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**Circulating free DNA (cfDNA) as biomarker of taxane
resistance in metastatic castration-resistant
prostate cancer (mCRPC)**

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To my Father – For having always been the shadow preceding all my steps

To my Grandfather – For bequeathing me his values and some of his talents

ABSTRACT

Introduction

Docetaxel (D) and cabazitaxel (C) are the standard chemotherapy for mCRPC. No biomarker predictive of resistance to D or C has been approved yet. Based on preliminary clinical data, we aimed to assess the association between ABCB1 amplification (ABCB1 amp) and primary resistance (RES) to D or C for mCRPC, using cfDNA.

Methods

A cohort (A) of 136 patients (pts) with at least 1 plasma sample drawn and stored within 12 months prior to starting D for mCRPC (2002-2014) and a cohort (B) of 42 pts with at least 1 plasma sample drawn and stored within 12 months prior to starting C for mCRPC (2010-2016) were identified from the Dana-Farber Cancer Institute IRB approved database. Whole genome sequencing (WGS) at 0.1x coverage, termed ultra-low pass WGS (ULP-WGS), was performed on the cfDNA extracted from the selected samples (1000 μ L/subject) and sequencing data were run through ichorCNA, a probabilistic model that allows to detect cases with sufficient tumor DNA content (>7%) and accurately identify copy number alterations (CNAs) including ABCB1 amp. Primary endpoint was assessing the association between ABCB1 amp and RES to D or C. Secondary endpoint was evaluating associations between any other CNAs with >10% prevalence and RES to D or C. RES was defined as the absence of a response, defined as PSA50 decline or radiologic response according to RECIST criteria version

1.1, within 16 weeks from treatment start. Odds ratio (OR) was used to compare odds of RES to D or C for pts with ABCB1 or exploratory CNAs and P-values were calculated by Fisher's exact test or Monte Carlo simulation.

Results

Of the selected 178 pts, 66 had sufficient tumor purity: 45 pts in cohort A and 21 in cohort B. The rates of men with ≥ 4 prior therapy lines were 22.2% in cohort A and 71.4% in cohort B. No significant association was noted between ABCB1 amp and RES to D (P=0.7123; OR=1.600) or C (P=1.000; OR=1.0606). RES was observed in 26 pts (57.8%) of cohort A and 18 (85.7%) of cohort B. ABCB1 amp was observed in 9 pts (20%; 95% CI, 9.6-34.6) in group A and 6 of them (66%) had RES to D. ABCB1 amp rate among D-resistant men was 23.1% (95% CI, 9.0-43.7). In group B, 2 pts (9.5%; 95% CI, 1.2-30.4) had ABCB1 amp and both of them had RES to C. ABCB1 rate among C-resistant pts was 11.1% (95% CI, 1.4-34.7). No significant association was found between exploratory biomarkers and RES to D or C.

Conclusion

In this study, ABCB1 amp does not predict for RES to D or C for pts with mCRPC. Future studies including ABCB1 amp in a suite of putative biomarkers and larger samples may aid drawing definitive conclusions.

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1. INTRODUCTION

1.1 Background

Prostate cancer is currently the most diagnosed cancer, with 174,650 estimated new cases in 2019, and the second cause of cancer-related mortality, in American men [1]. Prostate cancer has the highest incidence also among Italian men and is the third cause of cancer-related death [2]. A large part of patients with metastatic prostate cancer initially responds to androgen deprivation therapy. However, most of them will eventually relapse and develop metastatic castration-resistant prostate cancer (mCRPC) [3]. Docetaxel and cabazitaxel are currently the standard first and second, respectively, chemotherapy lines in mCRPC based on the results of the large phase 4 clinical trials TAX327, SWOG 9916, TROPIC, and FIRSTANA [4-7]. Both drugs belong to the category of the taxanes, cytotoxic agents which in the cytoplasm bind to the beta-tubulin dimers preventing microtubule depolymerization, which in turn results in the inhibition of mitotic cell division and apoptosis [8]. Only approximately half of the patients receiving docetaxel for mCRPC have a biochemical response, while the rest is primarily resistant to docetaxel [4]. The rate of innate resistance to cabazitaxel for men with mCRPC progressing on docetaxel is

even higher as only ~40% of them show a PSA decrease of 50% or greater in the cabazitaxel pivotal clinical trial [6]. Even initially responding patients, however, will progress in time. Several potential mechanisms of resistance to these taxanes, such as beta-tubulin isotypes alterations [9], overexpression of Bcl-2 [10] and induction of clusterin by pAkt [11,12], activation of central transcriptional factors such as NF-kB [13], alteration of multidrug resistant (MDR) genes [14], have been investigated in the past. However, no biomarker of taxane response or resistance for patients with mCRPC has been established yet. Both docetaxel and cabazitaxel are associated with non-neglectable rates of severe adverse events which, when not fatal, severely impact patients' quality of life [4-6]. Finding biomarkers able to predict resistance or response to docetaxel and/or cabazitaxel would allow the clinician to use the most efficient drug, avoiding administration of useless chemotherapy and thus sparing the patient relevant toxicity. Therefore, discovering biomarkers predictive of resistance or response to these agents is currently an unmet clinical need.

1.2 Potential biomarkers

The molecular mechanisms underpinning the primary or acquired resistance to either taxane remain yet to be completely understood. Nonetheless,

several studies reported a temporal correlation between the acquisition of docetaxel resistance and the upregulation of ABCB1 in ovarian, breast, and prostate cancer [15-17]. ABCB1 is a gene responsible for the induction of one of the major ATP-binding cassette (ABC) drug transporters, known for increasing drug efflux from tumor cells, ABCB1 [15,16]. Among the other forms of genomic instability so far postulated as mechanisms underlying the increased expression of ABCB1 (translocations, gene mutations, chromatin remodeling, etc.), amplification of it and of its locus 7q21 was demonstrated in ovarian and breast cancer cells resistant to docetaxel [16]. However, this correlation has not been proved in prostate cancer cells yet. Of note, cabazitaxel was identified from a screen for taxanes with improved pharmacologic properties, including a low affinity for MDRP (multidrug resistance or ABCB1 proteins). Additionally, a few studies investigating the activity of cabazitaxel in vivo and in vitro described only a mild resistance to ABCB1-overexpressing cells and low affinity to ABCB1 [18-20]. Therefore, the amplification of ABCB1 and of its locus 7q21 may be more reasonably associated with docetaxel resistance rather than cabazitaxel. In the Stand Up to Cancer (SU2C) database, an integrative landscape analysis of somatic and germline alterations in mCRPC obtained through DNA and

RNA sequencing of biopsies of 150 patients with mCRPC, the observed frequency of ABCB1 amplification was approximately 2% [21].

Furthermore, ERG was found overexpressed by 30 to 80 fold in at least 50% of prostate cancers as a result of recurrent gene fusions with TMPRSS2, in most cases, or with SLC45A3 and NDRG1 [22]. ERG rearrangements represent the most common form of genetic alteration detected in prostate cancer and seem to occur early in the course of the disease. In vivo and in vitro experiments reported that ERG has a role in starting prostate epithelium transformation and increasing cell invasion [23]. In a recent study, ERG overexpression resulted associated with decreased sensitivity to taxanes in prostate cancer cell lines in vivo and in vitro. Additionally, ERG overexpressing CRPC patients were found to have twice the probability to be taxane resistant compared to ERG negative ones [24]. Taxanes function by binding and stabilizing microtubule polymers, favoring their elongation and preventing the physiological removal of tubulin dimers (bundling). Presumably, ERG contributes to taxane resistance by binding the soluble tubulin dimers in the cytoplasm, thereby hindering their polymerization into microtubules and the taxane mediated bundling [24]. Therefore, ERG-TMPRSS2 rearrangement could be reasonably investigated as biomarker of

resistance to both docetaxel and cabazitaxel. In the SU2C database, the ETS-related gene (ERG) fusions observed rate was 43.3% and, as that the typical fusion partner for ERG is the androgen regulated gene TMPRSS2, we can assume a very similar rate for ERG-TMPRSS2 translocation.

