HHS Public Access

Author manuscript

Eur Urol. Author manuscript; available in PMC 2021 January 01.

Published in final edited form as:

Eur Urol. 2020 January ; 77(1): 14–21. doi:10.1016/j.eururo.2019.05.032.

Wnt-pathway Activating Mutations Are Associated with Resistance to First-line Abiraterone and Enzalutamide in Castration-resistant Prostate Cancer

Pedro Isaacsson Velho[a], **Wei Fu**[a], **Hao Wang**[a], **Nooshin Mirkheshti**[b], **Fahad Qazi**[b], **Fabiola A.S. Lima**[c], **Farah Shaukat**[a], **Michael A. Carducci**[a,d], **Samuel R. Denmeade**[a,d], **Channing J. Paller**[a], **Mark C. Markowski**[a], **Catherine H. Marshall**[a,d], **Mario A. Eisenberger**[a,d], **Emmanuel S. Antonarakis**[a,d,*]

[a]Johns Hopkins Sidney Kimmel Cancer Center, Johns Hopkins University, Baltimore, MD, USA

[b]MedStar Health, Baltimore, MD, USA

[c]Hospital Sirio Libanes, Sao Paulo, Brazil

[d]Brady Urological Institute, Johns Hopkins University, Baltimore, MD, USA

Abstract

Background: Wnt signaling is a cellular pathway involved in embryogenesis, development, and neoplasia. Wnt-pathway activation may accelerate prostate cancer androgen-independent growth and mediate antiandrogen resistance. Since 10–20% of advanced prostate cancers harbor Wnt-activating mutations, we aimed to characterize the clinical features and response to novel antiandrogens in such patients.

Objective: To determine whether men with metastatic castration-resistant prostate cancer (mCRPC) who harbor Wnt-pathway mutations have poorer responses to first-line novel hormonal therapies: abiraterone/enzalutamide.

Design, setting, and participants: Patients with mCRPC who received first-line abiraterone or enzalutamide were retrospectively evaluated. Using tumor DNA analyses, we queried for activating mutations in *CTNNB1* or inactivating mutations in *APC* or *RNF43,* all of which are

*Corresponding author. Johns Hopkins Sidney Kimmel Cancer Center, Skip Viragh Building—Room 9129, 201 North Broadway, Baltimore, MD 21287, USA. Tel. +1 410 955 8893; Fax: +1 410 614 7287. eantona1@jhmi.edu (E.S. Antonarakis).

***Author contributions*:** Emmanuel S. Antonarakis had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Velho, Antonarakis.

Acquisition of data: Velho, Mirkheshti, Qazi, Shaukat, Lima.

Analysis and interpretation of data: Velho, Fu, Wang, Carducci, Denmeade, Paller, Markowski, Marshall, Eisenberger, Antonarakis.

Drafting of the manuscript: Velho, Antonarakis.

Critical revision of the manuscript for important intellectual content: Velho, Antonarakis.

Statistical analysis: Velho, Fu, Wang.

Obtaining funding: Antonarakis.

Administrative, technical, or material support: Antonarakis.

Supervision: Antonarakis.

Other: None.

predicted to stimulate Wnt signaling. Presence or absence of at least one Wnt-activating alteration was correlated with clinical-pathologic characteristics and treatment outcomes.

Outcome measurements and statistical analysis: Time to prostate-specific antigen (PSA) progression, overall survival (OS), and PSA response were measured. Cox regression models were used to test associations between Wnt status and clinical-pathologic outcomes; Kaplan-Meier and log-rank analyses were used to compare time-to-event data.

Results and limitations: Of 137 patients evaluated, 11% (n = 15) had tumor DNA analysis showing at least one Wnt-stimulating alteration. Patients with Wnt-activating mutations had numerically fewer T3/T4 tumors than Wnt wild-type patients (31% vs 51%), but were otherwise generally balanced. Median time to PSA progression on first-line abiraterone/enzalutamide was shorter in Wnt-activated patients (6.5 vs 9.6 mo, hazard ratio [HR] 2.34, p = 0.003), as was OS (23.6 vs 27.7 mo, HR 2.28, p = 0.01). PSA responses were numerically worse in Wnt-activated patients (53% vs 75%, p = 0.12). Presence of Wnt-activating alterations (adjusted HR [aHR] 2.33, p = 0.007) and use of previous chemotherapy (aHR 1.83, p = 0.003) were both independently associated with increased hazard of progression.

Conclusions: Patients with somatic Wnt-pathway activating mutations have worse outcomes to first-line abiraterone/enzalutamide than Wnt wild-type patients. Our data suggest that additional genomically informed therapies are needed for this relevant subset of mCRPC patients.

Patient summary: In this report, we retrospectively examined outcomes of metastatic prostate cancer patients with or without Wnt-pathway mutations who received abiraterone or enzalutamide for the first time, in order to examine whether these mutations affect the prognosis. Our study suggested that patients who have Wnt-pathway activating mutations derived less benefit from abiraterone and enzalutamide when compared with patients without these mutations. We conclude that Wnt-pathway mutations might decrease the effectiveness of abiraterone and enzalutamide, and we propose that the Wnt pathway might be a good therapeutic target for these patients, in order to potentially reverse or prolong resistance to abiraterone and enzalutamide in men with Wnt mutations.

Abstract

Patients with metastatic castration-resistant prostate cancer who harbor Wnt-pathway–activating mutations have inferior outcomes with first-line abiraterone and enzalutamide when compared with patients without these abnormalities. Prospective validation of this finding is needed.

Keywords

Wnt; Prostate; Cancer; Antiandrogens; Abiraterone; Enzalutamide

1. Introduction

Prostate cancer has an intrinsic dependency on androgens and androgen receptor (AR) signaling, with androgen deprivation therapy (ADT) being the backbone of systemic treatment of metastatic disease [1]. Unfortunately, almost all patients invariably develop hormonal resistance over time, progressing to a state called metastatic castration-resistant prostate cancer (mCRPC) [2]. The most common mechanisms of escape include

amplification and/or mutation of the *AR* [3], expression of constitutively active AR splice variants [4], increased production of intracrine androgens, and changes in the activity or expression levels of AR coactivators and corepressors [3]. In this context, novel antiandrogens such as abiraterone [5,6] and enzalutamide [7,8] provide clinical and survival benefits, suggesting that mCRPC often remains addicted to androgens and AR signaling [2]. However, in addition to these AR-dependent mechanisms of escape, additional AR-independent mechanisms are involved in the emergence of castration resistance, as well as innate or acquired antiandrogen resistance; one of these relevant players is the Wnt signaling pathway [9,10].

