifferentiation and tumor immune microenvironment.

Preliminary data and hypothesis

We recently demonstrated that the chromatin remodeler Nsd2 is required for PCa metastasis, is associated with tumor progression, and that its silencing markedly reduced the metastatic burden and increased survival (*Nature Communications, 2018*). Our preliminary data indicates that Nsd2 antagonizes the SWI/SNF complex deregulating global chromatin accessibility and changes the landscape of the immune infiltrating microenvironment. Further, the remodeling of the immune infiltrate by epigenetic dysregulation is dependent on recurrently altered tumor suppressor genes in mCRPC. This data suggests that epigenetic remodeling altering chromatin accessibility in cancer cells impacts on the tumor immune microenvironment. We hence hypothesize that chromatin accessibility in treatment refractory mCRPC may act as a surrogate marker for epigenetic remodeling that can be correlated to distinct tumor immune infiltration phenotypes with implications for prostate cancer immune checkpoint inhibitors.

2. Keywords

Prostate cancer, immune microenvironment, transcriptional plasticity, immunoediting, single cell omics.

3. Accomplishments

3.1. What are the major goals of the project?

Specific Aim 1: To study how oncogenic drivers and treatment influence the tumor immune microenvironment preclinical models.			
Major Task 1: To correlate the dynamic changes in transcriptional identity with chromatin accessibility profiles.	Months	Responsible*	% of completion
Subtask 1: Generation of NPNsd2 tumor bearing cohorts	1-6	AYTES LAB technician	100%
Subtask 2: Enrollment of NPNsd2 mice in treatment cohorts, treatment administration, euthanasia, tissue harvesting and processing for single cell suspensions or FFPE for histological analysis	6-12	AYTES LAB technician/ postdoc	50%
Subtask 3: Single cell RNA and ATAC sequencing	6-18	HEYN LAB postodoc	50%
Subtask 4: Histological characterization of tumor phenotypes and immune microenvironment	6-18	AYTES LAB Postdoc	75%
Milestone(s) Achieved: *To define the structure, composition, and antigenicity of the tumor-immune microenvironment in mCRPC samples and to stablish correlations between chromatin accessibility and distinct immune populations in this GEM model.*	18	AYTES LAB PIULATS LAB HEYN LAB	75%
Major Task 2: To Investigate mechanisms of immunoediting in the emergence of castration resistant PC.	Months	Responsible	% of completion
Subtask 5: Time course in Nu/J and syngeneic mice of growth dinamics of NPNsd2-Allo tumors	6-9	AYTES LAB Technician/ Postdoc	0%

Subtask 6: Immunoediting cohort at Tel, Teq and Tes timepoints. Harvesting of tissues and process through the 28-color panel by spectral flow cytometry. Includes optimization.	6-18	AYTES LAB Technician/ Postdoc	0%
Subtask 7: Validation in vivo by antibody-based depletion studies	18-24	AYTES LAB PIULATS LAB Technician/ Postdoc	0%
Milestone(s) Achieved: *To validate the role of specific immune populations in the immunoediting process in castration resistant prostate cancer*	24	AYTES LAB PIULATS LAB HEYN LAB	0%
Specific Aim 2: Cross-species analysis of immune microenvironment in human treatment refractory mCRPC.			
Major task 3: Prospectively collect bone and soft tissue biopsies from patients progressing approved therapies.	Months	Responsible	% of completion
Subtask 8: Collection and processing to single cell suspension of human PC tissue samples from metastatic CRPC. Cryopreservation and Sample transfer to Aytes Lab	1-24	BELTRAN LAB Technician	25%
Subtask 9: Single cell RNA and ATAC sequencing of human PC samples	6-30	HEYN LAB postdoc	25%
Milestone(s) Achieved: *To have a full dataset up no less than 20 human metastatic CRPC samples with full single cell RNA and ATAC profiling*	30	BELTRAN LAB HEYN LAB	25%
Major Task 4: To establish correlations between transcriptional ID and chromatin accessibility.	Months	Responsible	% of completion
Subtask 10: Pipeline optimization for correlation analysis between ATAC and immunophenotyping data.	1-6	AYTES LAB PIULATS LAB postdoc	25%
Subtask 11: Integration of GEM derived genomic with flow cytometry data	6-18	AYTES LAB Postdoc	25%
Subtask 12: Data analysis and integration from Aim 1.1 and and 2.1	27-36	AYTES LAB PIULATS LAB Postdoc	0%
Milestone(s) Achieved: Complete cross-species analysis of correlations between immune infiltrating subtypes and chromatin accessibility in PC. Provide tools and methods to test if ATACscore as predictor of CPI response	36	ALL LABS	25%
Specific Aim 3: To identify treatment combinations that increase tumor immunogenicity by synergizing with CPIs.			
Major task 5: To carry out a targeted CRISPR/Cas9 in vivo synthetic lethal screen.	Months	Responsible	% of completion
Subtask 13: Engraftment into the tibias of the NPNsd2 cells with the custom library of epigenetic remodelers.	22-26	AYTES LAB technician/ postdoc	0%
Subtask 14: Monitor tumor growth, excise and isolate sgRNAs for NGS	26-30	AYTES LAB technician/ postdoc	0%
Subtask 15: Drop out data analysis and interpretation	30-36	AYTES LAB technician/ postdoc	0%
Milestone(s) Achieved: *Prioritized list of candidate mechanisms for validation*	36	AYTES LAB	0%
Major task 6: To Assess the capacity for drugs to activate type-I interferon.	Months	Responsible	% of completion
Subtask 16: Validation of the responsiveness of IFN reporter human cell lines and transfer to Beltran Lab	10-12	AYTES LAB Technician	100%
Subtask 17: Drug screen optimization	12-16	BELTRAN LAB Technician	100%

