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European Association of Urology

Original Article

Prognostic Value of the Circulating Tumor DNA Fraction in Metastatic Castration-resistant Prostate Cancer: Results from the ProBio Platform Trial

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Article info

Article history:
Received 22 January 2025
Accepted 5 February 2025
Available online 21 April 2025

Abstract

Background and objective: The aim of this study was to evaluate the prognostic value of undetectable circulating tumor DNA (ctDNA) and the dose-response relationship between ctDNA levels and survival outcomes in metastatic castration-resistant prostate cancer (mCRPC).

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https://doi.org/10.1016/j.euo.2025.02.002

Editor-in-Chief:
Morgan Rouprêt

Keywords:
Circulating tumor DNA
Dose-response
Platform trial
Prognostic biomarker
Prostate cancer

Methods: We analyzed data for patients enrolled in the ProBio trial up to November 2022 who received an androgen receptor pathway inhibitor or taxane. We compared survival outcomes between patients with undetectable ctDNA and those with detectable ctDNA randomized to physician's choice or investigational arms. Time to no longer clinically benefiting (NLCB) and overall survival (OS) were assessed using Bayesian survival models, with results reported as survival time ratios (STRs). Dose-response relationships were estimated using spike-at-zero models.

Key findings and limitations: A total of 220 patients were included, of whom 139 had detectable ctDNA (56 in the physician's choice arm, 83 in investigational arms) and 81 had undetectable ctDNA. In comparison to the undetectable ctDNA group, the physician's choice arm had 60% shorter time to NLCB (STR 0.40, 90% credible interval [CrI] 0.31–0.51) and 51% shorter OS (STR 0.49, 90% CrI 0.38–0.61). Similar results were observed in comparison to the investigational arms. Dose-response analysis revealed that the undetectable ctDNA group had twofold longer time to NLCB (STR 2.05, 90% CrI 1.66–2.57) and 1.6-fold longer OS (STR 1.63, 90% CrI 1.33–2.05) in comparison to the subgroup with a ctDNA fraction of 2.5%. Every 10-point increment in the ctDNA fraction corresponded to a 10% reduction in NLCB and OS times.

Conclusions and clinical implications: Undetectable ctDNA at baseline predicts superior prognosis in mCRPC, suggesting potential for treatment de-escalation and less intensive monitoring for this subgroup of patients.

This trial is registered on ClinicalTrials.gov as NCT03903835.

ADVANCING PRACTICE

What does this study add?
This study demonstrates a promising role of circulating tumor DNA (ctDNA) as a biomarker for predicting prognosis in metastatic castration-resistant prostate cancer (mCRPC). It adds to the growing body of evidence that ctDNA can provide valuable insights into disease progression and response to treatment. By correlating ctDNA levels with clinical outcomes, the study highlights the potential for a more personalized approach to treatment decisions. The findings suggest that ctDNA could be integrated into clinical practice to better guide treatment strategies and monitor disease status in patients with mCRPC.

Clinical Relevance
This multicenter study validates Restriction Spectrum Imaging (RSI) as a quantitative MRI biomarker for prostate cancer. RSI outperformed ADC and matched expert PI-RADS interpretation for detecting clinically significant prostate cancer, providing an objective tool that could improve diagnostic consistency across centers. Editor-in-Chief: Morgan Rouprêt.

Patient Summary
This study looks at how tumor DNA circulating in the blood, called ctDNA, can help doctors in predicting how prostate cancer will progress. Our research shows that measurement of ctDNA levels can give important clues about how patients respond to treatment. This information could help doctors in making better decisions on how to manage prostate cancer.

1. Introduction

The circulating tumor DNA (ctDNA) fraction refers to the proportion of tumor-derived DNA present in the bloodstream relative to the total amount of cell-free DNA. Several studies have demonstrated the prognostic role of the ctDNA fraction in various cancers and provided valuable insights into tumor dynamics and patient outcomes [1–3]. In prostate cancer, several retrospective studies have shown that higher ctDNA levels at the start of treatment are associated with poorer prognosis, and rapid on-therapy reductions in ctDNA levels with better prognosis [2,4]. These findings suggest the potential utility of the ctDNA fraction as a biomarker for disease prognostication and treatment response. However, the majority of the existing evidence is from retrospective analyses that have certain methodological limitations. One challenge in assessing the prognostic value of the ctDNA fraction is the frequent use of categorical data with varying cutpoints. While informative, this approach can oversimplify the relationship between ctDNA levels and outcomes, and could potentially lead to loss of information, lower statistical power, and a higher risk of misclassi-

fication [5,6]. In addition, different cutpoints across studies hamper comparisons, complicating efforts to fully understand the prognostic significance of ctDNA [7].

The ProBio platform trial was designed to tailor of treatment decisions in metastatic prostate cancer [8]. In this trial, results for therapy classes are compared in groups predefined according to genetic mutations identified in ctDNA, with the aim of personalizing treatment strategies on the basis of molecular profiles [9]. Detection of ctDNA levels served as a prospective inclusion criterion for randomization in the ProBio study. Patients with undetectable ctDNA were monitored but treated according to the standard of care outside the ProBio trial.

