SPOP mutated/ CHD1 deleted lethal prostate cancer and abiraterone sensitivity.

Authors:
Gunther Boysen*[1], Daniel N. Rodrigues*[1], Pasquale Rescigno[2], George Seed[1], David Dolling[1], Ruth Riisnaes[1], Mateus Crespo[1], Zafeiris Zafeiriou[2], Semini Sumanasuriya[2], Diletta Bianchini[2], Joanne Hunt[2], Deirdre Moloney[2], Raquel Perez-Lopez[3], Nina Tunariu[2], Susana Miranda[1], Inês Figueiredo[1], Ana Ferreira[1], Rossitza Christova[1], Veronica Gil[1], Sara Aziz[1], Claudia Bertan[1], Flavia M. de Oliveira[1], Mark Atkin[1], Matthew Clarke[1], Jane Goodall[1], Adam Sharp[1], Theresa MacDonald, Mark A. Rubin[4,5,6], Wei Yuan[1], Christopher E. Barbieri[4,7], Suzanne Carreira[1], Joaquin Mateo[3], Johann S. de Bono[1,2]■

*These authors contributed equally.
■Corresponding author (johann.de-bono@icr.ac.uk)

Affiliations:
[1]Institute of Cancer Research, London, UK
[2]Prostate Cancer Targeted Therapy Group and Drug Development Unit, The Royal Marsden NHS Foundation Trust, London, UK
[3]Vall D'Hebron Institute of Oncology, Barcelona, Spain
[4]Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine, New York, USA
[5]Englander Institute for Precision Medicine, Weill Cornell Medicine, and New York-Presbyterian Hospital, New York, USA
[6]University of Bern, Switzerland
[7]Department of Urology, Weill Cornell Medicine, and New York-Presbyterian Hospital, New York, USA

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Abstract

Purpose:
CHD1 deletions and *SPOP* mutations frequently co-occur in prostate cancer (PCa) with lower frequencies reported in castration-resistant PCa (CRPC). We monitored CHD1 expression during disease progression and assessed the molecular and clinical characteristics of *CHD1* deleted/ *SPOP* mutated metastatic CRPC (mCRPC).

Experimental Design:
We identified mCRPC 89 patients who had hormone naive and castration resistant tumor samples available: these were analyzed for CHD1, PTEN and ERG expression by immunohistochemistry (IHC). *SPOP* status was determined by targeted next generation sequencing (NGS). We studied the correlations between these biomarkers and a) overall survival from diagnosis; b) overall survival from CRPC; c) duration of abiraterone treatment and d) response to abiraterone. Relationship with outcome was analysed using Cox-regression and Log-Rank analyses.

Results:
CHD1 protein loss was detected in 11 (15%) and 13 (17%) of HSPC and CRPC biopsies, respectively. Comparison of CHD1 expression was feasible in 56 matched, same patient HSPC and CRPC biopsies. CHD1 protein status in HSPC and CRPC correlated in 55 of 56 cases (98%). We identified 22 patients with somatic *SPOP* mutations, with 6 of these mutations not reported previously in PCa. *SPOP* mutations and/ or CHD1 loss was associated with a higher response rate to abiraterone (SPOP: OR=14.50 p=0.001; CHD1: OR=7.30, p=0.08) and a longer time on abiraterone (SPOP: HR=0.37, p=0.002, CHD1: HR=0.50, p=0.06).

Conclusion:
SPOP mutated mCRPCs are strongly enriched for CHD1 loss. These tumors appear highly sensitive to abiraterone treatment.

Translational Relevance:
The genomic heterogeneity of prostate cancer has been elucidated, enabling the study of how genomic sub-classification of these diseases associates with treatment outcome. These studies have identified SPOP mutations as early events in prostate tumorigenesis that commonly associate with CHD1 loss, and define a sub-class of this disease. SPOP mutations have recently been reported to associate with increased androgen receptor signaling. We therefore hypothesized that prostate cancers with SPOP mutations would be highly sensitive to AR blockade, and addressed this with regards to abiraterone treatment in efforts to develop predictive biomarkers of response to therapy. Abiraterone is a CYP17A1 inhibitor that improves survival from advanced prostate cancer. We found here that this subclass of prostate cancers has a very high sensitivity to AR signaling blockade with abiraterone, with most SPOP mutated/ CHD1 deleted cancers responding to this.

Introduction

Adenocarcinomas of the prostate comprise a heterogeneous collection of malignancies with distinct molecular underpinnings. Linking molecular background to clinical outcome, i.e. tumor progression and response to therapy remains an unmet need in prostate cancers. So far, genomic alterations are still not validated as molecular markers for patient stratification for current standard treatments in this disease. This is partially due to the lack of well-annotated patient cohorts, which cover disease evolution from localized, hormone naïve disease to metastatic castration resistant prostate cancer (mCRPC).

Deletions in the gene encoding the chromodomain helicase DNA binding protein 1 (CHD1) and mutations in the gene encoding the Speckle-Type POZ protein (SPOP) are among the most frequent genomic alterations in prostate cancer (up to 29%) (1-6). CHD1 is an ATPase-dependent helicase mediating a variety of biological processes including maintenance of open chromatin, DNA damage repair and transcription (7). SPOP is part of a E3-ubiquitin ligase complex that is involved in controlling protein stability of the androgen receptor (AR) and some of its transcriptional co-activators (8-10). In PCa, mutations specifically impact the MATH protein domain of SPOP leading to increased stability of its substrates and deregulation of diverse molecular pathways impacting transcription, invasion, genome instability and drug resistance (2, 10-14). Loss of *CHD1* significantly co-occurs with mutations in *SPOP* (2, 4, 5). Patients bearing these genomic alterations form a molecular subclass of prostate cancer with increased androgen receptor (AR) transcriptional activity, absence of *ERG* rearrangements and specific epigenetic pattern (4). Importantly, a recent study of multiparametric magnetic resonance imaging (mpMRI) in localized PCa suggests that *CHD1* deleted foci may associate with a less aggressive imaging phenotype (15). Whether and how *CHD1* loss and *SPOP* mutation impact progression to lethal metastatic disease is, however, still unclear. Recent next generation DNA sequencing studies suggest significantly different *CHD1* deletion and *SPOP* mutation frequencies when comparing localized PCa with metastatic castration-resistant prostate cancer (4, 5). This either suggests better outcome for these tumors to initial therapy or a more indolent clinical behavior. However these observations are difficult to interpret since disease molecular evolution during progression has not been monitored.

