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Serial Molecular Profiling of Low-grade Prostate Cancer to Assess Tumor Upgrading: A Longitudinal Cohort Study

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Abstract

Background—The potential for low-grade (grade group 1 [GG1]) prostate cancer (PCa) to progress to high-grade disease remains unclear.

Objective—To interrogate the molecular and biological features of low-grade PCa serially over time.

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Study concept and design: Salami, Tosoian, Marks, Palapattu.
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Design, setting, and participants—Nested longitudinal cohort study in an academic active surveillance (AS) program. Men were on AS for GG1 PCa from 2012 to 2017.

Intervention—Electronic tracking and resampling of PCa using magnetic resonance imaging/ultrasound fusion biopsy.

Outcome measurements and statistical analysis—ERG immunohistochemistry (IHC) and targeted DNA/RNA next-generation sequencing were performed on initial and repeat biopsies. Tumor clonality was assessed. Molecular data were compared between men who upgraded and those who did not upgrade to GG ≥2 cancer.

Results and limitations—Sixty-six men with median age 64 yr (interquartile range [IQR], 59–69) and prostate-specific antigen 4.9 ng/ml (IQR, 3.3–6.4) underwent repeat sampling of a tracked tumor focus (median interval, 11 mo; IQR, 6–13). IHC-based ERG fusion status was concordant at initial and repeat biopsies in 63 men (95% vs expected 50%, $p < 0.001$), and RNAseq-based fusion and isoform expression were concordant in nine of 13 (69%) ERG^+ patients, supporting focal resampling. Among 15 men who upgraded with complete data at both time points, integrated DNA/RNAseq analysis provided evidence of shared clonality in at least five cases. Such cases could reflect initial undersampling, but also support the possibility of clonal temporal progression of low-grade cancer. Our assessment was limited by sample size and use of targeted sequencing.

Conclusions—Repeat molecular assessment of low-grade tumors suggests that clonal progression could be one mechanism of upgrading. These data underscore the importance of serial tumor assessment in men pursuing AS of low-grade PCa.

Patient summary—We performed targeted rebiopsy and molecular testing of low-grade tumors on active surveillance. Our findings highlight the importance of periodic biopsy as a component of monitoring for cancer upgrading during surveillance.

Keywords

Prostate cancer; Low-grade cancer; Cancer progression; Tumor clonality; Gene fusions; Immunohistochemistry; Next-generation sequencing

1. Introduction

Acknowledging the minimal lethal potential of low-grade (grade group 1 [GG1]) prostate cancer (PCa) and the significant harms associated with treatment [1–3], active surveillance (AS) has widely been accepted as the preferred management approach for low-grade disease [4]. Still, adoption of AS has been modest in several parts of the world and varies widely by region and practice [5,6], with up to 70% of newly diagnosed low-risk cancers undergoing definitive treatment in some regions [7,8]. The limited use of AS can be ascribed, in part, to the prevailing concern that potentially lethal, high-grade cancers remain undetected during selection and monitoring [9].

Indeed, approximately one-third of men who initiate AS are found to have high-grade cancer (GG ≥2) on follow-up biopsies (ie, upgrading) [3], but the underlying mechanism of upgrading remains unclear [10–12]. Potential explanations of upgrading include the following: (1) clonal progression from low- to high-grade cancer; (2) detection of a

previously undersampled high-grade component of a known tumor focus; (3) detection of a previously unsampled, spatially distinct high-grade tumor focus; or (4) de novo development of a high-grade tumor focus during monitoring (Fig. 1). While undersampling of GG ≥2 disease present at diagnosis is thought to be a primary explanation, the answer to the critical question of whether low-grade, indolent-appearing cancers progress to high-grade disease is not known [13–16].

Emerging clinical tools have enabled improved characterization of the potential mechanisms of upgrading. A better understanding of these phenomena would have great impact on the clinical approach to AS, potentially reducing the intensity and morbidity of monitoring. We previously demonstrated that multiparametric magnetic resonance imaging/ultrasound (mpMRI/US) fusion-guided prostate biopsy can facilitate sampling of the same focus of cancer over time, regardless of whether a lesion is visible on MRI [17]. Here, we combined this technology with targeted next-generation sequencing (NGS) techniques to better inform the etiology of upgrading in a contemporary AS setting.

