Ultra-sensitive detection of circulating tumour DNA enriches for patients with greater risk of recurrence in clinically localised prostate cancer

Conflict of interest statement

Niall Corcoran:

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Table 1. Clinical and Pathological Characteristics Comparison: ctDNA-Positive vs. ctDNA-Negative Samples. The p-values indicate whether there is a significant difference between the positive and negative groups. For age and PSA at tumour collection, the p-value is computed by the Mann-Whitney U test. Differences in proportions across categories in pathological T stage and post prostatectomy PSA nadir are computed using a two-way Fisher's exact test. Differences in proportions across clinical T stage, biopsy ISUP grade group, prostatectomy ISUP grade group, Cambridge prognostic group, and salvage radiation were computed using a Chi-squared test. P-values less than 0.05 are in bold. ISUP = International Society of Urological Pathology; GG = Gleason grade; PSA = prostate-specific antigen. Medians were reported as median (quartile 1, quartile 3), and frequencies as percentages.

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Aims

Circulating tumour DNA (ctDNA) has clinical applications as a "liquid biopsy" due to its short half-life, non-invasive collection modalities, and propensity for sampling across populations of tumour cells [1]. While ctDNA detection has been successfully demonstrated in metastatic prostate cancer [2], localised disease yields low levels of ctDNA, making detection difficult using conventional methods [3]. In this study, we assessed the limits of detection of ctDNA in localised prostate cancer using the high-sensitivity INVAR method [4] and tested the hypothesis that ctDNA detection is associated with high-risk disease. Tumour information was used to create marker panels of patient-specific mutations which were used to identify ctDNA molecules in patient-matched plasma samples. After initial background error calculations and filtering steps, the detected ctDNA fraction was estimated using a global integrated mutant allele fraction (IMAF), using the background-subtracted mean allele fraction across the patient-specific loci in each sample. Patient-specific scores were compared to the threshold value for classification, which was calculated using data from control samples and set to 95% specificity.

Methods

A total of 118 individuals with clinically localised prostate cancer from Australia and the UK were analysed. All plasma samples were obtained pre-prostatectomy; immediately prior to surgery in Australia, and at the time of surgical consent in the UK. An additional 27 healthy, prostate cancer-free male individual plasma samples were used as controls. All primary tumour samples were derived from radical prostatectomy for pathologically-confirmed prostate cancer and were hormone-naïve at treatment. Blood plasma cell-free DNA samples were profiled with custom targeted sequencing panels using the Agilent SureSelect XT HS target enrichment system (Agilent Technologies), with a mean of 2380 patient-specific target loci per sample. We adhered to consistent standard operating procedures and implemented time intervals between cohort extractions to maintain sample integrity and minimise contamination risk. Survival analyses using biochemical recurrence and metastasis-free

survival as endpoints were assessed by Cox regression to understand the relationship between ctDNA detection and disease progression.

Results

Using the INVAR method, we detected ctDNA in 19 localised prostate cancer patients (16%), with an average IMAF of $2.55x10^{-4}$ with a range of $1.17x10^{-5}$ to $1.85x10^{-3}$. A comparison of the clinical characteristics of positive and negative samples is shown in Table 1. Within the cohort, there was a reasonably good representation of the clinical spectrum of localised disease, and a consistent association between ctDNA detection and more aggressive clinical features.

Kaplan-Meier curves were used to assess the relationship between ctDNA detection and disease progression (Figure 1). Comparing patients categorised according to the detection of ctDNA by INVAR at the time of prostatectomy, we observed a significant association for ctDNA positive individuals having both shorter biochemical recurrence-free survival ($p = 0.0001$, log-rank test; hazard ratio = 3.3, 95% CI = [1.4, 8.1]) and shorter metastasis-free survival ($p = 0.0055$, log-rank test; hazard ratio = 2.8, 95% CI = [1.1, 7.1])) compared with ctDNA negative individuals.

On multivariable analysis, the detection of ctDNA pre-treatment was positively associated with an increased risk of both biochemical recurrence and development of metastasis. This showed statistical significance for recurrence but not metastasis, where the ISUP grade group was dominant in the model. Despite this, ctDNA detection was a stronger predictor than pre-treatment PSA and pathological stage, which are both well-established prognostic variables for metastases. We interpret these results with caution however, given the relatively few metastatic events observed for the number of variables included in the model.

Conclusions

Our study provides clear insights into the required analytical sensitivity and potential utility of ctDNA mutation analysis in localised prostate cancer. We found that in ctDNA positive cases, there was a significant association with biochemical recurrence after surgical intervention. This raises the potential for including ctDNA detection as an additional tool for patient stratification post biopsy in future neo/adjuvant treatment trials aiming to assess the impact of treatment escalation in men at high risk of relapse with current standard of care treatment alone.

A limitation of our study is that the Australian cohort was enriched for individuals with high risk and was therefore poorly suited to find significant associations with disease recurrence in competition to already known variables such as high grade.

A limitation of INVAR is that it requires the primary tumour to have undergone WGS. However, as the cost of WGS continues to fall, it is increasingly being incorporated into routine clinical care. In addition, better upfront risk stratification may lead to treatment escalation/de-escalation, which may have economic benefits in the longer term.