Finally, an intriguing in vitro study recently demonstrated that KDM5D (Lysine-Specific Demethylase 5D or JARID1D) expression knock-down in LNCaP cells in the presence of dihydrotestosterone (DHT) leads to docetaxel resistance [25]. The report showed that KDM5D, encoded on the Y chromosome, modulates the androgen receptor (AR) transcriptional activity by demethylating H3K4me3 active transcription marks in the nucleus. This study also provided evidence that AR signaling impacts docetaxel sensitivity. DHT treatment on LAPC4 cell line inhibited docetaxel-induced apoptosis as it activates AR signaling. As a matter of fact, when LAPC4 cells were treated with the AR-targeted inhibitor enzalutamide, DHT induced AR activation was abolished and they started being sensitive to docetaxel. That does not occur in LNCaP cells presumably because they have KDM5D constitutively activated. In fact, KDM5D knockoff causes AR signaling activation, which in turn confers insensitivity to docetaxel. Dataset analysis from the publicly accessible gene expression

database Oncomine revealed significantly decreased KDM5D RNA expression in CRPC compared to hormone-sensitive primary cancer. Moreover, in the Grasso cohort, which extensively studied the copy number alterations (CNA) of CRPC patients, 27.1% of CRPC patients exhibited KDM5D deletion [26]. These data indicate that KDM5D deletion is at least partly responsible for KDM5D decrease and thus could be evaluated as a biomarker of resistance to docetaxel in mCRPC patients [25].

1.3 cfDNA and ichorCNA

The PCWG3 recommended use of direct biologic characterization of the tumor prior to considering start a new therapy, including blood-based diagnostics such as circulating DNA, to improve understanding of disease biology and detect potential predictive molecular biomarkers. In cancer patients, plasma derived circulating cell-free DNA (cfDNA) serves as a “liquid tumor biopsy” allowing for comprehensive tumor profiling including amplifications, deletions, and translocations [27]. Additionally, both the total quantity of cfDNA in the circulation and estimates of the tumor-derived contribution to cfDNA (tumor fraction) have been proposed as prognostic biomarkers [28] and indicators of response and resistance to therapy [29]. Of note, a study suggested an association between the total

cfDNA concentration in the circulation with clinical outcomes after taxane-based chemotherapy in mCRPC [30]. Through an existing collaboration between the Lank Center for Genitourinary Oncology Department of the Dana-Farber Cancer Institute – Harvard Medical School, the Blood Biopsy Team at the Broad Institute of MIT and Harvard, and the Koch Institute at MIT, it was recently developed a novel software platform, adaptation of the probabilistic model TITAN [31], called ichorCNA. Based on the assumption that cancer cells derive from a common precursor and thus have at least one truncal CNA (other alterations being subclonal), this algorithm is capable of quantifying the fraction of cfDNA derived from tumor (“tumor purity”) rather than normal tissues through CNAs detected by sparse ($\sim 0.1\times$ coverage) whole genome sequencing (technique termed Ultra-Low Pass Whole Genome Sequencing, ULP-WGS) [32]. Notably, measuring tumor content through quantification of the presence of individual alleles has demonstrated utility in assessment of response or resistance to therapy in cancers where those alleles are known clonal drivers [33]. The lower limit for detection of cfDNA using this computational tool was estimated to be approximately 3%. In the first 292 blood samples from patients with mCRPC tested, we observed tumor-derived DNA with purity $>3\%$ in $\sim 79\%$

and >10% in ~29% of patients (Figure 1A). There was no evidence for tumor-derived DNA from the 6 healthy donors analyzed [32]. The tumor purity estimate obtained from whole exome sequencing using a different method for deriving tumor content from somatic DNA alterations called ABSOLUTE [34] was similar to that achieved by ULP-WGS, which validates our method for quantification (Figure 1B).

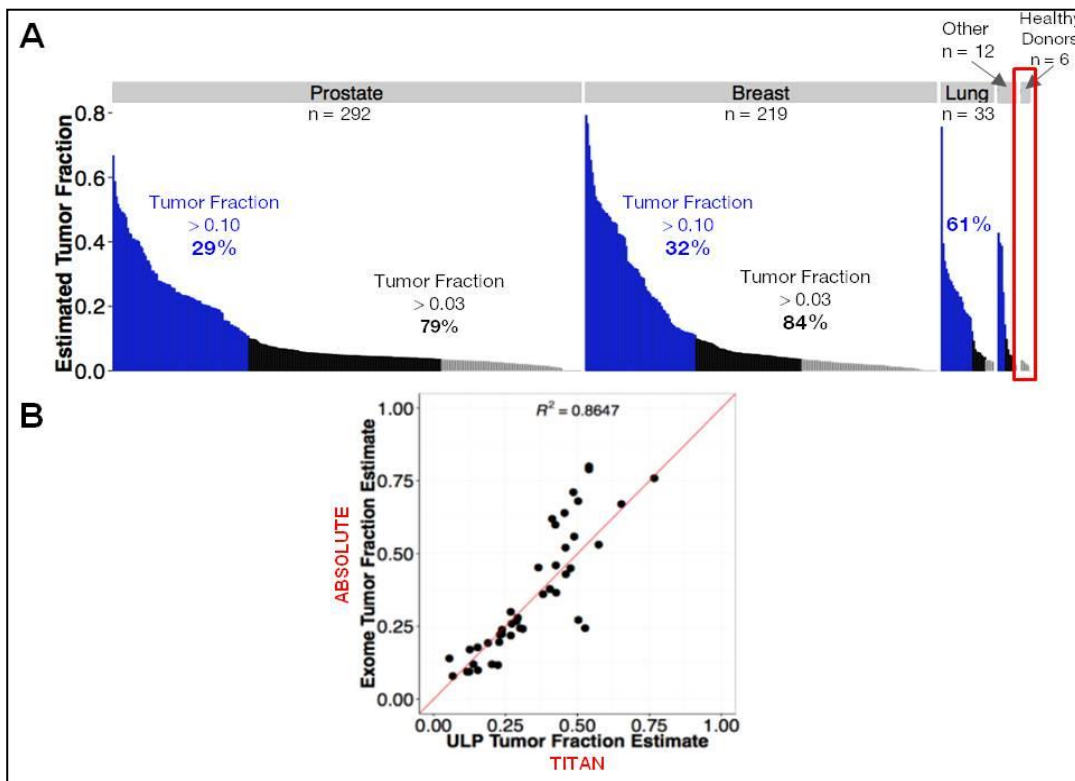


Figure 1. A. Tumor DNA Fraction as derived by ULP-WGS in plasma samples from patients with prostate, breast, lung and other cancers as well as healthy donors. **B.** Correlation of Tumor DNA Fraction derived from ULP-WGS using TITAN to that derived from WES using ABSOLUTE

Since ichorCNA does not account for translocations nor for detection of CNAs in chromosome Y, ERG-TMPRSS2 fusions and KDM5D deletions cannot be detected through this method. In this regard, the Broad Institute of MIT and Harvard designed a targeted sequencing panel which allows for identification of somatic single nucleotide variants (SSNVs) in all genes known to be recurrently mutated in mCRPC (<http://meetinglibrary.asco.org/content/179374-197>), as well as sequencing of intronic and intergenic regions of genes known to be translocated or have complex structural alterations in prostate cancer (i.e. TMPRSS2, ERG, AR, ETV1, ETV4, SLC45A3, RAF1, PTEN, MSH2, MSH6). This designed custom bait set including the above-mentioned set of candidate biomarkers will be applied on samples with estimated tumor purity >7% to further validate the results achieved through ULP-WGS and detect the presence of KDM5D deletions and ERG-TMPRSS2 translocations. In detail, this targeted sequencing panel will be applied on the same sequencing libraries constructed for ULP-WGS. Single nucleotide variants are identified using MuTect19, cancer cell fractions of these variants are derived using ABSOLUTE10, and rearrangement analysis is performed using BreakMer15. As this technique allows for probing many genes other than

the above-mentioned genetic alterations, it will also permit the detection of other mutations, copy number alterations, and mutational signatures that may be enriched in responders vs. non-responders or vice-versa and thus may represent alternative biomarkers.

1.4 Pilot analysis

In order to assess the feasibility of this project, a pilot analysis has been completed using ULP-WGS technique as relatively cheaper than the targeted sequencing panel [32]. Therefore, this analysis allowed for detecting only the putative biomarker ABCB1 amplification. Patients with at least 1 banked plasma sample drawn and banked within 12 months prior to docetaxel initiation were selected from the Lank Center for Genitourinary Oncology of the Dana-Farber Cancer Institute institutional review board (IRB) approved registry (termed CRIS). Of the 180 mCRPC patients treated with docetaxel (between 2001 and 2016) identified, 12 patients showing better biochemical response [measured in terms of % PSA decline = $(\text{baseline PSA} - \text{nadir PSA}) * 100 / \text{baseline PSA}$] (extreme responders) and 12 with worse response (extreme non-responders) within 4 months were selected and their corresponding banked plasma samples run through ULP-WGS. We found 4 extreme responders and 5 extreme non-responders

having tumor purity $\geq 10\%$ which is a pre-set value in ichorCNA for confidence. ABCB1 amplifications were observed in 3 extreme non-responders and only in 1 extreme responder. These preliminary data demonstrated that ABCB1 is a putative biomarker of docetaxel resistance. Given the data in the literature, ABCB1 was not investigated as biomarker of cabazitaxel resistance in this preliminary experiment.