Subtask 18: Drug screen and hit validation	16-18	BELTRAN LAB Technician AYTES LAB postdoc	25%
Milestone(s) Achieved: *Integrated prioritized list of candidate mechanisms and hit compounds for validation*	18-30	AYTES LAB BELTRAN LAB	50%

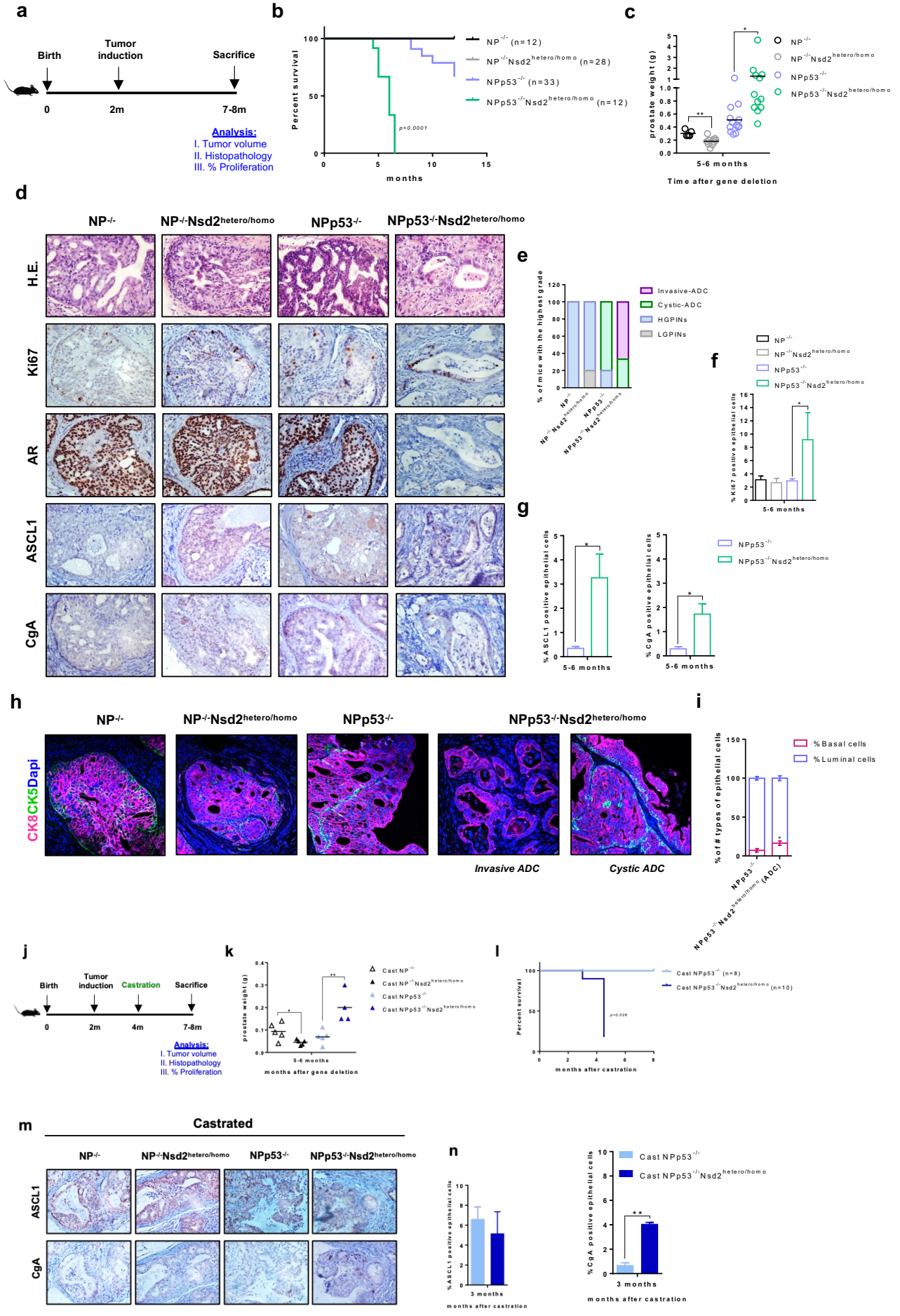
3.2. What was accomplished under these goals?

