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TITLE: High-Throughput Sequencing of Germline and Tumor From Men with Early-Onset Metastatic Prostate Cancer

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<b>14. ABSTRACT</b> This research project focused on genetic analysis of a rare subset of prostate cancer cases: men presenting with metastatic prostate cancer at a young age (before age 60 years). Whole exome sequencing identified a panel of germline variants that have biological as well as genetic evidence suggesting that they may be related to inherited susceptibility of early-onset aggressive prostate cancer. Comprehensive tumor genome and/or transcriptome sequencing of tumors from these patients identified an increased frequency of TP53 mutations compared to previously-reported unselected populations suggesting that this may be used as a prognostic marker for aggressive disease in the future. Finally, analysis of paired samples from unique patients confirmed the role of transdifferentiation as the mechanism for the development of NePC as well as CTNBN1 and PIK3CA mutations in adaptive resistance to treatment.						
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# 1. INTRODUCTION

In this project, next generation sequencing (NGS) approaches were used to analyze germline (blood) and prostate cancer tissue from men with newly diagnosed Stage 4 (Tx N1 or M1) prostate cancer. All participants were diagnosed with prostate cancer at or before age 60 years and had metastatic disease at (or within one year) of presentation. Men of European and African American descent were included in this study. The goal of this project was to identify germline variants that increase the risk of developing clinically significant prostate cancer, as well as novel driver somatic alterations. We hypothesized that men with early-onset aggressive prostate cancer are more likely to harbor such variants.

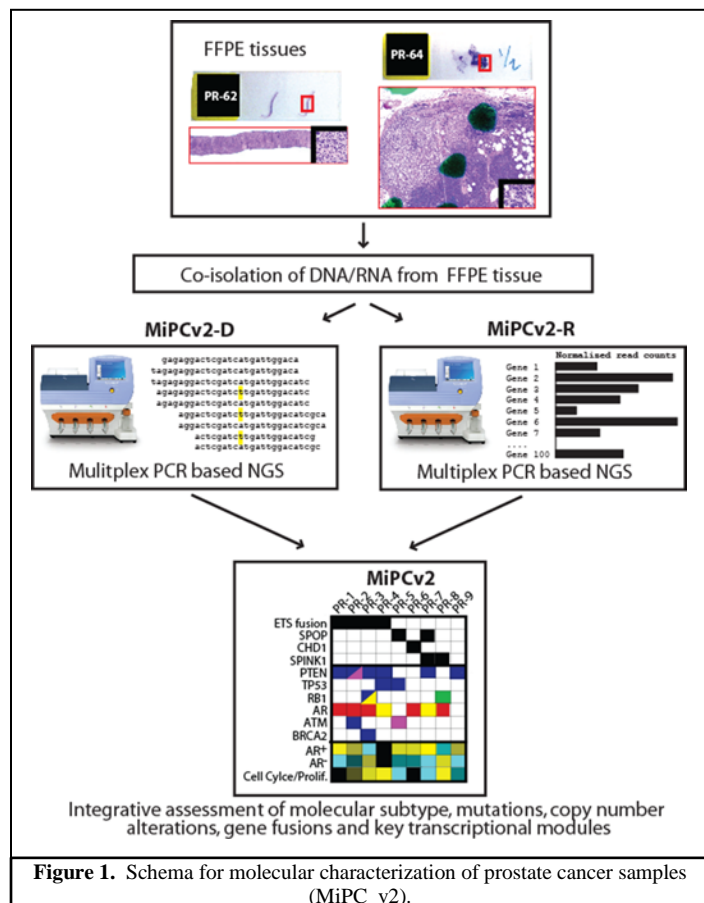
# 2. KEYWORDS

Prostate cancer, germline, somatic, susceptibility, metastatic, early-onset

# 3. OVERALL PROJECT SUMMARY

Over the course of the three grant period, we successfully recruited a cohort of men with early-onset, aggressive prostate cancer and completed germline and tumor sequencing to identify novel germline and somatic variants associated with prostate cancer. For **Major Task 1**, we identified and recruited 20 men with de novo metastatic prostate cancer presenting at or before age 60 years for this research project. We utilized a variety of recruitment methods to identify potential participants including announcements of our study to clinicians and distribution of an IRB-approved flyer. One participant indicated interest, but was subsequently lost to contact before providing consent so our total final sample size was 19.

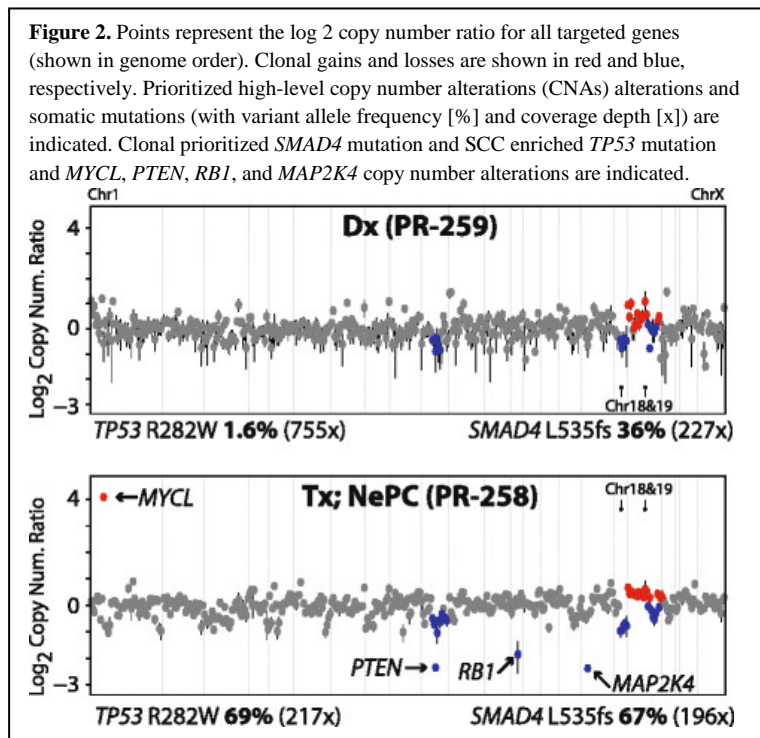
Regarding **Major Tasks 2 and 3**, which encompassed targeted, exome and/or transcriptome sequencing on germline DNA and prostate tumor DNA/RNA from men with early-onset stage 4 prostate cancer, whole-blood was collected and germline DNA isolated from all enrolled men. Whole-exome sequencing was conducted on the germline DNA samples. In addition to whole-blood, fresh frozen or formalin-fixed paraffin-embedded (FFPE) prostate tumor samples were collected for all participants and analyzed using methods below. As discussed in previous progress reports, most of the patients eligible for this study already had diagnostic procedures performed