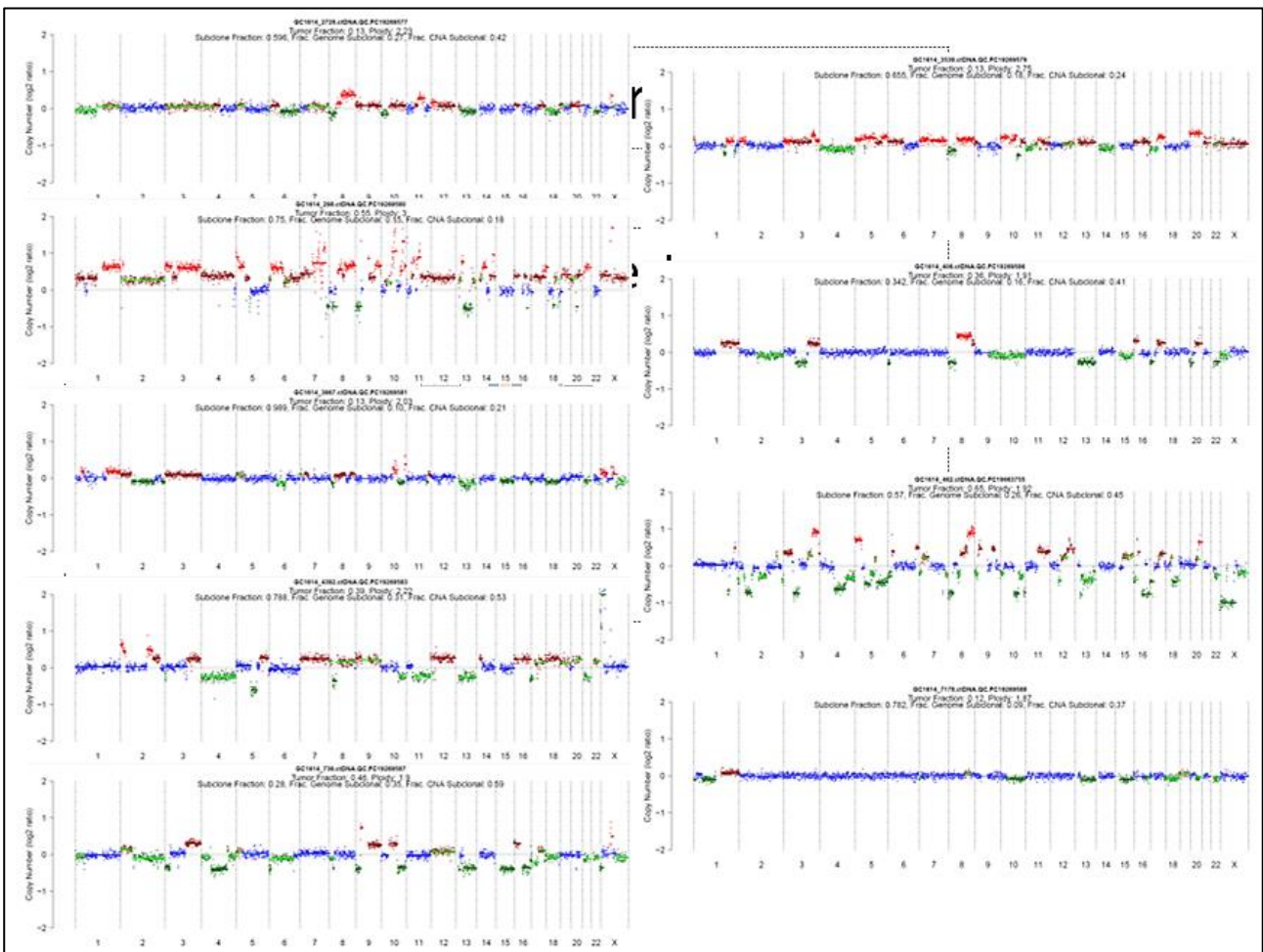


Figure 2. Pilot study: CNAs landscape plots of 5 extreme non responders (left) and 4 extreme responders (right) to taxane therapy.

1.5 Objectives

Therefore, in this first phase of the study, we sought to assess the association of ABCB1 amplification (established biomarker) and resistance to docetaxel or cabazitaxel therapy for mCRPC analyzing the cfDNA of a larger sample. Since ichorCNA allows for accurate detection of the sample copy number profile, a secondary endpoint was to evaluate the association of any other identified CNA (exploratory biomarkers) and resistance to either taxane for mCRPC. As it is often difficult to obtain metastatic tissue from patients for genomic testing, if successful, the present study would determine, without need for an invasive biopsy, a predictive factor for docetaxel and/or cabazitaxel resistance, which would enable us to perform a personalized therapy management.

2. METHODS

2.1 Study population

A cohort (A) of 180 patients with at least 1 plasma sample drawn and stored within 12 months prior to initiating docetaxel for mCRPC (between 2001 and 2016) and a cohort (B) of 62 patients with at least 1 plasma sample drawn and stored within 12 months prior to starting cabazitaxel for mCRPC (between 2010 and 2016) were identified from the Dana-Farber Cancer Institute IRB approved database CRIS. Patients who received cabazitaxel before docetaxel or in combination with other agents were excluded from cohort B. All patients were consented to trial 01-045 Collection of Specimens and Clinical Data for Patients with Prostate cancer or at High Risk for Prostate cancer. This protocol allows for banking of tissue and blood specimens for research use, including comprehensive genetic sequencing.

2.2 Study Design

We analyzed cfDNA of mCRPC patients who were treated with docetaxel and/or cabazitaxel and had plasma samples drawn and stored per protocol 01-045 within 12 months prior to treatment start. When multiple banked

samples were available for a patient we prioritized the closest to start of therapy. The DNA isolated from banked plasma samples was subjected to ULP-WGS through ichorCNA to identify cases with sufficient tumor content to build sequencing libraries and detect ABCB1 amplifications and any other CNA. The 9 cases with >7% tumor purity in the pilot study were also re-analyzed. The primary endpoint was to assess the correlation between ABCB1 amplification and primary resistance to docetaxel or cabazitaxel. The secondary endpoint was to evaluate the correlation between any detected CNA and resistance to docetaxel or cabazitaxel. Primary resistance was defined as the absence of a response within 16 weeks from treatment start. In turn, response was defined as the first of any of the following: 1) PSA decline $\geq 50\%$ from baseline 2) radiologic response according to RECIST criteria version 1.1 [35].

2.3 Study Procedures

The identified banked plasma samples (1000 μ L/subject) were requested from the genitourinary Gelb tumor bank. ULP-WGS was performed on the cfDNA extracted from these samples and sequencing information was run through ichorCNA to detect cases harboring detectable tumor DNA content and CNAs. Specifically, quantification of extracted cfDNA was performed

using the PicoGreen (Life Science Technologies) assay on a Hamilton STAR-line liquid handling system. Library construction of cfDNA was performed using the Kapa Hyper Prep kit with custom adapters (IDT). Generally, 5 ng of cfDNA input was used for ULP-WGS. A Hamilton STAR-line liquid handling system was used to automate and perform this method. Constructed sequencing libraries were pooled (2 uL of each x 96 per pool) and sequenced using 100bp paired-end runs over 1 x lane on a HiSeq2500 (Illumina) for ULP-WGS ($\sim 0.1\times$ coverage). The genome was divided into T non-overlapping windows, or bins, of 1Mb. Aligned reads were counted based on overlap within each bin using the tools in HMMcopy Suite. The read counts are then normalized to correct for GC-content and mappability biases using HMMcopy R package. This data was used to generate a Hidden Markov Model to derive CNAs, including ABCB1 amplification, and tumor DNA purity using ichorCNA. Samples passed a quality threshold (median absolute deviation score < 0.115) for accurate purity estimate. Considering the Broad Institute preliminary results [32], up to 40% of samples was estimated to yield $>7\%$ tumor purity which was set as threshold to guarantee the quality of the data. As ichorCNA does not account for subclonal events, to guarantee accuracy, a gene was defined

amplified or deleted when ≥ 5 copies or ≤ 1 copy, respectively, were found. The analyses were performed by Genomics Platform at the Broad Institute. In order to validate the output achieved with ichorCNA, GISTIC2.0 was rerun on the previously identified sequencing libraries. The GISTIC2.0 module, an evolution of the GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm, identifies probable CNAs by evaluating the frequency and amplitude of observed events [36]. GISTIC was applied to several cancer types [37,38] and aided identifications of several new targets of amplifications and deletions [39,40] and thus is an ideal tool to provide quality metrics for confidence.

2.4 Statistical analysis

As to the analysis of the established biomarker, the odds of docetaxel or cabazitaxel resistance (yes vs. no) for patients with ABCB1 amplification or without were compared by odds ratio (OR). An OR greater than 1 indicates a higher likelihood of taxane resistance for patients with ABCB1 amplification. Logit OR was used when there was 0 count for one of the resistance*amplification combination. P-values were calculated by Fisher's exact test.