2. Patients and methods

2.1. Study population

Study participants were identified from the men with low- and favorable intermediate-risk PCa managed with AS in the prospectively maintained, IRB-approved, HIPAA-compliant registry of University of California—Los Angeles (UCLA) [18]. All men underwent mpMRI prior to initial diagnostic biopsy (t_0) and subsequent confirmatory biopsy (t_1) at UCLA between 2012 and 2017. At both time points, biopsy GG was determined and Cancer of the Prostate Risk Assessment (CAPRA) scores were calculated to quantify risk [19]. We included patients who upgraded and those who did not upgrade (GG ≥2) during follow-up in a 1:1 ratio. Patients with a history of prostate ablative treatment, androgen deprivation therapy, or 5α-reductase inhibitor use were excluded.

2.2. Tracked prostate biopsy platform

Prostate mpMRI was performed using a 3 T (Siemens Medical Solutions, Malvern, PA, USA) magnet and a transabdominal coil prior to initial prostate biopsy [20]. Lesions suspicious for PCa were segmented by a genitourinary radiologist and graded according to the Prostate Imaging Reporting and Data System (PIRADS) [21]. All patients underwent a systematic 12-core biopsy. In patients with positive mpMRI (PIRADS 3–5), three to five targeted biopsy cores were obtained from each lesion (one core every 3 mm along the longest axis). A three-dimensional model of the prostate was generated, and all biopsy sites were electronically stored to facilitate subsequent resampling (Fig. 2A). We have previously validated this approach for tracking and resampling the same cancer focus over time [17], including mpMRI-invisible lesions. All patients underwent resampling of the tracked, previously positive biopsy site at t_1. Paired formalin-fixed paraffin-embedded (FFPE) cancer samples for each patient from the two time points (t_0 and t_1) were retrieved for molecular analyses.

2.3. Immunohistochemistry

Hematoxylin and eosin (H&E) slides of t_0 and t_1 tissue were reviewed by two anatomic pathologists (A.E.S. and S.A.T.) to identify tumor tissue for molecular analyses. Briefly, 11 FFPE sections (each 5 μm thick) were cut from each biopsy block. For each sample, H&E and ERG immunohistochemistry (IHC) staining were performed on the first and 10th slides, respectively [22,23]. ERG was considered positive in cases of diffuse moderate-to-strong nuclear immunoreactivity and heterogeneous in cases with patchy or weak nuclear immunoreactivity [17,24].

2.4. DNA and RNA targeted NGS

Next, we performed tumor macrodissection of each biopsy sample under a microscope (nine FFPE slides per time point). DNA and RNA were coisolated, and targeted NGS was performed using our custom pan-cancer DNA and RNA AmpliSeq panels as previously described and detailed in the Supplementary material [17,25,26]. High-confidence somatic DNA mutations at recurrent hotspot positions in oncogenes or tumor suppressors, or deleterious variants (ie, nonsense, frameshift, splice site, etc.) in tumor suppressors were considered prioritized variants. The RNAseq panel of 306 transcripts includes amplicons for relevant PCa molecular alterations, including genes comprising three commercially available prognostic tests (Myriad Prolaris cell cycle progression score, Oncotype DX genomic prostate score [GPS], and Decipher genomic classifier) from RNAseq data, as described [26].

DNA variant, copy number, and RNAseq analyses including quality control (QC) filters were performed as described in the Supplementary material. Briefly, targeted DNA and RNA NGS data analysis was performed using Torrent Suite software (Thermo Fisher Scientific) variantCaller and coverage Analysis plug-ins. Fusion isoforms and partner-level analyses were performed as previously described and detailed in the Supplementary material [25,26]. As part of an exploratory analysis, we derived and compared tissue-based prognostic scores as well PCa-relevant single gene expression levels (Supplementary material).

