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SLFN11 expression in advanced prostate cancer and response to platinum-based chemotherapy

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ABSTRACT

Expression of the DNA/RNA helicase schlafen family member 11 (SLFN11) has been identified as a sensitizer of tumor cells to DNA damaging agents including platinum chemotherapy. We assessed the impact of SLFN11 expression on response to platinum chemotherapy and outcomes in patients with metastatic castration-resistant prostate cancer (CRPC). Tumor expression of SLFN11 was assessed in 41 CRPC patients treated with platinum chemotherapy by RNAseq of metastatic biopsy tissue (*n*=27) and/or immunofluorescence in circulating tumor cells (CTCs) (*n*=20). Cox-regression and Kaplan-Meier methods were used to evaluate the association of SLFN11 expression with radiographic progression-free survival (rPFS) and overall survival (OS). Multivariate analysis included tumor histology (ie., adenocarcinoma or neuroendocrine) and the presence or absence of DNA repair aberrations. Patient-derived-organoids with SLFN11 expression and after knockout by CRISPR-Cas9 were treated with platinum and assessed for changes in dose response.

Patients were treated with platinum-combination (N=38) or platinum-monotherapy (N=3). Median lines of prior therapy for CRPC was two. Median OS was 8.7 months. Overexpression of SLFN11 in metastatic tumors by RNA-Seq was associated with longer rPFS compared to those without overexpression (6.9 versus 2.8 months, HR=3.72, 95% CI 1.56-8.87, $p<0.001$); similar results were observed for patients with SLFN11-positive versus SLFN11-negative CTCs (rPFS 6.0 versus 2.2 months, HR=4.02, 95% CI 0.77-20.86, $p=0.002$). A prostate specific antigen (PSA) decline of ≥50% was observed in all patients with SLFN11 overexpression. No association was observed between SLFN11 expression and OS. On multivariable analysis, SLFN11 was an independent factor associated with rPFS on platinum therapy. Platinum response of organoids-expressing-SLFN11 was reduced after SLFN11 knockout. Our data suggest that SLFN11 expression might identify CRPC patients with a better response to platinum-chemotherapy independent of histology or other genomic alterations. Additional studies, also in the context of PARP inhibitors, are warranted.

1. Introduction

Prostate cancer is the second leading cause of male cancer death worldwide (1). Patients with metastatic prostate cancer typically respond well to androgen deprivation therapy, often administered together with docetaxel or second-generation hormonal drugs, but invariably progression to castration-resistant prostate cancer (CRPC) occurs within a median of 16 months (2). Therapies for metastatic

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CRPC include potent androgen receptor (AR) targeted therapies, taxane chemotherapies, sipuleucel-T, radium-223, and a number of investigational drugs. With a growing armamentarium of approved and investigational agents, the identification of predictive biomarkers to help guide individual therapy and the optimal sequence of therapy for men with advanced prostate cancer is needed now more than ever. Even unapproved but widely available agents, such as platinum chemotherapy, may provide significant clinical benefit for select patients.

Prior clinical studies investigating platinum chemotherapy for molecularly unselected advanced prostate cancer patients showed that the traditional platinum compounds (ie., cisplatin, carboplatin, oxaliplatin), administered alone or in combination with other chemotherapies, resulted in moderate anti-tumor activity for the overall population (3). In a phase-3 trial of the oral platinum compound satraplatin for patients with metastatic CRPC, there was a delay in progression of disease but no significant overall survival benefit (4). Nonetheless, exceptional responders to platinum have been reported (5-7), and platinum is sometimes still used clinically for patients with metastatic CRPC, particularly those with aggressive disease and/or following resistance to approved drugs.

DNA repair alterations involving homologous recombination genes such as *BRCA1*, *BRCA2*, and *ATM*, are present in up to 20% of patients with CRPC (at either germline or somatic level) and have been found to mediate both platinum sensitivity (5,6,8) as well as poly(ADP-ribose) polymerase inhibitor (PARPi) response in metastatic CRPC (9,10,11). The Phase 3 PROfound clinical trial was recently reported as positive, demonstrating a rPFS benefit for the PARPi olaparib versus potent AR therapies for patients with CRPC with DNA repair aberrations (12). These observations have re-invigorated the field's interest and a number of additional clinical studies testing either platinum or PARPi for molecularly enriched CRPC patients with DNA repair alterations at various stages of prostate cancer progression are underway.

In addition to those with genomic alterations involving DNA repair genes, there may be other patients that might preferentially benefit from receiving platinum. For instance, patients that develop treatment-related small cell /neuroendocrine prostate cancer or those with aggressive variant prostate cancer (AVPC) clinical features respond to platinum-based chemotherapy regimens with similar response rates as seen as patients with small-cell lung cancer (13-17).

To explore other potential biomarkers of platinum sensitivity in prostate cancer, we investigated the role of Schlafen Family Member 11 (SLFN11). SLFN11 is a DNA/RNA helicase, first described for its

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role in normal thymocyte development and differentiation (18). SLFN11 is only expressed in vertebrates and especially in mammals, playing a critical role not only for thymocyte development, but also for immune response and cell proliferation (19). Later, SLFN11 expression was assessed in several cancer cell lines, including Ewing's sarcoma, small cell lung, ovarian, and colon cancers, and SLFN11 expression was found to be associated with response to DNA damaging agents including platinum compounds, topoisomerase I and II inhibitors, and alkylator chemotherapies (20). In addition, SLFN11 expression has been associated with sensitivity to PARPi in small cell lung cancer patient-derived xenograft models (21). In a recent randomized phase II trial of temozolomide in combination with either the PARPi veliparib or placebo in patients with relapsed-sensitive or refractory small-cell lung cancer (22), SLFN11 expression determined in tumor tissue by immunohistochemistry was associated with a significantly improvement in clinical outcomes for patients with SLFN11-expressing tumors treated with veliparib compared to those that were SLFN11-negative.