During this first period (months 1 to 12) we accomplished nearly all our objectives as detailed below:

Regarding subtasks 1, 2 and 4, At the Aytes Lab at IDIBELL, we carried out the characterization of the GEM models as shown below for mice at 6 months post induction (escape/adaptation). Briefly, as predicted from our previous and preliminary data, oncogenic Nsd2 expression results in accelerated aggressive phenotype and shorter survival (Fig 1a-e), accompanied by increased proliferation, focal neuroendocrine differentiation (Fig. 1f-g) and expansion of the basal cell compartment (Fig. 1h). In particular expression of the oncogenic NSD2 results in a modest increase in the percent of High-Grade PIN (HGPIN) in the Pten KO background but a remarkable increase in the percent of invasive adenocarcinomas by only 5 months after induction (Fig 1d), which is associated to a significantly shorter survival (Fig 1b) and increased tumor burden (Fig 1c). These Npp53Nsd2 tumors are highly proliferative (Fig 1f) and display clusters of focal expression of neuroendocrine markers ASCL1 and CgA (Fig. 1g)

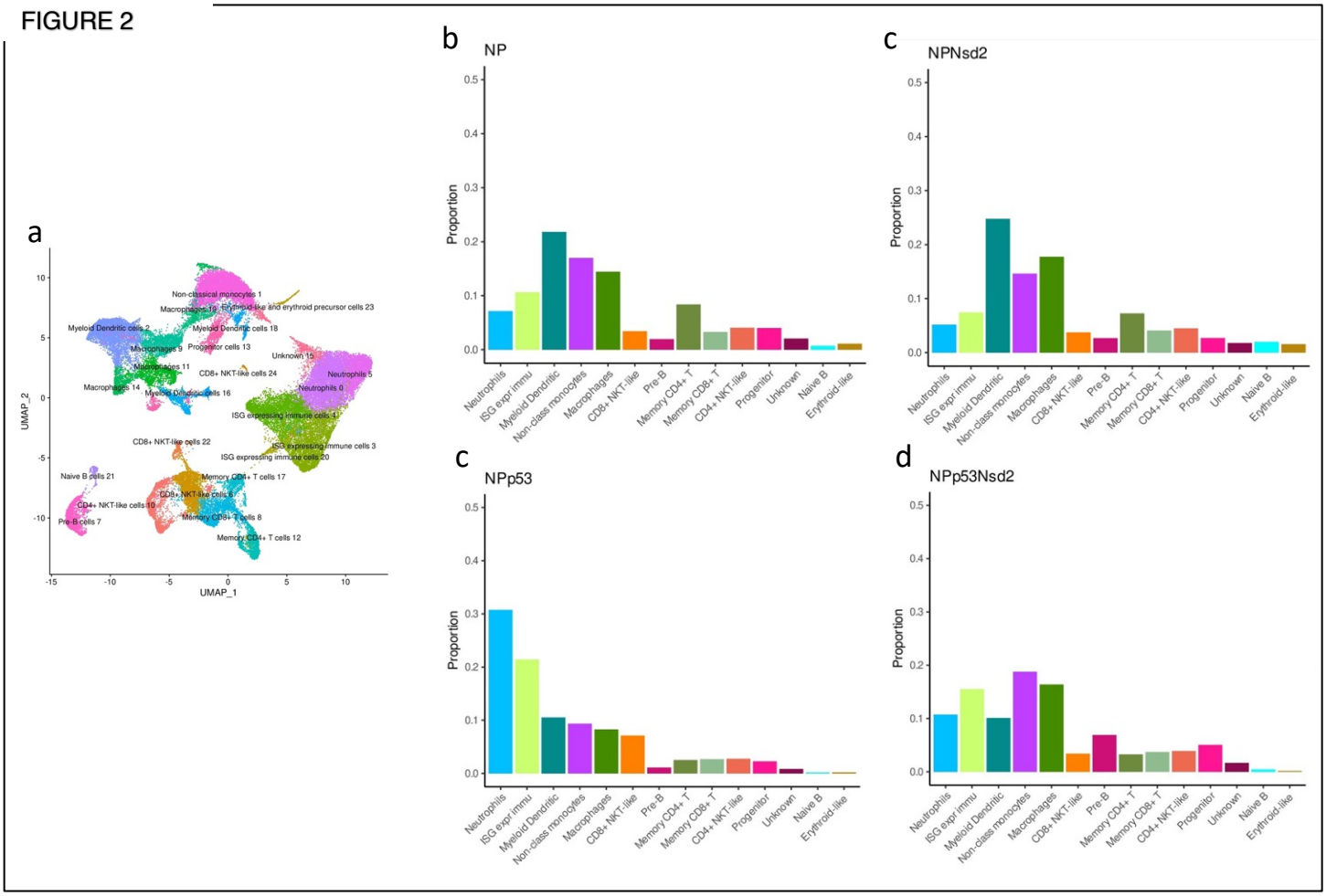
Importantly, oncogenic Nsd2 expression results in castration insensitivity as assessed by tumor weight response (Fig.1k) and survival (Fig. 1l). Similar to the intact mice, oncogenic Nsd2 expression enhanced the plastic phenotype in this GEM mice (Fig. 1n).

FIGURE 1


a
Birth
Tumor induction