Wnt signaling pathways are a group of three major signal transduction pathways that are physiologically responsible for many functions including cell growth, organ formation, stem cell renewal, cell-cycle progression, and survival [11,12]. The “canonical” Wnt pathway primarily comprises the cytoplasmatic protein β-catenin that is negatively regulated by the tumor suppressor protein APC. The “noncanonical” Wnt pathways include the complexes of planar cell polarity and Wnt calcium (Wnt/Ca^{2+}), which are responsible for morphogenetic cell movements [11,13]. Mutated Wnt-pathway genes cause multiple growth-related pathologies [14] and are implicated in the development, progression, and mechanisms of resistance of multiple nonprostatic cancers, including colorectal [15,16], breast [17], lung [18], and hematopoietic malignancies [19].

Somatic mutations in genes that regulate the Wnt signaling pathway, including activating mutations in *CTNNB1* and *RSPO2*, or inactivating mutations in *APC, RNF43* and *ZNRF3*, are present in approximately 10–20% of patients with mCRPC [20,21]. Preliminary clinical data have suggested an association between Wnt-pathway activation and higher Gleason grades [22], higher prostate-specific antigen (PSA) levels [22], earlier-onset prostate cancer (diagnosed at <50 yr of age) [20], and higher hazard of recurrence after radical prostatectomy [23]. Preclinical data have also demonstrated that the AR may have an intrinsic interaction with canonical and noncanonical Wnt signaling [24–26]. Hormonal modulations such as ADT, and even abiraterone and enzalutamide, may activate the canonical Wnt/β-catenin signaling pathway, leading to androgen-independent prostate cancer growth [24,25]. In addition, activation of the noncanonical Wnt signaling pathway has been implicated in resistance to AR antagonism, especially to enzalutamide [26,27].

In the present study, we hypothesized that mCRPC patients who harbor somatic Wnt-pathway activating mutations would have poorer responses to first-line novel hormonal therapies such as abiraterone and enzalutamide, due to the AR-independent mechanism of continued growth that this pathway may confer to these tumors. To resolve this biological question, we utilized clinical-grade tumor DNA sequencing results to interrogate for Wnt-activating alterations and to correlate these with sensitivity or resistance to abiraterone or enzalutamide treatment in the first-line mCRPC setting.

2. Patients and methods

Men who received first-line abiraterone or enzalutamide therapy for mCRPC at the Johns Hopkins Hospital, over a 9-yr period (from August 2009 to November 2018), formed the

study population. Genomic analyses were performed at each treating physician's discretion, and there was no systematic reason why some patients underwent or did not undergo genetic testing. Therefore, this is a genomic-centric analysis that is not capable of capturing information about all treated patients (ie, no clinical data were entered on patients without available genomic results). We utilized data from clinical-grade next-generation DNA sequencing assays (Foundation Medicine, or Personal Genome Diagnostics [PGDx]) to interrogate for somatic mutations resulting in activation of the Wnt pathway. Our genes of interest were *APC, CTNNB1, RNF43, RSPO2*, and *ZNRF3.* In the case of inactivating mutations in *APC* or *RNF43*, pathogenic alterations were defined as those that were predicted to result in a truncated protein (nonsense mutations, frameshift insertions or deletions, splice site mutations at conserved splice donor or acceptor sites, or genomic/exonic deletions). In the case of activating mutations in *CTNNB1*, only missense mutations at the six hotspot residues (codons 32, 33, 34, 37, 41, and 45) that are known to increase stabilization of the protein were defined as pathogenic. In the case of *RSPO2*, only activating gene fusions were classified as pathogenic. Of note, only Foundation Medicine interrogates *RSPO2* fusions, so this gene was only partially characterized in our cohort. *APC, CTNNB1*, and *RNF43* are interrogated by both Foundation Medicine and PGDx, while *ZNRF3* is not interrogated by either assay.

Tumor DNA analysis was performed using archival primary cancer specimens ($n = 70$), metastatic biopsies ($n = 52$), or circulating tumor DNA samples ($n = 24$). Men without available somatic DNA analysis were excluded from the study. All genomic analyses were performed retrospectively, and information about Wnt-pathway status was not used to influence the selection of therapy. The Johns Hopkins University Institutional Review Board and the Human Research Ethics Committee approved the conduct of this retrospective study.

Demographic, pathologic, and clinical characteristics of all patients were collected. We recorded the presence/absence of pathogenic or likely pathogenic somatic alterations predicted to result in Wnt-pathway stimulation (namely, activating mutations in *CTNNB1* or *RSPO2*, or inactivating mutations in *APC* or *RNF43)*, and classified patients into mutation-positive (Wnt-activated) and -negative (Wnt wild-type) groups. The primary endpoint was to estimate time to PSA progression on first-line abiraterone/enzalutamide treatment, defined as the time from starting the antiandrogen therapy to the second consecutive elevation in PSA above baseline or nadir. Secondary endpoints were overall survival (OS), defined as the time from starting abiraterone/enzalutamide therapy to death from any cause and PSA50 response, defined as a decline of ≥50% in PSA from baseline confirmed on a subsequent measurement at least 4 wk apart. In order for patients to be evaluable for PSA response, PSA data for a minimum of 12 wk were required following the initiation of treatment (all patients met this criterion). Fisher's exact test was used to test for the association between PSA50 response and Wnt-pathway status. Kaplan-Meier curves were used to estimate PSA progression-free survival and OS probabilities, and univariable Cox proportional-hazards model was used to compare differences in time to PSA progression and OS between the mutation-positive and mutationnegative groups. Multivariable Cox proportional-hazards models were used to estimate hazard ratios (HRs) and corresponding 95% confidence intervals (CIs), and to test for the association between Wnt-pathway status or types (APC/RNF43 vs CTNNB1) and PSA progression or OS, after adjusting for the clinical-pathologic

variables (age at diagnosis, time to castration resistance, Gleason grade group, PSA baseline level, and previous chemotherapy). Separate multivariable Cox proportional-hazards models were used to estimate the association between Wnt-pathway status and PSA progression or OS, adjusting for concurrent inactivating alterations in *TP53, RB1*, and *PTEN.* All statistical tests were two sided, and statistical significance was set at $p \leq 0.05$. Since this study was hypothesis generating, we did not perform corrections for multiple comparisons.