Genomic sequencing studies have enabled the molecular stratification of PCa identifying diverse subclasses with the promise of developing novel therapeutic strategies to deliver more precise patient care and improving outcome. Abiraterone improves survival in men with mCRPC but the benefit derived varies substantially between patients (16). Limited information is available for improving patient selection for such therapies. Because *CHD1* deletions and *SPOP* mutations reflect a common molecular PCa subclass with increased AR activity, we evaluated the clinical outcome from these cancers hypothesizing that these tumors would be highly sensitive to abiraterone treatment.

Material and Methods

Patient selection, clinical data and study design

Biopsies from bone, lymph node and liver metastases were obtained from patients with metastatic-castration resistant prostate cancer treated at the Royal Marsden Hospital between 2010-2016 and that were a) fit enough to participate in a clinical trial and b) consented for next generation DNA sequencing analysis. All patients gave their written informed consent and were enrolled on institutional protocols approved by the Royal Marsden NHS Foundation Trust Hospital (London, UK) ethics review committee (reference no. 04/Q0801/60). CRPC biopsies were acquired between 2010 and 2016 in accordance with the ethical guidelines of the Helsinki declaration. Clinical data including response to treatment was retrospectively collected from electronic patient records. Patients received abiraterone treatment in UK centers between November 2006 and December 2015. Response to therapy was defined based on Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 criteria and/or PSA falls ≥50% from baseline (17). This study was designed as a case-control study with SPOP mutant patients being selected based on DNA sequencing data and being matched with unselected SPOP wild type patients as controls.

Cell lines

22Rv1 (ATCC®CRL-2505), PC-3 (ATCC®CRL-1435) and NCI-H660 (ATCC®CRL-5813) prostate cancer cell lines were purchased from ATCC and cultured according to the manufacturer's protocol. CHD1 CRISPR clones were derived as described earlier (7).

siRNA transfection

siRNAs targeting CHD1 and non-targeting controls were purchased from Dharmacon (siGenome smartpool CHD1: M-008529-01-0005, siGenome smartpool non-targeting control: D-001206-13-05). siRNA oligonucleotides were transfected with lipofectamine 3000 (Life Technologies) at a final concentration of 100pmol and incubated for 48hrs.

Immunoblotting

22Rv1 isogenic CRISPR clones, siRNA transfected 22Rv1, PC-3 and NCI-H660 cells were harvested in RIPA buffer (Pierce, 89900) containing protease and phosphatase inhibitors (complete mini protease inhibitor cocktail tablets, Roche, 11374600). The soluble fraction was isolated using centrifugation and quantified by bicinchonininc acid assays (BCA) (Pierce, QG219588). Cell lysates were mixed with LDS sample buffer (Life Technologies, 1621149) and 30-50µg of total protein was loaded onto 4-12% gradient SDS acrylamide gels (Life Technologies, NP0322). Transfer to PDVF membrane (Millipore, IPV400010) was performed at 90V for 90 minutes at room temperature. Protein bands were detected using HRP-substrate (Millipore, WBLUC0500). Primary antibodies were anti-CHD1 rabbit monoclonal antibody (clone D8C2, Cell Signaling, 4351) and anti-GAPDH (clone 6C5, Millipore, MAB374). Secondary antibodies were anti-rabbit and anti-mouse horseradish peroxidase-conjugated whole antibody from sheep (GE, NA934V and NXA93).

Immunohistochemistry

Immunohistochemistry was performed on 3µM formalin-fixed and paraffin-embedded (FFPE) tissue sections. Heat based antigen retrieval was performed by boiling the tissue sections in pH6 citrate buffer (TCS Biosciences Ltd., HDS05, 1:100 dilution) for 18 minutes using a microwave. CHD1 immunostaining was done on the Launch i6000 IHC autostainer using a 1:50 dilution of primary antibody binding CHD1 (Cell Signaling, #4351) for 1hr. Visualization of antibody binding was achieved using the Novolink polymer detection method (Leica,

RE7200-CE). To avoid false negative results, we defined endothelial cell CHD1 expression as a necessary internal control for samples to be included in the analysis. Immunohistochemistry for PTEN and ERG was done as reported previously (18, 19). Tumor content, morphology and intensity of protein expression were evaluated by a pathologist (D.N.R.). H-score was defined as described earlier (18). The cut off for CHD1 protein loss was define as H-score ≤5.

Fluorescence in situ hybridization

FISH for assessing *CHD1* copy number status in formalin-fixed and paraffin-embedded tumor tissue was performed as described previously (18). The probes were: *CHD1* (RP11-58M12, chr. 5q21) and reference (RP11-429D13, chr. 5p13.1). Probes were amplified using the GenomiPhi v3 DNA amplification kit (Illustra, 25-6601-24) and directly labeled with CY3 (CHD1) and CY5 using the Bioprime DNA labeling system (Thermo Fisher Scientific, 18094011). Fluorescence images were taken using the Bioview Duet imaging system and copy number status of at least 50 non-overlapping tumor cell nuclei were determined by a pathologist (D.N.R).

DNA sequencing

DNA isolation: DNA from tumor tissue biopsies was extracted using the QIAamp DNA FFPE Tissue kit (Qiagen, Venlo, Limburg, Netherlands), quantified by Quant-iT Picogreen double-stranded DNA (dsDNA) Assay Kits (Invitrogen, ThermoFisher Scientific Co). The Illumina FFPE QC kit (WG-321-1001) was used for DNA quality control tests.

Targeted sequencing: Libraries were prepared using a customized Generead v2 DNAseq Panel (Qiagen) consisting of 113 genes including *SPOP* as previously described (20, 21). Libraries were read on the Illumina MiSeq™ platform.

Sequence alignment: FASTQ files were generated using the Illumina MiSeq Reporter v2.5.1.3. Sequence alignment and mutation calling was performed using BWA tools and the GATK variant annotator by the Qiagen GeneRead Targeted Exon Enrichment Panel Data Analysis web Portal.

Bioinformatic analysis

Mutation burden: The mutation burden was estimated from targeted next generation sequencing (NGS) panel data after filtering out of spurious and germline variants using methods reported previously (22). Genetic variants were called using the GATK pipeline (23). Low quality variants were removed (haplotype score >200, mapping quality<40, coverage depth <60, alternative allele <5% of reads, multi-allelic calls, indels, known poorly sequenced sites). Variants were then annotated using Oncotator (version 1.8.0)(24). Germline variants were defined when the allele frequency was >5% in our cohort or in two or more public databases (ExAC) (25), 1000 Genomes (The 1000 Genomes Project Consortium) (26) and dbSNP (27), or with more than 99.9% of the reads being the alternate allele. These germline variants were filtered out. Finally, point mutations described as somatic in the COSMIC database (28) at least 10 times or more were then ‘added’ back into the mutation count.