Sacrifice
0
2m
7-8m
Analysis:
I. Tumor volume
II. Histopathology
III. % Proliferation
b
Percent survival
months
p<0.0001
NP-/- (n=12)
NP-/-Nsd2hetero/homo (n=28)
NPp53-/- (n=33)
NPp53-/-Nsd2hetero/homo (n=12)
c
prostate weight (g)
5-6 months
Time after gene deletion
NP-/-
NP-/-Nsd2hetero/homo
NPp53-/-
NPp53-/-Nsd2hetero/homo
d
NP-/-
NP-/-Nsd2hetero/homo
NPp53-/-
NPp53-/-Nsd2hetero/homo
H.E.
Ki67
AR
ASCL1
CgA
e
% of mice with the highest grade
Invasive-ADC
Cystic-ADC
HGPINs
LGPINs
f
% Ki67 positive epithelial cells
5-6 months
g
% ASCL1 positive epithelial cells
% CgA positive epithelial cells
5-6 months
h
NP-/-
NP-/-Nsd2hetero/homo
NPp53-/-
NPp53-/-Nsd2hetero/homo
CK8CK5Dapi
Invasive ADC
Cystic ADC
i
% of # types of epithelial cells
% Basal cells
% Luminal cells
NPp53-/-
NPp53-/-Nsd2hetero/homo (ADC)
j
Birth
Tumor induction
Castration
Sacrifice
0
2m
4m
7-8m
Analysis:
I. Tumor volume
II. Histopathology
III. % Proliferation
k
prostate weight (g)
5-6 months
months after gene deletion
Cast NP-/-
Cast NP-/-Nsd2hetero/homo
Cast NPp53-/-
Cast NPp53-/-Nsd2hetero/homo
l
Percent survival
months after castration
Cast NPp53-/- (n=8)
Cast NPp53-/-Nsd2hetero/homo (n=10)
m
Castrated
NP-/-
NP-/-Nsd2hetero/homo
NPp53-/-
NPp53-/-Nsd2hetero/homo
ASCL1
CgA
n
% ASCL1 positive epithelial cells
3 months
months after castration
% CgA positive epithelial cells
3 months
months after castration
Cast NPp53-/-
Cast NPp53-/-Nsd2hetero/homo