As to the analysis of exploratory biomarkers, only amplifications or deletions with prevalence >10% (>4 and >2 patients in cohort A and B, respectively) were evaluated. OR of taxane resistance for patients with an amplification (or deletion) vs. no amplification (or no deletion) were calculated and raw P-values for all observed gene aberrations were generated using Monte-Carlo simulation and presented using volcano graphs [x-axis represents $\log(\text{OR})$ and y-axis represents $-\log_{10}(\text{raw p-value})$]. To control for false discovery rate, p-values were adjusted by Benjamini–Yekutieli procedure. The biomarker was considered promising if the adjusted p-value was <0.05 .

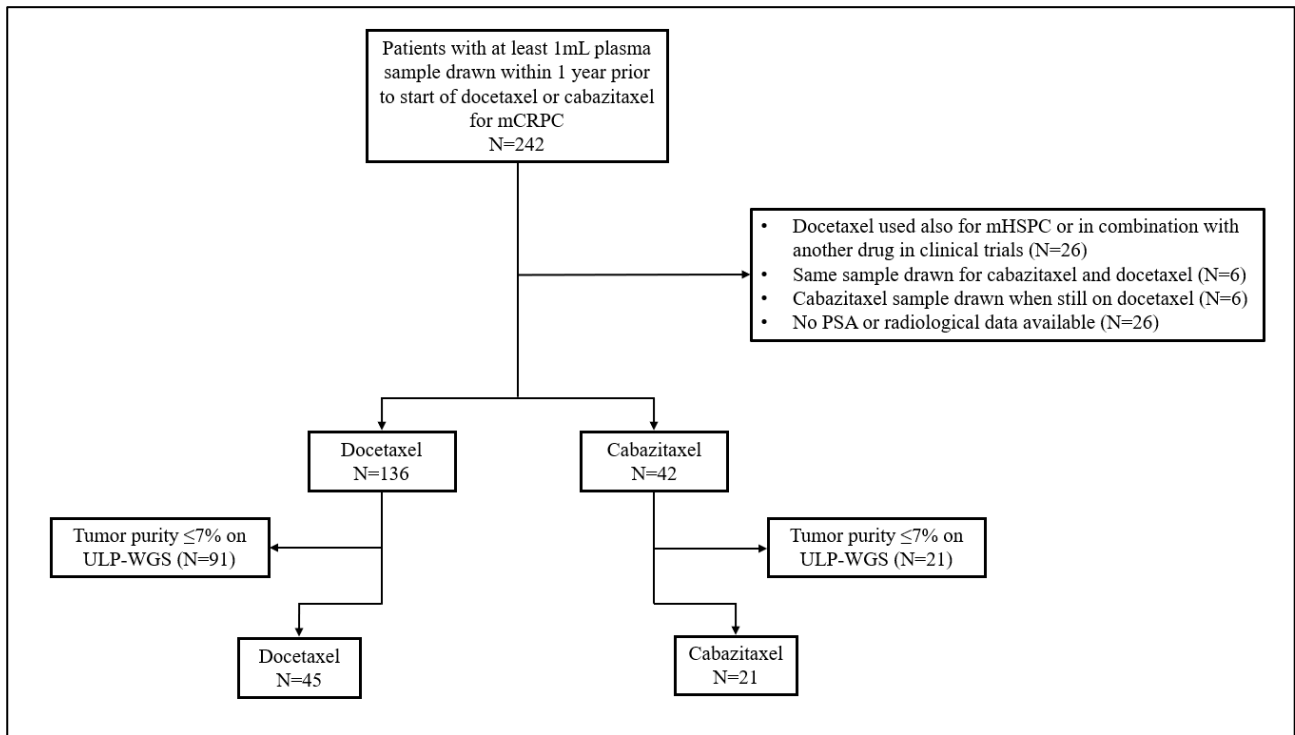
The prevalence of each biomarker, established or exploratory, was summarized as numbers and percentages with 95% confidence interval (CI).

3. RESULTS

3.1 Cohorts

Of the 242 patients initially selected from the Dana-Farber Cancer Institute registry, 180 in cohort A and 62 in cohort B, a total of 64 were excluded from the study (Fig. 1). Reasons for exclusion were the following: previous docetaxel administration for hormone sensitive disease or in combination with other drugs within clinical trials, plasma sample drawn and banked within 1 year prior to cabazitaxel being the same available for docetaxel or drawn when the patient was still on docetaxel, absence of efficacy (PSA or radiological) data. Of the 178 remaining patients, 136 had at least 1 plasma sample drawn and available within 1 year prior to docetaxel start and 42 prior to cabazitaxel start. Of these samples, the ULP-WGS identified a total of 68 with sufficient tumor purity (>7%) to confidently building sequencing libraries and detecting ABCB1 amplification and other CNAs. As 1 patient had 3 available samples, overall, 66 patients were eligible for this analysis: 45 patients in the docetaxel cohort and 21 in the cabazitaxel cohort, 33% and 50% of the original populations (136 and 42), respectively (Fig. 3). Four patients had 1 sample available prior to docetaxel and 1 prior to cabazitaxel and thus are counted both in cohort A and B.

Figure 3. Flow Chart



3.2 Patient characteristics

Patient clinical and radiological characteristics are described in Table 1. Year of taxane start spanned from 2002 to 2014 in the docetaxel cohort and from 2010 to 2016 in the cabazitaxel cohort. At the time of data annotation (May 2017), 97.8% of patients (44 of 45) in the cohort A and 71.4% (15 of 21) in cohort B were dead. More than half of men (51.1%) in the docetaxel group received at least 1 line of therapy for mCRPC prior to starting the taxane and more than 1/5 (22.2%; 10 of 45) had 4 or more prior treatments.

Abiraterone acetate, enzalutamide, or radium 223 were not commonly administered prior to docetaxel. More than 2/3 of men (71.4%, 15 of 21) in the cabazitaxel cohort progressed on ≥ 4 lines of therapies; all patients had had received docetaxel, 12 (57.1%) abiraterone acetate, and 7 (33.3%) enzalutamide, before receiving cabazitaxel. Most patients (70.0%; 26 of 45) in group A received at least 4 cycles of docetaxel and 60% (24 of 45) more than six. In group B, most patients (57.1%; 12 of 21) received less than 4 cycles of the taxane. A PSA decline $\geq 50\%$ was achieved in 42.2% (19 of 45 patients) and 14.3% (3 of 21 patients) of cohorts A and B, respectively. Two of 45 men (4.4%) in the docetaxel group had a radiologic response within 4 months of start of therapy, while none was observed in the cabazitaxel group. Primary resistance was observed in 26 of 45 patients (57.8%) in cohort A and in 18 of 21 (85.7%) in cohort B.

Table 1. Patient characteristics

	Cohorts	
	Docetaxel (A) (N=45)	Cabazitaxel (B) (N=21)
Still alive, n (%)		
N	44 (97.8)	15 (71.4)
N/A	1 (2.2)	6 (28.6)
Prior N. of treatments for mCRPC, n (%)		
0	12 (26.7)	0 (0.0)
1-3	23 (51.1)	6 (28.6)
≥ 4	10 (22.2)	15 (71.4)
Prior abiraterone acetate, n (%)		
N	39 (86.7)	9 (42.9)
Y	6 (13.3)	12 (57.1)
Prior enzalutamide, n (%)		
N	43 (95.6)	14 (66.7)
Y	2 (4.5)	7 (33.3)
Prior radium-223, n (%)		
N	44 (97.8)	19 (90.5)
Y	1 (2.3)	2 (9.5)
Prior docetaxel, n (%)		
Y	0 (0.00)	21 (100.0)
N	45 (100.0)	0 (0.00)
Resistance, n (%)		
N	19 (42.2)	3 (14.3)
Y	26 (57.8)	18 (85.7)
PSA decline ≥ 80% within 16 weeks after regimen start, n (%)		
N	36 (80.0%)	20 (95.2%)
Y	9 (20.0%)	1 (4.8%)
PSA decline ≥ 50% within 16 weeks after regimen start, n (%)		
N	26 (57.8)	18 (85.7)
Y	19 (42.2)	3 (14.3)
Radiologic response within 16 weeks from regimen start, n (%)		
N	42 (93.3)	21 (100.0)
N/A	1 (2.2)	0 (0.0)
Y	2 (4.4)	0 (0.0)
Total number of cycles of regimen, n (%)		
1-3	12 (30.0)	12 (57.1)
4-6	4 (10.0)	2 (9.5)
>6	24 (60.0)	7 (33.3)
N/A	5	0

Abbreviations: N, no; N/A, not available; Y, yes.