**Figure 1.** Schema for molecular characterization of prostate cancer samples (MiPC\_v2).

which impeded our ability to collect frozen cancer specimens as initially proposed. Concurrently, Dr. Tomlins developed state of the art technologies to use FFPE prostate cancer specimens for molecular studies to characterize the prostate cancer genome and transcriptome. These included: robust protocols for co-isolating DNA/RNA from FFPE tissues, capture based NGS and qPCR approaches, and multiplexed PCR NGS based approaches. Therefore, we modified our approach to use archived FFPE biopsy and prostatectomy tumor specimen using multiplexed PCR based NGS approaches to interrogate the prostate cancer transcriptome/genome. The platform, referred to as MiPC\_v2, is outlined in **Figure 1**.

We took the opportunity to perform a more comprehensive analysis of one of our more interesting patients to illustrate the molecular events that characterize the development of small cell/neuroendocrine carcinoma of the prostate (NePC). Participant DOD 3 presented with metastatic prostate cancer at age 47, progressed quickly with small cell carcinoma of the liver and eventually succumbed from this disease approximately one year from initial diagnosis. Using Dr. Tomlins' novel approaches to characterize small amounts of paraffin-embedded tissue, we had the opportunity to molecularly profile both the initial prostate cancer (PR-259, DOD\_03a) and the liver biopsy which contained histologically-confirmed small cell/neuroendocrine prostate carcinoma (NePC, PR-258, DOD\_03b). **Figure 2** shows the NGS genomic profiles from 409 genes in both cancers which demonstrates a *SMAD4* c1605delC p.L535fs frameshifting variant that was present in both PR-259 (36% variant allele frequency) and PR-258 (67% variant allele frequency). In contrast, a *TP53* c.C844T p.R282W non-synonymous variant was exclusively called in the NePC specimen (PR-258; 69% variant allele frequency). This variant was markedly enriched in PR-258, and was only present at a variant allele frequency of 1.6% (12/755 reads) in the diagnostic pre-treatment specimen (PR-259). These results are consistent with clonal origin and marked enrichment of the *TP53* R282W variant exclusively in the post-treatment NePC specimen. Exome sequencing of germline DNA isolated from white blood cells confirmed the *TP53* and *SMAD4* variants as somatic. Copy number analysis identified concordant, low-level alterations in both specimens, with focal *MYCL* amplification and homozygous *PTEN*, *RBI*, and *MAP2K4* losses identified exclusively in the NePC specimen. Integration with results from our retrospective profiling of 118 prostate cancer specimens spanning the disease spectrum including 8 small cell carcinomas (described below) identified *MYCL* as recurrently amplified in NePC. This is an example of an "N of 1" exceptional non-responder and highlights the ability to generate new knowledge about the molecular drivers of prostate cancer



through intricate tumor and germline studies of men presenting with metastatic prostate cancer at a young age.

Likewise, in preliminary research conducted in preparation for this DOD award we identified a truncating *KRAS* mutation (p.C180X) in a man presenting with metastatic prostate cancer at age 49 who was also characterized in our retrospective somatic analysis (see Fig 7 below). Functional studies in the Tomlins lab show this mutation to be partially activating and therefore likely contributing to the phenotype of early-onset prostate cancer (manuscript in preparation).

In fulfillment of **Major Task 2**, we completed and analyzed full exome sequencing of germline DNA samples from the 19 participants. We identified:

- 275,304 total variants in 19 men.
- 54,190 unique variants across all 19 men (counting variants present in multiple individuals only once).
- 939 unique variants presumed to be deleterious (frameshift deletion, frameshift insertion, stopgain, stoploss or nonframeshift substitutions).
  - 549 only occurred in one subject (“rare”).
- 115 unique variants labeled pathogenic in ClinVar.
  - An additional 61 variants were labeled pathogenic or probable-pathogenic by at least one source (but also labeled untested, nonpathogenic, etc. from other sources).

We closely reviewed the variants present in 85 genes previously implicated in the pathogenesis of prostate cancer from review of our internal data and the literature. In these 85 candidate genes, we identified:

- A total of 1,674 variants in 73 of these genes in the 19 men (averaging 88 variants per person or ~1 variant per gene per person).
- 342 unique variants in the 73 genes across all 19 men (counting variants present in multiple individuals only once).
- 4 unique variants were presumed to be deleterious (2 stopgain, 2 frameshift deletions (**Table 1**))
- 3 unique variants labeled pathogenic in ClinVar (indicated by \* in **Table 1**)

**Table 1 Deleterious and Pathogenic Variants Observed in Select Genes**

<b>Gene</b>	<b>Number of Subjects</b>	<b>Type</b>	<b>DNA Substitution</b>	<b>Protein Substitution</b>
<i>BLM*</i>	1	stopgain	c.C2695T	p.R899X
<i>CYP3A43</i>	1	frameshift deletion	c.74delA	p.Y25fs
<i>GEN1</i>	1	frameshift deletion	c.2515_2519del	p.K839fs
<i>RAD52</i>	1	stopgain	c.C806A	p.S269X
<i>HNF1A*</i>	14	nonsynonymous SNV	c.A1720G	p.S574G
<i>HSD17B4*</i>	7	nonsynonymous SNV	c.G263A	p.R88H

Bloom syndrome is an autosomal recessive disorder with features similar to Fanconi anemia and characterized by genome instability. There is conflicting evidence regarding the association between null alleles for *BLM* and prostate cancer (1, 2) and other common cancers (3-7). However, given the data implicating germline DNA repair gene mutations in metastatic prostate cancer (8, 9), this finding merits further investigation. Similarly, *RAD52* encodes a DNA repair protein but stop codons in this gene have not been described in men with prostate cancer.