In the present study, we evaluated the prognosis for patients with metastatic castration-resistant prostate cancer (mCRPC) and undetectable ctDNA before treatment initiation in comparison to patients with detectable ctDNA. Dose-response associations between quantitative ctDNA fraction levels and survival outcomes were assessed. By evaluating the association of ctDNA fraction levels with key clinical outcomes, such as time to no longer clinically benefiting (NLCB) and overall survival (OS), our aim was to provide robust evidence on the utility of ctDNA fraction quantification as a prognostic biomarker.

2. Patients and methods

The ProBio platform trial (ClinicalTrials.gov NCT03903835) was conducted in accordance with the Declaration of Helsinki and followed Good Clinical Practice guidelines. All patients provided written informed consent to participate in ProBio, including consent for future use of their data for auxiliary research objectives. In addition, patients enrolled in Sweden and Belgium who met the inclusion criteria but were not eligible for randomization (because of undetectable ctDNA, technical failure, or detection of microsatellite instability or a hypermutator genotype) also provided informed consent forms allowing use of their medical data for auxiliary research. Patients enrolled in Norway were not included in this substudy as there was no ethical approval for follow-up for patients with undetectable ctDNA.

2.1. Estimation of the ctDNA fraction

Somatic and germline alterations are identified via in-solution hybridization-based capture using a design tailored for prostate cancer [10]. This was followed by sequencing and processing using an updated version of the AutoSeq bioinformatic pipeline, which contains both in-house and publicly available bioinformatic tools [11]. The ctDNA fraction was estimated on a per-sample basis. In the initial step, an approximate value was determined via copy-number alterations (observable at a ctDNA fraction of 0.10, depending on the sequencing depth and technical quality) and/or the variant allele fraction (VAF) of any clonal drivers detected. The ctDNA fraction was then calculated using different approaches according to the estimated fraction levels. For samples with a ctDNA fraction $\geq$15%, the calculation incorporated ploidy and the cancer cell fraction using PureCN [12] according to the formula: ctDNA fraction = cancer cell fraction $\times$ ploidy / (cancer cell fraction $\times$ ploidy + (1 $-$ cancer cell fraction) $\times$ 2). For samples with a ctDNA fraction of 5–15%, the VAF of a clonal driver variant (eg, *TP53* hotspot mutation or *TMPRSS2-ERG* gene fusion) was used for estimation, assuming ploidy = 2 and the wild-type allele status. Specifically, when the wild-type allele was deleted, the ctDNA fraction was calculated as (2 / (1 / VAF + 1)). For cases with copy-number–neutral loss of heterozygosity, the ctDNA fraction was equal to the VAF. If the wild-type allele remained intact, the ctDNA fraction was determined as 2 $\times$ VAF. For samples with a ctDNA fraction $\leq$5%, the estimation was similarly performed using the VAF of a clonal driver variant under the assumption of ploidy = 2. In the case of tumor suppressors (eg, *PTEN*), assuming deletion of the wild-type allele, the ctDNA fraction was estimated as (2 / (1 / VAF + 1)), whereas for oncogenes (eg, *SPOP* hotspot mutations), assuming an intact wild-type allele, the ctDNA fraction was calculated as 2 $\times$ VAF. Samples with no observable copy-number alterations, mutations (single-nucleotide variants or insertions/deletions), or structural rearrangements were labeled as ctDNA-negative.

2.2. Study design

ProBio is an international, outcome-adaptive, multiarm, open-label, biomarker-driven randomized platform trial designed to tailor treatment selection for patients with metastatic prostate cancer [8]. ProBio uses predefined genetic biomarkers assessed via synchronous ctDNA and germline DNA analysis to randomize patients and compare the efficacy of different treatment classes versus the control group, which receives physician's choice of standard care [11]. For the present study, the prognostic value of undetectable ctDNA before treatment initiation was inferred using initial results from the ProBio trial for patients with mCRPC and detectable ctDNA who were randomized to either physician's choice (control group) or one of the investigational arms, which consisted of an androgen receptor pathway inhibitor (ARPI; either abiraterone acetate or enzalutamide) or a taxane (either docetaxel or cabazitaxel) [10].

The patient eligibility criteria for the ProBio trial have been published elsewhere [9]. The primary endpoint was the time to NLCB, defined as the time at which a patient experiences disease progression, leading to the clinical decision to discontinue a therapy [13]. Progression was identified via radiographic, biochemical, and clinical assessment. OS was a secondary endpoint. For patients with undetectable ctDNA, the randomization date was the date of communication of undetectable ctDNA status, which was the same approach as for the randomized patients. Whereas ProBio has a sequential, multiple-assignment, randomized design, we limited the analysis to data collected from randomization to the first NLCB endpoint.

2.3. Statistical analysis

The prognostic role of undetectable ctDNA levels and the dose-response relationship between the ctDNA fraction and the survival endpoints (NLCB and OS) were determined.

Undetectable ctDNA was defined as the absence of copy-number alterations, somatic small variants, and genomic structural rearrangements. The ProBio assay has a ctDNA detection limit of ~0.5% but the sensitivity may vary according to the amount of cell-free DNA available for library preparation and the number of reads obtained after sequencing [14,15].

