

Platinum Priority – Prostate Cancer

Editorial by Evgeny Izumchenko, Liat Shavit Grievink, Eli Rosenbaum and Mohammad Obaidul Hoque on pp. 313–314 of this issue

Serum Free Methylated Glutathione S-transferase 1 DNA Levels, Survival, and Response to Docetaxel in Metastatic, Castration-resistant Prostate Cancer: Post Hoc Analyses of Data from a Phase 3 Trial

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Abstract

Background: Glutathione S-transferase 1 (*GSTP1*) expression is inactivated in >90% of all prostate cancers in association with aberrant DNA methylation. Detection of serum free methylated *GSTP1* (*mGSTP1*) DNA is associated with overall survival (OS) and response to docetaxel in metastatic castration-resistant prostate cancer (mCRPC) in test and internal validation cohorts.

Objective: To assess the relationship between serum free *mGSTP1* and treatment outcomes in SYNERGY, a phase 3 multicentre randomised trial testing the addition of custirsens to first-line chemotherapy with docetaxel in mCRPC.

Design, setting, and participants: Serum free *mGSTP1* DNA was measured by a sensitive methylation-specific polymerase chain reaction assay in paired samples (baseline and after two cycles of docetaxel) from 600 patients.

Outcome measurements and statistical analysis: Associations between serum free *mGSTP1* at baseline, change in *mGSTP1* after docetaxel, OS, and time to prostate-specific antigen (PSA) progression were examined using Cox proportional hazards models and Kaplan-Meier methods.

Results and limitations: Serum free *mGSTP1* was detectable at baseline in 458 (81%) patients. Of those with detectable *mGSTP1* at baseline, *mGSTP1* became undetectable after two cycles in 243 (53%). Undetectable *mGSTP1* at baseline was associated with longer OS (hazard ratio [HR] 0.4, 95% confidence interval [CI] 0.29–0.55; $p < 0.00001$). The event of *mGSTP1* becoming undetectable after two cycles of chemotherapy was associated with longer OS (HR 0.36, 95% CI 0.29–0.46; $p < 0.00001$) and longer time to PSA progression (HR 0.44, 95% CI 0.35–0.56; $p < 0.00001$). Associations between *mGSTP1* and clinical outcomes were independent of other established prognostic variables. Analysis was limited by the lack of radiographic progression-free survival data.

Conclusions: This is the first study to externally validate the prognostic role of a circulating epigenetic biomarker in mCRPC. Further studies are needed to validate serum free *mGSTP1* as a surrogate endpoint for clinical trials and as a potential clinical decision tool.

Patient summary: In this study, we confirmed that a blood marker predicted outcomes after chemotherapy for metastatic prostate cancer. This marker may accelerate future clinical trials of new therapies and be useful in the clinic to guide treatment decisions.

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1. Introduction

Prostate cancer (PC) is the third leading cause of male cancer death in the developed world [1]. While androgen deprivation therapy is initially effective for metastatic disease, ultimately the disease progresses to the castration-resistant state. Over a decade ago, docetaxel was the first agent to provide a survival benefit in metastatic castration-resistant prostate cancer (mCRPC). While therapeutic options in this setting have improved significantly in recent years, docetaxel chemotherapy remains central to the treatment paradigm by providing symptomatic and survival benefits [2]. Despite the increasing use of docetaxel in metastatic castration-sensitive PC [3,4], around half of men with ultimately lethal PC present with localised disease, progress on androgen deprivation therapy, and require docetaxel in the castration-resistant setting [5].

However, not all patients benefit from docetaxel, and reliable predictive markers to guide systemic therapies are not yet available. The decision to cease ineffective chemotherapy is often based on prostate-specific antigen (PSA) levels; however, early levels can be unreliable, so at least three cycles of treatment are required before treatment decisions can be made accurately [2]. As a consequence, many patients suffer toxicities such as fatigue (43–53%), grade 3/4 neutropaenia (21–32%), and peripheral neuropathy (30%) [2,6], without gaining benefit. Early chemotherapy response markers are urgently needed to minimise unwarranted toxicity, expedite patient access to other life-prolonging therapies, and ultimately guide sequencing of systemic treatments.