To date, clinical studies investigating the role of SLFN11 as a potential mediator of sensitivity to DNA damaging agents has been largely investigated in lung and ovarian cancers. In this study, we sought to identify SLFN11 expression patterns in both tissue and blood from advanced prostate cancer patients and to elucidate its potential predictive and/or prognostic role in platinum-treated patients.

2. Material and methods

2.1 Patient Cohort

Patients with metastatic CRPC and evaluable metastatic tissue and/or CTCs treated with platinum-containing chemotherapy at a single institution were retrospectively identified. All patients had clinical features of castration-resistance defined according to Prostate Cancer Clinical Trials Working Group 3 (PCWG3) criteria (23) with a histology review confirming adenocarcinoma or small cell/ neuroendocrine prostate cancer using published morphologic criteria (24).

Previous systemic therapies for CRPC included AR-targeted therapies (e.g., enzalutamide, abiraterone), taxanes (e.g., docetaxel, cabazitaxel), radium-223, and investigational agents. Platinum-based treatments (monotherapy or combination therapy) were administered for a planned number of cycles according to regime-specific treatment protocols. The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. The protocol was approved by the

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WCM Institutional Review Board (IRB#1305013903 and IRB#0905010441) and patients provided written informed consent.

Clinical and demographic information was collected by medical record review. PCWG3 criteria (23) was used to assess clinical, biochemical and radiographic response.

2.2 Tissue biopsies

In patients with metastatic tumor biopsies and RNA sequencing (RNA-Seq) data available, mRNA expression of *SLFN11* was assessed (*n*=27). These RNAseq data were previously published (26) and are included in dbGaP phs000909.v.p1. Patients were stratified into two categories (high or low) according to the median value of *SLFN11* expression. We also mined our published datasets (7,25-27) to assess *SFLN11* expression across stages of prostate cancer progression [31 benign, 66 localized prostate cancer (PCA), 74 CRPC-Adeno, 35 NEPC] and in other cancer types. Reads were mapped to the human genome reference sequence (hg19/GRC37) using STAR v2.3.0e. For each sample, HTSeq and Cufflinks were utilized to produce read counts and FPKM values, respectively (28). In addition, when available, metastatic tumor genomic status of select genes (i.e., *AR, TP53, RB1, PTEN, BRCA2, BRCA1, ATM*) was collected from whole-exome sequencing (WES) data obtained through a clinical CLEP/CLIA compliant tumor/normal WES assay (EXaCT-1; IPM-Exome-pipeline, version 0.9) (29).

2.3 CTC collection and characterization

Whole blood (10 ml) from a subset of patients (n=20) was collected using Streck tubes and shipped to Epic Sciences for processing using protocols previously described (30). CTC enumeration was performed, and slides were stained for DNA (DAPI), whole blood cell linage marker (CD45), and epithelial cells marker (CK). SLFN11 protein expression in CTCs was assessed by immunofluorescence (IF). The images of nucleated cells were visualized using a multi-parametric digital pathology algorithm (28). The threshold for categorizing SLFN11 expression into “positive” (SLFN11+) and “negative” (SLFN11-) was locked at 6.0 and established following an analytical feasibility of the SLFN11 assay employing laboratory-derived samples (LDS). LDS consisted of healthy donor blood spiked with cell lines with varying levels of SLFN11 expression to mimic physiologically appropriate ranges of SLFN11 expression (**Supplementary Methods)**.

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2.4 *In vitro* studies

An organoid model (WCMO1388) derived from fresh metastatic tumor tissue using methods described in Puca et al (31) was assessed for SLFN11 expression by western blot analysis. To stably knockout SLFN11 in organoids, a single-guide RNA was cloned into lentiCRISPRv2 vector (from Feng Zhang (Addgene plasmid #52961; http://n2t.net/addgene:52961; RRID: Addgene_52961)) and transfected into 293FT cells using lipofectamine 3000 (ThermoFisher) to produce lentiviral supernatants. Organoids were dissociated into single cells using TrypLE (ThermoFisher) and infected with lentivirus harboring either CRISPRv2-sgGFP or CRISPR-sgSLFN11 (MOI=1) for 23 hours. After infection, cells were re-plated and cultured in media with 1ug/ml of puromycin for three days. After puromycin selection, protein lysates were collected and analyzed by immunoblot using anti-SLFN11 (Santa Cruz, 1:250) and anti-GAPDH (Cell Signaling) antibodies. To test the sensitivity to cisplatin and olaparib (both purchased from Selleckchem), organoids were dissociated to single cells, and 3,000 cells were plated in a 96-well plate coated with 1% collagen I; cisplatin or olaparib was added on the following day. Cell viability was measured using CellTiter-Glo assay (Promega) following the manufacturer's protocol after six days of treatment. The sequences of sgRNAs were: sgGFP:GGGCGAGGAGCTGTTCACC; sgSLFN11-1:CCCAATTCATGGATAGTGG; sgSLFN11-6:AGCCTGACAACCGAGAAAT.

2.5 Statistical analysis

The primary aim of this study was to assess the impact of SLFN11 expression on outcomes in patients with advanced prostate cancer treated with platinum-based chemotherapy. Radiographic progression-free survival (rPFS) was calculated from the first day of platinum-based therapy to the date of progression disease or death. Progression disease was defined using PCWG3 criteria (23). Overall survival (OS) was calculated from initiation of therapy to death from any cause. Patients still alive at time of last follow-up were censored. A multivariate Cox regression model was used to investigate potential predictors of rPFS and OS and to estimate hazard ratios (HR) and their 95% confidence intervals (95%CI).