3. Results

3.1. Baseline clinical and genomic characteristics

We identified 137 mCRPC patients with available somatic DNA sequencing results who had received first-line abiraterone or enzalutamide treatment at our institution. Of these, 11% (15 patients) harbored at least one Wnt-pathway stimulating alteration, including activating mutations in *CTNNB1* ($n = 8$), and inactivating mutations in *APC* ($n = 6$) and *RNF43* ($n = 3$). No *RSPO2* fusions were identified. Two patients had more than one Wnt-pathway alteration in their tumor (Table 1). Concurrent deleterious *TP53* mutations were seen in 33% (5/15) and 40% (49/122) of patients with and without Wnt-activating mutations, respectively. Of the 15 patients with Wnt-pathway alterations, 53% (8/15) had somatic DNA analysis from metastatic tissue, 33% (5/15) from the primary tumor, and 20% (3/15) from circulating tumor DNA (Table 2); one patient had analyses performed from both primary tumor and circulating tumor DNA.

The timing of collection of tumor DNA samples for genomic analysis was also evaluated. Of the 16 tumor DNA samples from 15 patients with Wnt-pathway alterations, 63% (10/16) were collected during castration-resistant disease and 38% (six/16) during hormone-sensitive disease. In patients without Wnt-pathway alterations, 53% (69/130) and 47% (61/130) of somatic DNA samples were collected during the castration-resistant and hormone-sensitive disease states, respectively (Supplementary Table 1). All somatic DNA analyses were performed from samples obtained prior to initiation of first-line abiraterone or enzalutamide.

The median follow-up for survivors only was 26.5 mo. Overall, 53% of patients had received prior taxane chemotherapy in our cohort, with 73% of Wnt-activated versus 51% of Wnt wild-type patients having received previous chemotherapy. Patients with Wnt-activating mutations had numerically fewer T3/T4 tumors (31% vs 51%) than Wnt wild-type patients. Ductal and intraductal histologies were seen at similar frequencies in Wnt-activated and wild-type groups, respectively (20% vs 14%). All demographic, clinical, and pathologic characteristics of our patients are detailed in Table 2. Groups generally appeared balanced with respect to common prognostic variables.

3.2. Efficacy of first-line abiraterone or enzalutamide

The median time to PSA progression in our overall cohort was 8.3 mo (range, 0.8–62.0 mo). Patients with Wnt-activating alterations had a median time to PSA progression of 6.5 versus 9.6 mo in patients without Wnt mutations (HR 2.34, 95% CI 1.32–4.17, $p = 0.003$; Fig. 1A). Multivariable analysis showed that the presence of a Wnt-activating mutation was

independently associated with increased hazard of PSA progression (adjusted HR [aHR] 2.33, 95% CI 1.25–4.32, $p = 0.007$). Previous taxane chemotherapy was also independently associated with higher hazard of PSA progression (aHR 1.83, 95% CI 1.21–2.76, $p = 0.004$; Table 3). The multivariable results for PSA progression are depicted in Table 3.

Exploratory analysis of the different types of Wnt-pathway mutations (inactivating *APC* or *RNF43* mutations vs activating *CTNNB1* mutations) showed that *APC/RNF43* mutations were independently associated with higher hazard of PSA progression than Wnt wild type (aHR 2.58, 95% CI 1.14–5.83, $p = 0.023$). Despite a strong trend in the same direction, *CTNNB1* mutations showed no statistically significant association with higher hazard of PSA progression (aHR 2.12, 95% CI 0.93–4.81, $p = 0.072$). Finally, after adjusting for concurrent inactivating alterations in *TP53, RB1*, and *PTEN*, presence of Wnt-activating mutations remained independently prognostic (aHR 2.38, 95% CI 1.33–4.24, $p = 0.003$; Supplementary Table 2).

With respect to PSA >50% responses to first-line abiraterone/enzalutamide treatment, Wnt-activated patients had numerically lower PSA responses than patients without Wnt mutations (53% vs 75%), but no statistical difference was observed between the two groups ($p = 0.12$). The PSA response waterfall plot is demonstrated in Figure 2.

3.3. Overall survival

The median OS of the whole cohort was 26.5 mo (range, 1.0–98.4 mo). Median survival time among patients with Wnt-activating mutations was shorter than those without Wnt mutations (median OS 23.6 vs 27.7 mo, HR 2.28, 95% CI 1.15–4.53, $p = 0.01$; Fig. 1B). Multivariable analysis confirmed that patients with Wnt-activating mutations had higher hazard of death than those without Wnt mutations (aHR 2.14, 95% CI 1.03–4.43, $p = 0.041$). Other factors were also independently associated with higher hazard of death in multivariable analysis, such as age at diagnosis and previous chemotherapy use (Table 2). Finally, after adjusting for concurrent inactivating alterations in *TP53, RB1*, and *PTEN*, presence of Wnt-activating mutations remained independently prognostic of OS (aHR 2.27, 95% CI 1.13–4.56, $p = 0.021$; Supplementary Table 3).

4. Discussion

The findings of our hypothesis-generating study support the rationale that tumors harboring Wnt signaling pathway activating mutations may be more resistant to antiandrogen therapies, such as abiraterone and enzalutamide. Our study showed that Wnt-activated tumors are more resistant to antiandrogen therapies in the first-line mCRPC context, translating into more rapid PSA progression in these patients. Moreover, patients with activating Wnt-pathway mutations have a trend for lower PSA responses, compared with patients without these abnormalities. Finally, our data suggest that OS may also be impaired in men harboring Wnt-activated tumors compared with those with Wnt wild-type tumors.

The rationale underlying this acquired resistance is that Wnt-activating mutations confer to these tumors an earlier androgen-independent pathway of growth [24,25] and resistance to AR antagonism [26]. Previous studies have analyzed the prognostic and predictive

significance of Wnt alterations in mCRPC, and have produced conflicting results. Geng et al [28] designed a study that correlated germline single nucleotide polymorphisms (SNPs) in Wnt-pathway genes with clinical outcomes in 465 patients with metastatic prostate cancer treated with ADT. This study found some protective alleles that reduced *APC* gene expression. The median time to castration resistance was 17 mo in patients without any of these protective alleles versus 29 mo in patients with three to four protective *APC* alleles. Multivariable analysis showed that two SNPs in *APC* were independently associated with decreased hazard of progression and also lower mortality [28].