Glutathione S-transferases (GSTs) are a family of enzymes that catalyse intracellular detoxification of a variety of electrophiles, including a number of xenobiotics and carcinogens [7]. Among five isozymes, the pi class enzyme (methylated glutathione S-transferase 1 [*GSTP1*]) is most widely distributed [8]. Many groups including our own have shown that *GSTP1* expression is inactivated in >90% of PCs [9,10] in association with aberrant DNA methylation of the CpG island region spanning the promoter and exons 1–3 [10]. Compared with PSA, serum free *mGSTP1* is more specific for PC [11], as it has not been observed in normal prostate tissue [12] and it is rarely seen elsewhere in the body [13]. In localised disease, preoperative serum *mGSTP1* predicts PSA recurrence, with higher levels seen in patients with metastatic disease [14]. While some evidence suggests that the presence of *mGSTP1* in the circulation is associated with tumour aggressiveness [15,16], levels may also reflect disease burden [17].

In test and internal validation cohorts, undetectable circulating *mGSTP1* DNA after one cycle of chemotherapy is correlated with PSA response following docetaxel treatment and improved overall survival (OS), while the baseline *mGSTP1* level also has prognostic value, even in the absence of treatment [17]. This study aimed to validate these prognostic findings through a post hoc analysis of a large prospective phase 3 cohort.

2. Patients and methods

2.1. Study population

The parent trial for this study was SYNERGY—a randomised, open-label, multinational, phase 3 trial across 134 study centres in 12 countries [6]. Detailed eligibility criteria, randomisation, and study procedures have previously been reported [6]. Patients with mCRPC who had not previously received systemic chemotherapy were randomly assigned (1:1) centrally to receive docetaxel alone ($n = 512$) or with custirsén ($n = 510$), an antisense oligonucleotide inhibitor of clusterin production [18]. Final results of the SYNERGY study revealed no OS benefit for the addition of custirsén to docetaxel [6]. Subsequent analyses were performed on the entire patient cohort with combined results across both arms.

All patients gave written informed consent before study enrolment.

2.2. Sample collection

A subset of patients (600/1022) enrolled in the SYNERGY study were randomly selected from the whole SYNERGY study population, blinded to outcome and stratified according to whether they were in the control or the treatment arm of the study (all treated with docetaxel), opiate use for PC-related pain, and evidence of radiographic progression (as per SYNERGY protocol [6]). Paired serum samples (baseline and cycle 3 day 1) were stored at -80°C and thawed for the *mGSTP1* assay.

2.3. Methylation-specific head-loop polymerase chain reaction *GSTP1* assay

Specimens received at the Garvan Institute of Medical Research were assigned an identification number. Paired samples were analysed in the same batch in a blinded fashion, and each batch was balanced for the treatment arm. There was limited batch-to-batch variability (Supplementary Table 1).

Assay details have previously been reported [17] and are included in Supplementary material.

2.4. Statistical analysis

Time to PSA progression and OS were calculated from the date of commencement of docetaxel. PSA progression was defined as a PSA rise of 25% or more above the nadir or baseline value (if no fall from baseline was observed) with an absolute increase of at least 2 ng/ml, confirmed by a second value 3+ wk later. If no fall in PSA was recorded or there was a 25% PSA rise from nadir prior to 12 wk, PSA progression was determined to occur at least 12 wk after commencing treatment, as per the Prostate Cancer Working Group 2 criteria [19]. Accordingly, a modified landmark analysis was performed for PSA progression-free survival. PSA response was defined as a fall in PSA of at least 50% at 12 wk from commencing docetaxel [20].

All analyses of serum free *mGSTP1* levels were dichotomised as detectable or undetectable, as defined by our prior phase I and II analyses [17].

Survival analyses were performed using the Kaplan-Meier method. Prognostic risk groups, utilising baseline clinicopathological variables, were defined by an exploratory post hoc analysis in the SYNERGY study (detailed in Supplementary material) [6]. The associations between *mGSTP1* DNA status, established prognostic variables [21] (Supplementary Tables 2 and 3), prognostic risk groups [6], OS, and time to PSA progression were analysed by Cox regression. Harrell's concordance index was used to measure the predictive discrimination of models for time-dependent outcomes [22]. Pearson chi-square test was used to examine for associations between: *mGSTP1* detectability and treatment arm, and between baseline *mGSTP1* and >50% fall in PSA at 12 wk.