We also reviewed all nonsynonymous mutations in our 85 candidate genes that were suspected to be pathogenic based on in silico prediction models (SIFT, Polyphen):

**Table 2. Nonsynonymous SNVs in Select Genes, Considered Deleterious by both SIFT and Polyphen**

<b>Gene</b>	<b>Number of Subjects</b>	<b>DNA Substitution</b>	<b>Protein Substitution</b>
<i>ATM</i>	1	c.A8734G	p.R2912G
<i>BRCA1</i>	5	c.A926G	p.Q309R
<i>CDK12</i>	1	c.C3797T	p.P1266L
<i>CYP3A43</i>	1	c.G389A	p.R130Q
<i>ELMO3</i>	1	c.G685A	p.V229M
<i>ELMO3</i>	1	c.C1105T	p.R369C
<i>EPCAM</i>	1	c.G267C	p.Q89H
<i>FGFR3</i>	1	c.G1242A	p.M414I
<i>MRE11A</i>	1	c.G1732T	p.G578C
<i>MSH2</i>	1	c.G913A	p.A305T
<i>NCOR2</i>	1	c.A6811G	p.K2271E
<i>ERCC2</i>	1	c.G1267A	p.D423N

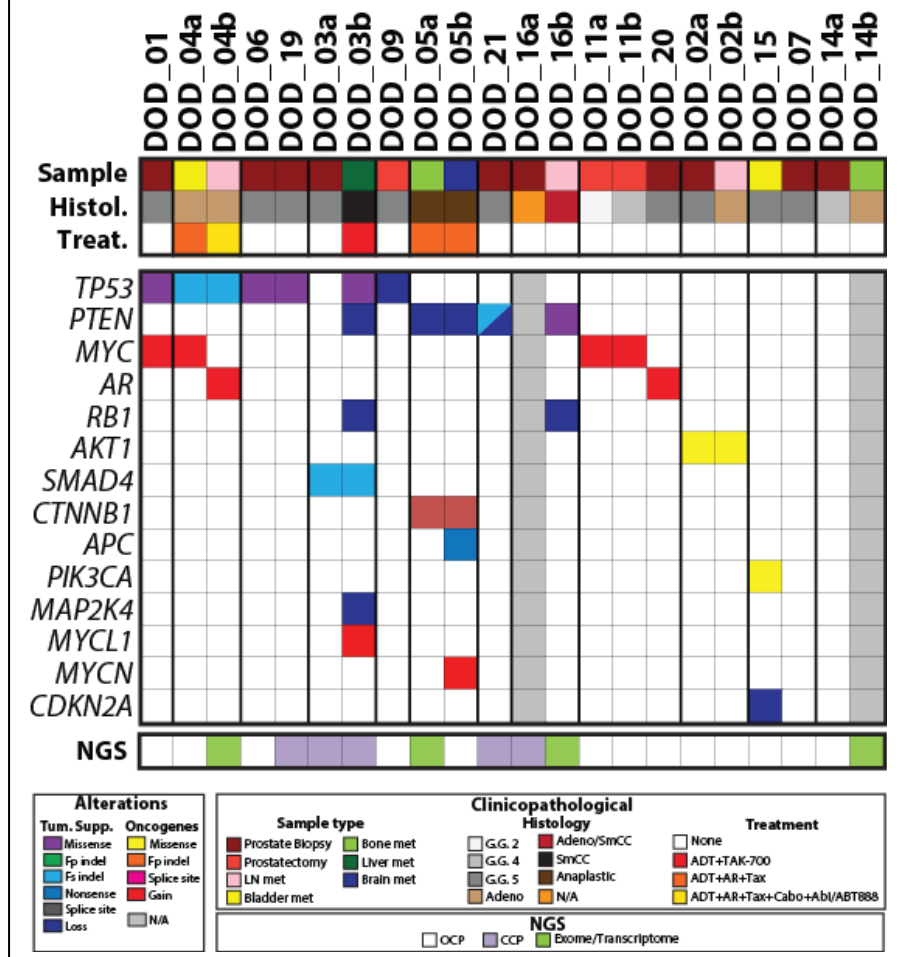
Germline *ATM* mutations have been associated with metastatic prostate cancer and appear to be the most commonly mutated gene in these patients after *BRCA2*. The *BRCA1* variant seen in 5/19 men in our study is located between the breast cancer cluster region 1 (BCCR1) and the ovarian cancer cluster region (OCCR). Despite the fact that it is predicted to be deleterious by SIFT and Polyphen, there is little clinical evidence that this is pathogenic. Lastly, the *ERCC2* p.D423N variant has been reported to impair ERCC2 mediated DNA repair capacity and dominant negative blocking of transcription (10).

In order to follow up on these germline findings, we developed a targeted gene panel to validate variants using germline DNA from a separate cohort of patients with early-onset, aggressive prostate cancer. This panel also includes GWAS SNPs and the assay was developed so it could be applied to both germline and tumor DNA samples in the future. This research is in progress and will be completed in the next 3 months and included in our final publication.

For **Major Task 3**, we completed genomic and/or transcriptomic sequencing from tumor material from 15 of the 19 enrolled men as shown in **Figure 3**; the remaining 4 men had insufficient archived FFPE material for any sequencing to be performed.

Hence in total, we profiled 22 individual samples from the 15 men, including diagnostic prostate biopsies, radical prostatectomy specimens, lymph node metastases and distant metastatic samples. Seventeen of the samples were from pretreatment diagnostic material (either prostate biopsies, prostatectomy specimens or lymph node biopsies to confirm metastatic disease), while five of the samples were collected after treatment (all treated with androgen deprivation therapy and at least one other agent). In addition to the paired diagnostic and small cell carcinoma samples from patient DOD 3 described in the previous section, we also profiled paired samples from six other men, including 1) paired diagnostic biopsy and near concurrent lymph node metastasis from patient DOD 2, 2) paired bladder and subsequent lymph node metastases from patient DOD4, 3) near concurrent bone and brain metastases biopsy samples from patient DOD 5, 4) paired low grade (Gleason score 3+4 = 7, Grade Group 2) and high grade (Gleason score 4+4 = 8 with intraductal carcinoma of the prostate, Grade Group 4) components of the radical prostatectomy specimen from patient DOD 11, 5) paired diagnostic biopsy and near concurrent bone metastasis from patient DOD 14, and 6) paired diagnostic prostate biopsy and near concurrent lymph node metastasis from patient DOD 16.