3.3 Laboratory and clinical outcomes

No statistically significant association was noted between detection of ABCB1 amplification and primary resistance in the docetaxel (P=0.7123; OR=1.600, 95% CI: 0.3451-7.4181) or in the cabazitaxel group (P=1.000; OR=1.0606, 95% CI: 0.0412-27.3003) (Table 2). The gene aberration was observed in 9 of 45 patients (20%; 95% CI, 9.6-34.6) in the cohort A and 6 of those 9 (66.7%; 95% CI, 29.9-92.5) showed innate resistance to docetaxel (Table 2). The rate of ABCB1 amplification among patients with docetaxel innate resistance was 23.1% (95% CI, 9.0-43.7). In cohort B, 2 of 21 patients (9.5%; 95% CI, 1.2-30.4) had ABCB1 amplification prior to starting the taxane and both of them showed primary resistance to cabazitaxel (Table 3). The rate of ABCB1 amplification among the patients with cabazitaxel innate resistance was 11.1% (95% CI, 1.4-34.7).

The analysis for exploratory biomarkers, after adjusting for false discovery rate, did not identify any CNAs (amplification or deletion) potentially predictive of docetaxel or cabazitaxel resistance (Fig. 4). The GISTIC2.0 output validated the CNA landscape plot obtained with ichorCNA.

Table 2. Association between ABCB1 amplification and resistance in cohort A (docetaxel)

	ABCB1 amplification, N (%)	No ABCB1 amplification, N (%)	Total, N (%)
Resistance, N (%)	6 (13.33)	20 (44.44)	26 (57.78)
No resistance, N (%)	3 (6.67)	16 (35.56)	19 (42.22)
Total, N (%)	9 (20.00)	36 (80.00)	45 (100.00)

P-value = 0.7123; Odds Ratio = 1.6000

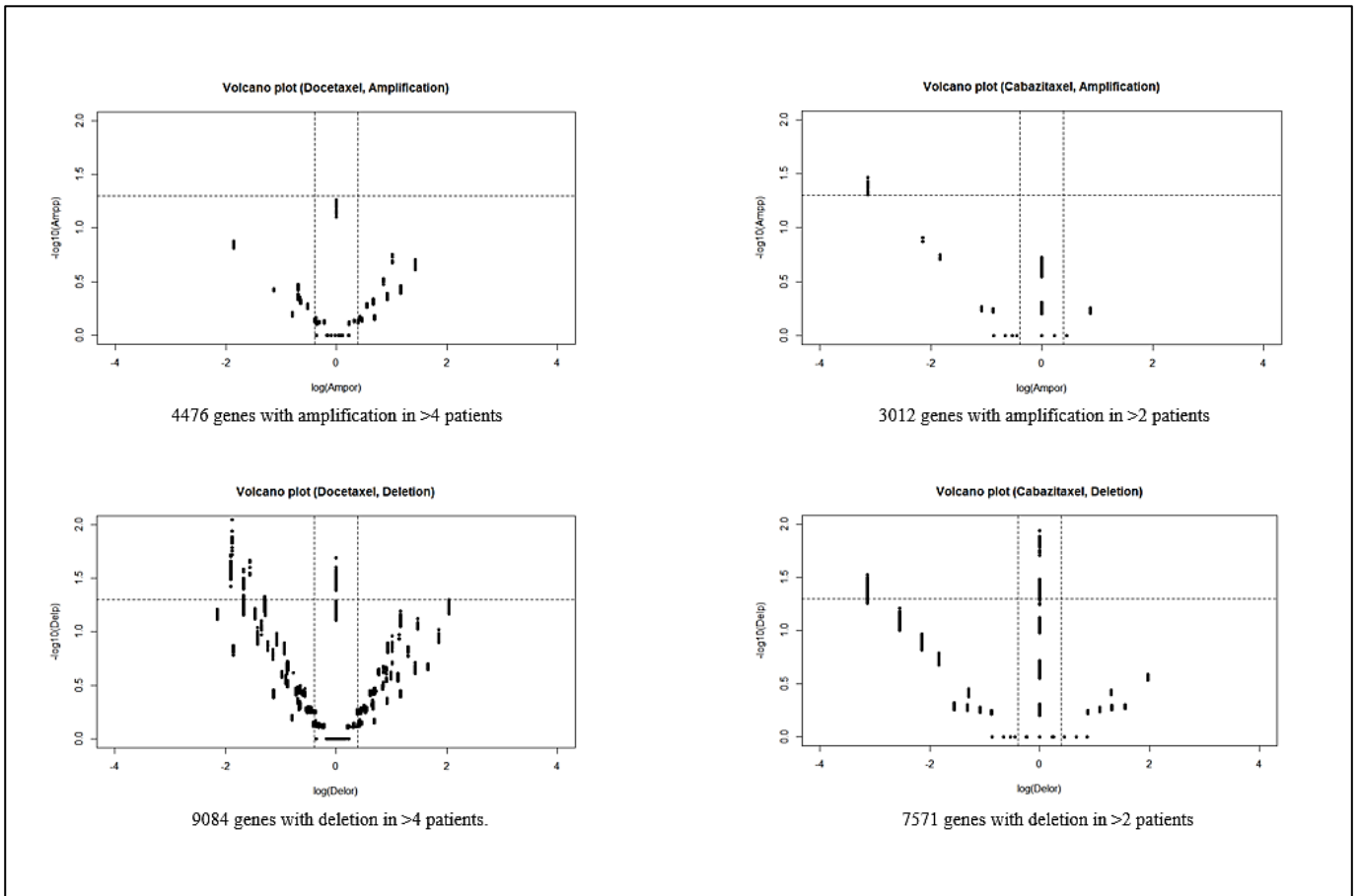
Table 3. Association between ABCB1 amplification and resistance in cohort B (cabazitaxel)

	ABCB1 amplification, N (%)	No ABCB1 amplification, N (%)	Total, N (%)
Resistance, N (%)	2 (9.52)	16 (76.19)	18 (85.71)
No resistance, N (%)	0 (0.00)	3 (14.29)	3 (14.29)
Total, N (%)	2 (9.52)	19 (90.48)	21 (100.00)

P-value = 1.0000; Odds Ratio* = 1.0606

* Logit odds ratio was used

Fig. 4. Volcano plots of exploratory biomarkers (amplifications, above; deletions, below) of resistance to docetaxel (left) or cabazitaxel (right)



Note: x-axis= $\log(\text{OR})$, y-axis= $-\log_{10}(\text{raw } p\text{-value})$. The vertical lines indicate an effect size (OR) of 2 and 0.5; the horizontal line indicates a (unadjusted) p-value of 0.05. The p-values are the raw p-value prior to false discovery rate adjustment.

4. DISCUSSION

Standard chemotherapy for patients with mCRPC has been represented by docetaxel and cabazitaxel for many years now. Despite their demonstrated efficacy for most patients with mCRPC, there is a portion of men which shows primary resistance to these agents [4-6]. Moreover, taxane administration is associated with relevant toxicity, particularly hematologic, which is detrimental to quality of life, when not life threatening. In the past, several studies aimed to correlate biological or genetic features in prostate cancer with resistance to taxane-based therapy [9-14]. However, no biomarker of resistance to taxane-based therapy has been approved yet. Prompted by the encouraging clinical data of a pilot study, in this analysis, first phase of the project, we sought to assess the correlation between the putative biomarker ABCB1 amplification or other CNAs, detected by ichorCNA applied on plasma extracted cfDNA, and innate resistance to docetaxel or cabazitaxel for mCRPC.

Primary resistance was observed in 57.8% of cohort A and in 85.7% of cohort B. Notably, these rates are quite similar to those reported in the key clinical trials of docetaxel and cabazitaxel [4-6], which confirms the validity of this database. Innate resistance could be more accurately evaluated in

cohort A, where 60% of men received more than 6 cycles of docetaxel, compared to cohort B, where only 1/3 of patients had > 6 cycles of cabazitaxel. GISTIC2.0 confirmed ichorCNA output providing quality metrics for confidence of the CNA plot in both cohort A and B.