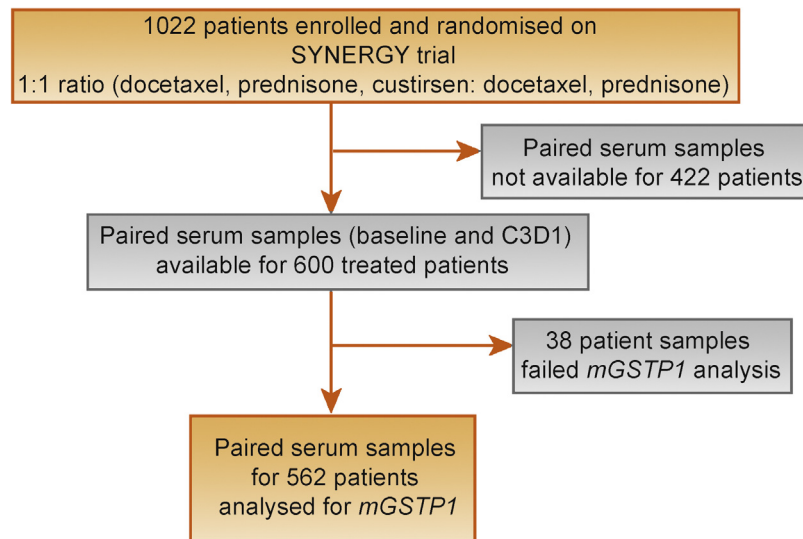


Fig. 1 – CONSORT diagram. C3D1 = cycle 3 day 1; mGSTP1 = methylated glutathione S-transferase 1.

In anticipation of a future adaptive design study to test the clinical utility of *mGSTP1*, a descriptive report of the clinical consequence of using *mGSTP1* was performed [23]. A lack of treatment benefit was defined as progression of PSA within 3 mo of commencing docetaxel, as it is known to predict OS [24]. Only patients with PSA response data at 12 wk ($n = 419$) were included. Using the established *mGSTP1* cutpoint of detectable versus undetectable at 6 wk, we assessed the potential of the biomarker to change treatment decisions.

Statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY, USA) and *somersd* package in STATA/SE version 9.2 (StataCorp, College Station, TX, USA).

3. Results

Patients ($n = 1022$) were recruited on the SYNERGY study between 10 December 2010 and 12 November 2012. Paired serum samples from 600 patients at baseline and at 6 wk after commencing treatment (cycle 3 day 1) were analysed. Analysis of free serum *mGSTP1* DNA failed in one sample of the pair for 38 patients, excluding them from further analysis (Fig. 1). Of the 562 patients included in the analysis, 283 (50%) were allocated to the study arm and 279 (50%) to the standard therapy arm. As there was no OS benefit for the addition of custirsen to docetaxel [6] and no significant effect on *mGSTP1* detectability at either time point by the treatment arm (baseline, $p = 0.6$; cycle 3 day 1, $p = 0.2$), subsequent analyses were pooled across both arms.

In this patient subset, median follow-up for surviving patients was 26 mo. At the time of primary analysis of the SYNERGY study, there were 341 deaths in the patient subset with median OS of 24 mo. The baseline clinical characteristics of our patient subset were similar to those in the overall SYNERGY study cohort (Table 1) [6].

3.1. OS by serum *mGSTP1* status at baseline

Baseline level of serum *mGSTP1* DNA had prognostic value. Irrespective of changes after chemotherapy, undetectable *mGSTP1* at baseline was associated with longer OS (Fig. 2;

hazard ratio [HR] 0.40, 95% confidence interval [CI] 0.29–0.55; $p < 0.00001$). As a continuous variable, *mGSTP1* (ng/ml) was also associated with OS on univariable analysis (HR 1.006, 95% CI 1.005–1.008; $p < 0.00001$). Prognostic risk groups defined in the SYNERGY study [6] utilising clinico-pathological variables were associated with OS in this subgroup of the overall study (SYNERGY prognostic model, good vs poor: HR 0.46, 95% CI 0.4–0.6; $p < 0.00001$).