The sample size calculations were performed for the ProBio primary analysis and were not specifically designed for this particular analysis [10]. Following the statistical analyses of the published source data [10], we modeled the survival outcomes using Bayesian Weibull accelerated failure-time models [16]. To assess the prognostic role of undetectable ctDNA levels, we considered the crude models with indicator variables for the groups under comparison, using undetectable ctDNA as the reference. We evaluated the differential prognostic value of ctDNA according to the therapy received (ARPI or taxane) using an interaction analysis. For the dose-response analysis, we defined a spike-at-zero model [17], which consists of an indicator variable for undetectable ctDNA and the ctDNA fraction levels multiplied by an indicator variable for having detectable ctDNA. We flexibly modeled ctDNA levels using restricted cubic splines with knots located at the 10th, 50th, and 90th percentiles of the observed distribution [18].

For the survival endpoints we considered three models: a crude model, a model adjusted for the therapy class received (ARPI, taxane, or other), and a fully adjusted model. The relevant confounder variables used in the latter model were Eastern Cooperative Oncology Group (ECOG) performance status (3 levels), location of metastases (lymph node only, bone ± lymph nodes, and viscera ± other sites), the timing of metastatic disease (metachronous or de novo M1), treatment line for mCRPC at trial enrollment, analgesic use, previous exposure to ARPIs and taxanes, log prostate-specific antigen, log lactate dehydrogenase, albumin, alkaline phosphatase, and hemoglobin, which were selected on the basis of a priori knowledge [19]. All continuous variables were modeled using restricted cubic splines, similarly to modeling of continuous ctDNA levels. The prognostic role

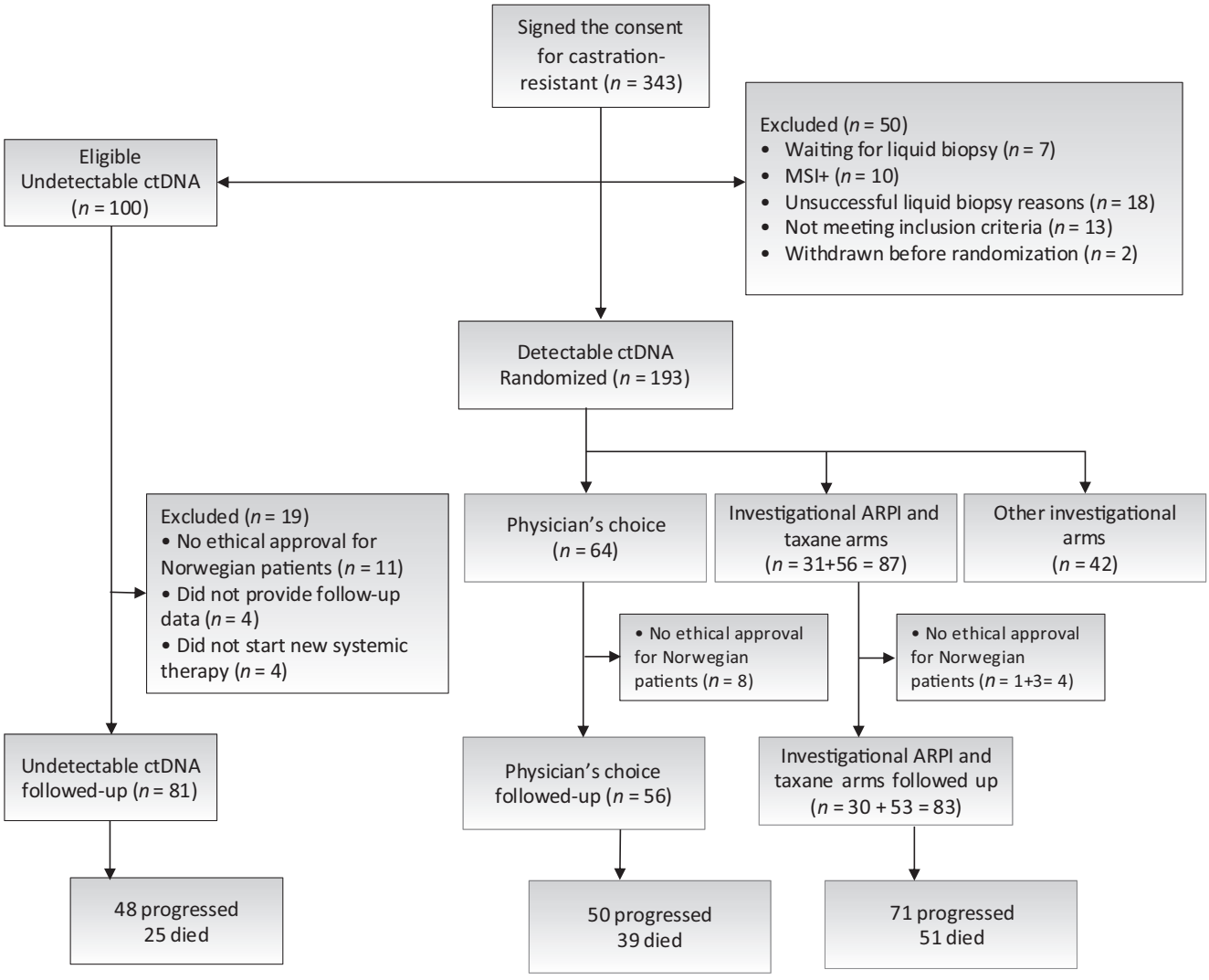

Fig. 1 – Consolidate Standards of Reporting Trials (CONSORT) flow diagram. ARPI = androgen receptor pathway inhibitor; ctDNA = circulating tumor DNA; MSI = microsatellite instability.

