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14. ABSTRACT

Transformation of castration resistant prostate cancer (CRPC) towards androgen signaling independence has emerged as a resistance mechanism in a subset of metastatic CRPC following exposure to androgen receptor (AR)-targeted therapies such as abiraterone or enzalutamide. Clinically, patients typically present with progression in the setting of a low or modestly rising serum prostate specific antigen (PSA) and metastatic biopsies can show pathologic or molecular features consistent with neuroendocrine prostate cancer (NEPC). NEPC is associated with low or absent AR expression, suppressed AR signaling, retention of early genomic mutations from its adenocarcinoma precursor, and acquisition of distinct genomic and epigenomic alterations (Beltran H, et al, Nature Medicine, 2016). The development of novel therapeutic approaches for patients with NEPC represents a clinical unmet need. Over the last seven years, our group has focused on characterizing the molecular landscape of NEPC and have identified and validated new therapeutic targets, including the N-Myc/Aurora A pathway and specific epigenetic modifiers such as (Enhancer of Zeste Homolog 2) EZH2 (Beltran H, Rickman DS et al Cancer Discovery 2011; Dardenne E, Beltran H, …. and Rickman DS, Cancer Cell 2016).

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TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Transformation of castration resistant prostate cancer (CRPC) towards androgen signaling independence has emerged as a resistance mechanism in a subset of metastatic CRPC following exposure to androgen receptor (AR)-targeted therapies such as abiraterone or enzalutamide. Clinically, patients typically present with progression in the setting of a low or modestly rising serum prostate specific antigen (PSA) and metastatic biopsies can show pathologic or molecular features consistent with neuroendocrine prostate cancer (NEPC). NEPC is associated with low or absent AR expression, suppressed AR signaling, retention of early genomic mutations from its adenocarcinoma precursor, and acquisition of distinct genomic and epigenomic alterations (Beltran H, et al, Nature Medicine, 2016). The development of novel therapeutic approaches for patients with NEPC represents a clinical unmet need. Over the last seven years, our group has focused on characterizing the molecular landscape of NEPC and have identified and validated new therapeutic targets, including the N-Myc/Aurora A pathway and specific epigenetic modifiers such as (Enhancer of Zeste Homolog 2) EZH2 (Beltran H, Rickman DS et al Cancer Discovery 2011; Dardenne E, Beltran H, …. and Rickman DS, Cancer Cell 2016).

Hypothesis: *N-Myc is an early driver of the NEPC phenotype, and that by tracking N-Myc signaling during the transition from adenocarcinoma to NEPC, we can identify cooperating factors that promote NEPC progression and define appropriate time points for early intervention.*

To address this hypothesis we formulated 3 Specific Aims:

Specific Aim 1: To assess the timing of N-Myc activation in patients and define how N-Myc and NEPC signaling impacts prognosis. The working hypothesis of this **Aim** is that there are specific molecular changes that serve as early markers of tumors that will evolve to NEPC. We will assess patient tumors at various timepoints during disease progression from prostate adenocarcinoma to NEPC and correlate N-Myc expression and NEPC signaling with clinical features and outcomes. We will also investigate MYCN and AURKA genomic amplification in tumors and circulating tumor DNA (ctDNA). Specifically we will evaluate: 1) localized prostate cancer patients treated with prostatectomy (see **letter of Support, GenomeDx**); 2) high risk localized patients treated with or without neoadjuvant docetaxel and androgen deprivation therapy (NCT00430183); 3) patients with metastatic CRPC treated with abiraterone, and enzalutamide (IRB1305013903, PI Beltran); 4) NEPC patients treated with the Aurora A inhibitor MLN8237 (NCT01799278, PI Beltran). Gene expression will be quantified using a custom-designed assay validated by our group and amenable to FFPE tissues. N-Myc, Aurora A, and AR signaling genes, stem cell, neuronal, and other NEPC markers will be assessed. AURKA and MYCN amplification will be evaluated in tumors and ctDNA by targeted exome sequencing and/or FISH. We predict that N-Myc/NEPC signaling may be detected early in a subset of high-risk patients and this represents the presence of treatment resistant cells, and that this signaling program is enhanced after both short and long term AR-targeted therapies. The detection and determination of the frequency of early NEPC-associated alterations will help molecularly define subsets of prostate adenocarcinomas as harbingers of NEPC. This has clinical implications towards the development of diagnostic, prognostic, and predictive biomarkers to be evaluated early (potentially at the time of prostate cancer diagnosis) to select individuals at high risk for progression for early intervention. These results will also help identify patients less likely to benefit from AR-targeted strategies and more likely to benefit from MLN8237 or other N-Myc directed approaches.

Specific Aim 2: To assess the impact of timing of N-Myc expression on the development of castration resistance and the NEPC phenotype. The working hypothesis of this **Aim** is that N-Myc drives the trans-differentiation of castration resistant adenocarcinomas towards the NEPC phenotype. In order to model the evolutionary acquisition of N-Myc signaling during

prostate cancer progression, we will temporally regulate N-Myc expression either at (3 months) or after (6 months and 9 months) the time of disease onset following Pten deletion both in intact mice and in mice castrated at 6 months. We will to monitor Pten mutant cells (using YFP) before N-Myc and after N-Myc (Td-Tomato) overexpression and lineage trace emerging clones that develop histological and molecular features of NEPC. We expect that castration will lead to a quicker N-Myc-induced onset of NEPC.

Specific Aim 3: To evaluate the influence of N-Myc timing on response to NEPC directed therapeutics. The working hypothesis of this **Aim** is that N-Myc over-expression sensitizes CRPC to NEPC-directed therapeutics. We will test N-Myc-early and N-Myc-late models with drugs that have demonstrated efficacy against NEPC including Aurora kinase A inhibition, platinum chemotherapy and EZH2 inhibitors. We will also evaluate the effect of the EZH2 inhibitors as priming agents with Aurora-A or AR-directed therapies (enzalutamide) in delaying or reversing AR-independent resistance *in vitro* and *in vivo*. We predict that early N-Myc expression results in comparable response to N-Myc targeted therapies as NEPC and early treatment with these agents modulates NEPC transcriptional programs. Results from this **Aim** will aid in biomarker selection for future trials.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Castration resistant prostate cancer, androgen receptor (AR)-targeted therapy, neuroendocrine prostate cancer, N-Myc transcription factor, Polycomb Repressive Complex 2-associated protein Enhancer of Zeste Homolog 2

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

a. Major goals of the project

1. Specific Aim 1: To assess the timing of N-Myc activation in patients and define how N-Myc signaling impacts response to aurora kinase A inhibition and clinical outcomes.
    a. Evaluate localized prostate cancer patients treated with prostatectomy (See letter of Support, GenomeDx).
    b. Evaluate high risk localized patients treated with or without neoadjuvant docetaxel and androgen deprivation therapy (NCT00430183).
    c. Evaluate pre-treatment metastatic biopsies from CRPC patients treated with abiraterone, and enzalutamide (IRB1305013903).
    d. Evaluate pre-treatment metastatic biopsies from NEPC patients treated with the Aurora A inhibitor MLN8237 (NCT01799278) on a Phase 2 clinical trial or the EZH2 inhibitor GSK126 (NCT02082977).