**Figure 3.** Integrative heat map of prioritized, somatic genomic alterations in our prospectively enrolled cohort. For each enrolled patient, all profiled samples are indicated according to patient number (e.g. DOD\_01), with paired samples from the same cases indicated by a and b. Sample type, histology and prior treatment are indicated in the top heat map according to the Clinicopathological legend (LN = lymph node, G.G. = grade group, SmCC = small cell carcinoma, ADT = androgen deprivation therapy, AR = androgen receptor antagonist, Tax = taxane-based chemotherapy, Cabo = cabozantinib, Abi = abiraterone). All prioritized somatic alterations are indicated per gene in the main heat map according to the alterations legend (samples DOD\_16a and DOD\_14b gave insufficient quality data to identify somatic alterations). The NGS platform used to profile somatic alterations is shown in the NGS row according to the bottom legend.



We profiled 4 of 22 samples using exome and transcriptome sequencing of fresh material, 5 FFPE samples using the Ion Torrent comprehensive cancer panel (CCP, as for patient DOD 3 described above), and 13 FFPE samples using the MiPC\_v2 (based on the Oncomine Comprehensive Assay [OCP]). High quality results were obtained for all specimens, except for



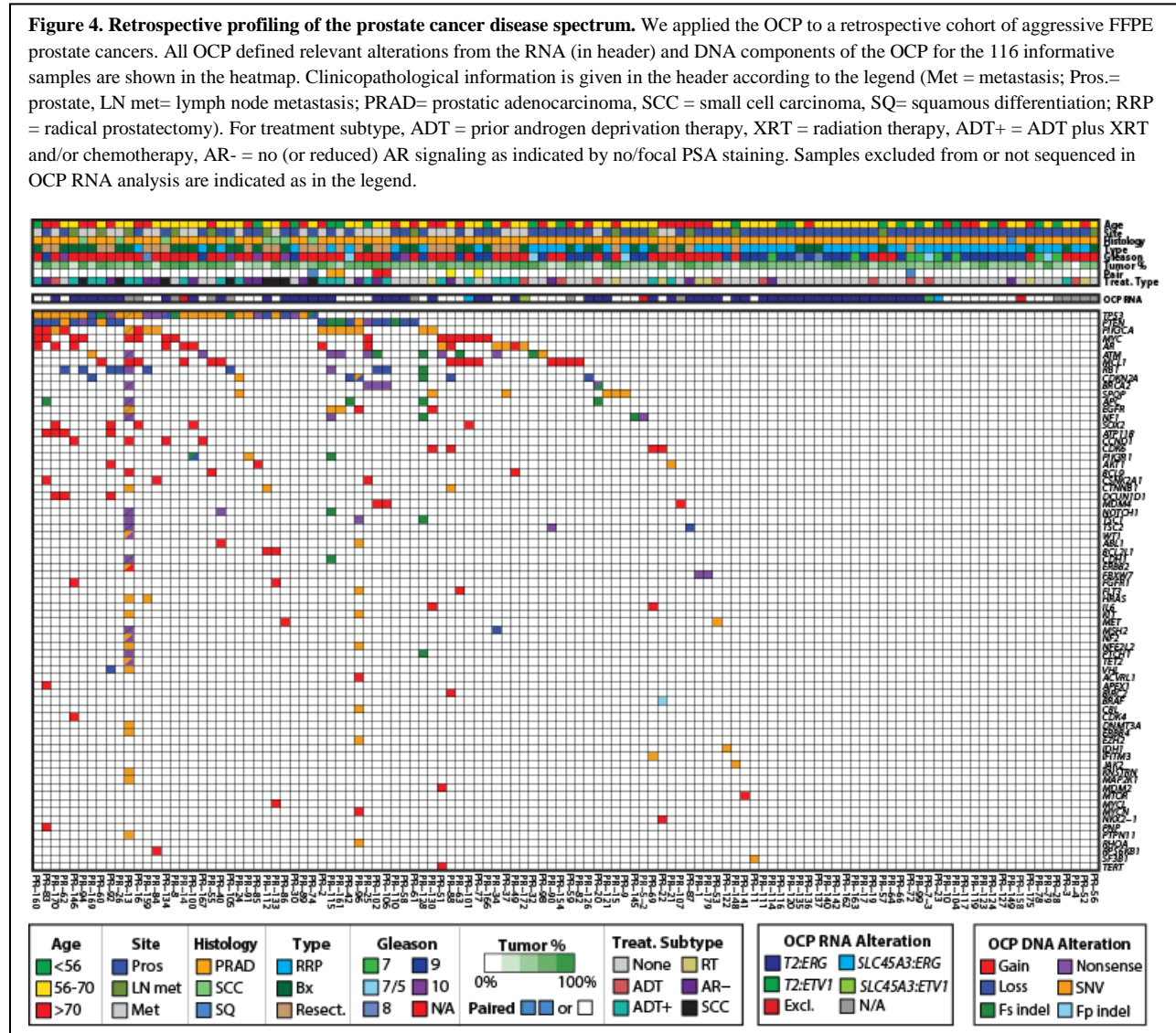
the pretreatment diagnostic biopsy from patient DOD 16 (samples DOD\_16a, where only a single unstained slide per each diagnostic biopsy was received and sequencing was performed without H&E assessment of tumor content) and a bone metastasis biopsy from patient DOD 14, which underwent exome and transcriptome sequencing and had an estimated tumor content less than 10%, precluding evaluation of genomic alterations (sample DOD\_14b, although an *ETVI* fusion was detected). Importantly, paired prostate cancer specimens—particularly those representing primary and distant metastases—are infrequently profiled due to the near exclusive availability of such samples as FFPE, representing a highly unique aspect of our study.