Copy number burden: Following assessment of copy number variation from targeted NGS, we calculated copy number burden as the proportion of evaluable genes (n=99) bearing any detectable change, i.e. a log2 ratio of greater or less than 0.4 and -0.4 respectively (21). Samples were included in the analysis if the total read count was >500K, >95% of properly paired reads, >99.9% reads on target and IQR >0.8.

Shannon Index: Given counts of FISH probes for minimum 50 individual cells on a per-sample basis, we calculated inter-cellular diversity by classifying each cell by the state of

both probes, totaling the number of each class, and applying the R package Vegan (v.2.4.4) to generate the Shannon-Weaver diversity index H.

Statistical analysis

Clinical characteristics at diagnosis (age, Gleason score, metastatic disease), and treatments received, were compared by CHD1 status at CRPC using Wilcoxon Rank-Sum tests or Fisher's Exact test if categorical. Baseline levels of PSA, hemoglobin, alkaline phosphatase, lactate dehydrogenase and albumin at the start of abiraterone treatment were compared by CHD1 and *SPOP* status at CRPC using t-tests or Wilcoxon Rank-Sum tests if considered to be non-normally distributed. Data on castration-resistance was determined retrospectively from patient's medical records. The change in CHD1 H-Score in patient matched HSPC and CRPC samples was compared using the Wilcoxon matched-pairs signed-ranks test. Overall survival (OS) from diagnosis, CRPC and the start of abiraterone treatment was compared by CHD1 and SPOP status using Kaplan-Meier plots. Univariate Cox models were used to evaluate the association of metastatic disease at diagnosis, *SPOP* mutation, CHD1 negativity and ERG expression at CRPC with OS from diagnosis, time on abiraterone and time from start of LHRH to CRPC. Univariate logistic regression analyses also evaluated the association of these characteristics with response to abiraterone, defined as radiographic response according to the Response Evaluation Criteria in Solid Tumours 1.1 (RECIST) and/or a ≥50% fall in PSA from baseline (17). All analyses were conducted using Stata v13.1.

Results

Establishing a CHD1 immunohistochemistry assay

To determine the frequency of CHD1 protein loss across a spectrum of clinical states, we established and validated an immunohistochemistry (IHC)-based assay for formalin-fixed and paraffin-embedded (FFPE) tissue. We first evaluated FFPE embedded human prostate cancer cell lines that either had an amplification (PC-3) or deletion (NCI-H660) of CHD1 confirmed by fluorescence *in situ* hybridization (FISH) (Figure 1A). To further ensure the specificity of the antibody, we depleted CHD1 in the CRPC cell line 22Rv1 transiently by siRNA as well as stably by CRISPR/ CAS9. Western blot analysis using the same CHD1 antibody revealed a major decrease of CHD1 protein after siRNA and CRISPR/CAS9 treatment (Figure 1B). The loss of CHD1 after CRISPR/CAS9 knockout in this isogenic model was further validated in FFPE embedded cells (Figure 1B). Lastly, we implemented the IHC assay in human tumor biopsies from 44 PCa patients (12 HSPC, 32 CRPC) representative of the most common sites of disease (13 bone, 9 lymph node, 5 liver, 3 soft tissue and 2 TURP biopsies) and compared *CHD1* expression with gene copy number determined by FISH. Overall, 3/44 (7%) had loss of both *CHD1* expression by IHC as well as gene loss by FISH. CHD1 protein expression by IHC and gene copy number status by FISH were strongly associated (Figure 1C, 1D).

Molecular features of CHD1 deleted CRPC

To explore the molecular features of *CHD1* deleted lethal prostate cancer we selected 89 patients for whom we had CRPC biopsies (Supplementary Figure 1A) enriching this cohort for *CHD1* loss CRPC by including tumors with known *SPOP* mutations based on data from prior molecular characterization studies. We performed IHC for CHD1 in paired, same patient, CRPC and HSPC biopsies from these 89 subjects. The IHC data were analyzable for 73 (82%) and 83 (93%) HSPC and CRPC biopsies, respectively. We identified 11 (15.1%) and 13 (16.9%) HSPC and CRPC biopsies, respectively, with complete loss of CHD1 protein (Figure 2A). Targeted or whole exome DNA sequencing data were available for 71 (79.7%) patients (CRPC biopsies: n=69, HSPC biopsies: n=2), with 22 of these carrying a mutation that affect SPOP (Figure 2B). We identified 6 mutations, which have not been previously described in PCa before, including one (p.A187T) located in the BTB domain in an area reported to be necessary for homodimerization (Figure 2B, Supplementary Table 1): two of these 6 mutations were located in residues (E50, R121) previously associated with endometrial cancer (29). The overall distribution of altered residues was, however, similar to other CRPC cohorts resulting in two distinct mutational hotspots in the MATH domain (Supplementary Figure 2B). Loss of CHD1 and/ or *SPOP* mutation ($CHD1^{Loss}$/$SPOP^{MUT}$) was found in a total of 22 patients (24.7%). All CHD1 loss cases that could be analyzed by targeted sequencing (11 out of 13 could be sequenced), had an *SPOP* mutation ($p<0.0001$) (Table 1, Figure 2A). We performed ERG IHC analysis in our CRPC cohort and confirmed a mutually exclusive relationship between *SPOP* mutations and CHD1 loss and ERG overexpression ($p<0.001$ for SPOP; $p=0.003$ for CHD1) (Figure 2A). Prior genomic studies in localized PCa have suggested a synthetic lethal relationship between *CHD1* loss and loss of the tumor suppressor *PTEN*. Surprisingly, our analysis identified 2 cases (2.4%) of combined CHD1 and PTEN protein loss suggesting that although the majority of $CHD1^{Loss}$/$SPOP^{MUT}$ tumors in our cohort do not have PTEN loss, certain genomic backgrounds tolerate the concurrent loss of these two proteins (Figure 2A, Supplementary Figure 2A). To investigate the impact of *SPOP* mutations on genome stability in CRPC we estimated mutation (SNV) burden and copy number burden based on targeted NGS data from 46 analyzable CRPC samples. *SPOP* mutation did not associate with differences in SNV burden (Figure 2C). However, *SPOP* mutation associated with increased copy number changes ($p=0.013$)

identified by targeted NGS similar to what we previously reported using whole genome DNA sequencing for localized PCa (Figure 2D).