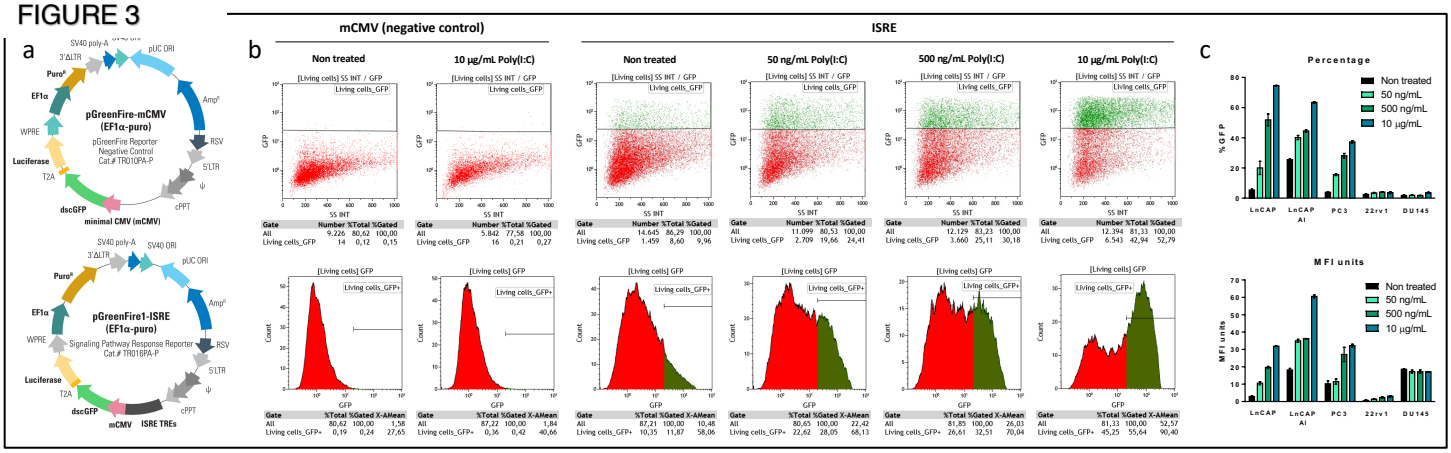
For **Major Task 1, Subtask 3, the Aytes and Heyn Labs at IDIBELL and CNAG** have initiated the single cell RNA and ATAC sequencing experiments planned. By month 12 after induction single cell suspensions for the different time points were collected for the CD45+ and CD45- fractions and froze in viability. The CD45 fraction of this cell models has first been sequenced for QC purposes together with the CD45+ fractions of the NP, NPNsd2, NPp53 and NPp53Nsd2 mice (Figure 2a). Notably, oncogenic NSd2 expression dramatically remodels the tumor immune microenvironment in the NPp53 (Fig 2c,d) but not in the NP background (Fig. 2b,c). We are now ready to go ahead and perform the planned sequencing and data analysis for the remaining CD45- fraction.