No statistically significant correlation was observed between the putative biomarker or any other exploratory CNA and primary resistance to docetaxel or cabazitaxel in this study. However, it should be noted that this analysis was quite underpowered due to the relatively small sample size (66 patients), even smaller considering the single cohorts (45 patients for A and 21 for B), which could have affected results. The decrease in the original population (N=178) was the result of the selection process operated by ichorCNA to identify cfDNA samples with sufficient tumor purity (>7%) to guarantee a good output quality. Of note, the rate of samples with tumor-derived cfDNA >7% detected in cohort A (33%) and in cohort B (50%) was in line with what reported in the original article describing ichorCNA [32], where cfDNA with purity >3% was observed in ~79% and >10% in ~29% of patients (Figure 1A), which provides validation to this analysis. Moreover, the database of this study is one of the largest registries of somatic CNAs for mCRPC currently reported in the literature. The Grasso

cohort includes somatic CNAs of 59 patients with prostate cancer. However, those with mCRPC were 48 while 11 patients had hormone-sensitive prostate cancer [26]. The SU2C database encompasses the somatic and germline genetic aberrations of 150 patients with mCRPC. Nonetheless, it is noteworthy that at the time of tissue collection, only 61 of them (41%) had received taxane therapy and 72 (48%) had progressed on either abiraterone acetate or enzalutamide [21]. In this respect, at the date of sample drawing, in cohort A no patient had received prior taxanes (per inclusion criteria) and few subjects had prior abiraterone acetate (13.3%) or enzalutamide (4.5%), probably due to the recruitment window (2002-2014), and yet 51.1% had progressed on 1 to 3 lines of therapies, prior to sample drawing. Furthermore, all 21 patients in cohort B had progressed on docetaxel and 71.4% had 4 or more line of therapies, prior to plasma sample drawing. As such, the population of our study on average had probably progressed on more lines of therapies compared to that of SU2C. This could partly explain the surprisingly greater rate of ABCB1 amplification observed in cohort A and B compared to that of SU2C database (~2%). In fact, the putative biomarker was reported ~10- and ~5-fold more frequently in cohort A (20%) and cohort B (9.5%), respectively. In fact, it is known

that the cancer mutational burden typically increases with the cumulation of resistance strategies to the therapies used [41-43]. Conversely, in the described pilot study of patients treated with docetaxel for mCRPC, ABCB1 rate was higher (44.4%; 4 patients of 9 with tumor purity >7%) than in cohort A (20%) or B (11.1%). However, it is worth noting that 3 of the 4 patients with ABCB1 amplification were in the group with extreme resistance to docetaxel and had PSA decline within 4 months of therapy start $\leq 10\%$ (vs. $\leq 50\%$ in the present analysis). The rate of extreme resistant patients with ABCB1 amplification was 60% (3 of 5 patients). In this respect, the rate of docetaxel- or cabazitaxel-resistant patients with ABCB1 amplification was 23.1% and 11.1% in cohort A and B, respectively. As such, despite not being significant, these data might portend a correlation between the degree of resistance to docetaxel and the frequency of ABCB1 amplification.

Given the small population analyzed, significant associations between the putative biomarker and docetaxel or cabazitaxel were not expected. However, in the docetaxel cohort, the frequency of ABCB1 (20%), the rate of patients with ABCB1 showing innate resistance to docetaxel (66.7%; 6 of 9 patients), and the rate of docetaxel-resistant patients with ABCB1

(23.1%) are promising data which should prompt further investigation in a larger population. Additionally, according to the second phase of this study, the Broad Institute designed targeted sequencing panel (see Introduction), including all the established biomarkers (ABCB1 amplification, ERG-TMPRSS2 translocation, and KDM5D deletion), will be applied on those sequencing libraries with estimated tumor purity >7% achieved using ichorCNA. This will allow for detecting the above mentioned candidate biomarkers and any other genomic aberration (exploratory biomarkers) included in the panel. Patients will be classified as positive vs. negative for the putative biomarker suite. A patient will be considered biomarker-positive if any of the putative genomic alterations is detected in his cfDNA sample. Considering the frequency of KDM5D deletion (~27%), ERG-TMPRSS2 translocation (~43%) and ABCB1 amplification (2% to 20%), observed in this and publicly available databases (SU2C and Grasso cohort) [21, 26] of mCRPC patients and the possible overlapping of these genomic aberrations in some patients, we expect to find a positive-biomarker status in at least ~60-70% of our study population (N=40/42 patients). The primary endpoint is to assess the correlation between positive biomarker status and innate resistance to docetaxel or cabazitaxel. Secondary endpoint is to

evaluate the association between any exploratory biomarker with prevalence >10% and primary resistance to docetaxel or cabazitaxel.

5. CONCLUSION

In a small population of patients treated with docetaxel or cabazitaxel for mCRPC, no statistical association could be observed between the putative biomarker ABCB1 amplification, identified using ULP-WGS on plasma-derived cfDNA, and primary resistance to docetaxel or cabazitaxel. However, data were encouraging and the second phase of this study will use a custom-designed targeted sequencing panel to evaluate the correlation between positive biomarker status, including ABCB1 amplification and the other putative biomarkers, and innate resistance to docetaxel or cabazitaxel.

6. REFERENCES

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7. SCIENTIFIC ACTIVITY DURING Ph.D.

7.1 Education

- Research Fellowship in the Lank Center for Genitourinary Oncology of the Dana-Farber Cancer Institute - Harvard Medical School, Boston (MA), USA. January 2016 – June 2017.
- Research Fellowship in Medical Oncology at the Harvard University, Boston (MA), USA. March 2016 – June 2017.
- National Scientific Qualification (ASN) as Associate Professor of Hematology, Oncology, and Rheumatology. April 6, 2017 – Present.
- Certificate in Applied Biostatistics – Harvard Catalyst Education Program, Harvard University, Boston (MA), USA. July 1, 2017.
- Medical Oncologist, final mark 70/70 Cum Laude, Sapienza Università di Roma, Rome, Italy. July 13, 2017.

7.2 Teaching experience

- Adjunct Professor at the School of Medical Oncology, Università degli Studi di Firenze, Florence, Italy. May 24, 2019 – Present.

- Adjunct Professor at the School of Hematology, Università di Pisa, Pisa, Italy. May 21, 2019 – Present.
- Research and Clinical Supervisor of resident physicians and medical students and interns at the Medical Oncology Unit inpatient service, Misericordia Hospital, Grosseto, Italy. October 2018 – Present.
- Oral presentations at the Dana-Farber Cancer Institute - Harvard Medical School and Brigham and Women's Hospital joint Journal Club, Boston (MA), USA. September 2016 – June 2017.

7.3 Professional activity

- Scientific coordinator, 3rd RIMOG State of the Art and Novelties in Geriatric Oncology. Siena (Italy) – September 20, 2018.
- Scientific coordinator, Il Paziente Oncologico Anziano Gestione e Trattamento. Siena (Italy) – November 23, 2018.
- Member of Meet-URO (Italian Network for Research in Urologic Oncology). November 2, 2018 – Present.
- Principal Investigator, “Clinical outcomes of abiraterone acetate + prednisone (AA) + bone resorption inhibitors (BRI) vs. AA alone as first-line therapy for castration-resistant prostate cancer (CRPC) with

bone metastases (BM) in an international multicenter database”, in collaboration with the following Institutions: Dana-Farber Cancer Institute - Harvard Cancer Center, Boston (MA), USA; Tom Baker Cancer Center, Calgary (AB), Canada; Mayo Clinic, Phoenix (AZ), USA; Fred Hutchinson Cancer Research Center, Seattle (WA), USA; Università Hospital Fundacion Jimenez Diaz, Autonomous University of Madrid, Madrid, Spagna; Ospedale Misericordia, Grosseto; Sapienza Università di Roma, Roma; Ospedale degli Infermi, Biella; AOUS Santa Maria alle Scotte, Siena. September 21, 2017 – Present.

- Member of AIMUT (Accademia Italiana Multidisciplinare per l’Urologia Territoriale). September 20, 2017 – Present.
- Co-Investigator, “A Phase 3, Randomized Study of Margetuximab Plus Chemotherapy vs Trastuzumab Plus Chemotherapy in the Treatment of Patients with HER2+ Metastatic Breast Cancer Who Have Received Prior Anti-HER2 Therapies and Require Systemic Treatment (SOPHIA)”, Parexel International. August 28, 2017 – Present.
- Co-Investigator, "Clinical outcomes of first-line abiraterone acetate or enzalutamide for metastatic castration-resistant prostate cancer after

androgen deprivation therapy (ADT) + docetaxel or ADT alone for metastatic hormone-sensitive prostate cancer" in collaboration with the following Institutes: Dana-Farber Cancer Institute - Harvard Cancer Center, Boston (MA), USA; Tom Baker Cancer Center, Calgary (AB), Canada Genitourinary (GU) Tumor Group del Tom Baker Cancer Center, Calgary (AB), Canada; St Vincent's Hospital, Sydney, Australia. February 1, 2017 – September 1, 2017.

- Member of ESMO (European Society for Medical Oncology). May 2017 – Present.
- Co-Investigator, “CABAzitaxel with or without prednisone in patients with metastatic CAstration REsistant prostate cancer (mCRPC) progressed during or after a previous docetaxel-based chemotherapy: a multicenter, prospective, two-arm, open label, non inferiority phase II study (CABACARE)”, Oncotech. February 2017 – Present.
- Co-Investigator, translational research project “Evaluation of prognostic markers in colon cancer patients” Mayo Clinic of Rochester and Università degli Studi di Siena. October 2016 – Present.