CHD1 status during disease progression

Since CHD1 loss co-occurred with *SPOP* mutations we hypothesized that the *CHD1* gene locus is under selective pressure throughout the evolution of *SPOP* mutant PCa. To estimate the stability of the *CHD1* gene locus during tumor evolution we evaluated copy number states at a single-cell level using fluorescence in situ hybridization (FISH) in matched HSPC and CRPC biopsies. For this purpose we determined copy number combinations of *CHD1* (5q21) and reference probes (5p13.1) in 4266 single cells in matched HSPC and CRPC biopsies from 36 patients and calculated the Shannon index (Figure 2E,F). This allowed us to perform a quantitative measurement of chromosome 5 copy number diversity at single cell level for each biopsy. This analysis indicates that PCa commonly has various degrees of intratumour genomic heterogeneity at 5p/5q with some dramatic outliers. However, *SPOP* mutant lethal PCa showed a significantly lower clonal diversity at chromosome 5 (Figure 2F, Supplementary Figure 2D,E) (HSPC: p=0.018, CRPC: p=0.0025). When comparing the Shannon index between HSPC and CRPC patient-matched biopsies, no significant changes of genomic clonal diversity occurred with progression on androgen deprivation therapy (ADT) from HSPC to CRPC (Figure 2F) suggesting that the loci containing *CHD1* are a) an important driver locus in SPOP mutant tumors and b) do not succumb to therapy-induced selection pressure. Thus, our analyses of patient-matched HSPC and CRPC biopsies from patients that developed lethal metastatic CRPC do not indicate a systematic depletion of *CHD1* negative tumor clones during progression on ADT.

To evaluate whether CHD1 protein expression changes during disease progression and development of resistance to ADT, we compared patient-matched HSPC and CRPC biopsies. Overall, 56 pairs of matched, same patient, HSPC and CRPC samples were available for CHD1 expression evaluation by IHC. When categorizing our samples into CHD1 IHC negative and CHD1 IHC positive we found that there was no change in CHD1 expression status between the matched tumor samples confirming our hypothesis that *CHD1* loss is under selective pressure during tumor progression; 55 out of 56 analyzable samples (98%) maintained their CHD1 status (Figure 2E). However, we found that overall, CHD1 expression level increases with progression from HSPC to CRPC in CHD1 IHC positive cases (p=0.010) (Supplementary Figure 2C). Tumors with CHD1 loss of protein expression had no detectable tumor cells expressing CHD1.

The CHD1 loss /*SPOP* ^{MUT} subclass and outcome from abiraterone

We investigated if this molecular subtype of prostate cancer, defined by CHD1 loss and *SPOP* mutations (CHD1^{Loss}/*SPOP*^{MUT}) impacts clinical outcome. We evaluated the prognostic value of CHD1 loss and *SPOP* mutation and found no significant association with overall survival (OS) from diagnosis for either variable (Table 2). As previously reported, we also found no association between ERG expression, detecting *ERG* rearrangements, and OS (Table 2). We also analyzed the performance of known prognostic variables including presence of metastasis at diagnosis, which associated with shorter OS (p=0.002). Furthermore, we did not find any association between CHD1^{Loss}/*SPOP*^{MUT} and time to resistance to androgen deprivation therapy by LHRHa (Table 2). We found no association of CHD1^{Loss}/*SPOP*^{MUT} with OS from CRPC (Figure 3A). However, we speculated that there might be a better response to second line treatments such as abiraterone since preclinical data suggest a direct impact of SPOP on AR protein stability and signaling (8, 10, 12). We found that *SPOP* ^{MUT} patients respond better to abiraterone when considering 50% PSA falls (SPOP: p=0.03) and are less likely to progress (OR=14.50, p=0.001). Moreover, SPOP mutations are associated with longer median duration of abiraterone treatment (SPOP:

HR=0.37, p=0.002). Similar trends were observed for CHD1 when considered as individual variable although not reaching statistical significance (absence of progression: OR=7.30, p=0.08; abiraterone treatment duration: HR=0.50, p=0.06) (Table 2, Figure 3B,C, D; Supplementary Figure 3A-C, Supplementary Table 2). In summary, our data suggest that CHD1^{Loss}/*SPOP*^{MUT} patients might respond better to abiraterone. These data need to be validated now in samples from randomized phase III clinical trials.

Discussion

Herein we describe key molecular features of *CHD1* deleted/ *SPOP* mutant mCRPC showing that *SPOP* mutations and CHD1 loss associate with a higher likelihood of benefit from abiraterone therapy. CHD1 loss significantly associated with *SPOP* mutations, while ERG rearrangements, as detected by ERG protein expression inversely correlated with CHD1 loss and *SPOP* mutation suggesting that these genomic loci underlie selective pressure during progression to PCa. We report PTEN loss largely in *CHD1* and *SPOP* wildtype backgrounds in mCRPC as reported for localized PCa but describe two patients with combined loss of CHD1 and PTEN suggesting that a synthetic essential relationship between these two proteins is not universal. Similar to previous reports most of the *SPOP* mutations identified affected the SPOP-MATH domain, which is responsible for the binding of protein substrates. When comparing these mutations with other cohorts, we identified a similar distribution with two major mutational hotspots including the residues Y83-F102 and F125-F133. Surprisingly, we also identified for the first time several SPOP mutations unreported in previous systematic prostate cancer studies including E50K, S105F, Q120R, R121P, G148E and A187T: these included two mutations affecting residues located in a less well-characterized portion of the MATH domain previously described in endometrial cancer. Whether these represent distinct functional impact unique to CRPC, or mechanistically have distinct substrate binding specificity, will need to be elucidated in future studies.

Recent genomic studies in prostate cancer suggested a decreased frequency of *SPOP* mutations in mCRPC when compared to localized disease (8% versus 11%) (4, 5). The frequency of *CHD1* homozygous loss was reported to be 4.7% versus 9% in mCRPC versus localized disease. It has been suggested that $CHD1^{Loss}$/$SPOP^{MUT}$ tumors are generally less aggressive in nature and that perhaps these data indicate a decreasing frequency of this sub-type in mCRPC. However, our analysis in same patient, matched, treatment-naïve and mCRPC biopsies indicate that the frequency of *CHD1* deletion does not change significantly with disease progression to mCRPC although we cannot completely exclude subclonal differences at 5q21 at a single cell level. Overall, our data indicate that this genotype is present from diagnosis and that there is no systematic selection generated by ADT, suggesting that the difference between the TCGA and SU2C series was most likely related to patient selection and not tumor evolution.