2. Specific Aim 2: Assess the impact of timing of N-Myc expression on the development of castration resistance and the NEPC phenotype.

a. Assess the influence of N-Myc on the onset of CRPC and NEPC and associated molecular changes.
b. Assess the role of N-Myc/EZH2 complex for N-Myc dependent transcriptional regulation in establishing the NEPC phenotype.
c. Assess the role of N-Myc/Aurora-A complex for N-Myc dependent transcriptional regulation in establishing the NEPC phenotype

3. Specific Aim 3: Determine the impact of the timing of N-Myc on response to NEPC directed therapeutics.
    a. Monitor the impact of the timing of N-Myc expression in response to NEPC-related monotherapies *in vivo* and *in vitro.*
    b. Monitor the impact of the timing of N-Myc expression in response to NEPC-related combination therapies *in vivo* and *in vitro.*
    c. Mechanistic studies to determine NEPC-related molecular changes associated with EZH2 and Aurora A inhibition *in vitro* and *in vivo*

b. Accomplishments under these goals

1. Specific Aim 1: To assess the timing of N-Myc activation in patients and define how N-Myc signaling impacts response to aurora kinase A inhibition and clinical outcomes.

Clinical NEPC is associated with neural lineage. In our previous studies, we demonstrated that N-Myc expression was associated with NEPC features[1], suggesting that N-Myc may be associated with poor clinical outcome. To directly address this, we assessed overall survival (OS) in 81 patients (57 CRPC-Adeno, 24 NEPC) with outcomes data. CRPC-Adeno patients with metastatic biopsies harboring high N-Myc expression showed a significantly worse OS compared to those with low N-Myc expression (37.7 versus 80.3 months, hazard ratio (HR) 1.95, 95% confidence interval (CI) 0.92-4.09, p-value=0.040, **Fig. 1a**), and this was also significant when also taking into consideration patients with NEPC alone (HR 3.31, 95% CI 1.22-9.09, p-value=0.006). or combined (34.0 versus 76.5 months, HR 2.27, 95% CI 1.24-4.11, p-value=0.002). While these data reveal a correlation between N-Myc expression and poor clinical outcome, the underlying mechanism remains poorly described. Although there is a spectrum within the pathologic subtype of NEPC, we have found that NEPC tumor cells defined by morphologic features often lose AR expression and express neuroendocrine markers[2]. We performed whole transcriptome analyses on an expanded cohort of patients including those for whom survival data were not available. Gene expression was assessed from metastatic tumor biopsies of patients with pathology confirmed NEPC (n=36) and CRPC-Adeno (n = 73), as well as localized prostate adenocarcinoma (PCa) (n = 66) and benign prostate (n= 29) from prostatectomy specimens. Relative to benign or PCa, NEPC tumors demonstrated a significant enrichment for stem cell genes associated not only with normal neuroendocrine cell precursors (neural crest stem cells (NCSC)), but also with activated neural stem cells (NSC) and embryonic stem (ES) cells (**Fig. 1b**). To validate the association with NSC genes, we purified murine NSC from the sub-ventricular zone and performed RNA-seq and histone-3 lysine-4 tri-methylation (H3K4me3) ChIP-seq. From these studies, we combined the upregulated genes marked by H3K4me3 with publicly available single-cell RNA-seq data from activated NSC[3] and revealed a similar enrichment of NSC pathways in NEPC patients samples versus benign prostate tissue samples (NES=1.37, FDR q-value=0.039, **Fig. 1b**) and versus PCa samples (NES=1.13, FDR q-value=0.23). Unsupervised clustering analysis of the NEPC versus PCa leading-edge genes from ESC, NCSC or NSC gene sets[4-6]

segregated patients according to their tumor histological classification (PCa, CRPC-Adeno or NEPC). Interestingly, in the subset of the CRPC-Adeno patients that expressed *MYCN* at comparable levels as NEPC, *MYCN* expression correlated with expression of neural lineage genes and inversely with AR signaling (**Fig. 1b).** We therefore sought to determine the precise mechanism that drives a prostate tumor epithelial cell to lose its luminal markers and gain a more neural-like lineage.

N-Myc associated with response to alisertib (MLN8237). In a phase 2 trial of alisertib for patients with castration resistant and neuroendocrine prostate cancer (NCT01799278), 60 patients were treated. Median PSA was 1.1, number of prior therapies was 3, and approx. 70% of patients had visceral metastases. Although six month progression free survival was 13.5%, four exceptional responders were identified, including complete resolution of liver metastases, all of which had evidence of N-myc overactivity. Further analysis of metastatic pre-treatment biopsies and N-myc signatures genes is ongoing. Patient derived organoids of two patients on study (PM154, PM155) were developed that demonstrated concordant response in vitro