3. Results

3.1 Patient cohort characteristics

Forty-one patients with metastatic CRPC treated with platinum-based chemotherapy with metastatic

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tumor tissue sufficient for RNA-seq and/or CTCs for analysis were identified. Median age was 67.1 years (range 50.6-90.7). Metastatic tumor pathology review revealed adenocarcinoma (*n*=21) or small cell/neuroendocrine (*n*=20) histology. Median lines of prior systemic therapy for CRPC was two (range 1-7). All patients underwent platinum-based chemotherapy treatment [38 in combination with etoposide (*n*=21) or taxanes (*n*=17)]. Data regarding indication or reasons for administering platinum chemotherapy was not available. Specimens were collected with a median of 85 days (range 0-707 days) between the time of specimen collection and platinum treatment. Clinical features are summarized in **Table 1**.

3.2 *SLFN11* expression by RNA-Seq across different prostate tumor subtypes

We first evaluated *SLFN11* mRNA expression by RNAseq across multiple solid tumors at our institution (7,25) and confirmed overexpression of *SLFN11* in a subset of lung and ovarian cancers (**Supplementary Fig. 1**) consistent with what has been previously reported (18-22). We also identified *SLFN11* overexpression in a subset of prostate cancers. When we looked across additional prostate cancer cohorts (n=197), *SLFN11* was overexpressed in approximately 45% of metastatic CRPC compared to 25% of primary prostate cancers (**Fig. 1**). *SLFN11* expression was lower in neuroendocrine prostate cancer compared to CRPC with adenocarcinoma histology; Wilcoxon test neuroendocrine (NE) versus castration resistant adenocarcinoma p=0.009, NE versus localized prostate adenocarcinoma PCA p=0.019, NE versus Benign p=0.002 (**Fig. 1**). In the 27 of 41 patients treated with platinum chemotherapy with metastatic tumor tissue RNA-seq data, 58.5% expressed high levels of *SLFN11* in metastatic biopsies (with a cutoff determined according to the median value of *SLFN11* mRNA expression) **(Fig. 2A)**. Of these 27 patients, we observed a longer rPFS when treated with platinum-chemotherapy in patients with *SLFN11* overexpression compared with those without *SLFN11* overexpression (6.9 versus 2.8 months, HR=3.72, 95% CI 1.56-8.87, p<0.001) (**Fig. 2B**). No significant association was observed between *SLFN11* expression and OS (**Fig. 2C**). A greater than 50% decline in serum PSA was observed in all patients with *SLFN11* overexpression (**Fig. 2F**).

3.3 SLFN11 detection in circulating tumor cells

We evaluated 23 blood samples from 20 CRPC patients treated with platinum chemotherapy. Seven of these patients also had matched metastatic tissue biopsies with RNA-Seq data. The median number of

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CTCs/mL was 23 (range 1-136). Detectable SLFN11 expression in CTCs (SLFN11+) by IF was identified in 16/23 samples (70%). Patients with SLFN11+ CTCs exhibited a dynamic range in their proportion of SLFN11+ CTCs from 4% to 100% (**Fig. 3**). SLFN11 expression in CTCs did not correlate with cytokeratin (CK) expression in CTCs, and SLFN11+ cells had a range of CK expression (**Supplementary Fig. 2).**

SLFN11 expression in CTCs of patients was concordant with *SLFN11* mRNA expression by RNA-Seq of matched metastatic tumor biopsies in 6/7 patients (85.7%). Only one discordant case was identified (with low *SLFN11* expression in tissue but positive CTCs); this case harbored heterogeneity of SLFN11 expression across individual CTCs (**Supplementary Fig. 3).**

Similar to what was observed in tumor tissues, PSA declines of $\geq$50% on platinum chemotherapy occurred in all patients with SLFN11+ CTCs. A longer rPFS was also observed in patients with SLFN11+ CTCs compared to those without (6.0 versus 2.2 months, HR=4.02, 95%CI 0.77-20.86, p=0.002) (**Fig. 2D**), and this was independent of the number of CTCs present. There was no association between SLFN11 expression in CTCs and OS (**Fig. 2E**).

Representative images (**Fig. 4**) showing CTC characteristics of one patient analyzed at two different time-points suggested a change of SLFN11 expression status. This patient transitioned from SLFN11 negative to SLFN11+ CTCs after approximately 24 months from the first SLFN11 assessment and four therapeutic lines (abiraterone, radium-223, docetaxel, enzalutamide) in between. SLFN11+ CTCs on the second blood collection was detected 10 days before starting cisplatin and etoposide as fifth-line therapy. He had a PSA decline of 16.7 ng/ml to 4.5 ng/ml and a radiographic partial response after six cycles of cisplatin and etoposide which was durable for 6 months. He did not have somatic DNA repair alterations, neuroendocrine histology, or clinical features of AVPC.

3.4 Multivariate analysis

We performed a multivariate analysis which included SLFN11 overexpression (by metastatic tumor or CTCs), age, histology (adenocarcinoma versus neuroendocrine), presence of visceral metastasis, serum PSA, serum chromogranin A, somatic alterations of *AR*, *TP53*, *RB1*, *PTEN*, DNA repair genes (*BRCA1/2* or *ATM*) (**Fig. 2G**). Frequencies of aberrations are listed in **Table 1**; 13/32 assessable patients had alterations in *AR*, 21 had loss of one tumor suppressor (*TP53*, *PTEN*, *RB1*), 15 had loss of two or more tumors suppressors, 11 patients had DNA repair gene aberrations. SLFN11 overexpression

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was independently associated with rPFS (HR=0.14, 95%CI 0.03-0.70, *p*=0.017). There was no association between SLFN11 expression and OS. Conversely, multivariable analysis identified neuroendocrine histology and genomic alterations involving *AR* and *TP53* as independent predictors of inferior OS (HR=0.11, 95%CI 0.02-0.57, *p*=0.009, and HR=0.02, 95%CI 0.01-0.24, *p*=0.002, and HR=0.09, 95%CI 0.01-0.79, *p*=0.030, respectively), but not PFS on platinum-based chemotherapy.