Despite the in-depth molecular characterization and sub-classification of prostate cancer, there is currently limited understanding of how this impacts benefit from established treatments including abiraterone. A detailed understanding of the molecular characteristics of the prostate cancers sensitive or resistant to these drugs is urgently needed to help minimize overtreatment with inactive drugs. We describe here that *SPOP* mutant and CHD1 IHC negative mCRPC respond substantially better and longer to abiraterone when compared to mCRPC that lack these alterations. Prospective clinical trials are now needed to validate this differential response to abiraterone in this subclass of PCa. Considering the early onset of these alterations in the history of PCa, *SPOP* mutation/ CHD1 loss may function as positive predictive biomarkers for abiraterone therapy.

This study does, however, have limitations since it is a retrospective, single center study conducted with an intentional selection bias to enrich for *SPOP* mutated PCa. These may limit translating these results to a general unselected population of prostate cancer patients. The impact of *SPOP*/*CHD1* status on abiraterone treatment outcome now needs prospective validation.

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Figure legends

Table 1: Demographic and clinical characteristics of patients in this cohort.

Table 2: Summary of univariate statistical analyses evaluating the association of CHD1 loss or *SPOP* mutation with response to LHRHa and abiraterone.

Figure 1: Development and validation of CHD1 immunohistochemistry assay.
A) Micrographs (20x) of formalin-fixed and paraffin-embedded (FFPE) prostate cancer cells negative (NCI-H660) or positive for CHD1 analyzed by CHD1 immunohistochemistry (top) and fluorescence in situ hybridization (FISH) (below). Signals from the FISH probes (CHD1: red; reference probe: green). **B)** Knockdown of CHD1 by siRNA or CRISPR/CAS9 decreases CHD1 protein levels in 22Rv1 cells by Western Blotting (left) and IHC on FFPE embedded cells using the same antibody as in A. **C)** Correlation of CHD1 copy number and expression level in 44 prostate cancer samples. Numbers for HSPC and CRPC are indicated. Protein expression was summarized as H-scores. p=0.002, one way Anova. **D)** Micrographs of representative examples of CHD1 loss (top) and CHD1 normal (below) by IHC (20x) and FISH (60x). Signals from the FISH probes (CHD1: green; reference probe: red).

Figure 2: Molecular characterization of the CRPC cohort.
A) Oncoprint summarizing CHD1, ERG and PTEN protein expression as well as *SPOP* mutation status in mCRPC biopsies from 89 patients. **B)** Lollipop blot representing the location of the affected amino acid changes in the SPOP protein corresponding to the 22 identified mutations. **C-D)** Genomic features of *SPOP* mutant CRPC estimated by a targeted NGS assay (n=46). The levels of mutational burden do not change in *SPOP* mutant CRPC (p=0.1) (C). Copy number burden estimated by the percentages of genes affected are significantly higher in *SPOP* mutant CRPC compared with *SPOP* wildtype disease (p=0.013) (D). p-values were calculated using unpaired t-test. **E)** Workflow describing how the Shannon Index was calculated. We counted the signals from a dual FISH assay containing probes for 5p and 5q on a single-cell level for each patient. FISH probes are indicated by red and green dots in the cell nucleus (blue). Different cell populations are then defined by possible probe combination reflecting the diversity of copy number differences at these genomic loci per tumor cell. The frequency of these different cell populations in a given biopsy reflects population diversity, which is estimated using the Shannon Index. **F)** *SPOP* mutant tumors have a significantly lower mean Shannon Index than *SPOP* wildtype tumors in both HSPC and CRPC biopsies (HSPC: p=0.018, CRPC: p=0.0025). Mean Shannon Indexes for each subclass do not change from HSPC to CRPC. p-values were calculated using unpaired t-test. n=36 **G)** CHD1 protein level in HSPC and CRPC biopsies. Heatmap summarizing CHD1 expression status (loss versus normal) in 56 patient-matched paired biopsies from HSPC and CRPC. Blue indicates protein loss. Grey indicates protein expression.

Figure 3: Association of CHD1 loss and *SPOP* mutation with clinical outcome. A) Kaplan Meier curve summarizing overall survival (OS) of $SPOP^{MUT}$ versus $SPOP^{WT}$ tumors from CRPC in this cohort. *SPOP* mutation is not prognostic. **B, C)** Kaplan Meier curves showing increased survival from start of abiraterone **(B)** and time on abiraterone **(C)** in *SPOP* mutant tumors. **D)** Waterfall plot showing increased PSA responses from start of abiraterone in $SPOP^{MUT}$ versus $SPOP^{WT}$ tumors. Each bar represents PSA nadir from start of treatment for an individual patient. Dashed line indicates PSA fall by 50%. PSA nadir data were available for 37 patients with known SPOP status.