When baseline *mGSTP1* and the SYNERGY prognostic risk group [6] were included in a multivariable analysis, both factors were independently associated with OS (baseline *mGSTP1*, undetectable vs detectable: HR 0.47, 95% CI 0.33–0.66; $p < 0.00001$, and SYNERGY prognostic model, good vs poor: HR 0.52, 95% CI 0.42–0.65; $p < 0.00001$). The concordance index for the prediction of OS using the SYNERGY prognostic risk group alone (C-index 0.62, 95% CI 0.59–0.65) was improved by the addition of baseline *mGSTP1* (undetectable vs detectable) to the model (C-index 0.65, 95% CI

Table 1 – Baseline clinical characteristics ($N = 562$)

Characteristic	Median (Q1, Q3) or number (%)
Age (yr)	69 (63, 74)
Karnofsky performance status	90 (80, 100)
Missing data	2 (0.3%)
Gleason sum at diagnosis	8 (7, 9)
Missing data	27 (5%)
PSA (ng/ml)	82 (26, 248)
Bone metastases	489 (87%)
Lymph node metastases	345 (61%)
Visceral metastases	147 (26%)
Lactate dehydrogenase	241 (190, 411)
Missing data	10 (2%)
Haemoglobin	127 (117, 136)
Missing data	2 (0.3%)
Baseline <i>mGSTP1</i>	
Detectable	458 (81%)
Undetectable	104 (19%)

mGSTP1 = methylated glutathione S transferase 1; PSA = prostate-specific antigen; Q1 = quartile 1; Q3 = quartile 3.

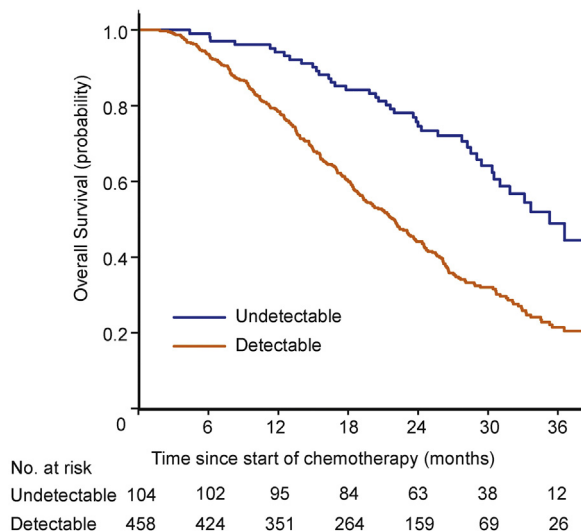


Fig. 2 – Kaplan-Meier estimates of overall survival for patients with undetectable baseline *mGSTP1* versus those with detectable baseline *mGSTP1*. *mGSTP1* = methylated glutathione S-transferase 1.

0.62–0.68; $p < 0.001$). The level of *mGSTP1* at baseline was not significantly correlated with subsequent PSA fall of at least 50% at 12 wk after commencing treatment ($p = 0.1$).

3.2. OS by serum *mGSTP1* status at 6 wk

Serum free *mGSTP1* DNA was detectable at baseline in 458 (81%) of patients (Table 1). Of those with detectable *mGSTP1* at baseline, *mGSTP1* became undetectable after two cycles of docetaxel in 243 (53%). In those whose *mGSTP1* became undetectable after two cycles of docetaxel, OS was significantly longer (Fig. 3A; HR 0.36, 95% CI 0.29–0.46; $p < 0.00001$). Serum *mGSTP1* DNA becoming undetectable after two cycles of docetaxel was more highly associated with OS than a PSA fall of $>50\%$ at 12 wk after commencing chemotherapy [20] (Fig. 3A and B; HR 0.36, 95% CI 0.29–0.46; $p < 0.0001$ vs HR 0.63, 95% CI 0.49–0.81; $p = 0.0003$).

On multivariable analysis including *mGSTP1* after two cycles of chemotherapy and the SYNERGY prognostic risk group [6], both factors were independently associated with OS (*mGSTP1* after two cycles of chemotherapy, undetectable vs detectable: HR 0.39, 95% CI 0.31–0.48; $p < 0.00001$; SYNERGY prognostic model, good vs poor: HR 0.53, 95% CI 0.43–0.66; $p < 0.00001$). The concordance index for the prediction of survival using the SYNERGY prognostic risk group alone (C-index 0.62, 95% CI 0.59–0.65) was improved by the addition of *mGSTP1* after two cycles of chemotherapy (undetectable vs detectable) to the model (C-index 0.68, 95% CI 0.66–0.71; $p < 0.001$). Serum *mGSTP1* becoming undetectable after two cycles of docetaxel was also associated with longer time to PSA progression (Fig. 3C; HR 0.44, 95% CI 0.35–0.56; $p < 0.00001$).