3.5 SLFN11 expression and sensitivity to platinum treatment *in vitro*

We utilized CRISPR-Cas9 to knockout SLFN11 in a human prostate cancer organoid, WCMO1388, and examine its effect on cisplatin sensitivity. This organoid demonstrated loss of multiple tumor suppressors (i.e., *PTEN, TP53, RB1*), and also expressed SLFN11; it did not harbor *BRCA1, BRCA2* or *ATM* genomic alterations (**Supplementary Table 1)**. Despite other possible mediators of platinum sensitivity, SLFN11 knockout with two independent sgRNAs resulted in a 3-fold increase in IC50 with cisplatin compared with control (sgGFP) (**Fig. 2H**). Moreover, SLFN11 knockout also resulted in enhanced resistance to cisplatin at lethal concentrations. Given the reported association between SLFN11 and PARPi sensitivity (21), we also performed the same experiments using the PARPi olaparib. We found that SLFN11 knockout also reduced response to olaparib with an approximate 1.5-fold increase in IC50 (**Supplementary Fig. 4**).

4. Discussion

Platinum-based chemotherapy has demonstrated moderate anti-tumor activity in unselected patients with metastatic CRPC but has been associated with significant clinical benefit and even exceptional responses in select patients particularly those with DNA repair alterations (5-8) or aggressive clinical features (15,16).

Here we report a cohort of 41 men with metastatic CRPC who received platinum chemotherapy after progression on standard therapies. We discovered that overexpression of SLFN11 was associated with longer rPFS on platinum compared to those without SLFN11 overexpression as well as significant PSA responses. This was regardless of the presence of DNA repair gene alterations or small cell/neuroendocrine carcinoma morphology.

We found that SLFN11 is overexpressed a subset of CRPC tumors but less so in primary prostate

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cancers and may be acquired in some cases. As repeat metastatic biopsies are invasive to perform in patients, we investigated the feasibility of using CTCs to detect SLFN11 expression. CTCs have been shown to capture disease heterogeneity in prostate cancer as well as identify the expression of key pathways and targets through in in situ analyses or single cell profiling (28,32-35). In the subset of cases where we had matched tumor tissue and CTCs, we demonstrated high concordance of SLFN11 expression.

Schlafen (from the word schlafen, which in German means sleeping) family genes were originally identified during screening for growth regulatory genes during lymphocyte development. In cancer, SLFN11 has been identified as mediator of platinum sensitivity, including in clinical studies of non-small cell and small cell lung cancer and ovarian cancer (22,36,37). In addition to platinum chemotherapy, SLFN11 expression has also been associated with response to PARPi in lung cancer (21,22). Our patient-derived organoid data with olaparib suggests the same may also be the case for prostate cancer. There are several PARPi trials currently underway in advanced prostate cancer that could further investigate this question.

The mechanisms by which SLFN11 is regulated in CRPC and how this contributes to platinum and/or PARPi sensitivity in prostate cancer require further study. SLFN11 is a DNA/RNA helicase that is actively recruited to sites of DNA damage and regulates replication stress. SLFN11 has been found to directly interact with replication protein A1 (RPA1) to destabilize RAP-single strand DNA complexes and inhibit homologous recombination (38). This may explain how overexpression of SLFN11 contributes to tumor cell response to DNA damaging agents. SLFN11 has also been found to extend cell cycle arrest at S phase, resulting in DNA double strand breaks and replication stalling in the presence of PARP inhibitors (39).

In small cell lung cancer, studies using patient derived xenografts models (40) have identified an EZH2-SLFN11 axis leading to a gene silencing of SLFN11 with marked deposition of H3K27me3, a histone modification placed by EZH2, contributing to platinum resistance. EZH2 inhibition also contributes to PARPi response in breast cancer through PARP-mediated poly-ADP ribosylation (41). It has also been shown that *SLFN11* gene promoter hypermethylation correlates with resistance to

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platinum agents, particularly in ovarian and lung cancers (37). These data suggest that the expression levels of SLFN11 may be epigenetically regulated. Targeting epigenetic modifications might serve as a means for re-expressing SLFN11 and potentially sensitizing tumors to DNA damaging agents(40).

Limitations of this study include a small sample size, retrospective design, and variability across patients in the clinical cohort including their number of prior and subsequent treatments. Additional mechanistic studies in prostate cancer are also warranted to better understand SLFN11 regulation and its impact on DNA repair processes and therapy response. Nonetheless, these data identify SLFN11 as a potential marker of platinum sensitivity in CRPC and pave the way for future studies. Additional studies are needed to translate these findings into biomarker-informed clinical decision making for men with advanced prostate cancer.

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Table 1. Patient characteristics

Variable	***n* (%)**
Age (years), median (range)	67.1 (50.6-90.7)
Radical radiotherapy	
No	26 (63.4)
Yes	15 (36.6)
Prostatectomy	
No	27 (65.9)
Yes	14 (34.1)
Histology	
CRPC-Adeno	21 (51.2)
CRPC-NE	20 (48.8)
Presence of metastases at diagnosis	
No	19 (46.3)
Yes	22 (53.7)
Bone metastases	
No	8 (19.5)
Yes	33 (80.5)
Lymph node metastases	
No	18 (43.9)
Yes	23 (56.1)
Visceral metastases	
No	19 (46.3)
Yes	22 (56.1)
Type of platinum chemotherapy	
Combination therapy	38 (92.7)
Monotherapy	3 (7.3)
Number of prior systemic therapies, median (range)	2 (1-7)
Prior AR-directed therapies	

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Variable	***n* (%)**
No	18 (43.9)
Yes	23 (56.1)
Presence of pain/opiates use at baseline	
No	30 (73.7)
Yes	11 (24.3)
Baseline PSA, ng/mL median (range)	10.5 (0.89-1500)
Baseline serum CGA before platinum therapy, ng/mL	
CGA < 95	19 (46.3)
CGA ≥ 95	22 (56.1)
Baseline serum LDH before platinum therapy, U/L	
LDH <192	14 (34.1)
LDH ≥192	27 (65.9)
Whole exome data available	
No	9 (22.0)
Yes	32 (78.0)
AR alterations	
No	19 (59.4)
Yes	13 (40.6)
TP53 alterations	
No	16 (50)
Yes	16 (50)
RB1 alterations	
No	17 (53.1)
Yes	15 (46.9)
PTEN alterations	
No	17 (53.1
Yes	15 (46.9)
DNA defect repair * alterations	

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Variable	*n* (%)
No	21 (79.7%)
Yes	11 (34.3%)

*DNA repair alterations included *BRCA1* and *BRCA2* mutation or deletion, and *ATM* mutation.