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Figure 1

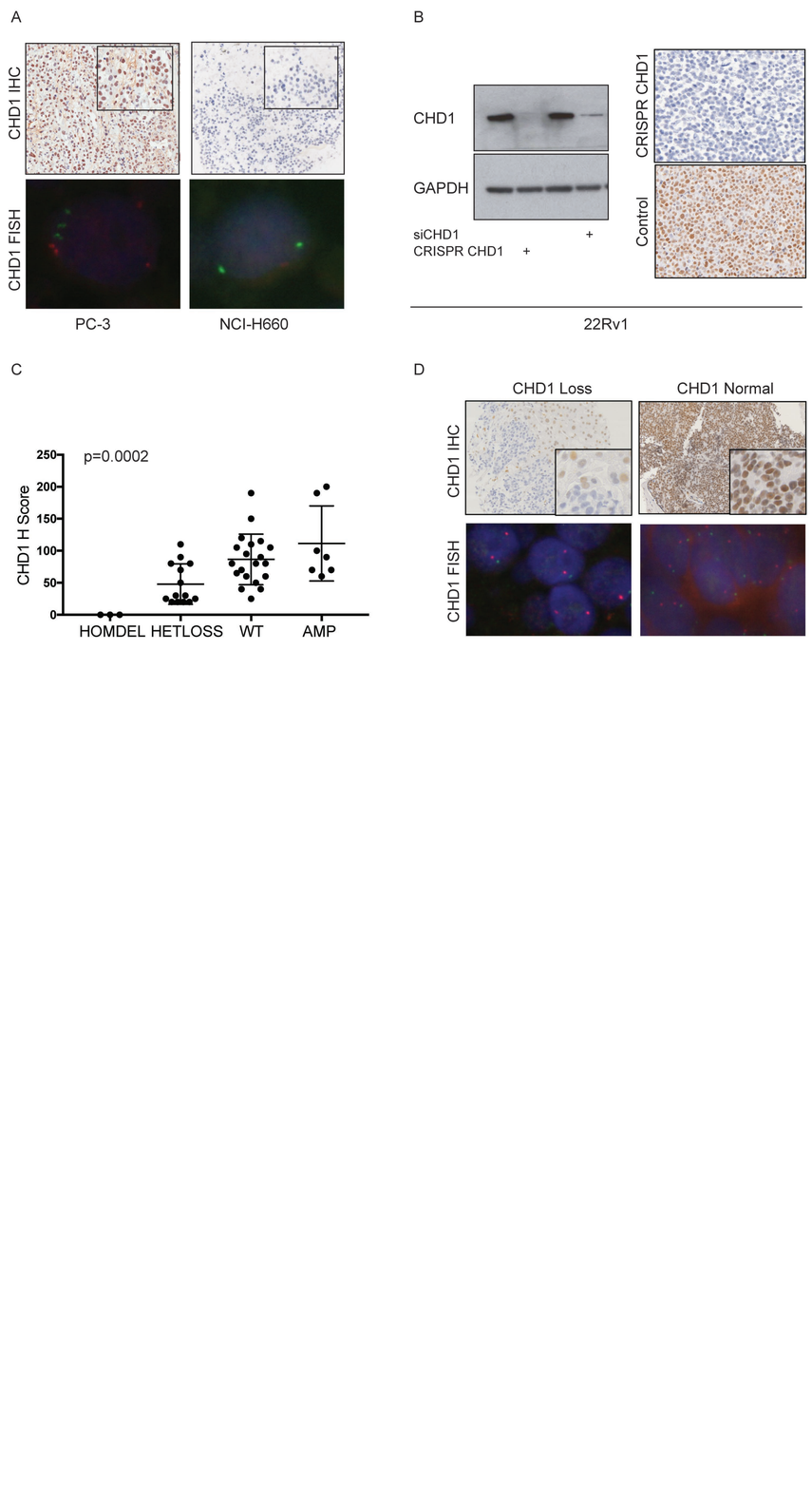
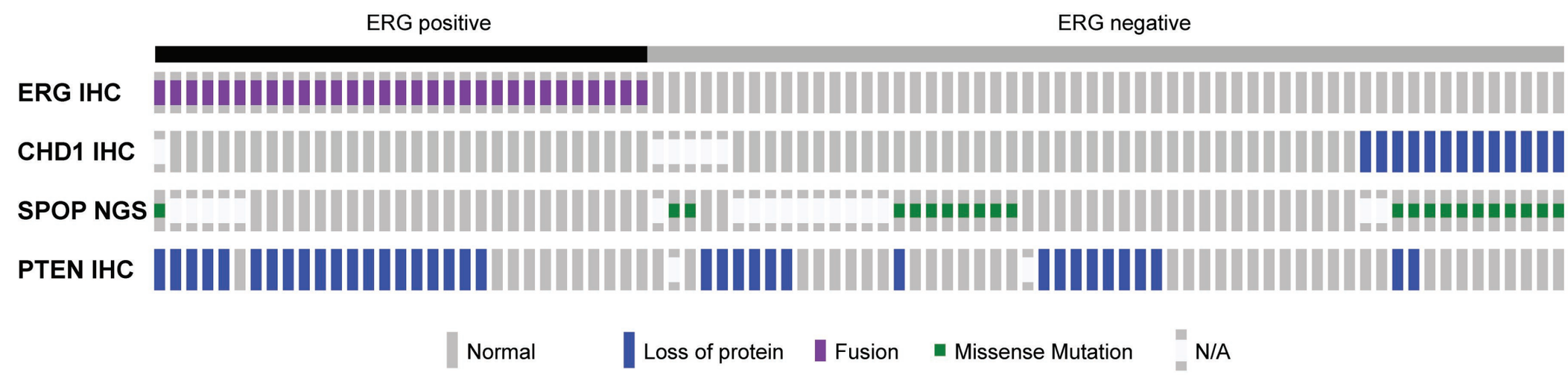