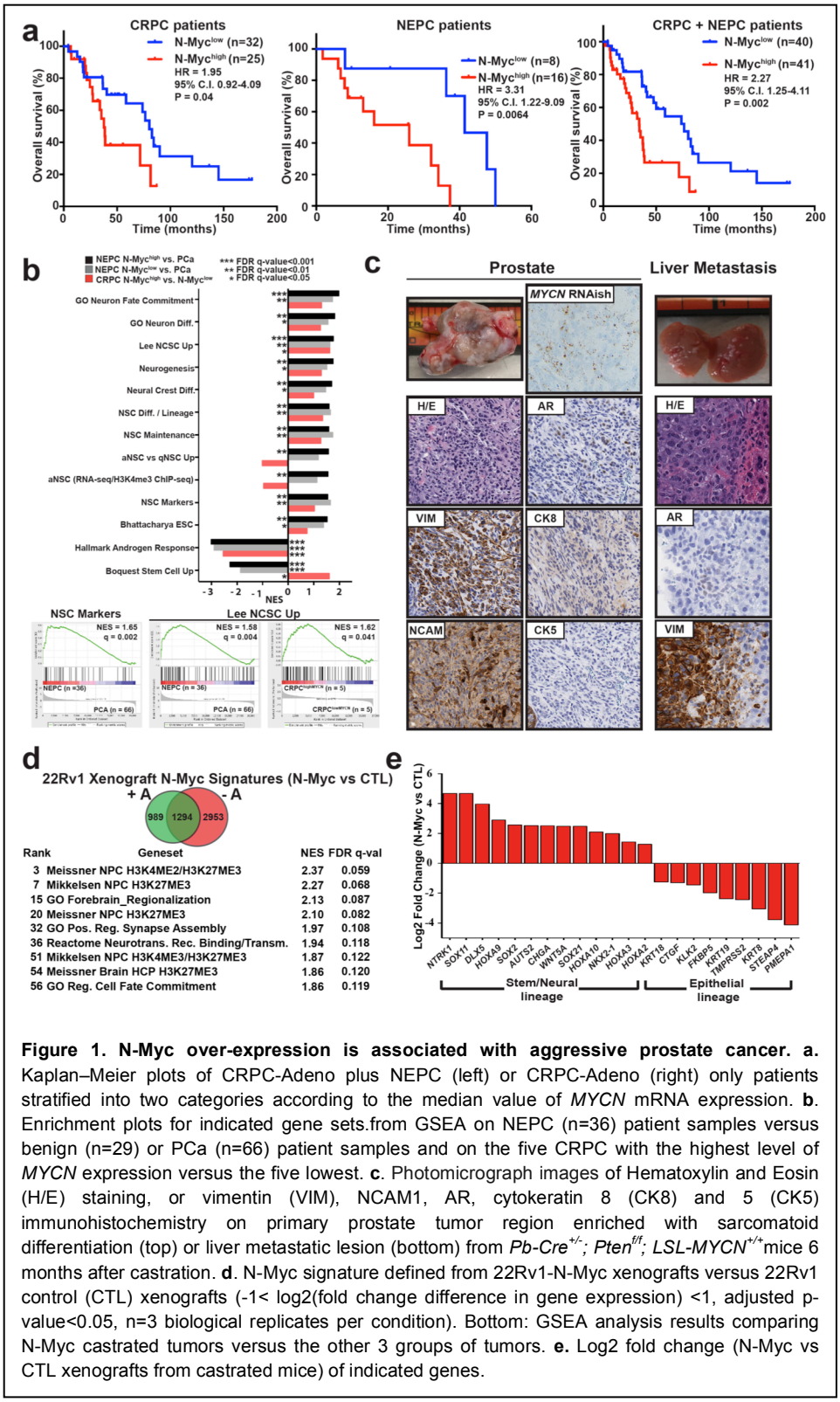

Figure 1. N-Myc over-expression is associated with aggressive prostate cancer. a. Kaplan–Meier plots of CRPC-Adeno plus NEPC (left) or CRPC-Adeno (right) only patients stratified into two categories according to the median value of *MYCN* mRNA expression. **b**. Enrichment plots for indicated gene sets.from GSEA on NEPC (n=36) patient samples versus benign (n=29) or PCa (n=66) patient samples and on the five CRPC with the highest level of *MYCN* expression versus the five lowest. **c**. Photomicrograph images of Hematoxylin and Eosin (H/E) staining, or vimentin (VIM), NCAM1, AR, cytokeratin 8 (CK8) and 5 (CK5) immunohistochemistry on primary prostate tumor region enriched with sarcomatoid differentiation (top) or liver metastatic lesion (bottom) from *Pb-Cre*$^{+/-}$*; Pten*$^{f/f}$*; LSL-MYCN*$^{+/+}$mice 6 months after castration. **d**. N-Myc signature defined from 22Rv1-N-Myc xenografts versus 22Rv1 control (CTL) xenografts (-1< log2(fold change difference in gene expression) <1, adjusted p-value<0.05, n=3 biological replicates per condition). Bottom: GSEA analysis results comparing N-Myc castrated tumors versus the other 3 groups of tumors. **e.** Log2 fold change (N-Myc vs CTL xenografts from castrated mice) of indicated genes.

as the patients did clinically and have been utilized for the dynamic testing of N-myc-Aurora complex formation and will be used for N-myc-EZH2 and other readouts.

N-Myc signaling in localized disease

In collaboration with GenomeDx, we analyzed transcriptome data of prostatectomy specimens from 9640 patients treated for localized prostate cancer with associated outcomes data. MYCN was expressed in approx 5% of localized prostate adenocarcinoma and did not associate with Gleason grade. MYCN expression correlated inversely with AR and positively with NEROG1, SOX11, AURKAIP1, and BRN2 expression. GSEA of this localized dataset also showed inverse correlation with EZH2 target genes, REST, and SUZ12 ($p<0.0001$; Pearson coeff >0.5). Further analysis, including neural genes, and correlation with clinical features and outcomes are ongoing.

2. Specific Aim 2: Assess the impact of timing of N-Myc expression on the development of castration resistance and the NEPC phenotype.

AR signaling alters the N-Myc transcriptome *in vivo.*

While we have previously shown that N-Myc expression in AR signaling-intact prostate cancer epithelial cells cooperates with Polycomb Repressive Complex 2 (PRC2)-associated protein Enhancer of Zeste Homolog 2 (EZH2) to abrogate AR signaling[1], the role of N-Myc in the context of low to no AR-signaling in driving lineage plasticity has not been characterized. Using our previously described *Pb-Cre*$^{+/-}$*; Pten*$^{f/f}$*; LSL-MYCN*$^{+/+}$ genetically engineered mouse model (GEMM)[1], we found that N-Myc overexpression *in vivo* induced a faster progression following castration. Although castration led to a slight increase in median survival (10.5 months compared to 7.5 for the non-castrated littermates), the mice developed invasive prostate tumors that metastasized to multiple locations including the liver at 6 months post-castration. We also noted an increase in poorly differentiated foci that lost expression of luminal (AR, cytokeratin 8 (CK8)) and basal (CK5) markers and gained expression of the epithelial-mesenchymal transition marker vimentin (VIM) and the NEPC marker neural cell adhesion molecule (NCAM1, **Fig. 1c**). Primary and metastatic lesions in castrated mice contained tumor foci with divergent differentiation (e.g., intestinal, squamous, sarcomatoid as previously described[1] or chondroid differentiation). In addition, we observed large foci of neural differentiation characterized by ganglion-like cells in both intact and castrated mice. These cells had abundant eosinophilic cytoplasmic and nuclei with prominent, centrally located nucleoli. These foci of tumor showed scattered cells with immunohistochemical staining for S100 (both nuclear and cytoplasmic), and they were negative for epithelial markers (e.g. AR, CK5, and CK8). The observed ganglion differentiation is also consistent with previous observations made in N-Myc-driven neuroblastoma models[7]. These data suggest that the removal of androgen signaling enables more aggressive tumors that display a variety of N-Myc-induced differentiation programs. To further define the transcriptional differences regulated by N-Myc in an androgen context-dependent manner, we performed RNA-seq on tumors derived from castrated or intact GEMM and on N-Myc-expressing 22Rv1 xenografts grown in castrated or intact recipients. Transcriptome-wide analyses revealed that castration was associated with a significant increase in the number of N-Myc-deregulated target genes (**Fig. 1d)**. In castrated 22Rv1 xenograft mice, the N-Myc-signature was enriched with neural-associated pathways (**Fig. 1e**), including neural progenitor cells (NPC) bivalent genes (H3K4me2/3 active and H3K27me3 repressive marks[8, 9], NES = 2.37, FDR q value < 0.059), in addition to other genes implicated in neural development (e.g. *SOX11, SOX21, NTRK1, NRXN2, NKX2-1, LRRN2*), expressed in adult stem cells (e.g. *FOXC1, HOXA2/A3/A9/A10, NKX2-3, PEG3, SOX6* and *WNT5A*), ES cells (e.g. *SOX2*) or NEPC (e.g. *CHGA*), while epithelial–lineage associated genes were downregulated (**Fig. 1e**). The same observation was made in the GEM model (**Fig. S1e**). These data suggest that the removal of circulating

androgen and AR signaling may impact N-Myc gene regulation. Since these datasets were generated from tumors following chronic androgen deprivation, we cannot rule out that these molecular changes did not evolve over time.

N-Myc cistrome is altered by AR-signaling and distinct from C-Myc. To directly assess the impact of acute removal of androgen on the N-Myc-induced molecular program, we performed, in replicate, RNA-seq and N-Myc chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) in isogenic LNCaP cells (+/- *MYCN*[1]) following short-term androgen withdrawal. LNCaP cells, a hormone-naive prostate cancer cell line with a well-characterized luminal/epithelial phenotype, were starved in androgen-deprived media for 72 hours, and subsequently re-exposed to androgen or maintained in androgen-deprived conditions for an additional 24 hours (**Fig. 2a**).

RNA-seq data revealed a distinct N-Myc driven transcriptional program with 8,585 genes differentially regulated in N-Myc cells compared to control (CTL) cells. Consistent with our observations *in vivo*, androgen withdrawal significantly altered the N-Myc signature *in vitro*. Thirteen percent and 42% of the N-Myc target genes are differentially regulated specifically in the presence or absence of androgen, respectively. In the absence of androgen, N-Myc-upregulated genes were enriched with stem cell signatures, including NPC bivalent genes (NES = 2.11 FDR q value = 6.8E-5, **Fig. 2b**). Furthermore, consistent with our previous study[1], in the presence of androgen, the N-Myc signature was de-enriched for AR target genes (NES = -1.52, FDR q value = 0.04) compared to LNCaP-CTL cells (**Fig. 2b**). To determine if the transcriptional changes that occur in the absence of androgen were reversible, we maintained cells in androgen-deprived condition for 41 days followed by a 24-hour androgen stimulation (**Fig. 2a**). After long-term withdrawal, the response to androgen stimulation was dramatically reduced (over 80% for *KLK4*, **Fig. 2b**). Altogether, these data support the hypothesis that there is interplay between AR and N-Myc signaling that propagates lineage plasticity and the transformation of prostate adenocarcinoma towards NEPC.