of the ctDNA fraction was quantified in terms of the posterior distribution of the survival time ratio (STR), which estimates the relative impact on expected survival time [20]. Using patients with undetectable ctDNA levels as the reference, STR value <1 suggest a shorter expected survival time. We summarized the posterior distributions using the median value and 90% credible interval (CrI). For the prognostic role of undetectable ctDNA, we complemented the survival results with posterior survival curves and the corresponding Kaplan-Meier estimates. We used normal vague priors for (log) STR, with a mean of 0 and standard deviation of 0.5. All analyses were performed in R v4.2.2 using the *rstanarm* package [16].

3. Results

Between February 2019 and November 2022, 343 patients with mCRPC from Sweden, Belgium, and Norway were enrolled in the ProBio trial. A total of 50 patients were excluded because of unsuccessful or pending liquid biopsy results, failure to meet the inclusion criteria, withdrawal before randomization, or detection of microsatellite instability. A further 100 patients were not randomized because of undetectable ctDNA, resulting in 193 patients with detectable ctDNA who were randomized (Fig. 1). According to the study protocol and design, only data with reported outcomes can be used, which in this case encompassed patients randomized to the control group (physician's choice, n = 64) or the investigational ARPI (n = 31) and taxane (n = 56) arms. Data for patients randomized to other treatments within the platform trial were not yet available at the time of the analysis. In addition, the Norwegian patients (8 in the control group, 4 in the investigational arms, and 11 with undetectable ctDNA) were excluded because of lack of ethical approval for the current analysis. Finally, eight patients with undetectable ctDNA were excluded because they had missing follow-up data or had not started systemic therapy. In total, 139 patients with detectable ctDNA (56 randomized to physician's choice and 83 to investigational arms) and 81 patients with undetectable ctDNA were included in the current analysis. The median follow-up was 9.5 mo (interquartile range [IQR]

Table 1 – Baseline patient characteristics by group

Characteristic	Undetectable ctDNA	Detectable ctDNA	
		Physician's choice	Investigational arms
Patients (*n*)	81	56	83
Median age, yr (range)	72 (67–75)	72 (68–76)	70 (65–74)
ECOG performance status score, *n* (%)			
0	67 (85)	33 (59)	62 (75)
1	12 (15)	19 (34)	16 (19)
2	0 (0)	4 (7.1)	5 (6.0)
Analgesic use, *n* (%)	21 (26)	22 (39)	39 (47)
Location of metastases, *n* (%)			
Lymph nodes only	13 (16)	2 (3.6)	8 (9.6)
Bone ± lymph nodes	55 (69)	47 (84)	60 (72)
Viscera (± other sites)	12 (15)	7 (13)	15 (18)
Metastatic disease at diagnosis, *n* (%)	28 (35)	32 (57)	47 (57)
Systemic therapy before castration resistance, *n* (%)			
ADT monotherapy	53 (65)	27 (48)	36 (43)
ADT + docetaxel	18 (22)	17 (30)	34 (41)
ADT + ARPI	10 (12)	12 (21)	13 (16)
Treatment lines, *n* (%)			
1 line	68 (85)	44 (79)	66 (80)
2 lines	11 (14)	11 (20)	16 (19)
3 lines	1 (1.3)	1 (1.8)	1 (1.2)
Previous ARPI therapy, *n* (%)	17 (21)	22 (39)	24 (29)
Previous taxane therapy, *n* (%)	23 (28)	19 (34)	37 (45)
Median lactate dehydrogenase, µkat/l (range)	3.20 (2.90–3.54)	3.59 (3.12–4.30)	3.60 (3.00–4.44)
Median hemoglobin, g/l (range)	134 (128–141)	133 (126–139)	131 (123–137)
Median serum albumin, g/l (range)	41.0 (38.8–44.0)	40.0 (37.5–43.0)	41.0 (37.0–43.0)
Median PSA, ng/ml (range)	10 (4–18)	28 (12–61)	17 (8–55)
Median alkaline phosphatase, µkat/l (range)	1.30 (1.10–1.50)	2.00 (1.43–4.18)	1.80 (1.38–2.98)
Therapy received, *n* (%)			
ARPI	57 (70)	22 (39)	30 (36)
Taxane	21 (26)	33 (59)	53 (64)
Other	3 (3.7)	1 (1.8)	0 (0)
ctDNA fraction, *n* (%)			
Undetectable	81 (100)	0 (0)	0 (0)
Low (<5%)	0 (0)	17 (30)	22 (27)
Medium (5–40%)	0 (0)	30 (54)	49 (59)
High (≥40%)	0 (0)	9 (16)	12 (14)

ADT = androgen deprivation therapy; ARPI = androgen receptor pathway inhibitor; ctDNA = circulating tumor DNA; ECOG = Eastern Cooperative Oncology Group; PSA = prostate-specific antigen.

4.6–18) for time to NLCB and 22.9 mo (IQR 15.4–31.8) for OS.