As shown in **Figure 3**, across the 20 informative samples from 15 men, the most frequently altered gene in our cohort was *TP53*, with prioritized, deleterious alterations occurring in 6 men (8 total samples), including two copy homozygous loss in the primary untreated prostatectomy specimen from patient DOD 9, as well as concordant frameshifting mutations in both the bladder metastasis and subsequent lymph node metastasis of patient DOD 4. Of note, three patients (DOD 1, DOD 6 and DOD 19), harbored *TP53* missense mutations in their diagnostic prostate biopsy specimens. *PTEN* and *MYC* were the next most frequently altered genes, with deleterious *PTEN* alterations observed in 5 samples from 4 cases, and *MYC* amplifications observed in 4 samples from 4 cases. *AR* and *RBI* were amplified and deleted, respectively, in two samples (each from 2 cases). Both of the *RBI* deleted samples showed prostatic neuroendocrine small cell (case DOD 3 described above) or overlapping adenocarcinoma and neuroendocrine small cell features (case DOD 16, specimen DOD16b). Of particular interest, DOD 20, which harbored a low level *AR* amplification, was from a primary diagnostic biopsy (Gleason score 4+5 = 9, Grade Group 5) in a patient who had not received antiandrogen therapy. To our knowledge, this is the first report of an *AR* amplification in the absence of antiandrogen therapy, with no such cases reported in the TCGA cohort of untreated primary prostate samples (333 cases) (11). Lastly, prioritized alterations in *AKT1*, *SMAD4* (described in DOD 3 above), *CTNNB1*, *APC*, *PIK3CA*, *MYCN* and *CDKN2A* were each observed in a single case. Transcriptome profiling demonstrated that all untreated specimens harbored active, intact *AR* signaling, with both small cell carcinoma specimens (DOD\_03b and DOD\_16b) and our paired anaplastic specimens (DOD\_05a and DOD\_05b, see **Figure 4**) showing loss of *AR* signaling and markedly high proliferation.

Taken together, the genomic findings in our cohort were consistent with previous profiling efforts in aggressive prostate cancer, however several alterations were observed at unexpected frequencies in this cohort of young patients with aggressive disease. For example, amongst the 13 cases where an untreated specimen was profiled, 4 (31%) had deleterious alterations in *TP53*, a significantly greater frequency than observed in the TCGA cohort of unselected primary prostatectomy specimens (25/333 [8%] with deleterious *TP53* alterations, two sided Fisher's exact test  $p=0.02$ ). Of note, the deleterious *TP53* alteration frequency in our untreated cohort was not significantly different than that observed in the SU2C cohort of CRPC specimens (75/150 [50%], two-sided Fisher's exact test  $p=0.25$ ) (8). These findings support *TP53* alterations as a marker of aggressive prostate cancer, in addition to their selection in transdifferentiation to small cell carcinoma as described in case DOD 3 described above.

Likewise, as shown in **Figure 3**, six cases harbored alterations affecting the PI3 kinase pathway, including 4 cases with *PTEN* alterations, one case with an *AKT1* p.E17K hotspot activating mutation (present in both the primary prostate biopsy and near subsequent metastatic lymph node

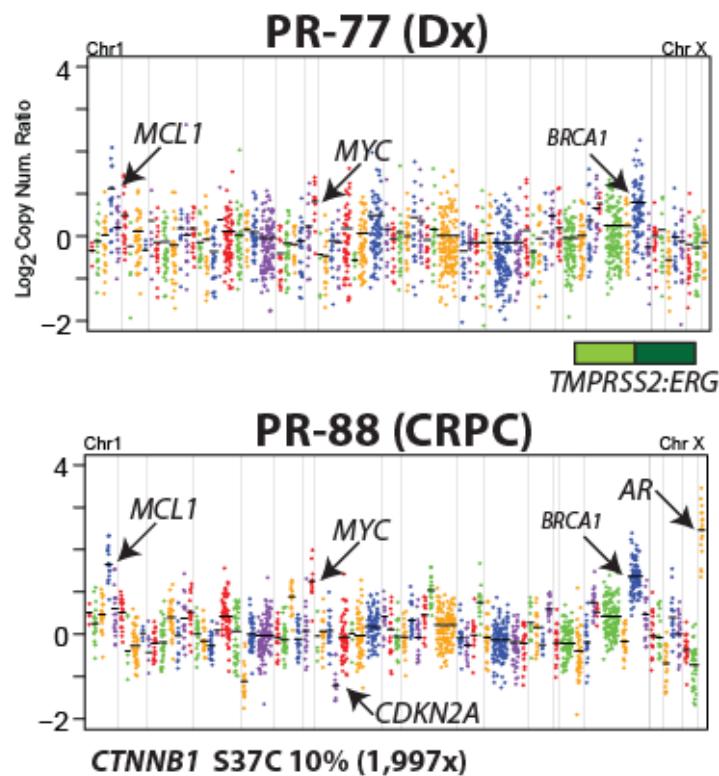
biopsy for patient DOD 2) and one case with a *PIK3CA* p.H1047Y hotspot activating mutation (DOD 15, a urinary bladder/prostatic urethra diagnostic pre-treatment resection specimen). Critically, although these *AKT1* and *PIK3CA* mutations are well-described recurrent mutations in other cancers, they are very rare in prostate cancer, with activating *AKT1* mutations being reported in 3/333 [1%] and 2/150 [1%] untreated and treated prostate cancers in TCGA and SU2C, cohorts respectively. Activating *PIK3CA* mutations have likewise been reported in only 5/333 [2%] and 5/150 [3%] of TCGA and SU2C samples, respectively.