In the presence or absence of androgen, we observed a narrow peak corresponding to N-Myc binding mostly within 2 kilobases (Kb) of gene transcription start sites (TSS, **Fig. 3a**). Among the N-Myc peaks, approximately 40%, 25% and 25% were within intronic, intergenic and promoter/TSS regions, respectively, in both conditions. To define N-Myc-specific binding in prostate cancer cells, we compared the N-Myc cistrome with the C-Myc cistrome generated from LNCaP cells and with publicly available N-Myc ChIP-seq data from a *MYCN*-amplified neuroblastoma model[10]. Approximately half of the N-Myc bound sites were shared with C-Myc in LNCaP cells and/or N-Myc in neuroblastoma cells (**Fig. 3b-d**). Not surprisingly, the majority of these shared binding sites are found at genes involved in cell cycle and metabolism (data not shown), corresponding to the most well characterized functions of Myc family proteins[11]. The genes uniquely bound by C-Myc in LNCaP cells were enriched with cell cycle and cell proliferation-related genes, while the genes uniquely bound by N-Myc in neuroblastoma cells were neural lineage-related. Despite these overlaps, we observed that 44% of the N-Myc bound sites are specific to the prostate cancer cells and that these prostate-specific N-Myc-bound genes are enriched for PRC2 targets and neural-lineage genes (**Fig. 3d**). The difference between N-Myc and C-Myc binding in prostate cancer cells is also in accordance with the different profiles of expression of *MYC* and *MYCN* mRNA prostate adenocarcinoma (high *MYC*, low *MYCN*) or NEPC (low *MYC*, high *MYCN*). These results suggest that Myc-family members are not functionally-redundant and have specific targets that are regulated in a cell type-dependent manner.

The AR-dependent change in gene regulation could be explained by a change in N-Myc binding to chromatin. From our ChIP-seq data, we found that, while there was a significant overlap (58%) of N-Myc binding in the absence and presence of androgen, 42% of N-Myc binding was dynamic, changing in different contexts of AR signaling (**Fig. 3e**). Upon androgen withdrawal, N-Myc exited or was depleted from 36% of bound sites while being significantly enriched or completely redirected to new sites (6%). These dynamic N-Myc peaks were less enriched at promoter/TSS regions and more enriched at intergenic and intronic regions. We found that 50% of the peaks closest to the TSS were within 2 Kb of the TSS for common peaks, and within 16 Kb and 24 Kb of the TSS for peaks specific to the condition with androgen and without androgen, respectively. Comparison of the genes uniquely bound by N-Myc in the presence or absence of androgen revealed an enrichment for AR signaling and stemness/oncogenic pathways, respectively.

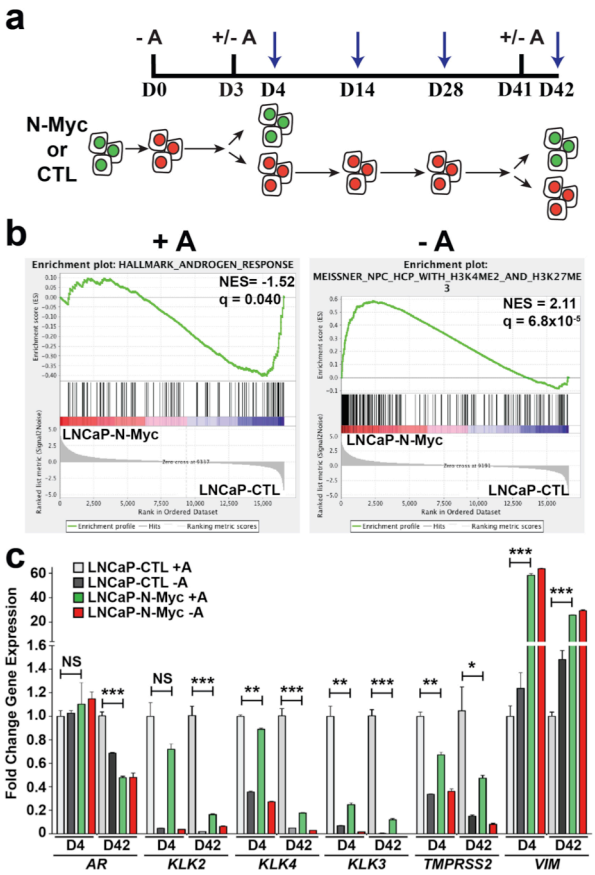

Figure 2. **a.** Schematic of experimental design with LNCaP-N-Myc or CTL cells and corresponding time points for RNA or ChIP-seq analyses (arrows). GSEA performed on +A or -A uniquely bound genes. Right: FC expression at indicated time points (n=3). **b.** Enrichment plots for the Androgen Response and the Meissner NPC H3K4me2/H3K27me3 gene sets from indicated conditions. **C.** Fold change expression of the indicated genes based on quantitative RT-PCR data performed on RNA extracted at the indicated time point in the indicated cells and conditions (n=3 technical replicates).

Differential N-Myc transcription co-factors or pioneering factors could explain this dynamic binding. Based on data from other cell types, it has been suggested that less than half of N-Myc binding sites on chromatin have consensus E-boxes and many do not even show variant E-boxes. This has formally been shown for C-Myc in two studies[12, 13]. Both studies conclude that the presence of an E-box enhances chromatin association but is not a prerequisite for binding. Most likely, therefore, protein/protein interactions play a large role in targeting N-Myc to sites on chromatin. To reveal such co-factors, we performed a *de novo* motif discovery in 200 bp regions surrounding all N-Myc binding sites identified by ChIP-seq. Motif analysis of the unique and enriched N-Myc peaks revealed that in addition to E-boxes, there was a significant enrichment for forkhead box (FOX) and homeobox (HOX) binding motifs (**Fig. 3f**). Using publicly available ChIP-seq data for AR and HOXB13 [14] in LNCaP cells or FOXA1 ChIPseq that we generated in the LNCaP-N-Myc cells, we compared the different classes of N-Myc binding sites (**Fig. 3e**). Interestingly, 61% of the unique N-Myc peaks in absence of androgen were also bound by FOXA1, 10% more compared to the overlap in the presence of androgen. Similarly we found that almost 30% of the unique N-Myc peaks without androgen were shared with HOXB13 peaks. This overlap reaches 45% when comparing our N-Myc ChIP-seq to HOXB13 CHIP-seq from clinical tumor samples [15].

In order to expand our observations to additional models of prostate cancer, we performed N-Myc ChIP-seq in 22Rv1-N-Myc xenografts and PM154 cells. ChIP-seq data from xenografts revealed similar proportions of dynamic peaks and distributions of N-Myc binding locations across the genome as LNCaP cells (**Fig. 3g**). Despite fewer numbers of N-Myc peaks in PM154, 79% of the peaks were in common with LNCaP-N-Myc and 22Rv1-N-Myc xenografts, including neural lineage-associated genes (*NREP, ULK2, RAB39A*) and bivalent genes. Moreover, we confirmed the N-Myc-dependent upregulation of these bivalent genes in 22Rv1 xenografts and NEPC patient-derived organoid models.