Clinical characteristics were similar among patients with detectable ctDNA, but differences were noted in comparison to patients with undetectable ctDNA levels (Table 1). The group with undetectable ctDNA tended to have lower ECOG scores, less analgesic use, metastases more often confined to the lymph nodes, and a higher incidence of metachronous metastatic disease. The latter was reflected in the treatment history, with greater prior use of androgen deprivation monotherapy before castration-resistant disease, and less previous ARPI or taxane therapy. Routine blood test results were comparable across groups, except for lower prostate-specific antigen and alkaline phosphatase levels in the undetectable ctDNA group. A larger proportion of patients in the undetectable ctDNA group received an ARPI (70%) in comparison to the detectable ctDNA group (39% in the physician's choice arm, 36% in the investigational arms; Table 1).

3.1. *Prognostic role of undetectable ctDNA*

Favorable times to NLCB and OS were observed for patients with undetectable ctDNA in comparison to patients with detectable ctDNA (Fig. 2). Specifically, time to NLCB was 60% shorter for patients with detectable ctDNA in the physician's choice arm (STR 0.40, 90% CrI 0.31–0.51), with a median time to NLCB of 8.5 mo (90% CrI 7.0–10.3) versus 21.2 mo (90% CrI 17.9–25.3) for patients with undetectable ctDNA. Similarly, OS was 51% shorter for patients with detectable ctDNA in the physician's choice arm (STR 0.49, 90% CrI 0.38–0.61) in comparison to patients with undetectable ctDNA, with median OS of 22.2 mo (90% CrI 19.2–26.1) versus 45.6 mo (90% CrI 38.6–55.1). Similar outcomes were observed when patients with undetectable ctDNA were compared to patients with detectable ctDNA in the investigational arms, with STRs of 0.46 (90% CrI 0.36–0.57) for NLCB and 0.57 (90% CrI 0.45–0.70) for OS (Table 2).

The treatment effects remained consistent, although they decreased to a 30–40% reduction for time to NLCB and a 20–36% reduction for OS after adjusting for therapy class and full adjustment (Table 2 and Supplementary Fig. 1).

We did not observe a strong difference in prognostic effect between the ARPI and taxane subgroups (Supplementary Figs. 2 and 3). For time to NLCB, the STRs for patients with detectable ctDNA in the physician's choice arm were