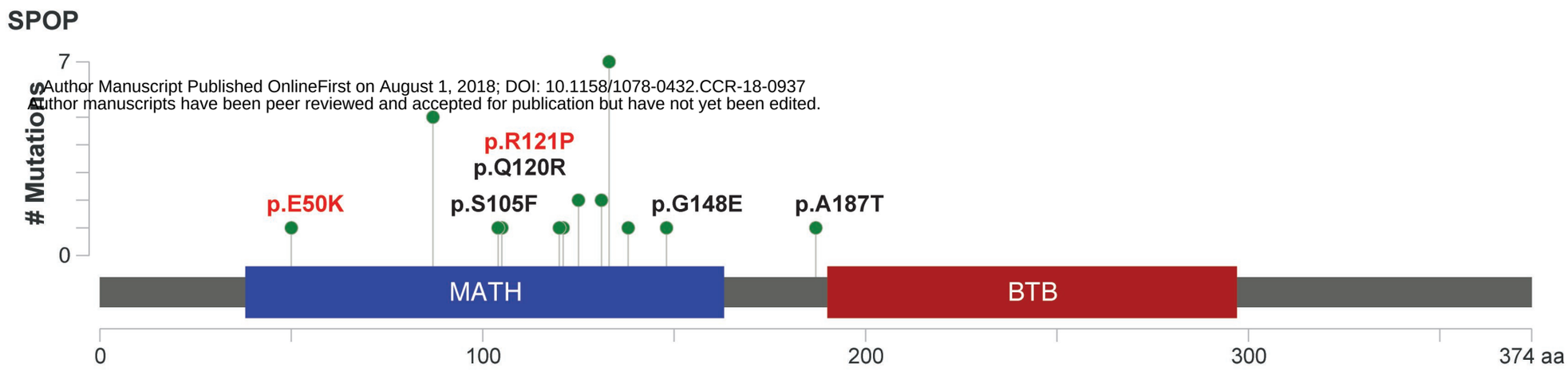
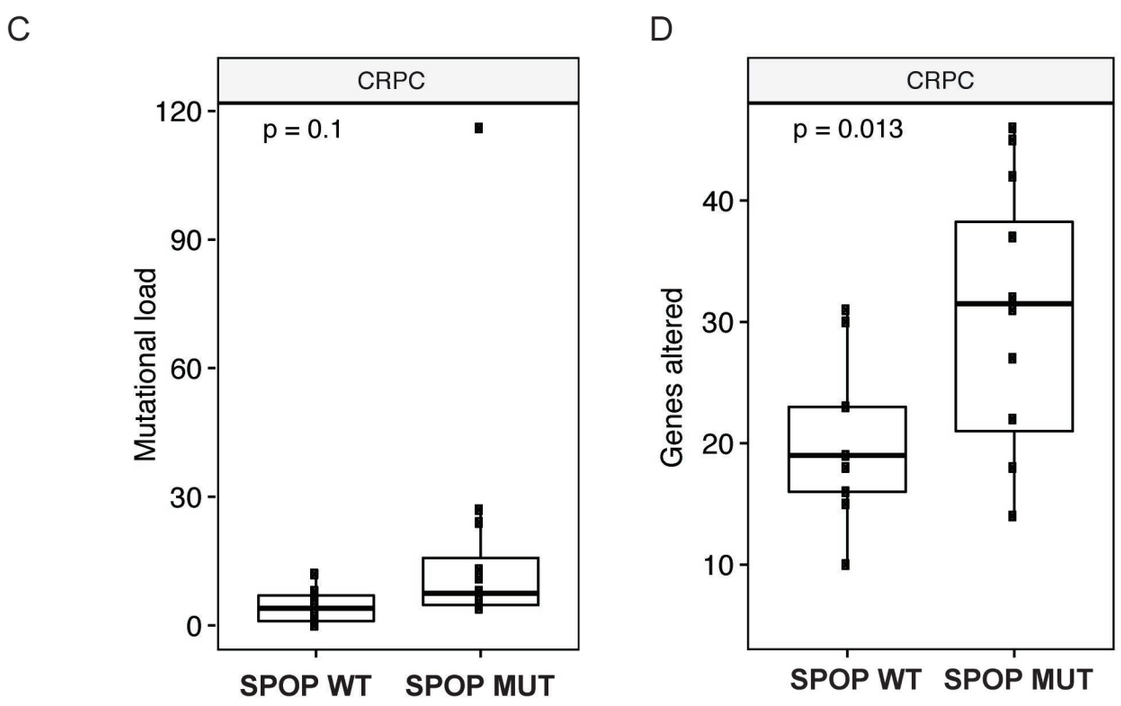
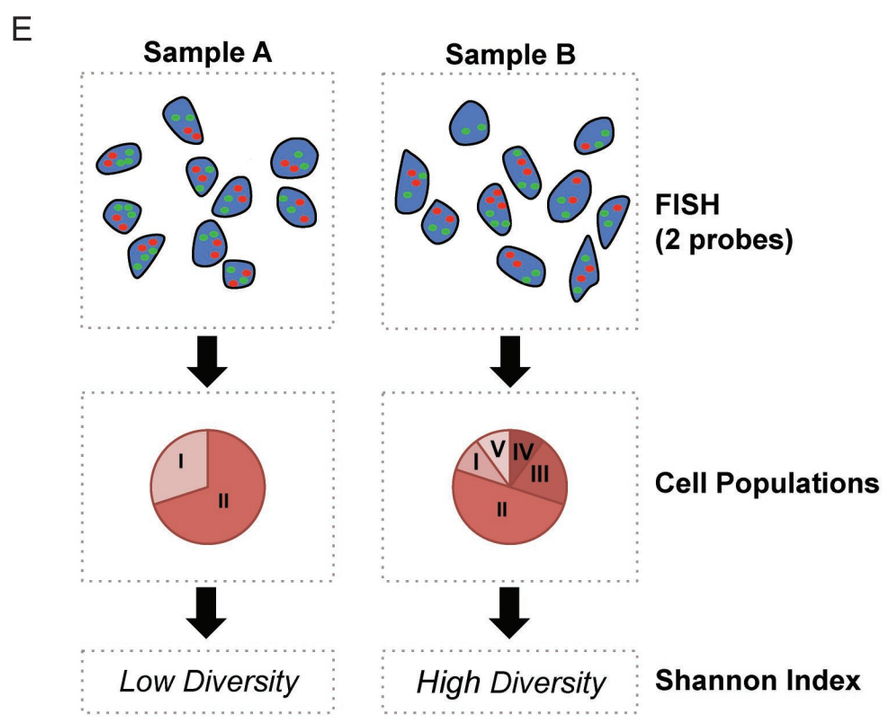