Abbreviations. AR, androgen receptor; *ATM*, ataxia telangiectasia mutated gene; *BRCA*, breast cancer gene; CGA, chromogranin A; CRPC-Adeno, castration-resistant prostate adenocarcinoma; CRPC-NE, castration-resistant prostate cancer with neuroendocrine features; LDH, lactate dehydrogenase; *n*, number; PSA, prostate specific antigen; *PTEN*, phosphatase and tensin homolog; *RB1*, retinoblastoma 1; *TP53*, tumor protein p53.

Figure Legends

Figure 1. SLFN11 expression in prostate cancer patient cohort by RNA-Seq. *SLFN11* mRNA expression by RNA-Seq evaluated as log2 (FPKM+1) across multiple disease stages (benign, PCA, CRPC-Adeno, CRPC-NE). Wilxocon test was performed. *Abbreviations.* PCA, localized prostate cancer; CRPC-Adeno, castration-resistant prostate adenocarcinoma; CRPC-NE, castration-resistant neuroendocrine prostate cancer.

Figure 2. Association of SLFN11 expression and response to platinum treatment. Flowchart of study population (A). Radiographic progression-free survival (rPFS) and overall survival (OS) in patients according to SLFN11 expression by RNA-Seq (n=27) (B,C) and/or immunofluorescence (IF) in CTCs (n=20) (D,E). Waterfall plot (n=20) showing the magnitude of PSA decline by SLFN11 status (F). Bars were clipped at maximum 100%. Multivariable analysis of rPFS and OS in platinum-treated patients (n=32), assessing clinical, pathological and molecular data (G). SLFN11 and sensitivity to platinum treatment *in vitro* (H). WCMO1388 organoids with SLFN11 knockout by two independent sgRNAs (sgSLFN11-1, sgSLFN11-6) significantly reduce the sensitivity to cisplatin and display resistance at lethal doses compared to control (sgGFP). ****p<0.0001, measured by 2-way ANOVA. *Abbreviations. AR*, androgen receptor; *ATM*, ataxia telangiectasia mutated gene; *BRCA*, breast cancer gene; CGA, chromogranin A; CI, confidence interval; CRPC, castration-resistant prostate adenocarcinoma; CRPC-Adeno, castration-resistant prostate adenocarcinoma; CRPC-NE, castration-resistant prostate cancer with neuroendocrine features; CTCs, circulating tumor cells; DDR, DNA defect repair; exp, expression; HR, hazard ratio; IF, immunofluorescence; LDH, lactate dehydrogenase; mets, metastasis; *n*, number; OR, odds ratio; OS, overall survival; rPFS, radiographic progression-free survival; PSA, prostate specific antigen; *RB1*, retinoblastoma 1; RNA-Seq, RNA sequencing; *TP53*, tumor protein p53; WES, whole-exome sequencing.

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Figure 3. Identification of SLFN11 status in circulating tumor cells. This image shows high expression of SLFN11 (SLFN11+) in CTCs of 23 patients tested with the SLFN11 Epic 4-color immunofluorescence (IF) assay. SLFN11 cRatio (signal to-noise ratio) is plotted along the y-axis, and patient ID is plotted along the x-axis. The x-axis also includes a data table with additional patient specific information including pathology (adenocarcinoma-green or neuroendocrine-orange), percentage of SLFN11+ CTCs, SLFN11 localization, SLFN11 expression by RNA-Seq, and IF/RNA-Seq concordance (concordant-blue or discordant-red), time on platinum response (days). In the graph, each dot represents a detected cell, and the dotted line at 6 along the y-axis indicates the analytical threshold of positivity for SLFN11.

Figure 4. Representative images of CTCs from patients evaluated by IF for SLFN11 expression. (A), Patient with two different time-points of CTCs sample collection [SLFN11- at time of metastatic prostate adenocarcinoma diagnosis and SLFN11+ after approximately 24 months of multiple treatments (abiraterone, radium-223, docetaxel, enzalutamide)].