2.5. Integrated temporal clonality assessment

For all tumor pairs for which both t_0 and t_1 samples passed RNAseq QC filters, DNAseq and RNAseq data were manually examined in an integrative manner by an experienced molecular pathologist (A.M.U.). Clonality was assessed utilizing stringent criteria, first using *ETS* gene fusion data. Any tumor pairs with discordant *ETS* gene fusion status (ie, positive vs negative) or discordant 5′ fusion partner (eg, *TMPRSS2* vs *SLC45A3*) were considered clonally discordant. In addition, *TMPRSS2-ERG* gene fusion positive tumor pairs with distinct transcript isoform patterns (eg, T1E2 vs EF194202/T1E4) were considered clonally discordant. Next, for tumor pairs with concordant *ETS* gene fusion status (including 5′ fusion partner and transcript isoform patterns), clonality was secondarily assessed using variant and copy number alterations. The presence of shared somatic variants (eg, *SF3B1* p.K700E mutation) or prioritized copy number alterations (eg, *AKT1* amplification) was considered evidence of clonally related tumors. In contrast, the presence of unique mutually exclusive oncogenic driver alterations (eg,, *SPOP* p.F125V mutation)

was considered evidence of clonal discordance. Importantly, the presence of unique non–mutually exclusive alterations, including prioritized oncogenic mutations (eg, *TP53* p.G245S), was not considered evidence of clonal discordance, as these alterations could represent spatial genomic heterogeneity and/or temporal clonal progression.

3. Results

3.1. Patient characteristics

Demographic and clinical data from t_0 and t_1 are presented in Table 1. Of 86 men meeting the inclusion criteria, 66 (77%) had sufficient tumor tissue from t_0 and t_1 for molecular analysis and comprised the study cohort. The median age at diagnosis was 64 yr (interquartile range [IQR], 59–69), and median prostate-specific antigen (PSA) was 4.9 ng/ml (IQR, 3.3–6.4). All men underwent mpMRI prior to initial biopsy. Fifteen men (23%) had negative mpMRI (PIRADS 1–2), and GG1 cancer was detected on systematic biopsy. The remaining 51 men (77%) had positive mpMRI (PIRADS 3–5). Among them, GG1 cancer was detected by systematic biopsy alone in 20 men (39%), by targeted biopsy alone in 12 men (24%), and by systematic and targeted biopsies in 19 men (37%). The median CAPRA score at diagnosis (t_0) was 1 (IQR, 1–2). The median interval from diagnostic (t_0) to repeat biopsy (t_1) was 11 mo (IQR, 6–13). Forty-eight patients (73%) underwent repeat mpMRI prior to confirmatory biopsy, which was positive in 34 (71%). Overall, upgrading to GG ≥2 was detected in 30 patients, including 12 of 18 (67%) who did not undergo repeat mpMRI, five of 14 (36%) who had negative repeat mpMRI, and 13 of 34 (38%) with positive repeat mpMRI. Men who upgraded had significantly higher measures of tumor volume at t_1 (ie, cancer core length). At t_1, the median CAPRA score was 2 (IQR, 1–2) in patients who did not upgrade and 4 (IQR, 3–5) in patients who upgraded. The extent of upgrading was further characterized in patients with GG2 cancer at repeat biopsy, with the most common Gleason pattern 4 morphology being poorly formed glands (Supplementary Table 1).

3.2. Temporal clonality assessment of paired biopsy samples by ERG fusion status on IHC

In order to assess clonal tumor sampling on repeat biopsy, we first utilized ERG IHC—a widely available clinical tool for detecting *ERG* gene fusions—to examine concordance of ERG protein expression in paired tumor samples (Fig. 2B and 3). Among the 66 patients, 63 (95%) had concordant ERG fusion status from t_0 to t_1. Of the three patients (56, 60, and 63) with discordant ERG fusion status, one (63) harbored both ERG^+ and ERG^- regions at t_1, suggesting sampling of two spatially adjacent but clonally distinct foci. Compared with an expected ERG fusion status concordance of 50% between two independent PCa foci [22], the observed concordance of 95% would suggest successful sampling of the same cancer focus over time (observed vs expected concordance: 95% vs 50%, $p < 0.001$).

