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Determining the Impact of Spatial Heterogeneity on Genomic Prognostic Biomarkers for Localized Prostate Cancer

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Abstract

Localized prostate tumors show remarkably diverse clinical courses, with some being cured by local therapy alone, while others rapidly relapse and have a lethal course despite precision surgery or radiotherapy. Many genomic biomarkers have been developed to predict this clinical behavior, but these are confounded by the extreme spatial heterogeneity of prostate tumors: most are multifocal and harbor multiple subclonal populations. To quantify the influence of spatial heterogeneity on genomic prognostic biomarkers, we developed a case-control high-risk cohort ($n = 42$) using a prospective registry, risk matched by clinicopathologic prognostic indices. Half of the cohort had early biochemical recurrence (BCR; ie, $\leq$18 mo), while half remained without evidence of disease for at least 48 mo after radical prostatectomy. We then genomically profiled multiple tumor foci per patient, analyzing 119 total specimens. These data allowed us to validate three published genomic prognostic biomarkers and quantify their sensitivity to tumor spatial heterogeneity. Remarkably, all three biomarkers robustly predicted early BCR, and all three were robust to spatiogenomic variability. These data suggest that DNA-based genomic biomarkers can overcome intratumoral heterogeneity: single biopsies may be sufficient to estimate the risk of early BCR after radical treatment in patients with high-risk disease.

Patient summary: We investigated whether heterogeneity between tumor regions within the prostate affects the accuracy of DNA-based biomarkers predicting early relapse after prostatectomy. We observed persistent accuracy in predicting disease relapse, suggesting that spatial heterogeneity may not hinder biomarker performance.

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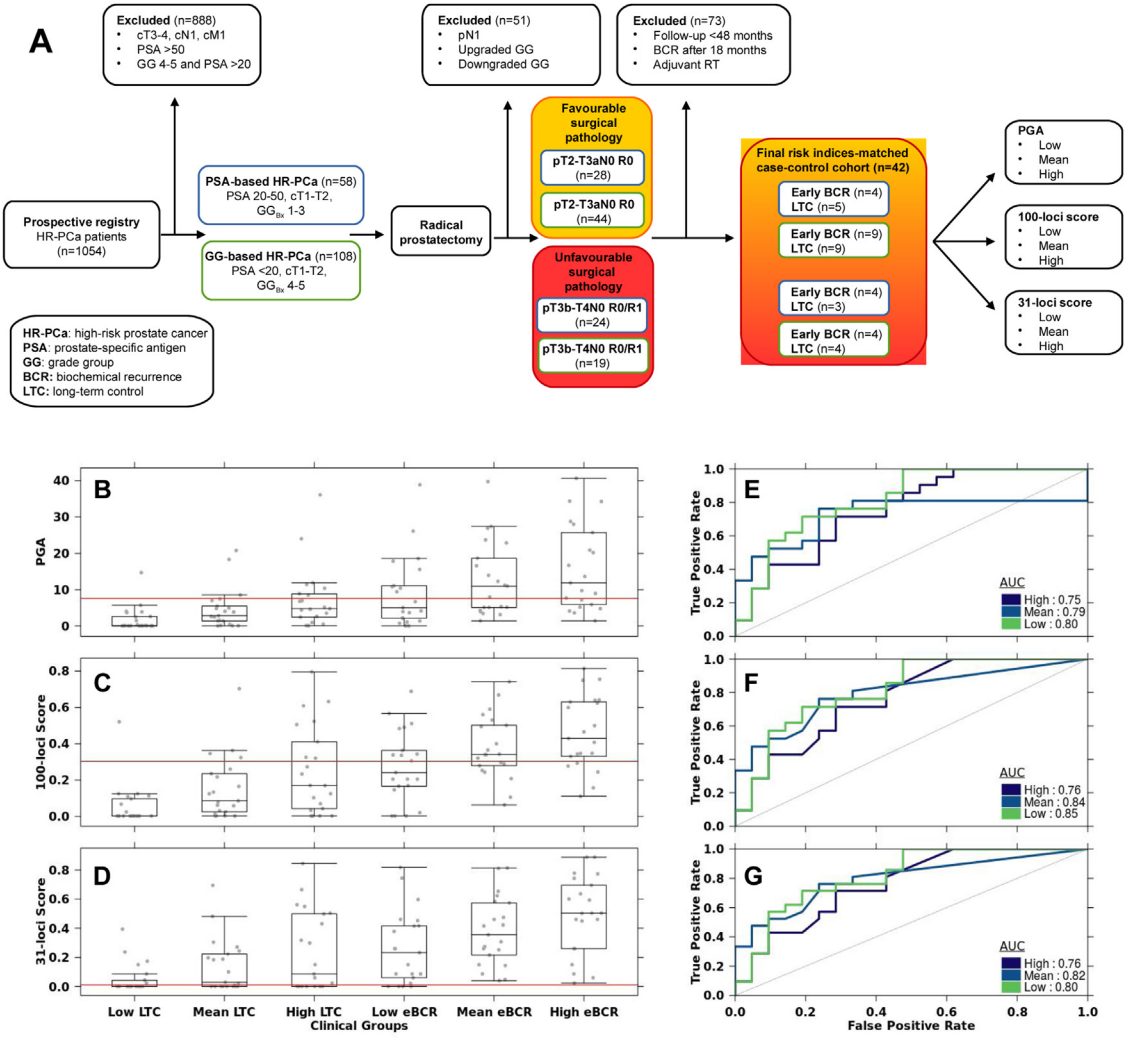