The hypothesis that somatically derived Wnt-pathway mutations would negatively influence novel hormonal therapy sensitivity was also recently tested in a single-center prospective study designed to identify predictive biomarkers of primary resistance to abiraterone using molecular analyses of tumor biopsies [29]. In that study of 82 mCRPC patients receiving first-line abiraterone, those with activating mutations in the canonical Wnt/β-catenin signaling pathway (eg, stabilizing *CTNNB1* alterations) had a higher chance of primary resistance to therapy (56% vs 17%, $p = 0.001$), suggesting that constitutively active Wnt/γ-catenin signaling might be responsible for this intrinsic antiandrogen resistance.

Another study also tried to evaluate sensitivity and resistance to first-line abiraterone and enzalutamide in 202 patients with mCRPC (7.9% of whom had Wnt-activating mutations), using whole-exome and deep targeted sequencing of plasma cell-free DNA [30]. Although this study showed greater hazard to disease progression on these drugs in patients who harbored different genomic abnormalities such as *BRCA2/ATM, TP53, RB1, PI3K, and AR* gain, no statistically significant differences were seen in patients with Wnt signaling pathway mutations in this study [30]. However, this study was limited by the fact that approximately one-third of patients did not have enough cell-free tumor DNA content to permit somatic genomic analyses.

Despite the clinical successes of abiraterone and enzalutamide as single-agent therapies for mCRPC, it is clear that additional non–AR-targeted therapeutic advances are needed. Further, now that antiandrogen therapies have been moved to earlier disease stages such as nonmetastatic CRPC (eg, apalutamide [31] and enzalutamide [32]) and hormone-sensitive metastatic disease [33,34] (eg, abiraterone), medical oncologists will possibly face the earlier development of different mechanisms of antiandrogen resistance. It is now clear that several of these mechanisms are acquired more frequently after the widespread and earlier use of abiraterone and enzalutamide [35,36], and knowledge of these potential resistance mechanisms (especially the AR-independent mechanisms) will be crucial to develop new strategies to improve outcomes in mCRPC moving forward.

Although our study offers some new insights into one important and emerging pathway of primary and acquired antiandrogen resistance, it also raises some clinical questions inherent to the limitations of its design. First, since Wnt-pathway mutations are generally not inherited in prostate cancer, at what stage of the disease are they somatically acquired? Second, is the development of Wnt-pathway mutations related to treatment selective pressures, and if yes, how could we avoid or delay these pressures? Third, once we detect a Wnt-pathway mutation, how should we change our practice, if at all? Fourth, given the

possibility of emerging drugs that specifically target activated Wnt signaling components [37], the question that arises is whether canonical or noncanonical Wnt signals are more critical in prostate cancer progression. Fifth, what is the inter-relationship between Wnt-pathway signaling mutations and other treatment-dependent mechanisms of antiandrogen resistance, such as AR overexpression and mutations, AR splice variants [4,35], and treatment-emergent small-cell neuroendrocrine prostate cancer [36]?

Our study has several limitations that should be considered when interpreting the results. First, this was a single-center retrospective analysis. While several provocative associations have been demonstrated between Wnt status and novel antiandrogen resistance, causal relationships are difficult to assess and the results may have been influenced by other clinical factors. For example, almost three-fourths of patients with Wnt-activating alterations (73%) had received previous chemotherapy, compared with only half (50%) of patients without these alterations. This difference may have impacted our results, since antiandrogens have lower efficacy after chemotherapy. Second, other inherent limitations of this type of study include the introduction of information bias. In addition, because this is a single-institution study, selection bias should be considered while interpreting our results. To this end, we tried to mitigate selection bias by including consecutive patients who received first-line abiraterone or enzalutamide and had next-generation DNA sequencing data available. In addition, we were unable to compare the clinical outcomes to abiraterone/enzalutamide between patients with and without available genomic information, because we do not routinely collect clinical data on patients who have not undfergone genetic analyses and this information is not readily available. Therefore, it is possible that outcomes in genomically selected patients might not be reflective of the broader mCRPC population at our institution. Finally, our assessment of somatic mutational status relied on multiple clinical-grade assays performed on primary or metastatic tumors; the use of primary tumor materials may have partially underestimated (or misclassified) Wnt-pathway mutations in these patients. In addition, not all relevant Wnt-pathway genes were interrogated by the commercial next-generation sequencing platforms used here; *RSPO2* fusions were evaluated only by the Foundation Medicine assay and not by the PGDx assay, and the *ZNRF3* gene was not evaluated in either assay. Therefore, defects in the full complement of genes potentially involved in Wnt-pathway signaling are likely underreported in our study, although probably only slightly.

5. Conclusions

In summary, our preliminary data suggest that mCRPC patients with Wnt-pathway activating somatic mutations have faster PSA progression on first-line abiraterone and enzalutamide, as well as worse OS. The negative prognostic impact of Wnt alterations remained after multivariable adjustments and after accounting for concurrent alterations in other key tumor suppressor genes (*TP53, RB1*, and *PTEN*). Our data support the rationale for testing Wnt-pathway inhibitors (eg, porcupine inhibitors or other agents), either alone or in combination with antiandrogen therapies, in clinical trials for Wnt-activated mCRPC patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial disclosures: Emmanuel S. Antonarakis certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Emmanuel S. Antonarakis is a paid consultant/advisor to Janssen, Astellas, Sanofi, Dendreon, AstraZeneca, Clovis, and Merck; has received research funding to his institution from Janssen, Johnson & Johnson, Sanofi, Dendreon, Bristol Myers-Squibb, AstraZeneca, Clovis, and Merck; and is the coinventor of a biomarker technology that has been licensed to Qiagen. The remaining authors report no relevant conflicts of interest.

***Funding/Support and role of the sponsor*:** This work was partially supported by National Institutes of Health Grant P30 CA006973 (Emmanuel S. Antonarakis) and Department of Defense grant W81XWH-16-PCRP-CCRSA (Emmanuel S. Antonarakis).

References

[1]. Mohler JL, Lee RJ, Antonarakis ES, Higano CS, Richey S. NCCN clinical practice guidelines in oncology—prostate cancer. Prostate Cancer 2018;151.