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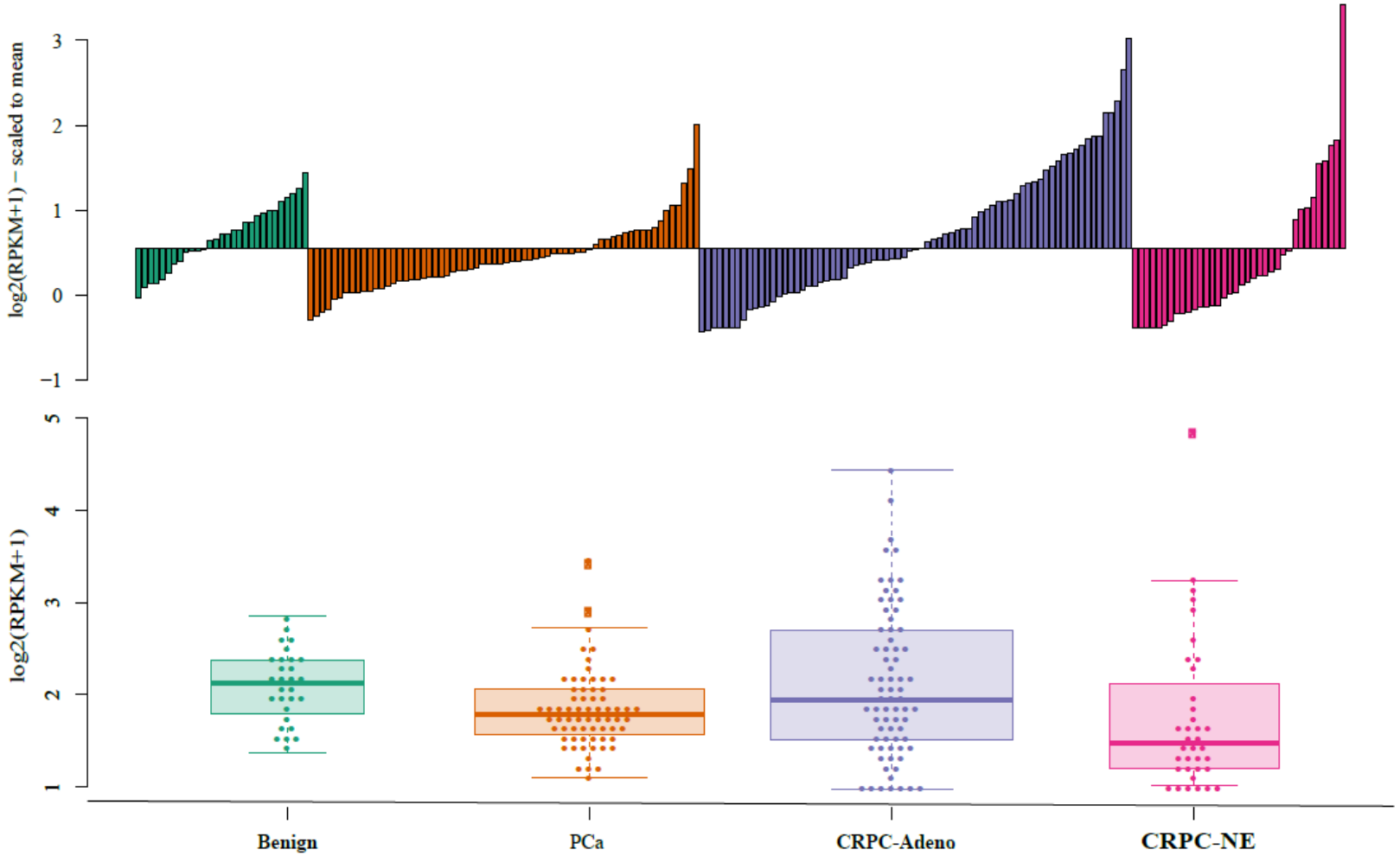

P-values (Wilcoxon test)

Comparison class	PCa	CRPC-Adeno	CRPC-NE
Benign	0.002	0.651	0.002
PCa		0.079	0.019
CRPC-Adeno			0.009

Figure 1

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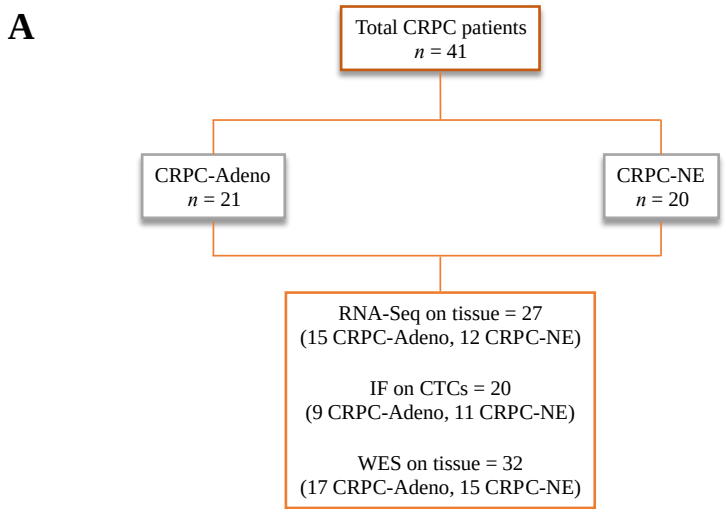