Figures

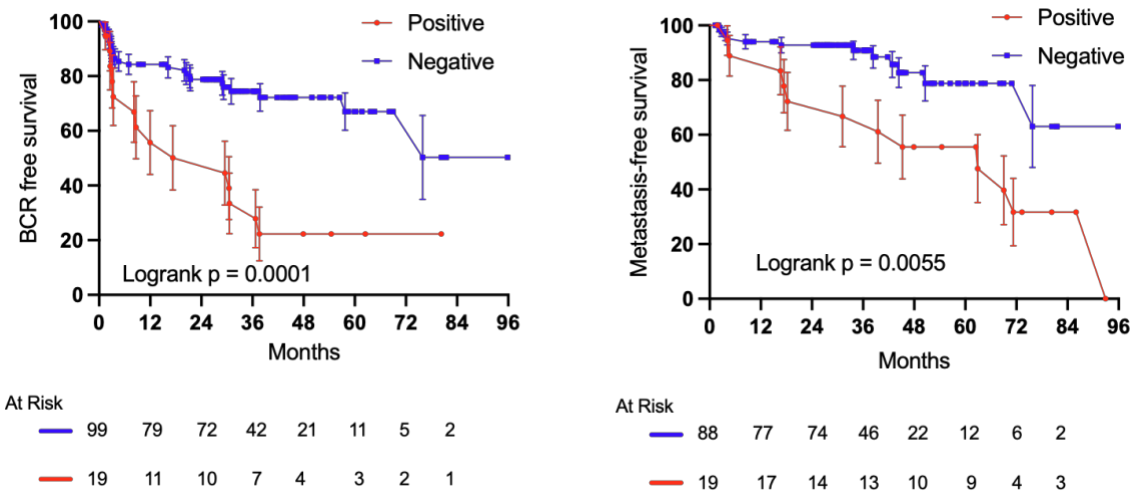

Figure 1: Kaplan-Meier curves demonstrating (A) biochemical- and (B) metastasis-free survival in patients categorised according to the detection of ctDNA by INVAR at the time of prostatectomy.

Tables

Table 1. Clinical and Pathological Characteristics Comparison: ctDNA-Positive vs. ctDNA-Negative Samples. The p-values indicate whether there is a significant difference between the positive and negative groups. For age and PSA at tumour collection, the p-value is computed by the Mann-Whitney U test. Differences in proportions across categories in pathological T stage and post prostatectomy PSA nadir are computed using Fisher's exact test. Differences in proportions across clinical T stage, biopsy ISUP grade group, prostatectomy ISUP grade group, Cambridge prognostic group, and salvage radiation are computed using Chi-squared tests. P-values less than 0.05 are in bold. ISUP = International Society of Urological Pathology; GG = Gleason grade; PSA = prostate-specific antigen. Medians were reported as median (quartile 1, quartile 3), and frequencies as “number (percentage)”.

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Table 1

Characteristic	Total (N=118)	ctDNA Positive (N=19)	ctDNA Negative (N=99)	*p-value*
Age	63 (58, 67)	68 (66, 70)	62 (58, 66)	**0.0001**
PSA at Tumour Collection (ng/ml)	9.0 (6.3, 13.9)	10.3 (6.6, 15.5)	8.7 (6.3, 13.8)	0.6
Clinical T Stage (n, %)				
cT1	77 (65)	8 (42)	69 (70)	
cT2	27 (23)	6 (32)	21 (21)	**0.037**
cT3	14 (12)	5 (26)	9 (9)	
Biopsy ISUP Grade Group (n, %)				
GG1	28 (24)	2 (11)	26 (26)	
GG2	38 (32)	3 (16)	35 (35)	
GG3	25 (21)	5 (26)	20 (20)	**0.042**
GG4	10 (9)	3 (16)	7 (7)	
GG5	17 (14)	6 (32)	11 (11)	
Cambridge Prognostic Group (n, %)				
1	16 (14)	1 (5)	15 (15)	
2	37 (31)	3 (16)	34 (34)	
3	27 (23)	4 (21)	23 (23)	**0.06**
4	15 (13)	3 (16)	12 (12)	
5	23 (19)	8 (42)	15 (15)	
Pathological T Stage (n, %)				
pT2	20 (17)	4 (21)	16 (16)	0.7
pT3	98 (83)	15 (79)	83 (84)	

Prostatectomy ISUP Grade Group (n, %)				
GG1	7 (6)	1 (5)	6 (6)	
GG2	54 (46)	5 (26)	49 (50)	
GG3	31 (26)	4 (21)	27 (27)	**0.02**
GG4	6 (5)	1 (5)	5 (5)	
GG5	19 (16)	8 (42)	11 (11)	
Missing	1 (1)	0 (0)	1 (1)	
Low-Intermediate Grade (GG < 4)	92 (79)	10 (53)	82 (84)	**0.005**
High-Grade (GG >= 4)	25 (21)	9 (47)	16 (16)	
Biochemical Recurrence (n, %)				
Yes	40 (34)	14 (74)	26 (26)	
No	78 (66)	5 (26)	73 (74)	
Metastasis (n, %)				
Yes	24 (20)	12 (63)	12 (12)	
No	83 (70)	7 (37)	76 (77)	
Missing	11 (9)	0 (0)	11 (11)	
Follow Up (months)	35 (18, 51)			
Post Prostatectomy PSA Nadir (n, %)				
< 0.2 ng/ml	104 (88)	16 (84)	88 (89)	0.6
≥ 0.2 ng/ml	14 (12)	3 (16%)	11 (11)	
Salvage Radiation (n, %)				
Yes	37 (31)	5	32	
No	79 (67)	14	65	0.6
Missing	2 (2)			