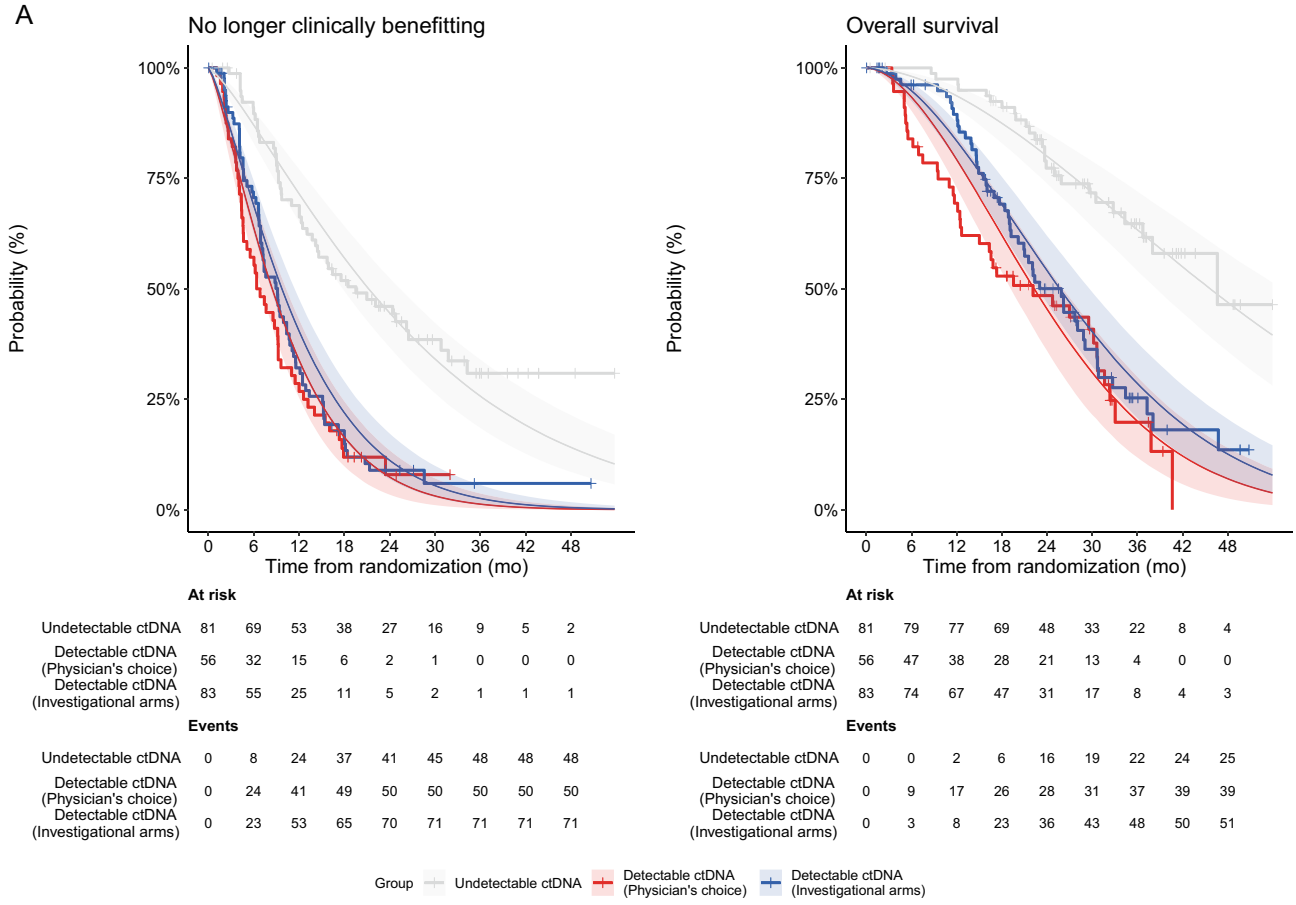

Fig. 2 – Posterior survival curves (smooth) and Kaplan-Meier estimates (stepped) for (A) time to no longer clinically benefiting (left panel) and (B) overall survival (right panel) are shown for each group. Tick marks on the Kaplan-Meier curves denote censored patients. Survival estimates were generated using Weibull accelerated failure-time models. Colored lines represent the median of the posterior distribution, while shaded areas indicate the 90% credible interval. ctDNA = circulating tumor DNA.

0.5 (90% CrI 0.38–0.70) for ARPI treatment and 0.55 (90% CrI 0.40–0.75) for taxane treatment. Similarly, the STRs for patients with detectable ctDNA in the investigational arms were 0.61 (90% CrI 0.46–0.80) for ARPI treatment and 0.62 (90% CrI 0.45–0.82) for taxane treatment. A similar pattern was observed for OS.

3.2. Dose-response relationship

Even with a flexible dose-response model, the spike-at-zero analysis revealed a linear relationship between the ctDNA fraction and the STRs for time to NLCB and OS (Fig. 3). Using a ctDNA fraction of 2.5% as the reference, patients with undetectable ctDNA had a twofold longer time to NLCB (STR 2.05, 90% CrI 1.66–2.57) and 1.6-fold longer OS (STR 1.63, 90% CrI 1.33–2.02; Supplementary Table 1). Furthermore, higher ctDNA fractions were associated with shorter expected times to NLCB and OS. Specifically, for every 10-percentage point increment in ctDNA fraction, the STR decreased by an average of 8.8% for NLCB and 10% for OS (Fig. 3 and Supplementary Table 1). In particular, with a ctDNA fraction of 2.5% as the reference, ctDNA fractions of 20%, 50%, and 70% were associated with 14% (STR 0.86, 90% CrI 0.79–0.95), 35% (STR 0.65, 90% CrI 0.52–0.83), and 46% (STR 0.54, 90% CrI 0.39–0.77) shorter time to NLCB. Similar to the previous analysis, the results remained stable after multivariable adjustment, with consistent estimated dose-response curves indicating an inverse linear association between increasing ctDNA fraction and survival endpoints (Supplementary Table 1 and Supplementary Fig. 4).

4. Discussion

We evaluated the prognostic value of undetectable ctDNA in patients with mCRPC enrolled in the ProBio trial. Our findings show that patients with detectable ctDNA had a 60% shorter time to NLCB and 40% shorter OS. With a ctDNA fraction of 2.5% as the reference, patients with undetectable ctDNA experienced a twofold increase in time to NLCB and a 1.6-fold increase in OS. Furthermore, each 10-point increment in the ctDNA fraction was associated with 10% decreases in the time to NLCB and in OS. The clinical validity of undetectable ctDNA in patients with mCRPC was prospectively evaluated using data from the ProBio trial, in which ctDNA detection was a predefined criterion for randomization.

Our findings are consistent with previous studies that explored the prognostic value of the ctDNA fraction in prostate cancer [2,4,21–28]. However, the retrospective or observational nature of these studies, along with their limited sample sizes, heterogeneous patient populations, and varying therapeutic contexts, limit the generalizability of the findings. A common limitation across these studies is the prevalent use of assay-driven and nonreproducible cut-points for categorization of detectable ctDNA fractions levels. This approach can oversimplify the complex relationships between the ctDNA fraction and clinical outcomes, complicating the interpretation and comparison of results across studies. More recently, Fonseca et al [29] also observed a nonlinear relationship between ctDNA fractions and all-cause mortality using a model that included the ctDNA fraction as a continuous variable, highlighting the importance of considering ctDNA fractions beyond cut-points. However, the methodology used in their analysis relied on hazard ratios. While hazard ratios are a common and valuable metric, they can be challenging to interpret, as they do not directly convey how much longer or shorter one group is expected to survive in comparison to another, impeding translation of these results into meaningful survival benefits for patients [30].