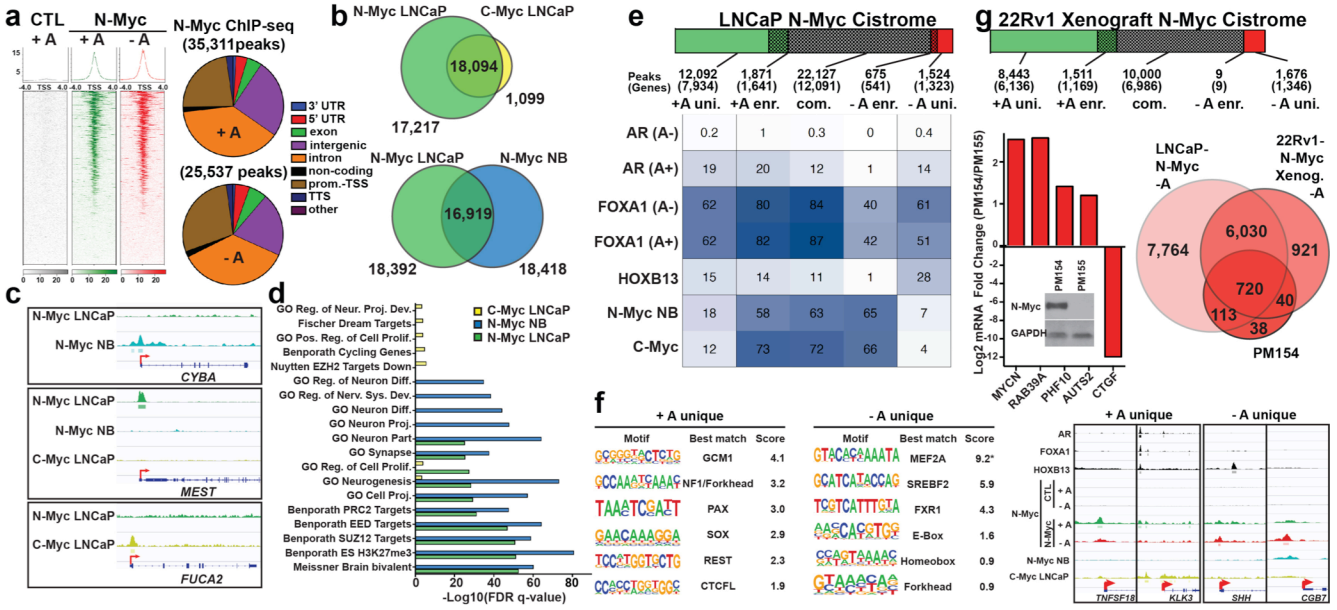

Figure 3. N-Myc binding is distinct from C-Myc in prostate cancer cells. a. Distributions and heatmaps of N-Myc ChIP-seq data generated from cells at day 4 (D4, n=2 biological replicates per condition). Right: Pie charts indicating the percent of N-Myc-bound sites at the indicated genomic annotation. **b.** Venn diagrams showing overlap of Myc family member binding in prostate cancer cells (LNCaP, top) or N-Myc in prostate cancer cells versus published N-Myc in neuroblastoma cells (LNCaP/BE2C, bottom). **c.** Examples of ChIP-seq tracks for indicated genes. **d.** GSEA performed on the uniquely bound genes as identified in **b**. **e.**Top: representation of N-Myc binding peaks determined by ChIP-seq in LNCaP-N-Myc cells with and without androgen, and their distribution throughout the genome (n=2 biological replicates per condition). Bottom: comparison of N-Myc peaks with AR and FOXA1 peaks with or without androgen, HOXB13, N-Myc in BE2C neuroblastoma cells and C-Myc peaks. Numbers express the percentage of N-Myc peaks in each condition overlapping with the indicated co-factor. **f.** Motif analysis of unique N-Myc peaks with or without androgen obtained by ChIP-seq in LNCaP-N-Myc cells. The scores correspond to the log2 (%target / %background). All the motifs shown are enriched with a p-value<10^{-5}. * % background of MEF2A motif was 0% and was subsequently adjusted to 0.001% to calculate a score. Right: ChIP-seq tracks for the indicated protein near the TSS of the indicated gene as representative examples of genes uniquely bound by N-Myc in the presence or absence of androgen in LNCaP cells. **g**. Representation of N-Myc binding peaks determined by ChIP-seq in 22Rv1-N-Myc xenografts with and without androgen (n=2 biological replicates per condition), and their distribution throughout the genome. Below: Overlap of N-Myc binding in LNCaP-N-Myc, 22Rv1-N-Myc and PM154 cells. Log2 fold change (N-Myc vs CTL xenografts from castrated mice, left) and Log2 fold change (PM154 versus PM155, right) of indicated genes after validation of N-Myc expression by Western Blot.

3. Specific Aim 3: Determine the impact of the timing of N-Myc on response to NEPC directed therapeutics.

By integrating the RNA-seq and ChIP-seq data, we identified 492 N-Myc-bound target genes that are deregulated specifically in the absence of androgen. GSEA analyses of these 492 genes showed enrichment for bivalent genes in neuronal cells (Meissner Brain HCP Bivalent (H3K4me3/H3K27me3), data not shown, NES = 1.45, FDR q-value = 0.65), consistent with the data from the 22Rv1 *in vivo* model (**Fig. 1d**). Genes associated with bivalent histone marks are well characterized in ES cells and have been shown to be essential for development and lineage-determination[16, 17]. The bivalent mark changes during the differentiation of the cells,

through the action of histone modifiers, and biases gene expression towards activation (H3K4me3 only) or repression (H3K27me3 only) depending on the identity of each cell.

Bivalent genes in N-Myc cells were enriched for PRC2, neurogenesis and neural lineage pathways while bivalent genes in control cells were associated with gene regulation and stress response**.** Moreover, androgen withdrawal dramatically increased the level of enrichment of PRC2 and neural-associated genes. Among the N-Myc-bound, bivalent genes, a subset showed a decrease of H3K4me3 and an increase of H3K27me3 levels in the absence of androgen. An example of this was observed for the desmocollin 3 gene (*DSC3)*, which has been implicated in epithelial cell junctions[18]. Inversely, many genes involved in neural lineage specification, such as the NK2 homeobox 2 gene (*NKX2-1)*, became bivalent by gaining H3K4me3 mark in the N-Myc cells, suggesting the activation of gene expression. To determine the clinical relevance of these findings, we queried the RNA-seq from our patient cohort and performed a targeted GSEA on the bivalent-associated genesets. NEPC clinical samples were enriched for neural lineage expressed bivalent genes compared to PCa or benign samples (**Fig. 4a**). Interestingly, this enrichment was also observed in CRPC-Adeno samples with high levels of N-Myc expression when compared to CRPC-Adeno samples with low levels of N-Myc expression. We then focused on the N-Myc-bound bivalent neural-associated genes defined in LNCaP cells, and found that they were up-regulated in NEPC compared to PCa or CRPC-Adeno patient samples (**Fig. 4a**). We also observed an upregulation of these genes during the transition from benign to NEPC, as illustrated with *NKX2-1*, Insulinoma-associated 1 *(INSM1)* and homeobox C9 (*HOXC9*). A corresponding downregulation was observed for epithelial lineage-linked genes such as *FGFRL1* and *DSC3*. Finally, the 966 bivalent genes that we identified in androgen-deprived LNCaP-N-Myc cells successfully classified the PCa, CRPC-Adeno and NEPC patient samples (**Fig. 4b**), suggesting that these genes associated with