Fig. 1 – (A) Consort diagram of clinical cohort of patients with localized high-risk prostate cancer with early biochemical recurrence (<18 mo) and long-term control (>48 mo). Every patient had two to three tumor regions profiled, and three scores were assigned for each biomarker: the lowest and highest scores among foci and the mean score across all foci. Interfocal differences in the biomarker scores between least aggressive focus (low), the average biomarker score across foci (mean), or the most aggressive focus (high) for (B) PGA, (C) 100-loci DNA biomarkers, and (D) 31-loci DNA biomarkers. The threshold of each signature stratifying patients at a higher risk of recurrence is depicted with the corresponding horizontal red line (eg, 7.49%, 0.30321, and 0.01027 for the PGA, and 100-loci and 31-loci DNA biomarkers, respectively). ROC analysis for predicting eBCR and LTC by means of (E) PGA, (F) 100-loci, and (G) 31-loci scores. AUC = area under the receiver operator curve; BCR = biochemical recurrence; GG = grade group; eBCR = early biochemical recurrence (<18 mo); HR-PCa = high-risk prostate cancer; LTC = long-term control; PGA = percentage of the genome with a copy number aberration; PSA = prostate-specific antigen; RT = radiation therapy.

Localized prostate cancer (PCa) shows remarkably diverse clinical courses, with some patients remaining stable for years after local therapy, while others relapse rapidly despite precision surgery or radiotherapy. Those who suffer biochemical recurrence (BCR) shortly after local therapy, often defined as within 18–24 mo, have very poor outcome. Indeed, early BCR (eBCR) is a surrogate for PCa-specific mortality [1,2]. Genomic prognostic biomarkers have been developed to enhance decision making for PCa patients, highlighting those harboring lethal disease [3–6]. Despite promising initial studies, the clinical validity of these biomarkers is uncertain, in part because of the profound spatial heterogeneity of most prostatic adenocarcinomas, which leads to large genomic variation between biopsy specimens [7–9]. To quantify the robustness of existing genomic prognostic biomarkers, we developed a "best" case-control design and performed spatiogenomic characterization of 119 distinct tumor regions. We then scored three DNA-based biomarkers of eBCR: percentage of genome with a copy number aberration (PGA), a 100-loci biomarker [3], and an optimized 31-loci biomarker derived from the previous one [4]. For each of these, we determined the robustness to intratumoral heterogeneity and association with the respective clinical phenotype.