In addition to our prospective cohort of young men with aggressive prostate cancer, we also profiled a cohort of 118 retrospective FFPE prostate cancer specimens using the OCP as shown in **Figure 4**. This cohort represented the entire disease spectrum from untreated diagnostic biopsies and prostatectomy specimens (nearly all with aggressive disease [Gleason score  $\geq 8$ ], pT3b and/or N1) to CRPC and small cell carcinoma. In this cohort, 50/118 (42%) and 20/118 (17%) samples were post-treatment and from patients younger than 56, respectively. Similar to our prospective cohort, *TP53* (27%) and *PTEN* (18%) were the most frequently altered genes in

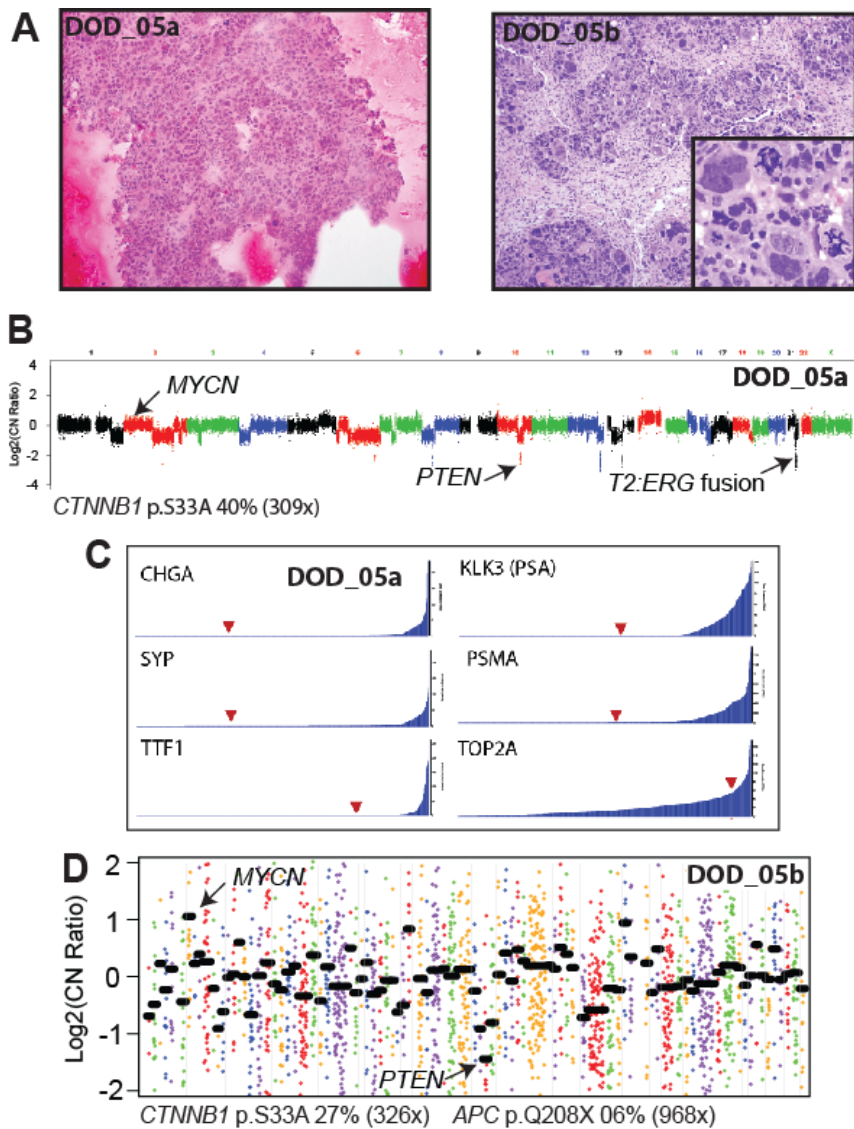
this cohort. This cohort also included several pre- and post-treatment specimens enabling the identification of specific alterations potentially associated with resistance to therapy that are potentially informative in our prospective cohort. For example, PR-77 and PR-88 represented an untreated diagnostic biopsy and a posttreatment AR signaling negative lymph node metastasis, respectively. As shown in **Figure 5**, we observed an *AR* amplification exclusively in PR-88, consistent with adaptation to previous androgen deprivation therapy. Importantly, PR-88 also exclusively harbored an activating *CTNNB1* p.S37C mutation, similarly supporting *CTNNB1* activation as an adaptive mechanism.

**Figure 5.** OCP profiling of paired pre-/post-therapy prostate cancer specimens identifies *CTNNB1* mutation as an adaptive (or selected) response to therapy. PR-77 is an untreated diagnostic (dx) primary Gleason score 9 prostate cancer and PR-88 is a subsequent castration resistant prostate cancer (CRPC) bladder metastasis obtained after ADT, XRT and chemotherapy that had AR<sup>-</sup> phenotype. OCP profiling demonstrates shared high level *MCL1* and *MYC* CNAs (and non-prioritized high level *BRCA1* amplification), consistent with clonality; however a *TMPRSS2:ERG* fusion (exons T2E2) was only identified by the OCP RNA-seq panel in PR-77, consistent with the AR<sup>-</sup> phenotype in PR-88. PR-88 uniquely harbored *AR* amplification (a known ADT resistance mechanism) and *CDKN2A* deletion, as well as a *CTNNB1* S37C (variant allele frequency 10%). No read support for *CTNNB1* S37C was present in PR-77 (>5,000 reads).