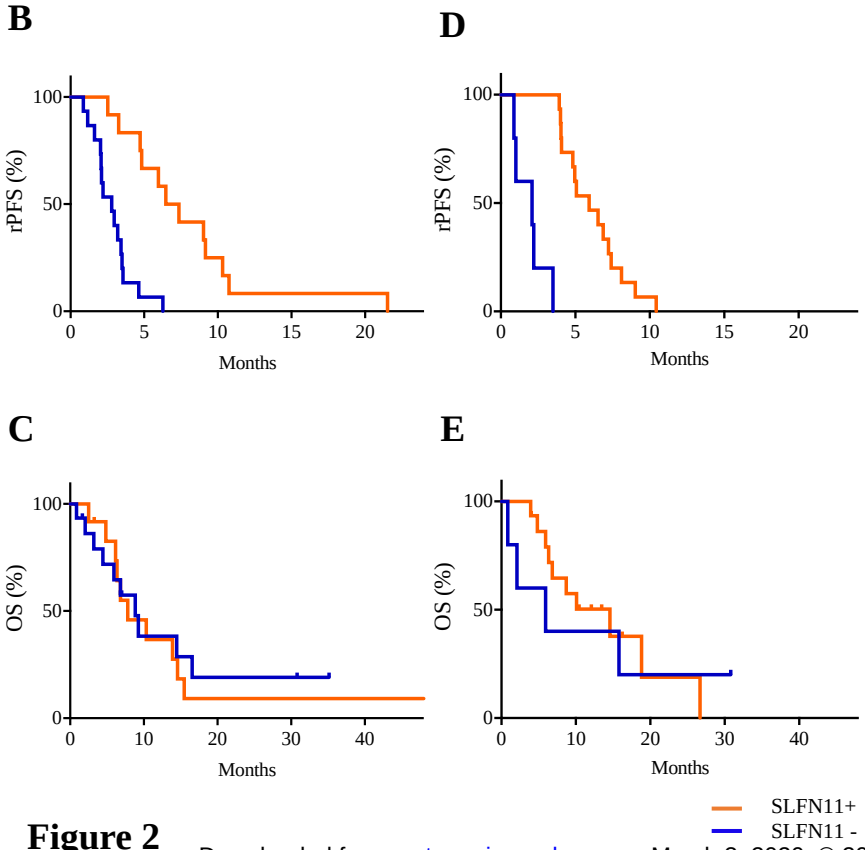

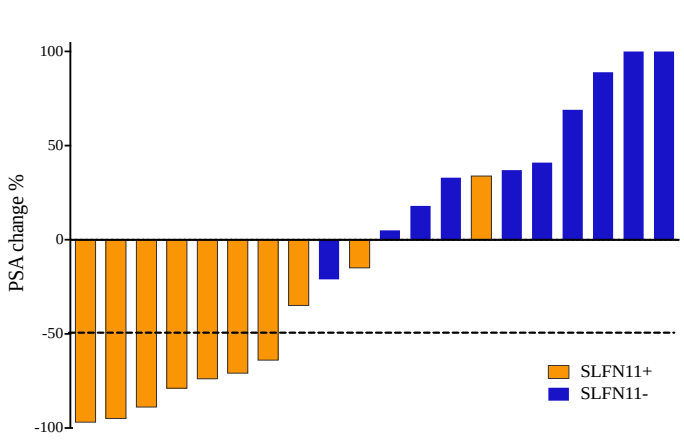

G

	rPFS		OS	
	HR (95% CI)	*p*	HR (95% CI)	*p*
SLFN11 (high vs low exp)	0.14 (0.03-0.70)	0.017	0.72 (0.12-4.45)	0.726
Age (continuous variable)	1.079 (0.964-1.206	0.185	1.101 (0.947-1.281)	0.211
CRPC-NE vs CRPC-Adeno	1.75 (0.47-6.56)	0.406	0.11 (0.02-0.57)	0.009
Visceral mets (yes vs no)	0.52 (0.11-2.41)	0.404	0.28 (0.04-1.98)	0.201
PSA (continuous variable)	1.001 (1.000-1.002)	0.121	1.002 (1.000-1.003)	0.054
CGA (continuous variable)	1.000 (0.998-1.002)	0.724	1.000 (0.997-1.002)	0.801
***AR* alterations (yes vs no)**	0.42 (0.10-1.74)	0.232	0.02 (0.01-0.24)	0.002
***TP53* alterations (yes vs no)**	0.62 (0.10-3.81)	0.602	0.09 (0.01-0.79)	0.030
***RB1* alterations (yes vs no)**	1.17 (0.17-7.96)	0.870	8.25 (0.68-100.01)	0.097
***DDR* alterations (yes vs no)**	0.74 (0.19-2.87)	0.669	0.34 (0.07-1.61)	0.172

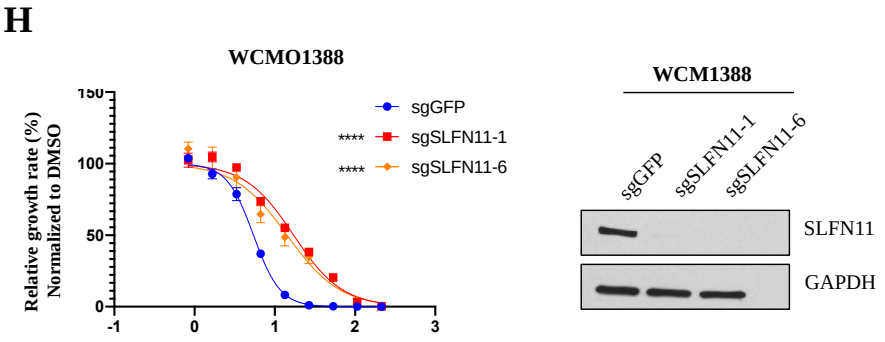

Figure 2

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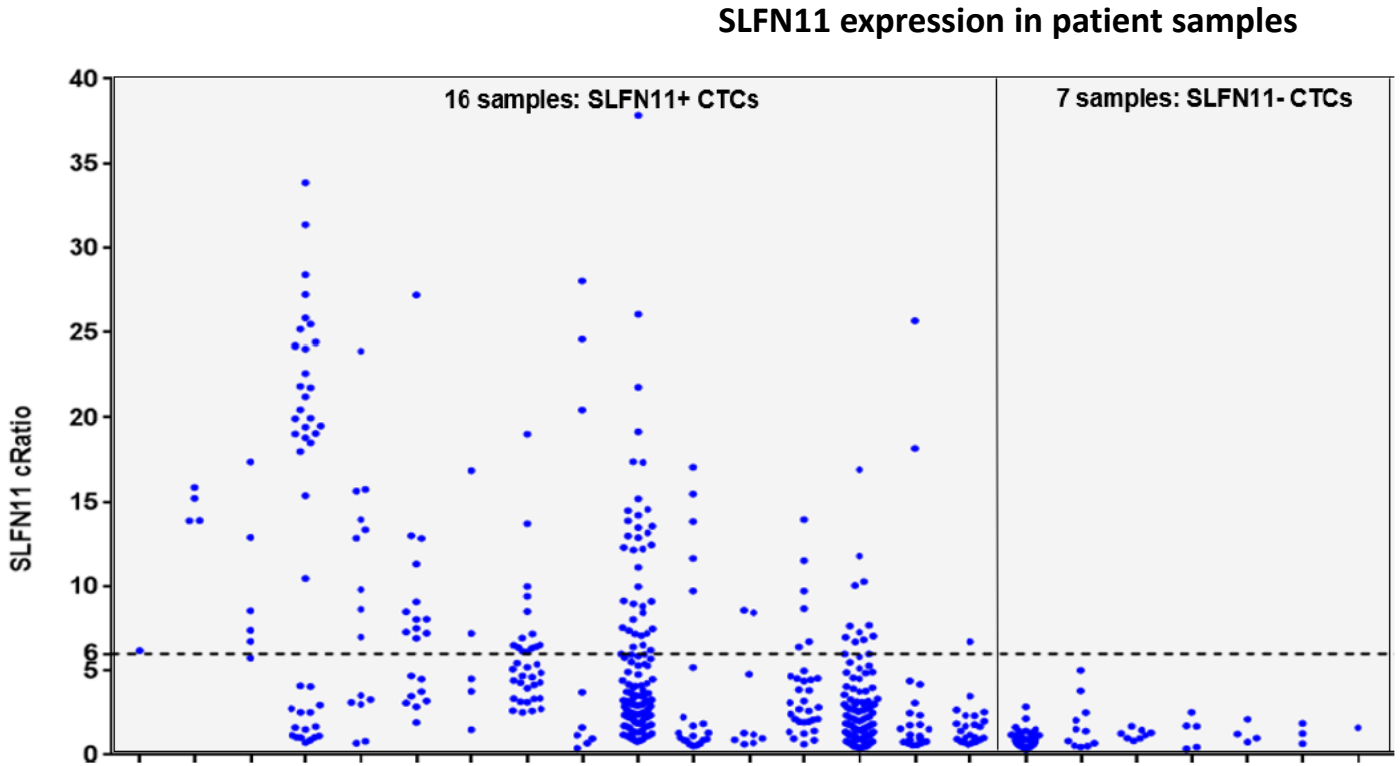