[2]. Armstrong AJ, Antonarakis ES, Taplin M-E, et al. Naming disease states for clinical utility in prostate cancer: a rose by any other name might not smell as sweet. Ann Oncol 2018;29:23–5. [PubMed: 29088323]

[3]. Wang Q, Li W, Zhang Y, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 2009;138:245–56. [PubMed: 19632176]

[4]. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371:1028–38. [PubMed: 25184630]

[5]. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005. [PubMed: 21612468]

[6]. Ryan CJ, Smith MR, de Bono JS, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138–48. [PubMed: 23228172]

[7]. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424–33. [PubMed: 24881730]

[8]. Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187–97. [PubMed: 22894553]

[9]. Yokoyama NN, Shao S, Hoang BH, Mercola D, Zi X. Wnt signaling in castration-resistant prostate cancer: implications for therapy. Am J Clin Exp Urol 2014;2:27–44. [PubMed: 25143959]

[10]. Murillo-Garzón V, Kypta R. Wnt signalling in prostate cancer. Nat Rev Urol 2017;14:683–96. [PubMed: 28895566]

[11]. Croce JC, McClay DR. Evolution of the Wnt pathways. Methods Mol Biol 2008;469:3. [PubMed: 19109698]

[12]. Acebron SP, Niehrs C. β-Catenin-independent roles of Wnt/LRP6 signaling. Trends Cell Biol 2016;26:956–67. [PubMed: 27568239]

[13]. Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 2017;169:985–99. [PubMed: 28575679]

[14]. Clevers H Wnt/β-catenin signaling in development and disease. Cell 2006;127:469–80. [PubMed: 17081971]

[15]. Nishisho I, Nakamura Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 1991;253:665–9. [PubMed: 1651563]

[16]. Tenbaum SP, Ordóñez-Morán P, Puig I, et al. β-Catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. Nat Med 2012;18:892–901. [PubMed: 22610277]

[17]. Pohl S-G, Brook N, Agostino M, Arfuso F, Kumar AP, Dharmarajan A. Wnt signaling in triplenegative breast cancer. Oncogenesis 2017;6:e310. [PubMed: 28368389]

[18]. Teng Y, Wang X, Wang Y, Ma D. Wnt/beta-catenin signaling regulates cancer stem cells in lung cancer A549 cells. Biochem Biophys Res Commun 2010;392:373–9. [PubMed: 20074550]

[19]. Peterson LF, Turbiak AJ, Giannola DM, et al. Wnt-pathway directed compound targets blast crisis and chronic phase CML leukemia stem progenitors. Blood 2009;114:2168. [PubMed: 19589924]

[20]. Robinson D, Van Allen EM, Wu Y-M, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161:1215–28. [PubMed: 26000489]

[21]. Beltran H, Yelensky R, Frampton GM, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol 2013;63:920–6. [PubMed: 22981675]

[22]. Jung SJ, Oh S, Lee GT, et al. Clinical significance of Wnt/β-catenin signalling and androgen receptor expression in prostate cancer. World J Mens Health 2013;31:36–46. [PubMed: 23658864]

[23]. Huang S-P, Ting W-C, Chen L-M, et al. Association analysis of Wnt pathway genes on rostatespecific antigen recurrence after radical prostatectomy. Ann Surg Oncol 2010;17:312–22.

[24]. Lee E, Ha S, Logan SK. Divergent androgen receptor and beta-catenin signaling in prostate cancer cells. PLoS One 2015;10:e0141589. [PubMed: 26509262]

[25]. Terry S, Yang X, Chen M-W, Vacherot F, Buttyan R. Multifaceted interaction between the androgen and Wnt signaling pathways and the implication for prostate cancer. J Cell Biochem 2006;99:402–10. [PubMed: 16741972]

[26]. Miyamoto DT, Zheng Y, Wittner BS, et al. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. Science 2015;349:1351–6. [PubMed: 26383955]

[27]. Chen WS, Aggarwal R, Zhang L, et al. Genomic drivers of poor prognosis and enzalutamide resistance in metastatic castration-resistant prostate cancer. Eur Urol. In press. 10.1016/j.eururo.2019.03.020

[28]. Geng J-H, Lin VC, Yu C-C, et al. Inherited variants in Wnt pathway genes influence outcomes of prostate cancer patients receiving androgen deprivation therapy. Int J Mol Sci 2016;17:1970.

[29]. Wang L, Dehm SM, Hillman DW, et al. A prospective genome-wide study of prostate cancer metastases reveals association of Wnt pathway activation and increased cell cycle proliferation with primary resistance to abiraterone acetate-prednisone. Ann Oncol 2018;29:352–60. [PubMed: 29069303]

[30]. Annala M, Vandekerkhove G, Khalaf D, et al. Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. Cancer Discov 2018;8:444–57. [PubMed: 29367197]

[31]. Smith MR, Saad F, Chowdhury S, et al. Apalutamide treatment and metastasis-free survival in prostate cancer. N Engl J Med 2018;378:1408–18. [PubMed: 29420164]

[32]. Hussain M, Fizazi K, Saad F, et al. Enzalutamide in men with nonmetastatic, castration-resistant prostate cancer. N Engl J Med 2018;378:2465–74. [PubMed: 29949494]