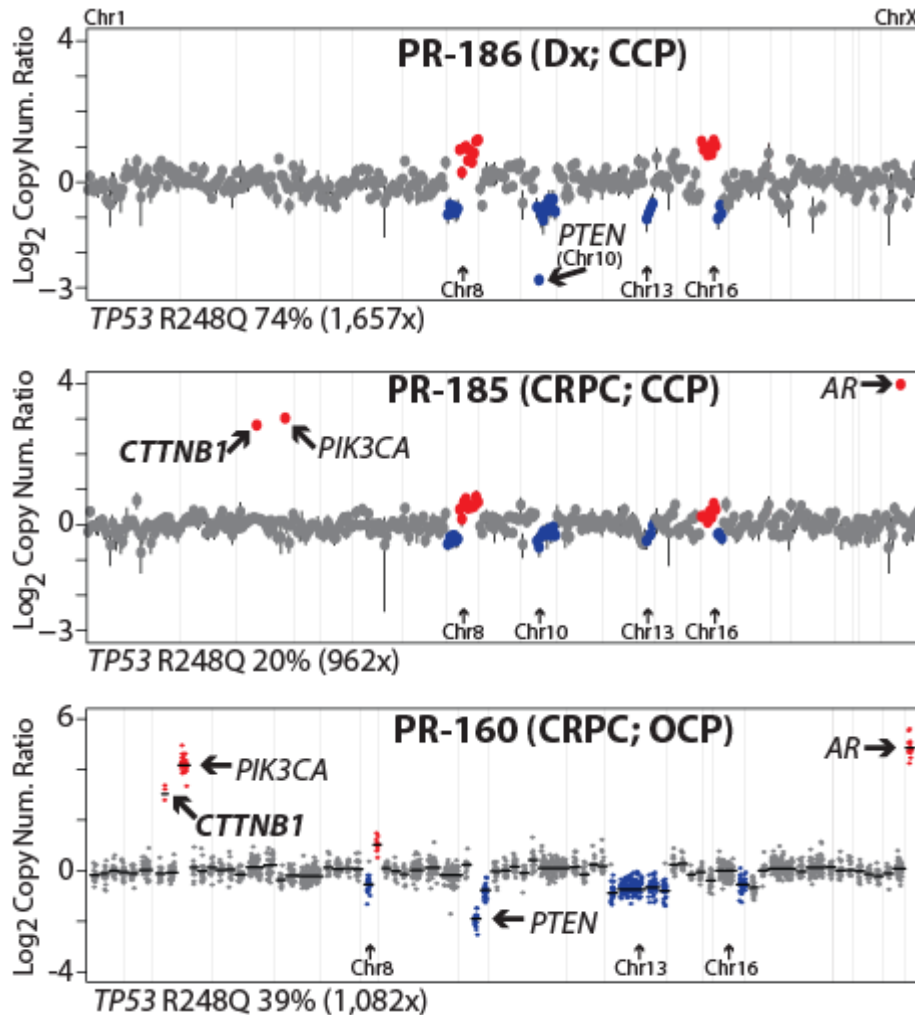
In our prospective cohort, as shown in **Figure 6**, we identified concordant activating *CTNNB1* p.S33A mutations in both post androgen deprivation therapy specimens from case DOD 5 (representing a bone metastasis and near concurrent brain metastasis). Of note, these specimens showed remarkable anaplastic morphology, a particular rare variant of prostate cancer, and also were AR signaling negative (as evaluated by *KLK3* and *PSMA* expression by transcriptome profiling) and highly proliferative (as evaluated by high mitotic index and *TOP2A* expression). These findings support *CTNNB1* mutations as adaptive alterations that may confer resistance to androgen deprivation therapy and portend an extremely aggressive androgen receptor signaling negative phenotype.

**Figure 6.** Comprehensive molecular profiling of paired post-t brain and bone metastasis samples from prospective patient DOD 5 demonstrates activating *CTNNB1* mutations and loss of AR signaling in anaplastic prostate cancer. Near concordant bone (DOD\_05a) and brain metastases (DOD\_05b) from a patient status/post androgen deprivation therapy, AR antagonist therapy and docetaxel were profiled by comprehensive exome/transcriptome (DOD\_05a) and OCP approaches (DOD\_05). **A.** Anaplastic morphology was observed in both specimens. **B.** Comprehensive genomic copy number profile for DOD\_05a demonstrates homozygous *PTEN* deletion, genomic evidence of a *T2:ERG* fusion, and an activating *CTNNB1* p.S33A mutation (40% variant allele frequency; 309x coverage). **C.** Comprehensive transcriptome profiling demonstrates that DOD\_05a lacks expression of neuroendocrine/small cell prostatic carcinoma (low *CHGA*, *SYP* and *TTF1* expression), is AR signaling negative (no detectable *KLK3*, *PSMA* or *T2:ERG* expression) and is highly proliferative (high *TOP2A* expression). Expression of each marker in this sample is indicated by the red triangle on a histogram of expression across a compendium of advanced/metastatic cancer samples. **D.** OCP profiling of the near concordant brain metastasis (DOD\_05b) demonstrating concordant *PTEN* deletion and *CTNNB1* mutation. A discordant *MYCN* amplification was observed exclusively in DOD\_05b, along with a subclonal *APC* mutation.



Likewise, as shown in **Figure 7**, in paired diagnostic and post androgen deprivation therapy brain and liver metastatic specimens (PR-186, PR-185, and PR-160) from the patient in our retrospective cohort that harbored the *KRAS* truncating germline variant described above and progressed to lethal castration resistant prostate cancer, we observed focal, high level *AR*, *CTNNB1* and *PIK3CA* amplifications exclusively in the posttreatment brain and liver metastases. Taken together, these results support both *CTNNB1* and *PIK3CA* alterations as adaptive alterations (like *AR* amplification) potentially conferring treatment resistance. These findings support future investigation into the prognostic and predictive significance of these alterations

**Figure 7.** Comprehensive profiling of paired pre-and post-treatment specimens from our retrospective cohort support *CTNNB1* and *PIK3CA* amplifications as adaptive alterations. Using the Ion Torrent Comprehensive Cancer Panel (CCP), which targets all coding exons of 409 cancer related genes, we profiled the diagnostic prostate biopsy tissue (PR-185, top) from a 49 year old man presenting with M1 (lymph node and liver metastases) prostate cancer. After rapidly developing CRPC after ADT and chemotherapy, liver biopsy of a metastasis (PR-185, middle) and an epidural metastasis resection specimen (PR-160, bottom) were obtained. PR-185 was profiled on the CCP and PR-185 was profiled using the OCP. All three tumors were gene fusion negative by the RNA component of the OCP. Integrative profiles for each tumor are shown as in Figure 5, except for CCP copy number plots, gene level copy number ratios are plotted as points with 95% confidence intervals indicated. Shared *TP53* R248 mutations and broad low level CNAs (shown in red and blue points/amplicons, including 1 or 2 copy *PTEN* loss) were present in each sample, consistent with clonal progression. High level, focal *AR*, *PIK3CA* and *CTNNB1* amplifications were present in both CRPC specimens but not the pretreatment sample, consistent with adaptive (or selected) alterations in response to therapy.



both prior to androgen deprivation therapy (such as DOD 15 in our prospective cohort) and in the 2<sup>nd</sup> or 3<sup>rd</sup> line setting after androgen deprivation therapy and potentially chemotherapy.