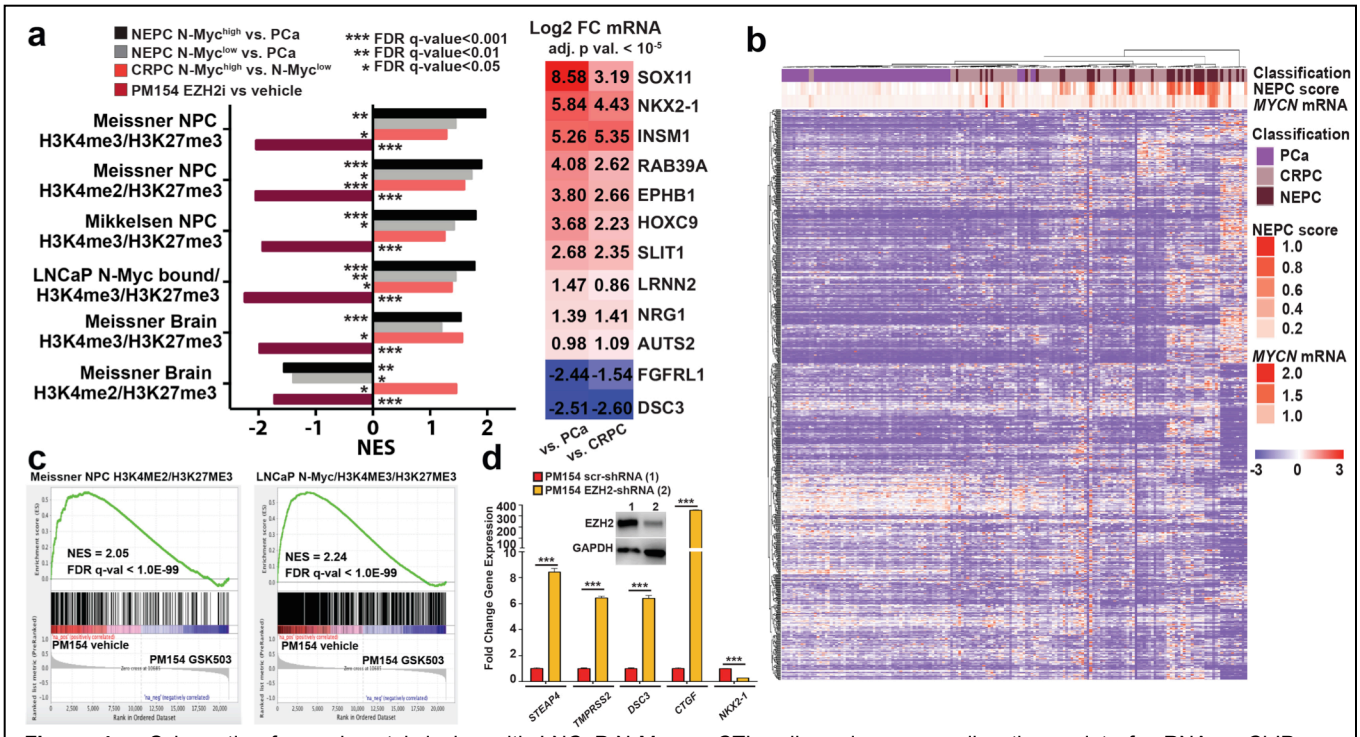

Figure 4. **a** Schematic of experimental design with LNCaP-N-Myc or CTL cells and corresponding time points for RNA or ChIP-seq analyses (arrows). GSEA performed on +A or -A uniquely bound genes. Right: FC expression at indicated time points (n=3). **b.** Unsupervised clustering of clinical samples based on the expression level of the 966 bivalent and N-Myc bound genes in LNCaP-N-Myc cells without androgen. **c.** GSEA of indicated gene sets in PM154 treated with vehicle vs EZH2 inhibitor GSK503. **d.** Fold change expression of the indicated genes based on quantitative RT-PCR data (n=3) in PM154 following EZH2 knockdown.

bivalent marks in N-Myc-expressing cells play a critical role in prostate cancer evolution and may help to identify patients who are most likely to develop NEPC.

We have previously shown that, in the context of active AR signaling, N-Myc cooperates with EZH2 (H3K27me3 writer) and redirects its activity to downregulate AR target genes[1]. Using an *in situ* proximity ligation assay (PLA) to monitor EZH2 and N-Myc complex formation, we confirmed the presence of the EZH2/N-Myc complexes in LNCaP, 22Rv1-N-Myc (with and without AR-targeted treatment) cells and in an AR-negative, NEPC patient-derived organoid (PM154, **Fig. 5a**). This suggests that N-Myc and EZH2 maintain a protein-protein interaction in the absence of AR that may regulate the H3K27me3 status of the bivalent genes. To address

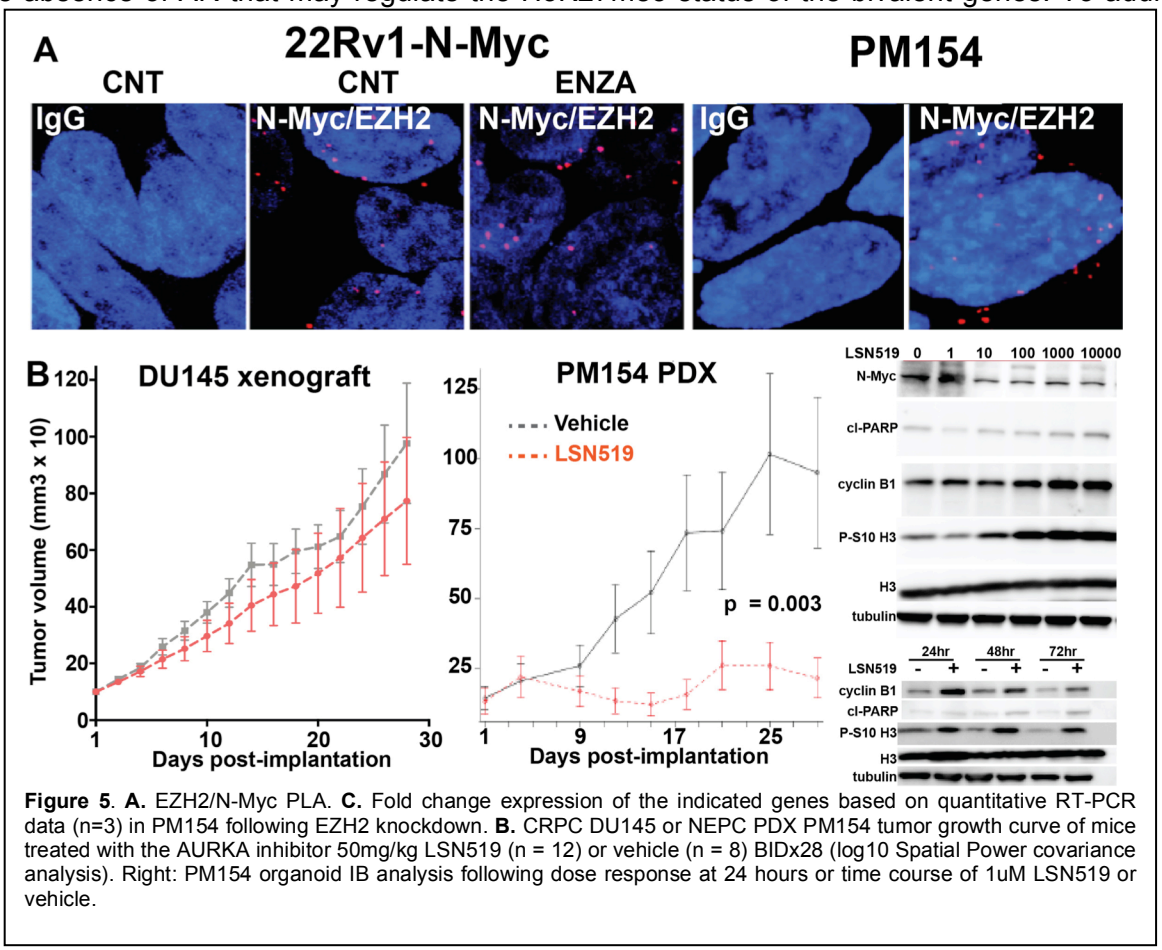

Figure 5. **A.** EZH2/N-Myc PLA. **C.** Fold change expression of the indicated genes based on quantitative RT-PCR data (n=3) in PM154 following EZH2 knockdown. **B.** CRPC DU145 or NEPC PDX PM154 tumor growth curve of mice treated with the AURKA inhibitor 50mg/kg LSN519 (n = 12) or vehicle (n = 8) BIDx28 (log10 Spatial Power covariance analysis). Right: PM154 organoid IB analysis following dose response at 24 hours or time course of 1uM LSN519 or vehicle.