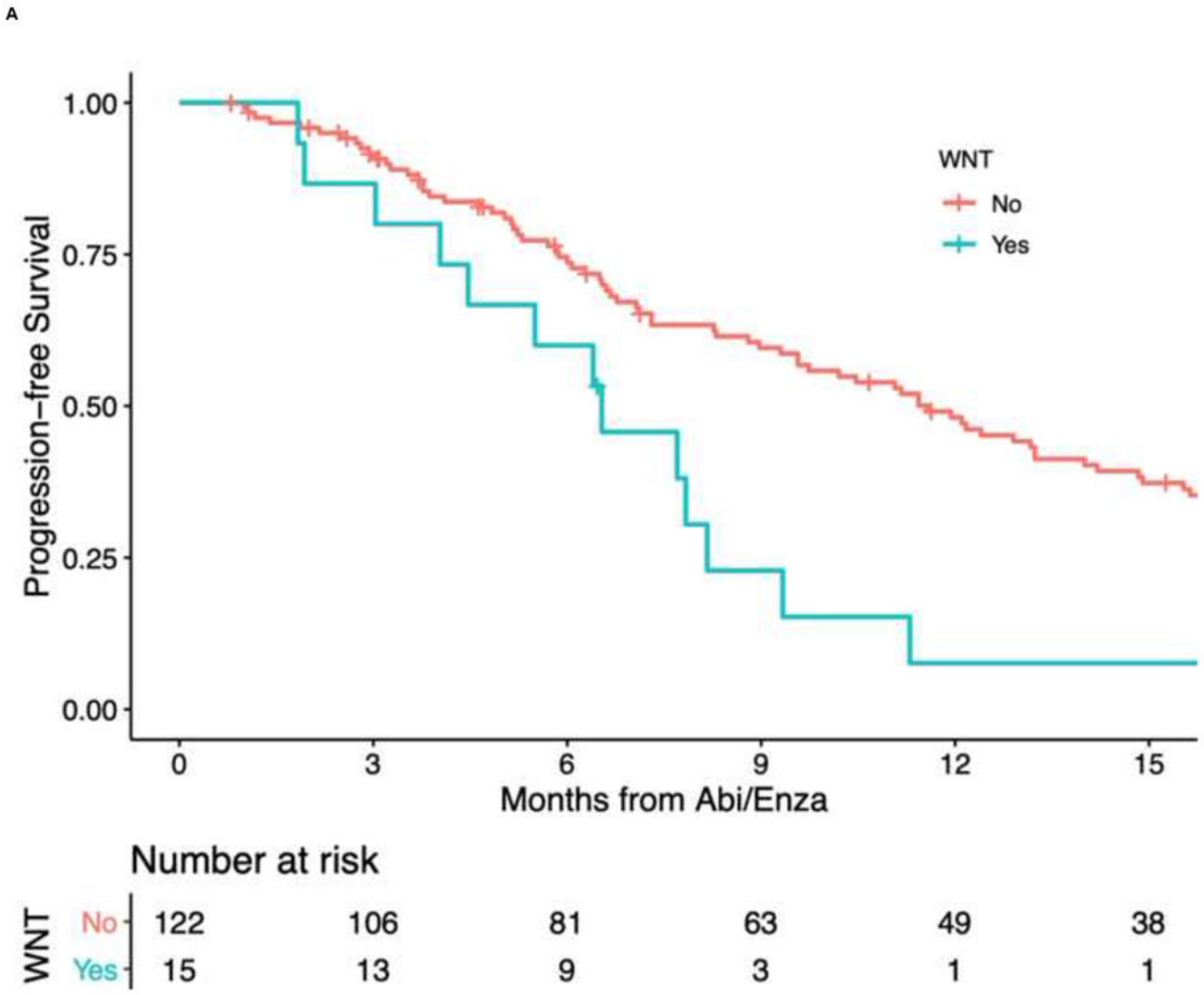
[33]. Fizazi K, Tran N, Fein L, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 2017;377:352–60. [PubMed: 28578607]

[34]. James ND, de Bono JS, Spears MR, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. N Engl J Med 2017;377:338–51. [PubMed: 28578639]

[35]. Scher HI, Lu D, Schreiber NA, et al. Association of AR-V7 on circulating tumor cells as a treatmentspecific biomarker with outcomes and survival in castration-resistant prostate cancer. JAMA Oncol 2016;2:1441–9. [PubMed: 27262168]

[36]. Aggarwal R, Huang J, Alumkal JJ, et al. Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: a multi-institutional prospective study. J Clin Oncol 2018;36:2492–503. [PubMed: 29985747]

[37]. Zhang Z, Cheng L, Li J, et al. Inhibition of the Wnt/β-catenin pathway overcomes resistance to enzalutamide in castration-resistant prostate cancer. Cancer Res 2018;78:3147–62. [PubMed: 29700003]



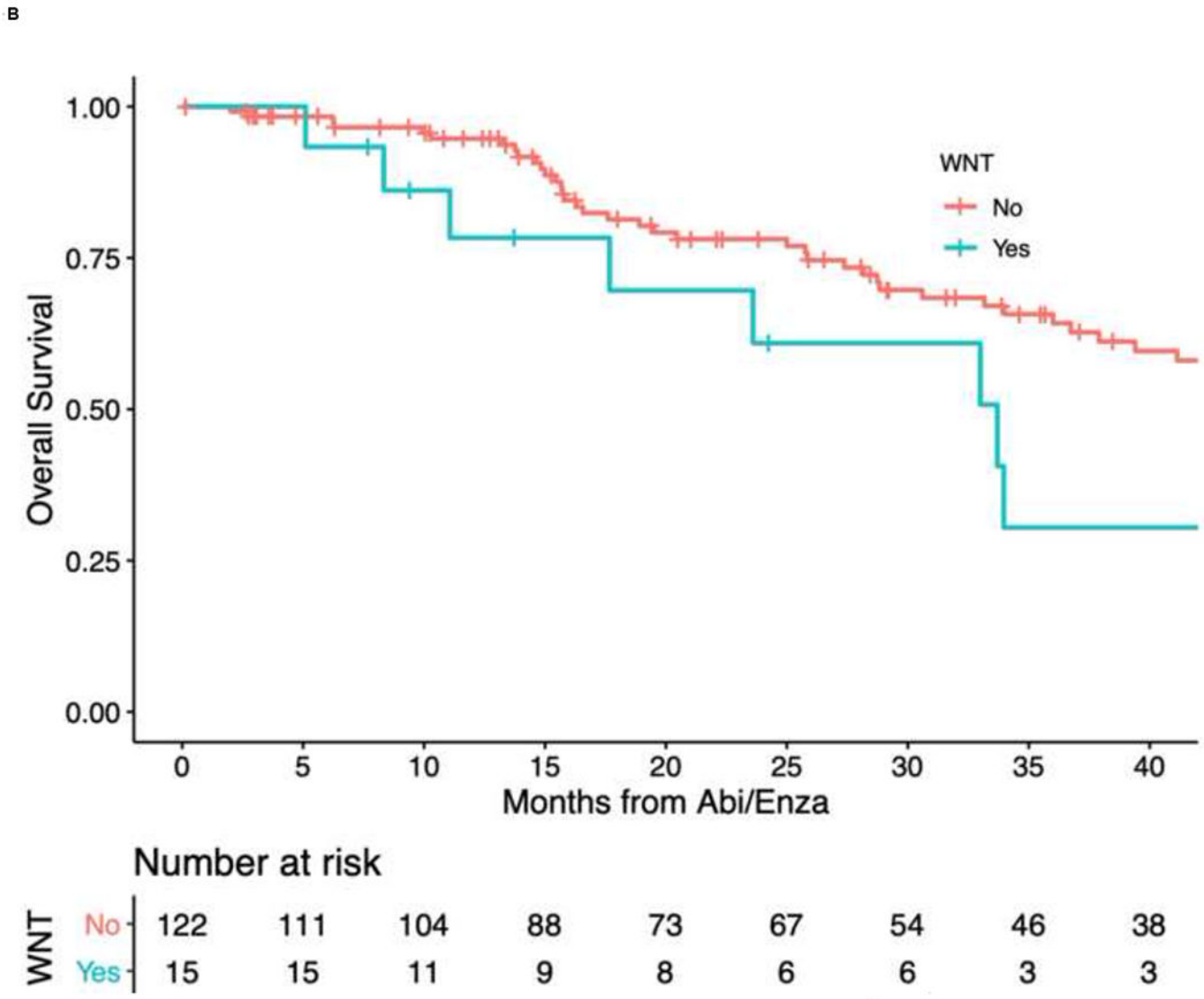


Fig. 1 – .