After obtaining institutional approval, we queried a prospective registry comprising 1054 patients with high-risk PCa who underwent radical prostatectomy (RP) between 2001 and 2013. We excluded 888 patients with clinically advanced disease (local, regional, or distant) or clinicopathologic indices for the highest risk of failure

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(Fig. 1A). The remainder were stratified based on PSA- or Gleason grade–determined high-risk, and those harboring pN1 disease were excluded. Finally, we distinguished two pathologically defined groups: pT2-pT3aN0, margins negative (R0) disease (eg, favorable pathology; $n = 72$), and pT3b-T4N0, R0/R1 (eg, unfavorable pathology; $n = 42$). Based on our aim to compare unequivocally differing clinical scenarios (eg, early vs no BCR), we excluded patients with short follow-up (<48 mo) and those receiving adjuvant radiotherapy or developing BCR after 18 mo. Our final case-control risk-matched cohort comprised 21 patients who developed eBCR and 21 patients with long-term control (LTC; ie, >48 mo) after RP (Fig. 1A). The clinical characteristics are given in Supplementary Table 1.

An expert uropathologist (T.v.d.K.) demarcated tumor regions within each RP specimen (eg, distinct tumors and/or regions of the primary lesion in different tissue blocks; Supplementary Table 2). Most patients had three foci discriminated, leading to 119 samples for genomic profiling. For each focus, global CNA profiles were generated and the three genomic biomarkers calculated as previously described [3,4,7] (Supplementary material). Subsequently, for each patient and biomarker, we considered three scenarios: sampling of only the lowest-score region, sampling of only the highest-score region, and sampling of all foci and use of the mean score across them. These scenarios simulate the extremes of biased sampling, along with the value of evaluating multiple regions.

These data allowed us to provide the first quantitation of the distribution of interfocal genomic heterogeneity in a well-defined curative scenario with follow-up outcomes. We evaluated, for each patient, how much genomic divergence there was between its least and most altered tumor region (Supplementary Fig. 1). For the average tumor, this divergence represented 6.15% of the genome: a magnitude approximating gain or loss of an entire chromosome. Nevertheless, this distribution was highly skewed, with six tumors showing >10% of the genome differentially altered between foci, while for nine others it was <1%. Unsurprisingly, then, there were statistically significant differences in the biomarker scores between tumor foci from a single patient (Friedman test, $p < 0.001$; Supplementary Table 3). Figs. 1B–D show the difference in the distribution of biomarker scores for patients with LTC versus eBCR. These differences persist irrespective of whether we select the least aggressive focus (low), the average biomarker score across foci (mean), or the most aggressive focus (high).

Next, we tested whether the significant spatial intratumoral heterogeneity affected the biomarker correlation with clinical phenotype. Rather encouragingly, all three biomarkers distinguished LTC from eBCR successfully in this cohort, independent of which focus or way of summarizing foci was used (Fig. 1E–G). For example, PGA scores separated the two groups with an area under the receiver operator curve (AUC) ranging from 0.75 to 0.80 (Fig. 1E). This was mirrored for the 100- and 31-loci signatures, with AUCs ranging from 0.76 to 0.85 and 0.76 to 0.80, respectively. In all cases, there was no statistical difference between AUCs, independent of how they were summarized across foci (Supplementary Table 4).

The use of AUC inherently discretizes patient outcomes, so we sought to confirm these results using time-to-event analyses. Using Cox proportional hazards modeling, we found that PGA was significantly associated with BCR-free survival, again independent of how different foci were summarized (hazard ratio [HR] range: 2.56–6.22; $p < 0.05$; Fig. 2A). This was replicated for the 100-loci (HR range: 3.55–5.23; $p < 0.05$; Fig. 2B) and the 31-loci (p value range: 5.1×10^{-4} to 5.9×10^{-3}; Fig. 2C) signatures scores, the latter evaluated with the log-rank test due to a detected violation of the proportional hazards assumption.