Pt ID	44	20_1	50	34	4	43	5	49_3	6_2	29	39	46	23	31	35	10_1	26	10_2	36	27	6_1	21	49.4
# Cells	1	4	6	42	15	20	5	35	9	136	21	9	32	102	21	23	64	11	8	5	4	3	1
# SLFN11+ Cells	1	4	5	26	9	12	2	14	3	37	5	2	6	11	2	1	0	0	0	0	0	0	0
% SLFN11+ Cells	100	100	83	62	60	60	40	40	33	27	24	22	19	11	10	4	0	0	0	0	0	0	0
SLFN11 Localization	C	N	C	N	N/C	C	N/C	C	N	N	N/C	N	N/C	N/C	N	N							
SLFN11 exp by RNAseq			+						+	+	+		-				-				-	-	
IF & RNAseq concordance																							
Days on platinum	122	121	348	118	178	313	131	243	145	149	122	145	155	155	120	N/A	109	75	30	63	N/A	26	N/A

*

Figure 3

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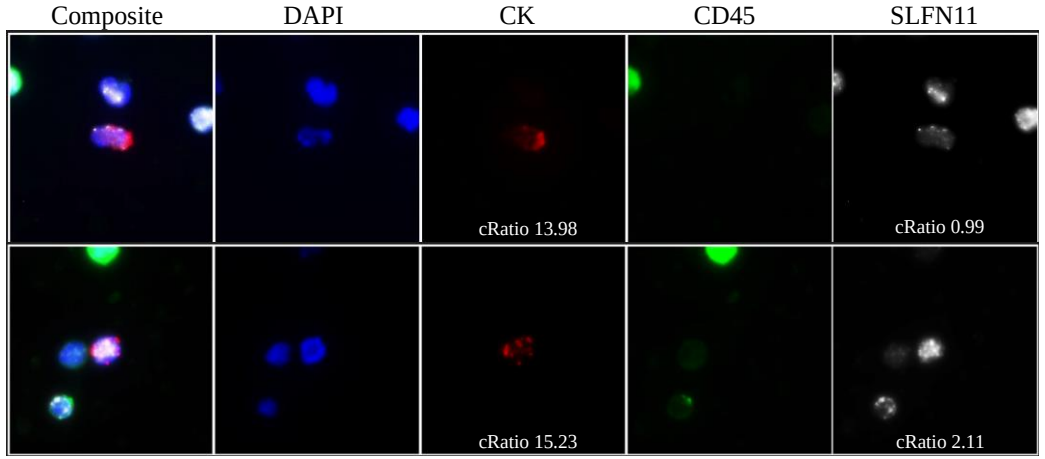
Time 0

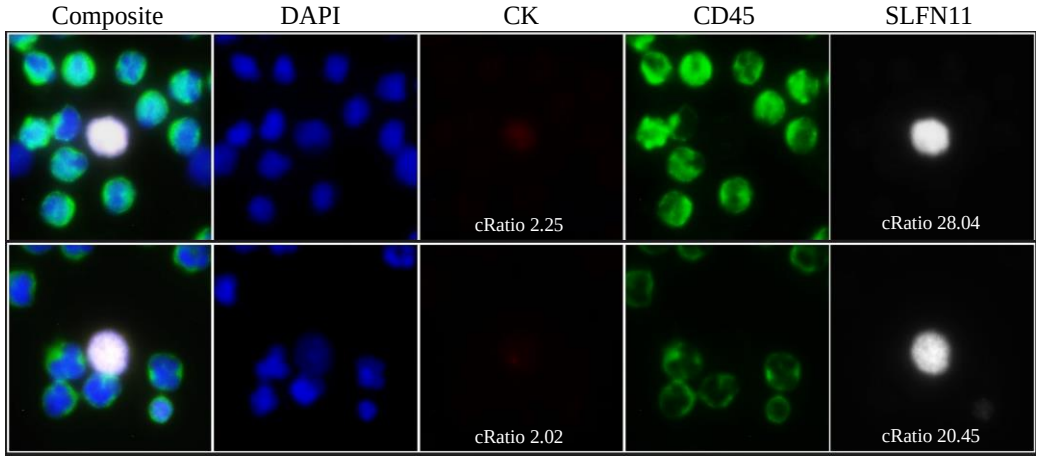
I. Abiraterone
II. Radium-223
III. Docetaxel
IV. Enzalutamide



CK+, SLFN11- cells

Time ~24 months

V. Cisplatin + Etoposide



CK-, SLFN11+ cells

Figure 4

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Vincenza Conteduca, Sheng-Yu Ku, Loredana Puca, et al.

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