Kaplan-Meier curves of (A) time to PSA progression and (B) overall survival, by Wnt status. Abi = abiraterone; Enza = enzalutamide; PSA = prostate-specific antigen.

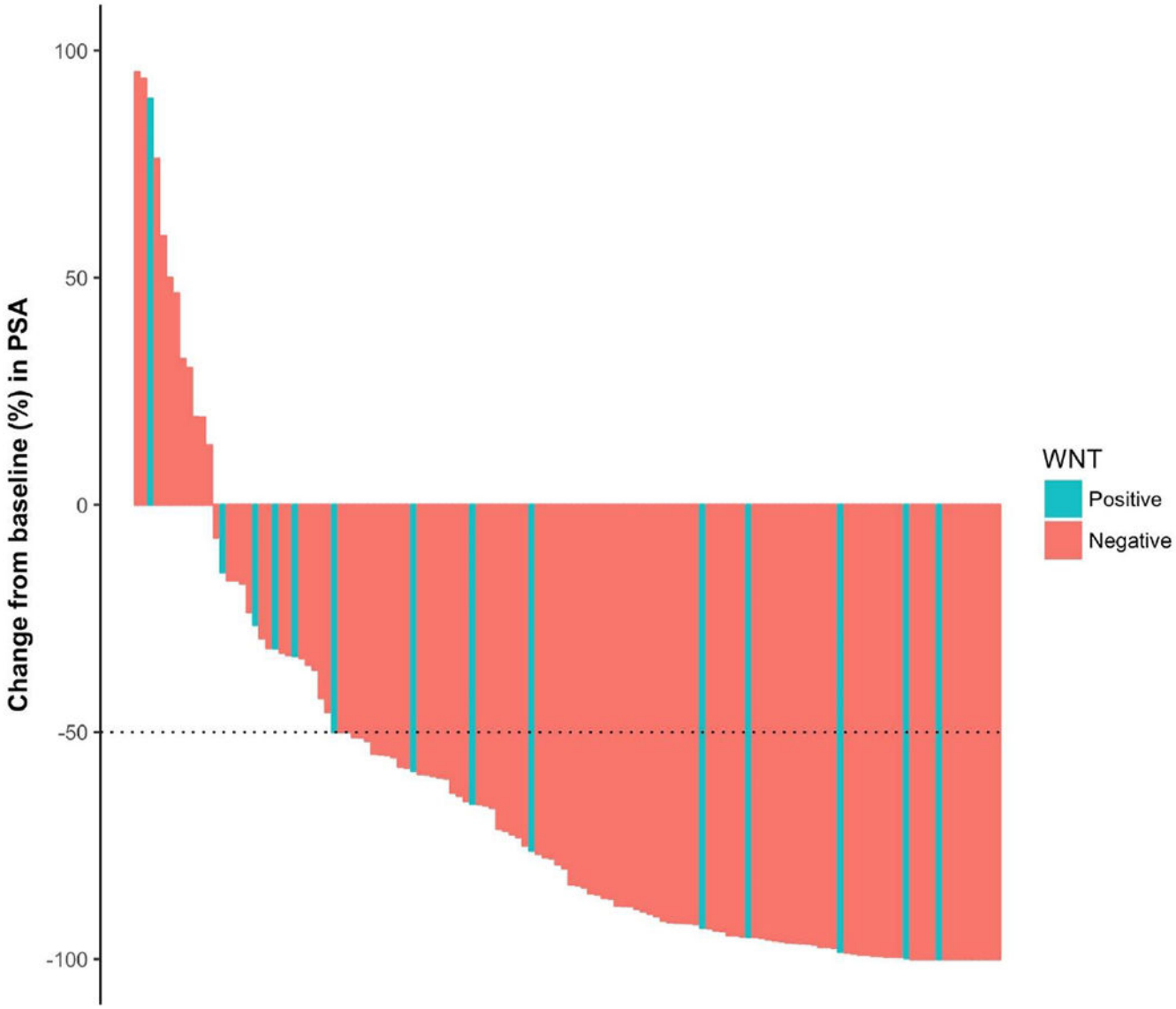


Fig. 2 – .

PSA waterfall plot (best PSA response), by Wnt status.

PSA = prostate-specific antigen.