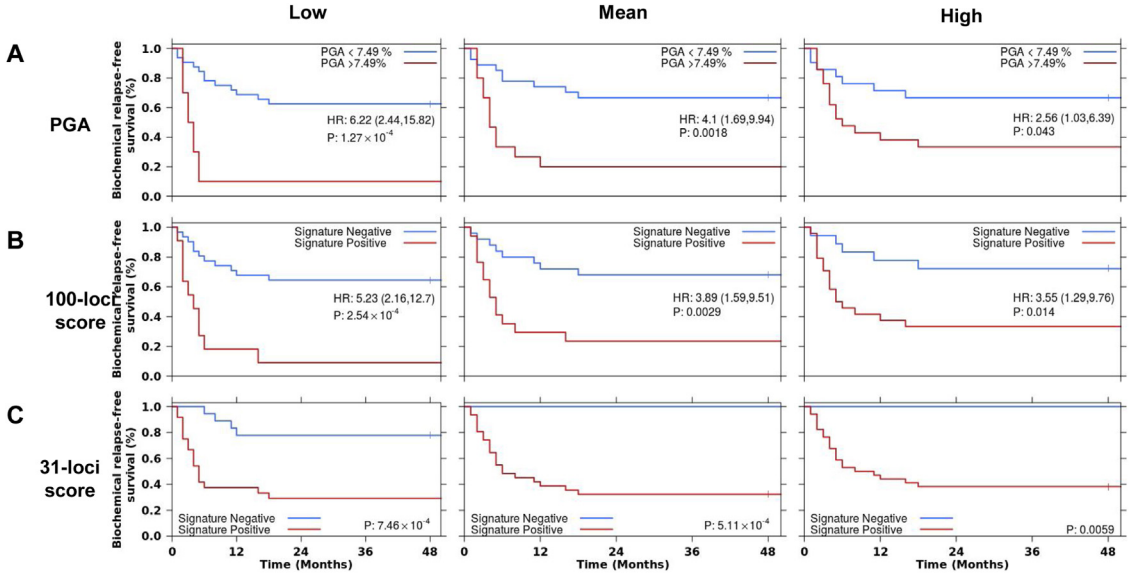

Fig. 2 – DNA-based biomarkers prognostic performance. Increased risk of early biochemical recurrence was identified by all three biomarkers and scores. Estimated biochemical relapse-free survival stratified by the three DNA biomarkers: (A) PGA, (B) 100-loci, and (C) 31-loci scores. HR = hazard ratio; PGA = percentage of genome with a copy number aberration.

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To our knowledge, this is the first report quantifying the impact of intraprostatic spatial heterogeneity on the accuracy of genomic-based biomarkers in predicting outcomes after curative-intent surgery for localized PCa. It is limited by its retrospective nature and the discreet sample size, but has the advantage of being a nested case-control cohort with well-defined and dissimilar outcomes in patients with a high risk of recurrence after RP, based on well-established clinicopathologic indices. We generated one of the first large-scale characterization of genomic heterogeneity across different PCa regions, creating a resource of 42 patients with long-term clinical follow-up and 119 genomic profiles that will be of significant future research value.

In this study, we quantified the influence of spatiogenomic heterogeneity on three published DNA-based prognostic biomarkers. The overall extent of heterogeneity within patients is very large, approximating a full chromosome difference in the average patient. Nonetheless, despite the theoretical impact on prognostication, all three evaluated genomic biomarkers predicted eBCR after RP accurately, and the accuracy of tests was not affected by whether we used the most or the least aggressive focus, or simply averaged the set used. While prior studies have suggested that analyses of multiple samples are necessary to counteract interfocal heterogeneity [10], our research provocatively suggests that individual samples may be adequate to determine eBCR risk accurately using DNA-based biomarkers in patients with high-risk disease. The validity and implications of these findings for RNA-based biomarkers, and in patients with low- and intermediate-risk PCa will be of great interest.

***Author contributions*:** Alejandro Berlin 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: Murgic, Chua, Bristow, Boutros, Berlin.
Acquisition of data: Brastianos, Murgic, Salcedo, Chua, Meng.
Analysis and interpretation of data: Brastianos, Boutros, Berlin.
Drafting of the manuscript: Brastianos, Boutros, Berlin.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Brastianos, Salcedo, Boutros.
Obtaining funding: Bristow, Brundage, Boutros.
Administrative, technical, or material support: Salcedo, Fraser, Meng, Fleshner, Berlin.
Supervision: Boutros, Berlin.
Other: Meng, van der Kwast (pathology processing and analyses).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euo.2020.06.005.

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