3.3. Potential clinical utility of *mGSTP1* status at 6 wk

A key question for future studies is as follows: can *mGSTP1* affect clinical decision making? The classic scenario is a

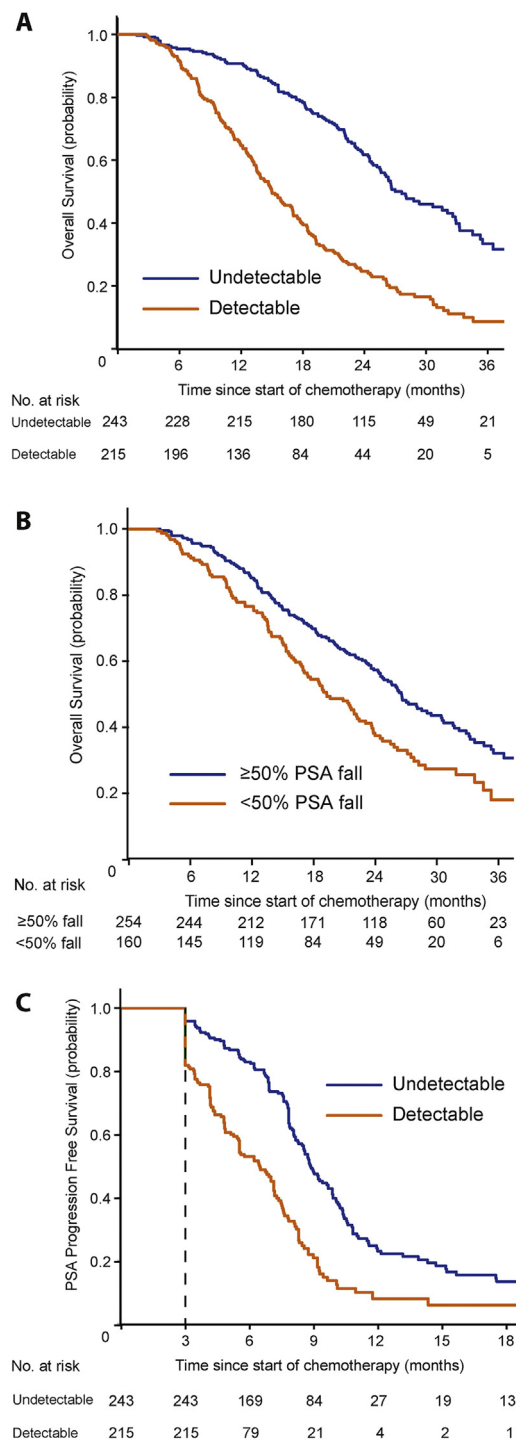


Fig. 3 – Kaplan-Meier estimates of survival in patients with a detectable *mGSTP1* at baseline. (A) OS according to *mGSTP1* detection after two cycles of docetaxel. (B) OS according to a fall in PSA by 12 wk from commencing docetaxel. (C) PSA PFS according to *mGSTP1* detection after two cycles of docetaxel. PSA progression was defined as at least 12 wk from the commencement of chemotherapy. *mGSTP1* = methylated glutathione S-transferase 1; OS = overall survival; PFS = progression-free survival; PSA = prostate-specific antigen.