Table 1 –

Wnt pathway alterations in our patient cohort

Patient ID	Wnt mutation	Mutant allelic fraction (%)	Function	Other somatic mutations	Source of somatic DNA
Patient 27	*CTNNB1* (G34E)	3	Activating	*AKT1* (D323N), *MLH1* (E230V), *ROS1* (A1381T)	Metastasis
Patient 46	*APC* (Q215fs*)	15	Inactivating	*PTEN* (loss exons 3–9), *RB1* (V654fs*14), *TP53* (S215I)	Metastasis
Patient 48	*APC* (H668Tfs*6)	63	Inactivating	*BRCA2* (N8991fs*5), *PDGFRA* (A384S), *SMO* (R726Q), *SRC* (R208H), *TSC2* (A678T)	Metastasis
Patient 136	*CTNNB1* (D32G)	34	Activating	*TP53* (E271V), *DDR2* (D621Y), *FANCA* (L278I), *KIT* (P467Q), *BRCA2* (P3189H), *TSC1* (Q898H)	Metastasis
Patient 157	*APC* (D1425fs*)	31	Inactivating	*ARID1A* (E1780fs*)	Prostate
Patient 180	*APC* (R1450X*)	26	Inactivating	*AR* (T878A), *AKT1* (E17K), *NF1* (Q1993X*)	Plasma
Patient 197	*CTNNB1* (T41A)	7	Activating	*BRCA1* (Q1111fs*), *NF1*	Prostate
	RNF43 (G659fs*)	41	Inactivating	(K1387fs*), *PTEN* (T319fs*), *CIC* (P1116fs*), *CSF1R* (E317fs*), *JAK1* (P430fs*), *TMPRSS2* (- *ERG* fusion)	
Patient 263	*CTNNB1* (D32H)	37	Activating	*ARID2* (S1500X*), *NTRK1* (A5T), *SF3B1* (E622D), *STAG2* (S1075X*), *TP53* (H115fs*34)	Metastasis
Patient 266	*CTNNB1* (S37F)	12	Activating	*ASXL1* (P808Lfs*10), *POLH*	Metastasis
	APC (Q1621K)	48	Inactivating	(R371H), *RUNX1* (D160Y), *BRCA2* (C1913G), *BLM* (P126Q),), *MET* (Q826K), *NOTCH2* (C1060S), *ERCC4* (D762V)	
Patient 268	*CTNNB1* (S33F)	8	Activating	*CDKN2A* (S12L)	Plasma
Patient 271	*RNF43* (truncation exon 8)	Cannot be determined	Inactivating	*PTEN* (H123fs*2), *TMPRSS2* (- *ERG*)	Prostate
Patient 276	*CTNNB1* (S37F)	13	Activating	*ATM* (D2708N),), *FLT3* (C368F), *TP53* (A76Vfs*55), *TMPRSS2* (- *ERG*)	Metastasis
Patient 277	*CTNNB1* (S45Y)	43	Activating	*CDKN1B* (S175X*)	Prostate
Patient 283	*APC* (D1532fs*33)	24	Inactivating	*AR* (H875Y), *BRCA2* (loss exons 19-20), *ASXL1* (deletion exon 2), *BCORL1* (c.3608-lG>C), *CDKN2A* (c.151-1G>C), *VHL* (L188V)	Metastasis
Patient 294	*RNF43* (G659Vfs*41)	45	Inactivating	*ASXL1* (Q976X*), *TP53* (C238G, M237I)	Prostate

Table 2 –

Baseline clinical and pathologic characteristics, by Wnt status

	Wnt mutation (*N* = 15)	No Wnt mutation (*N* = 122)
Age at diagnosis, median (Q1, Q3)	60 (58–66)	62 (56–68)
Age at ADT starting, median (Q1, Q3)	64 (59–66)	65 (58–69)
Race, *N* (%)		
White	13 (87)	98 (81)
Black	2 (13)	17 (14)
Other	0 (0)	6 (5)
Baseline PSA (ng/ml) prior to starting abiraterone or enzalutamide, median (Q1, Q3)	21.8 (6.3–75.8)	14.5 (5.6–46.2)
Time from diagnosis to first-line abi/enza, mo (Q1, Q3)	56.3 (22.6–79.7)	41.9 (19.4–83.4)
Time to castration-resistance, mo (Q1, Q3)	14.6 (8.3–33.4)	17.3 (11.9–28.5)
Gleason grade group, *N* (%)		
Grade 1	0 (0)	2 (1.7)
Grade 2	1 (6.7)	11 (9.6)
Grade 3	3 (20)	13 (11)
Grade 4	2 (13)	20 (17)
Grade 5	9 (60)	69 (60)
Radical prostatectomy, *N* (%)	9 (60)	46 (38)
Pathologic/clinical T stage, *N* (%)		
T1/T2	9 (69)	52 (49)
T3/T4	4 (31)	55 (51)
Pathologic pN+, *N* (%)	3 (33)	8 (18)
Metastasis at diagnosis, *N* (%)	4 (27)	50 (42)
Ductal or intraductal histology, *N* (%)	3 (20)	15 (14)
Perineural invasion, *N* (%)	8 (57)	63 (61)
Lymph vascular invasion, *N* (%)	1 (7.1)	13 (13)
Previous taxane chemotherapy, *N* (%)	11 (73)	62 (51)
Taxane chemotherapy for mHSPC, *N* (%)	2 (13)	16 (13)
Concurrent p53 mutation, *N* (%)	5 (33)	49 (40)
Sites of metastatic disease, *N* (%)		
Bone	12 (80)	109 (89)
Lymph node	9 (60)	83 (68)
Visceral	7 (47)	38 (31)
Source of somatic DNA, *N* (%) [a]		
Prostate	5 (33)	65 (53)
Metastasis	8 (53)	44 (36)
Plasma tumor DNA	3 (20)	21 (17)

abi = abiraterone; ADT = androgen deprivation therapy; enza = enzalutamide; mHSPC = metastatic hormone-sensitive prostate cancer; PSA = prostate-specific antigen.

[a]The sum of percentages exceeds 100% because some patients had more than one test.

Table 3 –

Multivariable analyses of time to PSA progression and overall survival

	Time to PSA progression			Overall survival		
	HR	**95% CI**	***p* value**	**HR**	**95% CI**	***p* value**
Model 1						
Any Wnt-activating mutation	2.33	1.25–4.32	0.007	2.14	1.03–4.43	0.041
Age at diagnosis	1.03	1.01–1.06	0.015	1.05	1.01–1.09	0.01
Time to castration resistance	0.56	0.41–0.75	0.0001	0.65	0.42–1.0	0.050
Gleason score	1.07	0.66–1.74	0.8	1.56	0.76–3.19	0.2
Baseline PSA	0.99	0.87–1.12	0.8	1.17	0.99–1.39	0.061
Previous chemotherapy	1.83	1.21–2.76	0.004	2.49	1.33–4.65	0.004
Model 2						
APC/RNF43 (ref. wild type)	2.58	1.14–5.83	0.023	2.39	0.83–6.90	0.10
CTNNB1 (ref. wild type)	2.12	0.93–4.81	0.072	1.98	0.79–4.96	0.14
Age at diagnosis	1.03	1.01–1.06	0.015	1.05	1.01–1.09	0.01
Time to castration resistance	0.56	0.41–0.76	0.0002	0.64	0.41–1.00	0.050
Gleason score	1.08	0.67–1.74	0.8	1.57	0.77–3.21	0.2
Baseline PSA	0.99	0.88–1.12	0.9	1.18	1.34–4.69	0.062
Previous chemotherapy	1.84	1.22–2.78	0.004	2.51	1.34–4.69	0.004

CI = confidence interval; HR = hazard ratio; PSA = prostate-specific antigen; ref. = reference.

Model 1 evaluated the effect of any Wnt-activating mutation versus wild type, and model 2 evaluated the effect of various types of Wnt-activating mutations.