7.4 Organization, direction, and coordination or participation to national and international research groups

1. Organization, direction, and coordination of the research group for the clinical project “Clinical outcomes of abiraterone acetate + prednisone (AA) + bone resorption inhibitors (BRI) vs. AA alone as first-line therapy for castration-resistant prostate cancer (CRPC) with bone metastases (BM) in an international multicenter database” including the following Institutions: Dana-Farber Cancer Institute - Harvard Cancer Center, Boston (MA), USA; Tom Baker Cancer Center, Calgary (AB), Canada; Mayo Clinic, Phoenix (AZ), USA; Fred Hutchinson Cancer Research Center, Seattle (WA), USA; Università Hospital Fundacion Jimenez Diaz, Autonomous University of Madrid, Madrid, Spagna; Ospedale Misericordia, Grosseto; Sapienza Università di Roma, Roma; Ospedale degli Infermi, Biella; AOUS Santa Maria alle Scotte, Siena. Since 09/01/2017 to present.
2. Participation to the research activities of the Genitourinary (GU) Tumor Group, Tom Baker Cancer Center, Calgary (AB), Canada. Since 02/01/2017 to present.

3. Participation to the research activities of the Gastrointestinal Oncology Tumor Group, Mayo Clinic, Rochester (MA), USA. Since 10/1/2016 to present.

7.5 Grants

- Roche liberal donation for the project “Circulating cell-free DNA (cfDNA) of biomarkers predictive of resistance or response to taxane treatment in metastatic castration-resistant prostate cancer (mCRPC)” in collaboration with Dana-Farber Cancer Institute – Harvard Medical School and Broad Institute of MIT and Harvard, Boston (MA), USA – November 27, 2018.
- GOGI sponsorship for the project “Circulating cell-free DNA (cfDNA) of biomarkers predictive of resistance or response to taxane treatment in metastatic castration-resistant prostate cancer (mCRPC)” in collaboration with Dana-Farber Cancer Institute – Harvard Medical School and Broad Institute of MIT and Harvard, Boston (MA), USA – September 26, 2018.

7.6 Peer-reviewed publications

1. Petrioli R, Marrelli D, Roviello F, d'Ignazio A, Torre, Chirra M, Savelli V, Ambrosio MR, Francini G, Calomino N, Farsi M, Vernillo R, **Francini E** Feasibility of modified docetaxel, oxaliplatin, capecitabine (DOC) followed by capecitabine as maintenance chemotherapy as first-line therapy for patients with metastatic gastric or gastroesophageal cancer. SUBMITTED to Anticancer Drugs.
2. Petrioli R; Mazzei MA; Giorgi S; Cesqui E; Gentili F; Francini G; Volterrani L; **Francini E**. Hyperprogressive disease in advanced cancer patients treated with nivolumab: a case series study. Anticancer Drugs. IF:1.801 IN PRESS
3. Caraglia M, Correale P, Giannicola R, Staropoli N, Botta C, Pastina P, Nesci A, Caporlingua N, **Francini E**, Ridolfi L, Mini E, Roviello G, Ciliberto D, Agostino RM, Strangio A, Azzarello D, Nardone V, Falzea AC, Cappabianca S, Bocchetti M, D'Arrigo G, Tripepi G, Tassone P, Addeo R, Giordano A, Pirtoli L, Francini G, Tagliaferri P. GOLFIG chemo-immunotherapy in metastatic colorectal cancer (mCRC) patients. A critical review on a long lasting follow up. Journal: Frontiers in Oncology, section Cancer Immunity and

Immunotherapy Front Oncol. IF: 4.137 IN PRESS

4. Petrioli R, Marrelli D, Roviello F, d'Ignazio A, Torre, Chirra M, Savelli V, Ambrosio MR, Francini G, Calomino N, Farsi M, Vernillo R, **Francini E**. Pathological response and outcome after neoadjuvant chemotherapy with DOC (docetaxel, oxaliplatin, capecitabine) or EOF (epirubicin, oxaliplatin, 5-fluorouracil) for clinical T3-T4 non-metastatic gastric cancer. Surg Oncol. IF: 3.000 IN PRESS
5. Petrioli R, Torre P, Pesola G, Paganini G, Paoletti L, Miano ST, Martellucci I, Francini G, **Francini E**. Gemcitabine plus nab-paclitaxel followed by maintenance treatment with gemcitabine alone as first-line treatment for older adults with locally advanced or metastatic pancreatic cancer. J Geriatr Oncol. 2019. doi: 10.1016/j.jgo.2019.08.008. [Epub ahead of print] IF: 3.164
6. **Francini E**, Gray KP, Shaw GK, Evan CP, Hamid AA, Perry CE, Kantoff PW, Taplin ME, Sweeney CJ. Impact of New Systemic Therapies on Overall Survival of Patients with Metastatic Castration Resistant Prostate Cancer in a Hospital-based Registry. Prostate Cancer Prostatic Dis. 2019 Sep; 22(3):420-427. doi: 10.1038/s41391-018-0121-2. IF: 4.099

7. Miracco C, **Francini E**, Torre P, Baldino G, Chirra M, Cinotti E, Francini G. Extramammary invasive Paget's disease and apocrine angiomatous hamartoma: an unusual association. *Eur J Dermatol*. 2018 Dec; 28(6):853-855. doi: 10.1684/ejd.2018.3438. IF: 1.944
8. Choudhury AD, Werner L, **Francini E**, Wei XX, Ha G, Freeman SS, Rhoades J, Reed SC, Gydush G, Rotem D, Lo C, Taplin ME, Harshman LC, Zhang Z, O'Connor EP, Stover DG, Parsons HA, Getz G, Meyerson M, Love JC, Hahn WC, Adalsteinsson VA. Tumor fraction in cell-free DNA as a biomarker in prostate cancer. *JCI Insight*. 2018 Nov; 3(21). doi: 10.1172/jci.insight.122109. IF: 6.014
9. Petrioli R, **Francini E**, Cherri S, Torre P, Fiaschi AI, Miano ST, Marrelli D, Rovello F, Francini G. Capecitabine plus oxaliplatin and bevacizumab, followed by maintenance treatment with capecitabine and bevacizumab for patients aged > 75 years with metastatic colorectal cancer. *Clin Colorectal Cancer*. 2018 Dec; e663-e669. doi: 10.1016/j.clcc.2018.07.002. IF: 3.861
10. **Francini E**, Gray KP, Xie W, Shaw GK, Valença L, Bernard B, Albiges L, Harshman LC, Kantoff PW, Taplin ME, Sweeney CJ. Time of metastatic disease presentation and volume of disease are

- prognostic for metastatic hormone sensitive prostate cancer (mHSPC).
Prostate. 2018 Sep; 78:889-95. IF: 3.347
11. Petrioli R, Chirra M, Messuti L, Fiaschi AI, Savelli V, Martellucci I, **Francini E**. Efficacy and safety of regorafenib with schedule 2/1 for patients ≥ 75 years with metastatic colorectal cancer (mCRC) after failure of two lines of chemotherapy. Clin Colorectal Cancer. 2018 Dec; 17(4):307-312. doi: 10.1016/j.clcc.2018.02.005. IF: 3.861
 12. **Francini E**, Yip S, Ahmed S, Li H, Ardolino L, Evan CP, Kaymakcalan M, Shaw GK, Kantoff PW, Taplin ME, Alimohamed NS, Joshua AM, Heng DYC, Sweeney CJ. Clinical outcomes of first-line abiraterone acetate or enzalutamide for metastatic castration-resistant prostate cancer after androgen deprivation therapy + docetaxel or adt alone for metastatic hormone-sensitive prostate cancer. Clin Genitourin Cancer. 2018 Apr; 16(2):130-134. IF: 2.539
 13. Moreira RB, Debiasi M, **Francini E**, Nuzzo PV, De Velasco G, Maluf FC, Fay AP, Bellmunt J, Choueiri TK, Schutz FA. Differential side effects profile in patients with mCRPC treated with abiraterone or enzalutamide a meta-analysis of randomized controlled trials. Oncotarget. 2017 Aug; 8:84572-84578. IF: 5.168

14. **Francini E**, Taplin ME. Prostate cancer: developing novel approaches to castration-sensitive disease. *Cancer*. 2017 Jan; 123(1):29-42. IF: 6.537