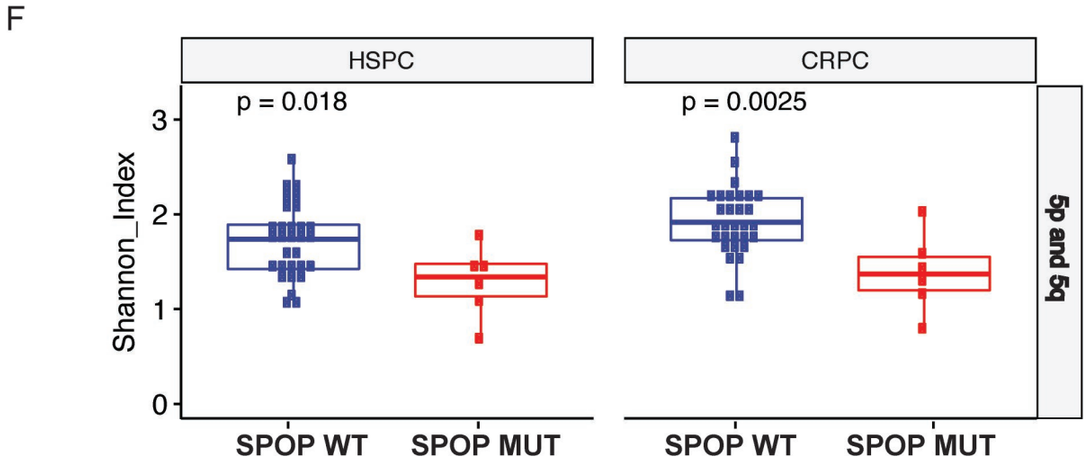
Figure 2











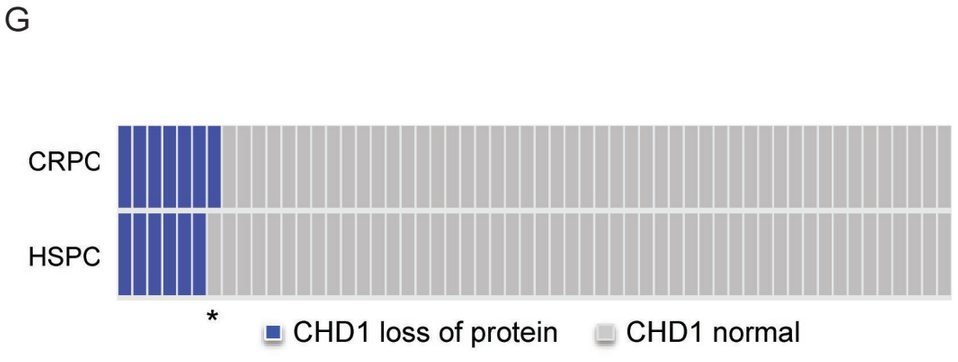


Figure 3

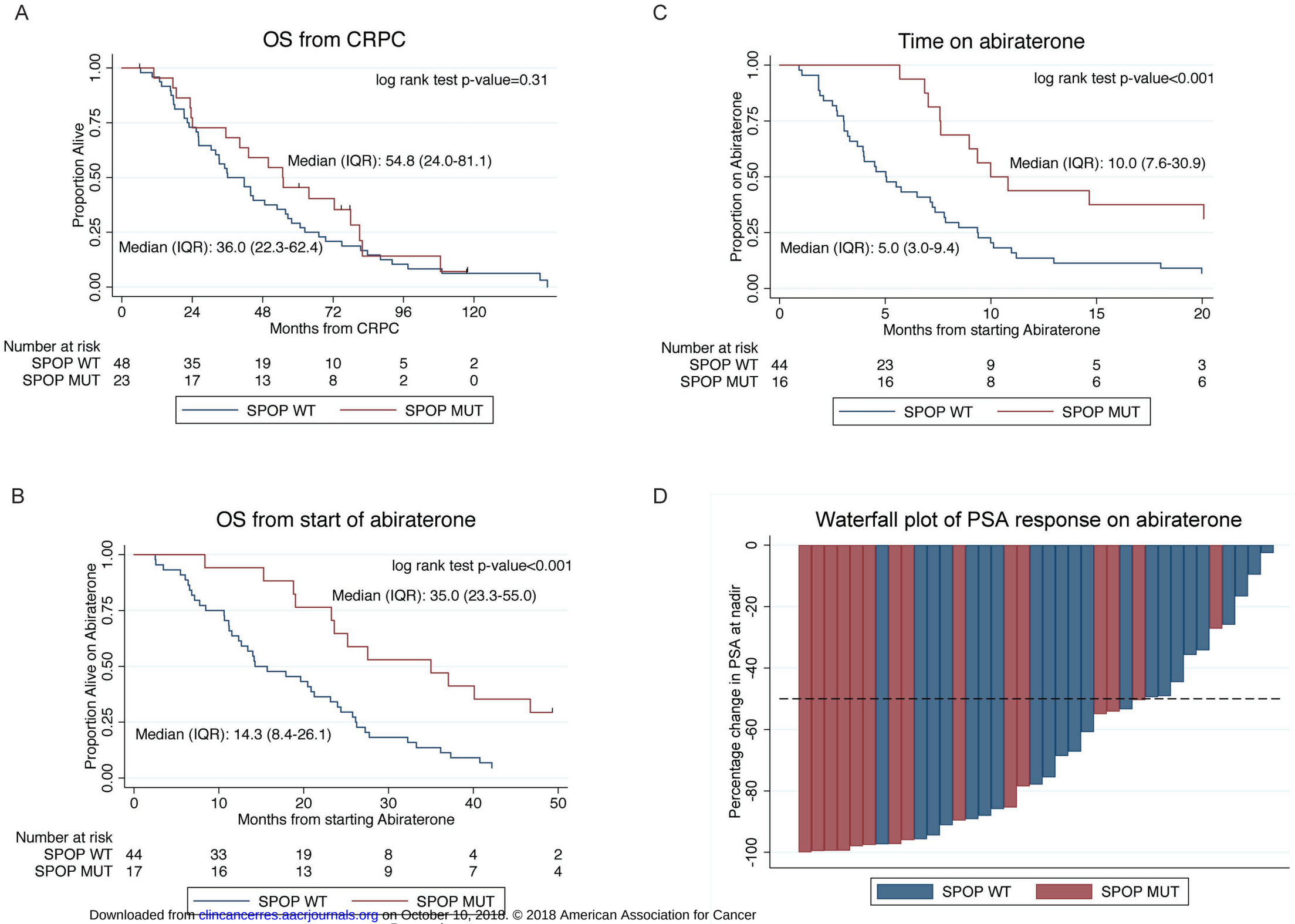


Table 1 Demographic and clinical characteristics of patients

Overall (n=89) **Lacking CHD1 IHC n=6 (6.7%)**	**CHD1 negative in CRPC n=13 (14.6%)**	**CHD1 positive in CRPC n=70 (78.7%)**	**P-value**
Age at diagnosis, years (mean(SD))	62.7 (6.8)	59.9 (13.1)	p=0.46[1]
Gleason at diagnosis 6 7 8-10 NA	 2 (15%) 2 (15%) 8 (62%) 1 (8%)	 5 (7%) 16 (23%) 44 (63%) 5 (7%)	p=0.71[2]
Metastasis at diagnosis M0 M1	 5 (39%) 8 (62%)	 43 (61%) 27 (39%)	p=0.14[2]
PSA at diagnosis, ln(µg/l) (mean (SD))	4.8 (1.6)	5 (1.6)	p=0.82[1]
Treatments for CRPC Docetaxel Abiraterone	 11 (85%) 9 (69%)	 64 (93%) 62 (90%)	 p=0.31[2] p=0.07[2]

[1]T-test
[2]Fisher's Exact Test

Table 2

Overall Survival from Diagnosis	**Univariate HR**	**95% CI**	**p-value**
Metastatic at diagnosis	2.07	1.32-3.24	0.002
SPOP mutation at CRPC	0.80	0.46-1.38	0.43
CHD1 negative IHC at CRPC	0.81	0.42-1.58	0.54
ERG at CRPC	1.14	0.72-1.78	0.58
Time on Abiraterone	**Univariate HR**	**95% CI**	**p-value**
Metastatic at diagnosis	1.00	0.63-1.60	0.99
SPOP mutation at CRPC	0.37	0.20-0.69	0.002
CHD1 negative IHC at CRPC	0.50	0.25-1.02	0.06
ERG at CRPC	1.25	0.77-2.02	0.37
Time from start LHRH to CRPC	**Univariate HR**	**95% CI**	**p-value**
Metastatic at diagnosis	2.11	1.36-3.28	0.001
SPOP mutation at CRPC	1.13	0.68-1.87	0.64
CHD1 negative IHC at CRPC	0.86	0.44-1.69	0.66
ERG at CRPC	1.16	0.75-1.79	0.52
Response to Abiraterone	**Univariate OR**	**95% CI**	**p-value**
Metastatic at diagnosis	0.72	0.29-1.80	0.49
SPOP mutation at CRPC	14.50	2.92 -71.94	0.001
CHD1 negative IHC at CRPC	7.30	0.82-65.11	0.08
ERG at CRPC	0.71	0.28-1.82	0.47

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SPOP mutated/ CHD1 deleted lethal prostate cancer and abiraterone sensitivity.

Gunther Boysen, Daniel Nava Rodrigues, Pasquale Rescigno, et al.

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