In our prospective evaluation, patients with undetectable ctDNA underwent similar follow-up management with consistent recording of NLCB and OS data, which allowed a robust assessment of the utility of the ctDNA fraction as a prognostic marker for patients with mCRPC. Unlike previous studies that retrospectively assessed the ctDNA fraction, the ProBio trial involved prospective integration of ctDNA analyses, with undetectable ctDNA serving as an exclusion criterion for randomization. This allowed us to directly compare survival outcomes between patients with detectable and undetectable ctDNA, both treated with a physician's choice for standard of care. In addition, we extended our analysis to include patients treated in the ARPI and taxane investigational arms, further validating

Table 2 – Prognostic role of undetectable ctDNA for time to NLCB and overall survival [a]

Outcome and group	*n*	Events	Median time, mo (90% CrI)	STR (90% CrI)		
				Crude	Adjusted	Fully adjusted
NLCB						
Undetectable ctDNA	81	48	21.2 (17.9–25.3)	Reference	Reference	Reference
Detectable ctDNA						
Physician's choice	56	50	8.5 (7.0–10.3)	0.40 (0.31–0.51)	0.52 (0.42–0.66)	0.61 (0.47–0.78)
Investigational arms	83	71	9.7 (8.2–11.4)	0.46 (0.36–0.57)	0.61 (0.49–0.76)	0.68 (0.54–0.85)
Overall survival						
Undetectable ctDNA	81	25	45.6 (38.6–55.1)	Reference	Reference	Reference
Detectable ctDNA						
Physician's choice	56	39	22.2 (19.2–26.1)	0.49 (0.38–0.61)	0.53 (0.42–0.67)	0.74 (0.58–0.93)
Investigational arms	83	51	25.8 (22.7–29.7)	0.57 (0.45–0.70)	0.62 (0.50–0.77)	0.80 (0.64–0.99)

CrI = credible interval; ctDNA = circulating tumor DNA; NLCB = no longer clinically benefiting; STR = survival time ratio.
[a] Patients with undetectable ctDNA serve as the reference group for treatment effects, measured as STR with 90% CrI. Results from three analyses are presented: crude, adjusted by therapy class, and fully adjusted for relevant confounders.

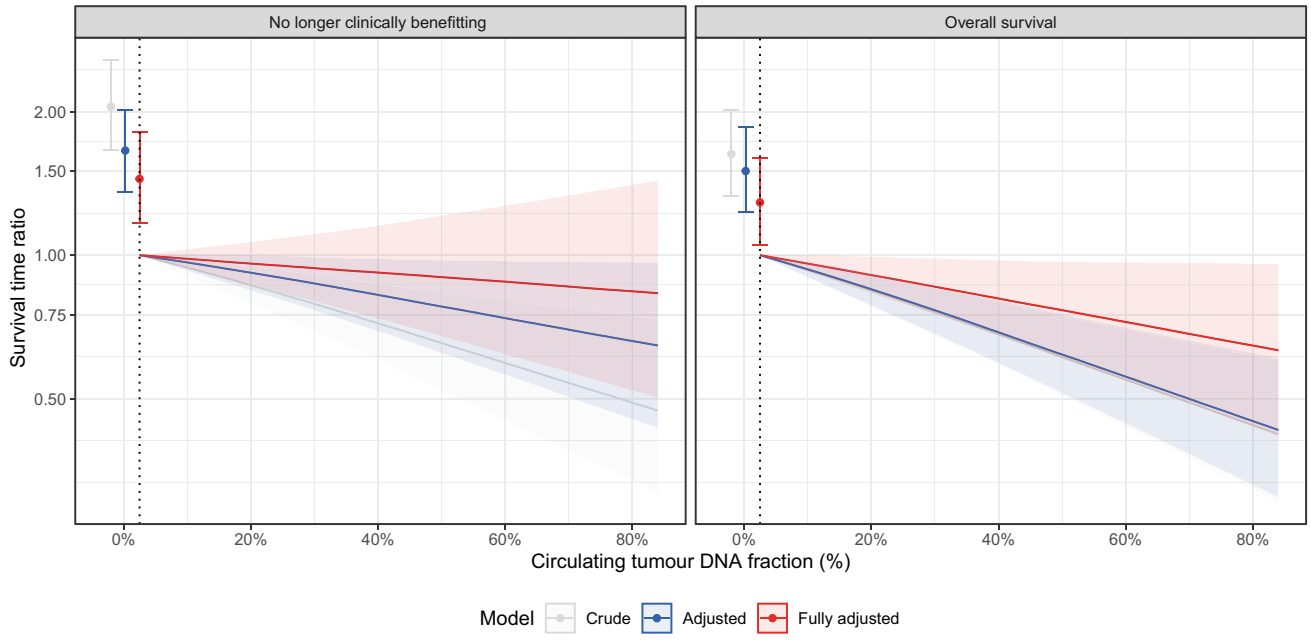

Fig. 3 – Posterior dose-response curves of the relationship between the ctDNA fraction and the survival time ratio for (A) time to no longer clinically benefiting and (B) overall survival. The curves were generated using a Bayesian spike-at-zero model with a detectable ctDNA level of 2.5% as the reference. Colored lines depict the median survival time ratio and shaded areas the 90% credible interval. Different colors indicate results from various models: crude, adjusted by therapy class, and fully adjusted for relevant confounders. ctDNA = circulating tumor DNA.