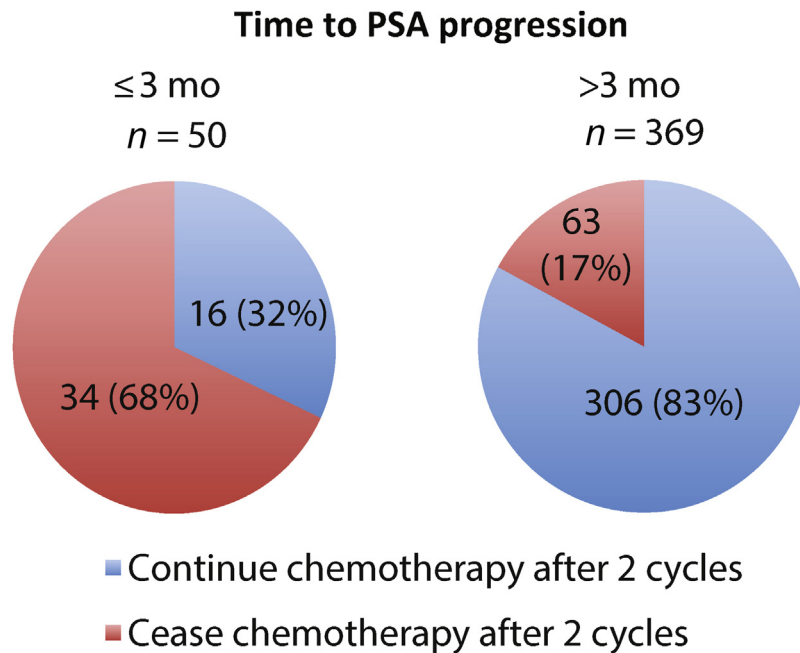


Fig. 4 – Exploratory analysis of potential clinical utility. Number of patients who would cease or continue chemotherapy after two cycles depending on changes in *mGSTP1* and PSA grouped according to time to PSA progression of ≤3 or >3 mo. After two cycles of chemotherapy, patients with stable or rising PSA and detectable *mGSTP1* would cease chemotherapy (red) and those with PSA response and/or undetectable *mGSTP1* would continue chemotherapy (blue). *mGSTP1* = methylated glutathione S-transferase 1; PSA = prostate-specific antigen.

patient without a clear PSA response (stable or rising PSA) after two cycles of docetaxel, who has mild to moderate side effects, and it is unclear whether further treatment with chemotherapy will provide benefit or only worsen toxicity. After two cycles of docetaxel, there were 212 (38%) patients whose PSA was stable (<30% fall and <25% rise from baseline; $n = 147$, 26%) or rising ($\geq 25\%$ increase from baseline; $n = 65$, 12%) [19]. Of these patients, serum *mGSTP1* remained detectable at the same time point in 111 (52%).

Treatment decisions are informed by multiple factors including symptoms, toxicity, radiographic findings, and PSA; however, only data on PSA were available, so approximate assumptions were required. As early changes in PSA are unreliable and should not be used alone to make treatment decisions [19], an assumption was made that using PSA alone, chemotherapy would be continued until at least 12 wk. Alternatively using PSA and *mGSTP1* data, treatment could be ceased at 6 wk if PSA was stable or rising and serum *mGSTP1* remained detectable. Of the patients in this analysis, 97/419 (23%) may have ceased docetaxel at 6 wk. Of these, 34/50 (68%) patients had PSA progression within 3 mo, whereas 63/369 (17%) patients did not have PSA progression within 3 mo (Fig. 4). Ultimately, many of these patients may be spared unnecessary toxicity and have the opportunity to be treated sooner by potentially effective alternative agents.

In patients whose PSA was stable or rising at 6 wk, the fact that *mGSTP1* remained detectable was associated with shorter OS (HR 3.2, 95% CI 2.1–4.8; $p < 0.0001$). While PSA change at 6 wk was associated with OS ($p < 0.001$, Supplementary Fig. 2A), the addition of *mGSTP1* detectabil-

ity was a better discriminator for OS ($p < 0.00001$, Supplementary Fig. 2B).

4. Discussion

This post hoc analysis of a large prospective phase 3 clinical trial of first-line docetaxel ± custirsen has validated our previous findings that early changes in serum free *mGSTP1* DNA levels after treatment strongly correlate with survival outcomes. Detection of serum *mGSTP1* at baseline before docetaxel is also prognostic for OS.