this, we performed shRNA-mediated knockdown of EZH2 in PM154 cells. After validation on genes that have been previously shown to be regulated by EZH2 (*STEAP4, TMPRSS2*), we observed that knockdown of EZH2 led to a dramatic upregulation of the bivalent genes that were previously downregulated by N-Myc (**Fig. 5g**). In addition, we have previously shown that N-Myc down-regulated AR signaling genes were significantly up-regulated following EZH2 inhibition (genetically and pharmacologically (GSK343)) suggesting phenotype reversion [1]) similar to the DKO GEM data [19]. Moreover, shRNA-mediated knockdown of EZH2 or pharmacological inhibition of EZH2 activity with GSK503 in the AR negative, NEPC PM154 cells resulted in a dramatic upregulation of the bivalent and AR target genes that were previously downregulated by N-Myc (**Fig. 4c,d**). *Interestingly, AR and glucocorticoid receptor*

expression [20] did not change and remained low to negative (not shown) suggesting another mechanism of EZH2-related AR target gene regulation. Based on this data, we predict that EZH2 inhibition will revert cells to more of a luminal phenotype that could potentially enhance enza sensitivity at earlier stages of CRPC to NEPC transformation but not bone fide AR negative NEPC cells ("terminal NEPC").

Recently, we have found that the orally available and specific AURKA inhibitor LSN3199519 (LSN519, 50mg/kg, BID x 28 days) from Eli Lilly is effective in abrogating tumor growth *in vivo* using the alisertib-resistant NEPC PDX PM154 (**Fig. 5b**) and shows on-target catalytic inhibition (compensatory induction of histone 3 serine 10 phosphorylation (P-S10 H3) induces cell cycle arrest (cyclin B1 induction) and apoptosis (cleaved PARP (cl-PARP) in vitro. Based on blood analysis at day 1 and day 28, LSN519 exposure averaged 3.6 and 3.7 uM blood concentration versus 9.9 and 6.5 nM unbound LSN519, respectively which in line with it in other mouse efficacy studies. In support of this, Dr. Beltran has completed a screen with Cure First (http://www.curefirst.org/) with human NEPC organoids to identify FDA-approved drugs that can synergize EZH2 inhibitor GSK503 and identified alisertib as one of the top hits (z score = -0.97 (PM154); z score= -1.80 (PM155)). *More work is needed to determine genetic or pharmacologic interventions (and at what point during the transformation process) can fully or partially reverse the transformation from CRPC towards NEPC.*

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

Drs Beltran and Rickman are fully committed to furthering the training and professional development of the post-doctoral fellows and students affiliated to this project. This has including presentations at the AACR – Prostate Cancer December 2017, Orlando, FL; Prostate Cancer SPORE programs Annual Retreat, February 2018, Ft. Lauderdale, FL; AACR April 2018; Society of Basic Urologic Research, Palm Beach, CA Nov 2018. Postdoc fellows also participate in the following:
NIH T32 MTOR Course. 15 lectures, especially designed for MTOR, will be presented by our preceptors jointly with their clinical collaborators to showcase the principles of translational cancer research and team science.

Transitioning to Research Independence seminar series. A seminar series geared towards postdoctoral researchers and fellows who plan to set up their own laboratory and develop their own research program at an academic institution. Trainees will comprehend the faculty application process; understand strategies for funding, mentoring and leadership to effectively run their own research group.

Biostatistics Lecture Series through the Clinical and Translational Science Center. This is a monthly lecture series geared towards clinical investigators, research staff, and students who are interested in gaining insight into fundamental research design and statistical concepts with a focus on practical knowledge.

How were the results disseminated to communities of interest?

We presented this initial data at the Multi-institutional Prostate Cancer Meeting in Ft Lauderdale in February 2018 which is organized and led by the SPORE programs as an oral presentation. This data was also presented at AACR – Prostate Cancer December 2017, Orlando, FL; Prostate Cancer SPORE programs Annual Retreat, march 2018, Ft. Lauderdale, FL; AACR April 2018; Society of Basic Urologic Research, Palm Beach, CA Nov 2018. The following manuscripts are related to this project:

1. Puca, L., et al., Patient derived organoids to model rare prostate cancer phenotypes. Nat Commun, 2018. **9**(1): p. 2404.

2. Beltran, H., et al., A phase II trial of the aurora kinase A inhibitor alisertib for patients with castration resistant and neuroendocrine prostate cancer: efficacy and biomarkers. Clin Cancer Res, 2018.

3. Berger A, Brady NJ, Bareja R, Dardenne E, Robinson B, Vincenza Conteduca V, Augello MA, Ahmed A, Puca L, Hwang I, Bagadion AM, Sboner A, Elemento O, Paik J, Barbieri CE, Dephoure N, Beltran H and Rickman DS. N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer (Nature Cell Biology, in revision).

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Related to Aim 1, we continue to recruit CRPC and NEPC patients for this study. Given the limited availability of fresh/frozen samples especially of archival primary tumors, we have been developing a custom-designed targeted mRNA assay to evaluate N-myc target gene expression amenable to formalin fixed paraffin embedded tissue samples using Nanostring approach (an approach previously described - Beltran et al, CCR 2017). This will allow for assessment of dynamic changes in N-Myc and N-Myc target gene expression, AURKA, EZH2, and other genes during disease progression towards the NEPC phenotype. We will correlate these changes with genomics (including TP53 and RB1 loss). We continue ongoing analyses of clinical cohorts with outcomes data including MLN8327 trial patients (pre-treatment and progression biopsies), pre-treatment biopsies CRPC patients treated with abi/enza (SU2C-PCF Dream Team cohort of 500 patients, in process) and localized cohorts including those with GenomeDx data and an addition cohort from CALGB90203 (nanostring data in process).

Related to Aim 2, we will continue our analyses of the N-Myc cistrome and how N-Myc impacts transcriptomic reprogramming interacting co-factors that account for the induction of a lineage plastic state that favors the development of NEPC in our models (GEM, xenograft, cell lines and patient-derived NEPC organoids),. We will perform genetic experiments (knock-in/knock-out) of specific cofactors to determine their cooperative role with N-Myc in driving this phenotype. These experiments will be done in the context of Aim 1.

Related to Aim 3, we will, focus on compounds that have been shown to be stable in animals. In addition to early stage drug development, we are also exploring additional opportunities for clinical trial development for NEPC, specifically around EZH2, given number of new EZH2i drugs now in later stages development (Eli Lilly, Constellation, Epizyme, GSK).