FIGURE 2



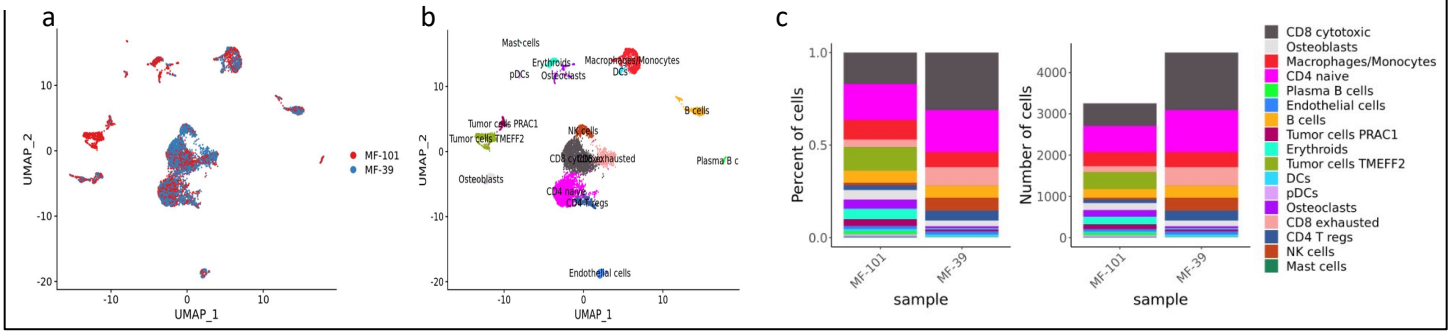
For **Major Task 6, subtasks 16 to 18, the Aytes and Beltran Labs at IDIBELL and DFCI** have been working closely to meet the planned aims. First, control and interferon reporter plasmids were obtained (Fig 3a) and the expression of the luciferase and GFP optimized using Poly(I:C) on HEK293 cells (Fig. 3b). As shown below, treatment with 10ug/ml of Poly(I:C) did not trigger the expression of either luciferase ort GFP in cells transduced with minimal CMV promoter control vectors. On the contrary, ISRE transduced cells show a dose dependent induction of GFP/luciferase expression. Nex, we introduced these control and ISRE vectors into a panel of prostate cancer cell lines, including LNCaP, LNCaP-AI, 22Rv1, DU145 and PC3 cells. Notably, treatment with Poly(I:C) only induced robust GFP/luciferase expression in the LNCaP, LNCaP-Ai and PC3 cells but not in DU145 cells. As expected, 22Rv1 cells fail to activate Interferon-mediated transcriptional responses as they these cells carry mutant JAK1/2 copies and are insensitive to the JAK/STAT mediated activation of interferon response.

FIGURE 3



For **Major task 3, the Beltran Lab at DFCI and Aytes and Heyn Labs at IDIBELL and CNAG** have collected nearly 30 bone biopsies from patients progressing on Enzalutamide or Abiraterone. Histopathological analysis and targeted exome sequencing for cancer drivers has been done in these biopsies using alternative funds. So far 10 biopsies have been subjected to single cell RNAseq, ATACseq and/or TCR sequencing. Data is available for the first two samples bellow (Fig. 4a-c).

FIGURE 4



3.3. What opportunities for training and professional development has the project provided?

Nothing to report

3.4. How were the results disseminated to communities of interest?

Nothing to report

3.5. What do you plan to do during the next reporting period to accomplish the goals?

During year 2 we will proceed with the execution of aims as described in the Statement of Work above. In particular, we will be completing the sequencing and analysis related to Major Task 1 and 3
Subtasks associated with Major Task 2 are also scheduled mostly for year 2 and will be carried pout as planed
Data integration and analysis to meet Major Task 4 will also be carried out in year 2. This will allow to know complete the delayed tasks mentioned above during the next reporting period.

4. Impact

4.1. What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

4.2. What was the impact on other disciplines?

Nothing to report

4.3. What was the impact on technology transfer?

Nothing to report

4.4. What was the impact on society beyond science and technology?

Nothing to report

5. Changes/Problems.

5.1. Changes in approach and reasons for change
Nothing to report

5.2. Actual or anticipated problems or delays and actions or plans to resolve them.
During Year one we experience technical issues with our equipments in the flow cytometry core facility at IDIBELL. As a result, most of the experimental work related to Major Task 2, subtasks 5 and 6, which were planned for this reporting period could not be done. To ensure the project would move forward and milestones met, we instead prioritized subtasks 16 to 18 of Major Task 6, which were mostly planned for the second reporting period, on year 2

5.3. Changes that had a significant impact on expenditures
Nothing to report

6. Products
Nothing to report

7. Participants & Other Collaborating Organizations

7.1. What individuals have worked on the project?

Name	Javier Sigüenza Andrade
Project role	Technician
Researcher Identifier	NA
Nearest person month worked	12
Contribution	Carried out ex vivo work, including primary cultures, scRNA and ATACseq, assays as well as in vitro assays
Funding Support	This Award

Name	Adrian Martinez Tebar
Project role	Postdoc
Researcher Identifier	NA
Nearest person month worked	4
Contribution	Carried out all the mouse work including breeding, colony maintenance, genotyping as well as necropsy and tissue processing
Funding Support	This award

7.2. 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

7.3. What other organizations were involved as partners?
Nothing to report

8. Special Reporting Requirements

9. Appendices