With burgeoning therapeutic options in advanced PC and improved survival outcomes, an early surrogate marker is urgently needed to streamline PC clinical trials and expedite implementation into clinical care. Conversion of *mGSTP1* from being detectable to undetectable after docetaxel implies a treatment effect on this biomarker and translates into significantly improved survival, suggesting that it may have value as a surrogate endpoint for the use in early phase clinical trials. Although PSA changes at 3 mo are of moderate surrogate value, the association is not sufficient to supplant OS [20], requiring longer follow-up. Our study confirms that conversion of *mGSTP1* from being detectable to undetectable at 6 wk is more highly correlated with OS than PSA changes at 3 mo. On this basis, early *mGSTP1* changes may be sufficient for surrogacy. However, our study was limited by the lack of a positive experimental arm; therefore, we are unable to measure the proportion of treatment effect reflected by *mGSTP1*. The post hoc retrospective nature of the analysis was another limitation. A prospective study in a randomised clinical trial with a positive experimental arm is now warranted to evaluate *mGSTP1* as a surrogate outcome measure.

In the clinic, *mGSTP1* provides promise as an additional tool to direct treatment decision making and sequencing. For instance, clinical decisions are often unclear in patients whose PSA has not fallen after two cycles of docetaxel, especially in those suffering significant toxicities from chemotherapy or who have rapidly progressing disease where a delay in potentially effective therapies would be deleterious. Our data suggest that in these patients, addition of *mGSTP1* to the decision algorithm may be advantageous, although it is not yet ready for clinical implementation. This study was limited by the absence of data on radiographic progression; however, our analysis demonstrates a clear link between *mGSTP1*, PSA progression, and OS. Clinical utility of *mGSTP1* warrants further study in a prospective adaptive design trial where early changes in *mGSTP1* are incorporated as a treatment decision aid.

Circulating tumour cell (CTC) enumeration at baseline and during treatment of mCRPC predicts OS and is more robust than changes in PSA [25–27]. In patients receiving abiraterone after docetaxel for mCRPC, a biomarker panel combining CTC counts and lactate dehydrogenase levels after 12 wk of treatment fulfilled the Prentice [28] criteria as an individual patient surrogate endpoint [29].

Like CTCs, our study demonstrates that both baseline and early changes in *mGSTP1* after first-line docetaxel are significantly correlated with OS. The *mGSTP1* assay has several technical advantages over CTC measurement. The methylated DNA marker is stable and can reliably be detected in small-volume (500 µl) frozen samples of serum or plasma [17]. In our study, where serum had been frozen for up to 6 yr, only 38/1200 (3%) samples failed analysis. This is unlike the CTC measurement where 7.5 ml of whole blood is required and analysis must be performed within 96 h of collection [30]. Serum free *mGSTP1* is measured using a polymerase chain reaction assay kit, which has potential for easy commercialisation to allow testing in most laboratories using standard equipment. Conversely, CTCs must be measured using specialised equipment that is not easily accessible in many centres. In measuring a treatment effect, detection of both *mGSTP1* and CTCs is required at baseline. Notably, *mGSTP1* was detectable in 81% of this study cohort at baseline compared with only 51–57% having an unfavourable CTC count (≥ 5 CTCs/7.5 ml blood) in similar patient cohorts [25–27]. This suggests that changes in *mGSTP1* may be informative and useful in a greater number of patients. Our study was limited by a lack of comprehensive CTC data, precluding a direct comparison with *mGSTP1*.

5. Conclusions

Our study validates free serum *mGSTP1* DNA as a robust early response and prognostic biomarker in patients with mCRPC receiving first-line docetaxel. This post hoc analysis was conducted using a representative subset of patient samples from a large, prospective, multinational phase 3 study. A prospective adaptive design study using *mGSTP1* as a clinical decision tool is warranted. In addition, serial *mGSTP1* analysis should be incorporated into prospective

treatment studies in mCRPC to confirm its role as an early surrogate endpoint for OS in clinical research.

Author contributions: Lisa G. Horvath had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Mahon, Chi, Horvath, Clark.

Acquisition of data: Mahon, Qu, Lin, Spielman, Cain, Jacobs.

Analysis and interpretation of data: Mahon, Qu, Jacobs, Chi, Clark, Horvath.

Drafting of the manuscript: Mahon, Horvath.

Critical revision of the manuscript for important intellectual content: Lin, Stockler, Higano, de Bono, Chi, Clark, Horvath.

Statistical analysis: Mahon.

Obtaining funding: Mahon, Horvath, Clark.

Administrative, technical, or material support: Cain, Jacobs.

Supervision: Chi, Clark, Horvath.

Other: None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.eururo.2018.11.001>.

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