the prognostic value of ctDNA. This approach allowed a more nuanced evaluation of the prognostic role of ctDNA across different therapeutic contexts by comparing patients with undetectable ctDNA to those with any detectable ctDNA, and by estimating a flexible dose-response model that comprised all patients, including those with undetectable ctDNA. As the STR is expressed as a ratio, the linear relationship observed for ctDNA fractions suggests an underlying exponential biological mechanism by which higher ctDNA fractions could drive shorter survival outcomes. Presentation of our findings in terms of STR values enhanced the clarity of the associations, and offers a direct and clinically meaningful translation of the prognostic value of the ctDNA fraction into longer survival outcomes, which is highly relevant during patient-physician communication. We believe that the quantitative analysis of ctDNA fraction levels, which avoids arbitrary categorization, and the translational applicability of the STR as a more intuitive measure of the treatment effect represent advances over the existing evidence in the literature. In particular, the reductions in time to NLCB and OS observed with increasing ctDNA fractions, relative to a low but detectable ctDNA fraction, provide a unique, more informative, and previously unavailable insight into the prognostic value of ctDNA.

Some limitations should be considered when interpreting our results. First, as undetectable ctDNA was an exclusion criterion for ProBio, the randomized patients may differ from those with undetectable ctDNA. Specifically, we observed that the randomized patients tended to have more aggressive and advanced disease characteristics. However, the primary objective of this study was to assess the prognostic role of undetectable ctDNA, without necessarily ensuring comparability across other prognostic markers. To address this, we also examined the independent prognostic value of ctDNA by adjusting our survival models for multiple relevant confounding clinicopathological variables, including therapy received and selected variables related to disease aggressiveness. Even after these adjustments, the treatment effects remained stable, reinforcing the independent prognostic value of the ctDNA fraction. However, our multivariate analyses could not fully eliminate residual confounding. Second, this a secondary analysis within the ProBio platform trial, which was originally powered for the primary analyses. Nevertheless, the sample size in our analysis was adequate for detection of differences across groups, primarily because of the large effect sizes according to the STR values observed. Furthermore, use of NLCB as the primary endpoint is recommended by the Prostate Cancer Working Group 3 but may be influenced by subjectivity and a lack of standardization, which could introduce potential bias, particularly in open-label trials. While radiographic progression-free survival is a common endpoint in metastatic prostate cancer trials and has been associated with OS, it does not fully align with how treatment discontinuation decisions are made in routine clinical practice. In addition, use of NLCB offers advantages in pragmatic clinical trials, such as reducing costs and the logistics for centralized imaging review. In this study, analysis of OS as a complementary endpoint provides further support for the robustness and validity of the NLCB results. Lastly, while other studies have explored similar research questions, our study offers novel insights because of its prospective design, inclusion of patients with both undetectable and detectable ctDNA, and the Bayesian inference framework for analysis.

These elements contribute to a deeper understanding of the utility of ctDNA quantification as an independent prognostic marker for patients with mCRPC.

We believe that as genetic testing becomes increasingly common, our findings have important implications for future trial design and current patient management. Clinical trials should consider comparing ctDNA fraction levels across treatment arms and potentially using ctDNA detection and ctDNA fractions as stratification variables for randomization. In terms of patient management, identification of a patient population with metastatic disease and undetectable ctDNA may provide a rationale for novel trial designs that address de-escalation treatment strategies [31–33] or care pathways with lower monitoring intensity.

5. Conclusions

Patients with mCRPC and undetectable ctDNA had better prognosis than patients with detectable ctDNA, regardless of the therapy received and other prognostic variables. Higher ctDNA fraction levels were associated with shorter times to NLCB and OS. Given the better prognosis observed for patients with undetectable ctDNA, they may be suitable candidates for de-escalated treatment strategies and less intensive monitoring.

Author contributions: Henrik Grönberg 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.

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Administrative, technical, or material support: Larsson, Persson, Johansson, D'hondt.
Supervision: None.
Other: None.

Financial disclosures: Henrik Grönberg certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: This study was supported by Karolinska Institutet. The sponsor played a role in the design and conduct of the study.

Acknowledgments: We thank all of the patients for their willingness to participate in this study. We thank the biobanks of the Karolinska Institutet (Stockholm, Sweden) and HIRUZ (Ghent, Belgium) for weekly processing of the biomaterial for genomic analysis. We also thank Clinical Genomics (Stockholm, Sweden) and National Bioinformatics Infrastructure Sweden (Uppsala, Sweden) for providing timely and cost-efficient DNA sequencing and analysis services. The ProBio consortium thanks the following funding organizations for the research grants: ALF Medicine (20190087 to H.G.), Swedish Cancer Society (211610P to H.G.; 232988Pj to J.L.), The Cancer Research Funds of Radiumhemmet (234102 to J.L.), Prostatacancerförbundet (Icke-invasiv blodbaserad biopsi för spridd prostatacancer to J.L.), Swedish Research Council (2021-00331 to H.G.), Krebsliga beider Basel (KLbB-5580-02-2022 to A.M.), and Kom Op Tegen Kanker/Stand up to Cancer–Flemish Cancer Society (STI.VLK.2020.0006.01 to P.O.; STI.VLK.2022.0005.01 to B.D.L.). We extend our gratitude to all the investigators, co-workers, and staff involved in the ProBio trial.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euo.2025.02.002.

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