Additionally, we sought to integrate findings from the germline and tumor profiling in our prospective cohort. Importantly, all prioritized germline mutations were confirmed in the profiled tumor specimens (when targeted by the profiling performed in the tumor). Likewise, integrating results allowed us to infer potential functional significance of uncharacterized mutations through identification of loss of heterozygosity of the wild type allele in the tumor. For example patient DOD 4 harbored a germline *MSH2* p. A305T mutation as shown in **Table 2** at 45% variant allele frequency. This variant showed loss of heterozygosity through a one copy deletion (81% variant allele frequency in the bladder metastasis profiled by MiPC\_v2 and 75% variant allele frequency in the lymph node metastasis profiled by conference of exome sequencing), demonstrating the utility of combined germline and somatic sequencing to support potential functional significance of germline alterations undergoing a “second hit”.

Most recently, we have developed targeted RNA sequencing based expression profiling, which is compatible with even less material than required for qRT-PCR. Profiling of 8 of the 18 FFPE samples using this 309 transcript panel has been completed, with the remaining specimens to be completed to include more detailed transcriptomic assessment in the final publication.

#### **4. KEY RESEARCH ACCOMPLISHMENTS**

- Enrollment and germline sequencing and analysis of 19 men with early-onset metastatic prostate cancer.
- Comprehensive serial molecular profiling of a patient with metastatic prostate cancer progressing to small cell carcinoma on treatment. Our findings are consistent with the concept of transdifferentiation in the development of NePC and this is the only study to date with tumor samples from both primary cancer and metastatic lesions to confirm this concept. Our data also supports the roles of *TP53* mutation, *MYCL* amplification and homozygous *PTEN*, *RBI*, and *MAP2K4* losses in the development of NePC.
- Development of state-of-the-art technologies to use formalin-fixed paraffin-embedded (FFPE) prostate cancer specimens for molecular studies to characterize the prostate cancer genome and transcriptome.
- Whole exome sequencing identified a panel of variants that have biological as well as genetic evidence suggesting that they may be related to early-onset aggressive prostate cancer. We are validating these findings in an additional sample of 96 young men with aggressive and/or metastatic prostate cancer.
- Comprehensive tumor genome and/or transcriptome sequencing from 22 specimens from 15 enrolled men.
- Comprehensive sequencing of driving alterations in 118 retrospective men representing the entire prostate cancer spectrum from diagnostic material to advanced lethal disease.
- Comprehensive profiling of paired pre- and post-treatment specimens in our prospective and retrospective cohorts supports *CTNNB1* and *PIK3CA* alterations as adaptive resistance mechanisms.
- *TP53* mutations are more frequent in untreated prostate cancer in our prospective cohort of young patients with aggressive disease compared to unselected populations.
- Compiled list of driving genes with germline and somatic variants.



## 5. CONCLUSION

This research project focused on genetic analysis of a rare subset of prostate cancer cases: men presenting with metastatic prostate cancer at a young age (before age 60 years). We identified a panel of germline mutations that may be related to inherited susceptibility to prostate cancer. Confirmatory molecular analysis is underway and will be completed in several months. We are also in process of reviewing all publically-available datasets as well as data from a large sequencing effort conducted by the International Consortium for Prostate Cancer Genetics (Dr. Cooney is a member of the Steering Committee). The comprehensive analysis of tumors from these patients identified an increased frequency of *TP53* mutations compared to previously-reported unselected populations suggesting that this may be used as a prognostic marker for aggressive disease in the future. Finally, analysis of paired samples from unique patients confirmed the role of transdifferentiation as the mechanism for the development of NePC as well as *CTNNB1* and *PIK3CA* mutations in adaptive resistance to treatment.

## 6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

### a. Manuscripts

#### (1) Lay Press

None

#### (2) Peer-Reviewed Scientific Journals

- Hovelson DH, McDaniel AS, Cani AK, Johnson B, Rhodes K, Williams PD, Bandla S, Bien G, Choppa P, Hyland F, Gottimukkala R, Liu G, Manivannan M, Schageman J, Ballesteros-Villagrana E, Grasso CS, Quist MJ, Yadati V, Amin A, Betz B, Knudsen KE, Cooney KA, Feng FY, Roh MH, Nelson PS, Liu C, Beer DG, Wyngaard P, Sadis S, Rhodes DR, Tomlins SA. Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. *Neoplasia* 17(4):385-99, 2015. PMID: 25925381. DOI: 10.1016/j.neo.2015.03.004
- Kadakia KC, Tomlins SA, Sanghyi SK, Cani AK, Omata K, Hovelson DH, Liu CJ, Cooney KA. Comprehensive serial molecular profiling of an "N of 1" exceptional non-responder with metastatic prostate cancer progressing to small cell carcinoma on treatment. *J Hematol Oncol* 8:109, 2015. PMID: 26444865. DOI: 10.1186/s13045-015-0204-7

#### (3) Invited Articles

None

#### (4) Abstracts:

None

### b. Presentations

None

## 7. INVENTIONS, PATENTS AND LICENSES

Nothing to report.

## 8. REPORTABLE OUTCOMES

Nothing to report.

## 9. OTHER ACHIEVEMENTS

Nothing to report.

## 10. REFERENCES

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7. A. Sassi, M. Popielarski, E. Synowiec, Z. Morawiec, K. Wozniak, BLM and RAD51 genes polymorphism and susceptibility to breast cancer. *Pathol Oncol Res* **19**, 451-459 (2013).
8. D. Robinson *et al.*, Integrative clinical genomics of advanced prostate cancer. *Cell* **161**, 1215-1228 (2015).
9. C. C. Pritchard *et al.*, Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *New England Journal of Medicine* **0**, (2016).
10. A. Rump *et al.*, Identification and Functional Testing of ERCC2 Mutations in a Multi-national Cohort of Patients with Familial Breast- and Ovarian Cancer. *PLoS Genet* **12**, e1006248 (2016).
11. Cancer Genome Atlas Research, The Molecular Taxonomy of Primary Prostate Cancer. *Cell* **163**, 1011-1025 (2015).

## 11. APPENDICES

None.