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Patient derived organoids were developed from patients on the MLN8237 trial and the GEMs being generated represent new models of NEPC. To study the underlying biology of the *MYCN* over-expression in prostate cancer, we have created several in vitro (isogenic LNCaP and 22Rv1 [1]), in vivo (e.g. GEM models, GEM-derived allografts, isogenic xenografts [1] and PDXs [21]) and ex vivo models (mouse and human cell lines and organoids [1, 21-23]. Dr. Beltran has established 3 CRPC and 4 NEPC organoids. Two of the NEPC organoids (PM154 and PM155 [21]) are from patients enrolled on the Phase 2 Aurora kinase A (AURKA) inhibitor (alisertib) clinical trial, have been characterized extensively and retain all genomic alterations from their matched tumor [24]. Clinically, PM154 progressed after 3 cycles of alisertib expresses high levels of N-Myc and EZH2 (see **Fig 3g**); whereas, PM155 demonstrated significant symptomatic and radiographic response with near complete remission maintained for 18 months. Genomic analyses of the other organoids are pending. In addition to the human organoids, we have also developed *in vivo* (e.g. GEM, xenos, murine allografts) and *in vitro* (e.g. GEM prostate cancer organoids, isogenic cells) models that show that N-Myc drives the NEPC phenotype [1]. In order to recapitulate N-Myc over-expression observed in clinical prostate cancer samples (i.e. after onset of disease initiation) and to track N-Myc expressing cells following other genetic alterations (e.g. *Pten* or *Rb1* loss) we are generating improved GEMs. These GEMs will allow for temporal control of N-Myc expression after disease onset and castration, cell lineage tracing and cell enrichment for further downstream molecular characterizations and drug testing (**Fig. 6**). We have engineered the mice to have a prostate epithelial cell specific-Cre-dependent floxed *Pten* with or without floxed *Rb1* [25] alleles and with or with a doxycycline (dox)-inducible human *MYCN* allele [26]. Pilot experiments are ongoing to determine optimal dosing of dox in the drinking water [27] or in the chow [28]. The *Pten* null only and DKO cells will be tracked using an incorporated Rosa26-YFP (green) locus. N-Myc expressing cells will be tracked with Td-Tomato (red) using an independent dox-inducible allele upon Dox withdrawal [29]. All lines of mice are bred on the same mixed genetic background (C57/Bl6/129x1/SvJ).

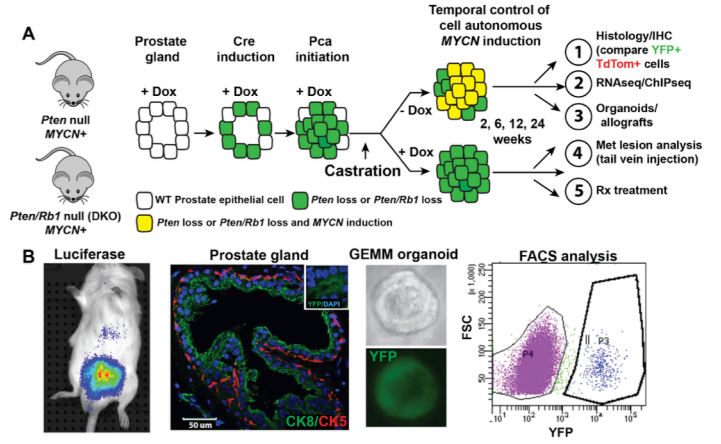

Figure 6. A. Experimental design for cell autonomous N-Myc over-expression following Pten loss or Pten/Rb1 double loss induced prostate adenocarcinoma in GEMs to generate 4 genotype conditions and indicated downstream analyses. **B.** Example of an non-induced (Dox-) 5-week old Pb-Cre+; TRE-MYCN-luc; Rosa26-LSL-YFP/ztTA mouse showing high levels of luciferase activity (luciferin injected/imaged), and YFP –positive (insert) prostate luminal cells (CK8+ (luminal) vs CK5+ (basal)), YFP-positive GEM derived organoids and FACS analysis on dissociated organoids showing YFP-positive specific population.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Dr. Beltran moved to Dana Farber Cancer Institution. No other changes/problems to report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications.

1. Puca, L., et al., Patient derived organoids to model rare prostate cancer phenotypes. Nat Commun, 2018. **9**(1): p. 2404; yes

2. Beltran, H., et al., A phase II trial of the aurora kinase A inhibitor alisertib for patients with castration resistant and neuroendocrine prostate cancer: efficacy and biomarkers. Clin Cancer Res, 2018; yes

3. Berger A, Brady NJ, Bareja R, Dardenne E, Robinson B, Vincenza Conteduca V, Augello MA, Ahmed A, Puca L, Hwang I, Bagadion AM, Sboner A, Elemento O, Paik J, Barbieri CE, Dephoure N, Beltran H and Rickman DS. N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer (Nature Cell Biology, in revision); yes

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

We presented this initial data at the Multi-institutional Prostate Cancer Meeting in Ft Lauderdale in February 2018 which is organized and led by the SPORE programs as an oral presentation. This data was also presented at AACR – Prostate Cancer December 2017, Orlando, FL; Prostate Cancer SPORE programs Annual Retreat, march 2018, Ft. Lauderdale, FL; AACR April 2018; Society of Basic Urologic Research, Palm Beach, CA Nov 2018.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

- Nothing to report.

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	*Himisha Beltran, MD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	*Work on Aim 1,2,3*
Funding Support:	
Name:	*David Rickman, PhD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Work on Aim 1,2,3*
Funding Support:	
Name:	*Brian Robinson, MD*
Project Role:	*co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	*Work on Aim 1,2*
Funding Support:	
Name:	*Nicholas Brady*
Project Role:	*Post-doctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Work on Aim 2*
Funding Support:	*This award and as of Sept. 2018 T32 training award*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not

necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

2017 PCF Challenge Award (Zoubeidi / Beltran)
12/31/2017– 12/31/2019 0.48 calendar
Prostate Cancer Foundation
Targeting BRN2 in Neuroendocrine Prostate Cancer
Goals/Aims: To assess if BRN2 inhibitors should be deployed alone or in combination with current standard-of-care to block the emergence and/or progression of NEPC.

P50 CA211024 Specialized Programs of Research Excellence (SPORE) (Rubin)
National Cancer Institute
08/01/2017-07/30/2022
Weill Cornell Medicine (WCM) SPORE in Prostate Cancer
Project 1: *Non-invasive clinical assay for early detection of treatment resistance in patients with metastatic prostate cancer* (Beltran/Demichelis)
Project 2: *Targeting N-MYC and EZH2-Driven Castrate Resistant Prostate Cancer* (Project Leaders: Rickman / Beltran)
Effort: 1.2 calendar (Project 1); 1.2 calendar (Project 2)
Goals/Aims Project 1: The goals of this project are to determine tumor dynamics and the clinical impact of circulating alterations in predicting response to AR-directed therapy and define the spectrum of circulating DNA alterations in patients with metastatic CRPC.
Goals/Aims Project 2: The goal of this project is to develop more effective targeting strategies for a biomarker-selected subgroup of late stage CRPC driven by N-Myc and less dependent on the AR.

W81XWH-17-PCRP-IA (Tagawa/ Beltran/ Bander)
07/01/2018-06/30/2021 0.36 calendar
Department of Defense: Prostate Cancer Research Program
Molecular and clinical correlates with prostate-specific membrane antigen (PSMA)-targeted radionuclide therapy
The goal of this project is to determine the best genomic, clinical, and imaging characteristics for successful PSMA-TRT and described immune response from PSMA-TRT. Specific Aims: 1. Prospectively and retrospectively assess genomic biomarkers and gene expression changes associated with outcome from anti-PSMA targeted radionuclide therapy. 2. Prospectively and retrospectively assess clinical parameters associated with outcome from anti-PSMA- TRT. 3. Prospectively and retrospectively assess PSMA expression as determined by PSMA molecular imaging associated with response to anti-PSMA -TRT. 4. Evaluate generation of an immune response following anti-PSMA-TRT in association with clinical outcome.

8

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.