7.7 Book chapters, posters/abstracts, editorials

1. **Francini E**, Ou FS, Rhoades J, Wolfe EG, O'Connor E, Ha G, Gydush G, Kelleher K, Bhatt RS, Balk SP, Sweeney CJ, Adalsteinsson V, Taplin ME, Choudhury A. Circulating free DNA (cfDNA) as biomarker of taxane resistance in metastatic castration-resistant prostate cancer (mCRPC). Abstract SUBMITTED at Genitourinary Cancers Symposium 2020.
2. **Francini E**, Montagnani F, Nuzzo PV, Gonzalez-Velez M, Alimohamed NS, Cigliola A, Moreno-Candilejo I, Rubio-Perez J, Crivelli F, Shaw GK, Petrioli R, Bengala C, Francini G, Garcia-Foncillas J, Sweeney CJ, Higano CS, Bryce AH, Harshman LC, Lee-Ying R, Heng DYC. Clinical outcomes of abiraterone acetate + prednisone (AA) + bone resorption inhibitors (BRI) vs. AA alone as first-line therapy for castration-resistant prostate cancer (CRPC) with

- bone metastases (BM) in an international multicenter database.
Abstract SUBMITTED at Genitourinary Cancers Symposium 2020.
3. **Francini E**, Ou FS, Lazzi S, Petrioli R, Multari AG, Pesola G, Messuti L, Colombo E, Livellara V, Bazzurri S, Cherri S, Miano ST, Wolfe EG, Alberts SR, Hubbard JM, Yoon HH, Francini G. CD3+ tumor-infiltrating lymphocytes (TILs) are prognostic in patients (pts) with stage II colon cancer (CC) not treated with adjuvant chemotherapy (ADJ). Abstract SUBMITTED at Gastrointestinal Cancers Symposium 2020.
 4. Spini A, Roberto G, Bellan C, Rosellini P, Pascucci A, Donnini S, Caldarella A, Barchielli A, Furiesi F, Leoncini L, **Francini E**, Bartolini C, Giorgi S, Zappa M, Ziche M, Gini R. Using Linked Administrative And Pathology Database To Describe Treatment Patterns Of Non-small-cell Lung Cancer Patients At The Azienda Ospedaliera Universitaria Senese. Abstract at 35th ICPE, Philadelphia (PA), USA.
 5. **Francini E**, Montagnani F, Nuzzo P, Alimohamed NS, Moreno I, Rosellini P, Gonzalez-Velez M, Rubio-Perez J, Zhang L, Shaw GK, Crivelli F, Petrioli R, Bengala C, Francini G, Garcia-Foncillas J, Bryce

- AH, Sweeney CJ, Harshman LC, Lee-Ying R, Heng DYC. Efficacy of bone resorption inhibitors (BRI) + abiraterone acetate + prednisone (AA) vs. AA alone as first-line therapy for men with castration-resistant prostate cancer (CRPC) and bone metastases (BM) in an international multicenter hospital-based registry. Abstract at 2019 ASCO Annual Meeting, Chicago (IL), USA. *J Clin Oncol* 37, 2019 (suppl; abstr e16508).
6. Miano ST, Francini G, Petrioli R, Civitelli S, **Francini E**. Clinical outcomes of sunitinib (Su) for patients (pts) with desmoid tumors (DT). Poster at 2019 ASCO Annual Meeting, Chicago (IL), USA. *J Clin Oncol* 37, 2019 (suppl; abstr 11052).
7. Frullanti E, Palmieri M, Baldassarri M, Fava F, Fabbiani A, Gelli E, Tita R, Torre P, Petrioli R, Hadijstilianou T, Galimberti D, Cinotti E, Mencarelli MA, Pinto AM, **Francini E**, Marsili S, Francini G, Renieri A. Specific clonal expansion at disease progression (PD) in solid cancers pinpointed by cell free DNA analysis. *J Clin Oncol* 37, 2019 (suppl; abstr e13144).
8. Spini A, Roberto G, Ciccone V, Pascucci A, Rosellini P, **Francini E**, Donnini S, Gini R, Ziche M. Relevance of Gender In the Drug-

- utilization pattern of Non Small Cell Lung Cancer patients at University Hospital of Siena, Italy (UHS). Abstract at OSSD 2019, Washington (DC), USA.
9. Boccardo F, **Francini E**, Francini G, Zanardi E. Neoplasie prostatiche. COMU Collegio degli Oncologi Medici Italiani, editor. Manuale di oncologia medica. Torino: Edizioni Minerva Medica; 2018; 246-64.
 10. Zhang L, Gray KP, Shaw G, Evan C, **Francini E**, Sweeney CJ. Bone targeted therapy and skeletal related events in the era of modern therapies for castration resistant prostate cancer with bone metastases. Poster at 2018 ASCO Annual Meeting, Chicago (IL), USA. J Clin Oncol 36, 2018 (suppl; abstr 5049).
 11. **Francini E**, Gray KP, Shaw G, Evan C, Hamid AA, Perry CE, Kantoff PW, Taplin ME, Sweeney CJ. Impact of New Systemic Therapies on Overall Survival (OS) of Patients with Metastatic Castration Resistant Prostate Cancer (mCRPC) in a Hospital-based Registry. Poster at Genitourinary Cancers Symposium 2018, San Francisco (CA), USA. J Clin Oncol 36, 2018 (suppl 6S; abstr 203).
 12. **Francini E**, Yip S, Ahmed NS, Li H, Ardolino L, Evan CP, Kaymakcalan M, Shaw GK, Kantoff PW, Taplin ME, Alimohamed

- NS, Joshua AM, Heng DYC, Sweeney CJ. Outcomes of Prechemotherapy (preCRx) Abiraterone Acetate (AA) or Enzalutamide (E) for Metastatic Castration-Resistant Prostate Cancer (mCRPC) after ADT + Docetaxel (D) or ADT Alone for Metastatic Hormone Sensitive Prostate Cancer (mHSPC) in a Multi-Institution Hospital-based Registry. Poster at ESMO 2017 Congress, Madrid (Spain). *Ann Oncol* 28, 2017 (suppl 5; abstr 806).
13. Kantoff PW, Spratt DE, **Francini E**. Androgen Blockade and Salvage Radiation Therapy in Prostate Cancer: Cautious Optimism Amid Unanswered Questions. Invited Editorial in *ASCO Post*. June 10, 2017.
14. **Francini E**, Gray KP, Evan C, Kaymakcalan MD, Shaw GK, Kantoff PW, Taplin ME, Sweeney CJ. Outcomes of prechemotherapy (pCHT) abiraterone (AA) or enzalutamide (E) for metastatic castration-resistant prostate cancer (mCRPC) after androgen deprivation therapy (ADT) + docetaxel (D) or ADT alone for metastatic hormone sensitive prostate cancer (mHSPC) in a hospital-based registry. Abstract at 2017 ASCO Annual Meeting, Chicago (IL), USA. *J Clin Oncol* 35, 2017 (suppl e16515).

15. **Francini E**, Gray KP, Xie W, Kantoff PW, Valenca L, Bernard B, Albiges L, Shaw G, Harshman LC, Taplin ME, Sweeney CJ. Prior Therapy and Volume of Disease are Prognostic for Metastatic Hormone Sensitive Prostate Cancer (mHSPC) in a Hospital-based Database. Poster at Genitourinary Cancers Symposium 2017, Orlando (FL), USA. J Clin Oncol 35, 2017 (suppl 6S; abstr 221).

7.8 Invited presentations/seminaries

1. **Francini E**. Il Paziente Oligometastatico: Il Ruolo dell'Ormonoterapia. Invited oral presentation at "Prostate Cancer Match Point" – Naples, Italy, 11-12 October 2019.
2. **Francini E**. Il Ruolo della Chemioterapia nel Carcinoma della Prostata. Seminary at the School of Medical Oncology, Università degli Studi di Firenze – Florence, Italy, 24 July 2019.
3. **Francini E**. Role of Chemotherapy in Prostate Cancer. Invited oral presentation at "First International congress of Bioimmunotherapy of cancer An International multidisciplinary meeting" – Reggio Calabria, Italy, 12-14 April 2019.

4. **Francini E.** Trattamento del mHSPC ed Implicazioni Terapeutiche Successive. Invited oral presentation at Il Paziente Oncologico Anziano Gestione e Trattamento. Sienna, Italy, 23 November 2018.
5. **Francini E.** Carcinoma della Prostata: Quali Prospettive per l'Immunoterapia? Invited oral presentation at Immuno-Oncologia: Le Nuove Possibilità Terapeutiche. Bari, Italy, 27 November 2017.
6. **Francini E.** Nuovi Orizzonti nel Trattamento del Carcinoma Prostatico Castration Naive. Invited oral presentation at Approccio Multidisciplinare al Paziente con Carcinoma della Prostata: Specialisti a Confronto. Rieti, Italy, 29 September 2017.
7. **Francini E.** Evoluzione delle Linee di Trattamento del Cancro Prostatico Metastatico: Note per l'Urologo Territoriale. Invited oral presentation at V Congresso Nazionale AIMUT. Pisa, Italy, 15-16 September 2017.