3.3. Temporal clonality assessment by integrative targeted NGS

While ERG IHC is sensitive and specific for the presence of ERG fusions in clinical specimens, it cannot distinguish between distinct ERG fusion transcripts, limiting its utility for clonality assessment [24]. We therefore combined RNAseq-based *ETS* gene fusion

expression with DNAseq-based detection of somatic variants and prioritized copy number alterations as a more stringent assessment of clonality.

Overall, paired samples from 27 patients passed RNAseq and DNAseq QC (Supplementary Table 2) at both time points and were included in the integrated analysis. Of these, two patients (56 and 63) were ERG fusion discordant (ie, positive/negative) on targeted RNAseq (Fig. 3). A total of 13 patients were found to be ERG fusion positive by targeted RNAseq at both time points. Of these, three patients (40, 89, and 92) expressed different predominant ERG fusions at t_0 and t_1 (*SLC45A3-ERG* vs *TMPRSS2-ERG*), indicating sampling of clonally distinct PCa foci (Fig. 3 and Supplementary Fig. 1). Another patient (53) had different predominant *TMPRSS2-ERG* isoforms at t_0 and t_1 (*TMPRSS2-ERG.T1E4/T1E5* vs *TMPRSS2-ERG.T1E2*), which could be due to either sampling of clonally distinct foci or differential fusion isoform expression of the same clonal focus [27]. As a conservative measure, we classified such cases as discordant, such that nine of 13 (69%) ERG fusion–positive patients were classified as concordant based on integrated sequencing data.

3.4. Clonality assessment of cancer upgrading

Fifteen patients passed targeted DNA/RNA NGS QC at both time points and upgraded during follow-up. Of them, four men (27%) had evidence of clonal discordance between t_0 and t_1 (56, 63, 89, and 92), suggesting that a clonally distinct region of tumor was sampled at repeat biopsy (Table 2). Of the remaining 11 patients, four (61, 66, 88, and 91) were ERG fusion positive by targeted RNAseq and had temporally concordant fusion and isoform expression. Additionally, all prioritized mutation and copy number variant calls in the cohort are summarized in Supplementary Tables 3 and 4, respectively. Of the seven RNAseq-based ERG-negative patients, one man (67) had a somatic driver *SF3B1* mutation (p.K700E) present in similar frequencies at t_0 and t_1 (23.8% and 24.1%, respectively), supporting a clonal relationship between the GG1 tumor detected at t_0 and the GG4 tumor detected at t_1. Although the possibility of undersampling of a heterogeneous tumor at t_0 exists in these cases, at least five men (33%) who upgraded and had DNA and RNAseq data available showed evidence of shared clonality.

Patient 91 upgraded from GG1 to GG5 (Fig. 2B). The 56-yr-old man initially presented with PSA 6.6 ng/ml (PSA density 0.13) and abnormal digital rectal examination. Multiparametric MRI revealed a PIRADS 5 lesion, and fusion biopsy detected GG1 cancer in six cores at t_0. Repeat mpMRI prior to surveillance biopsy at t_1 (11 mo later; PSA 7.5 ng/ml) showed a PIRADS 4 lesion in the same anatomic location. Tracked biopsy of this site revealed GG5 cancer. Interestingly, the GG5 tumor detected at t_1 harbored a hotspot *TP53* mutation (p.G245S) that was not identified in the GG1 tumor at t_0 (Supplementary Fig. 2). Targeted sequencing data, including *ERG* fusion isoforms, were otherwise concordant from t_0 to t_1. It is unclear whether this indicates undersampling at initial biopsy or temporal clonal grade progression.

3.5. Expression-based molecular prediction of cancer upgrading

Unsupervised hierarchical clustering of targeted genes revealed no distinct clustering pattern based on biopsy time point (t_0 or t_1) or upgrading status (Supplementary Fig. 3A and 3B). In

exploratory analyses, we found that derived prognostic scores and PCa-relevant single gene expression data were not associated with cancer upgrading (Supplementary material and Supplementary Fig. 4A–C).

4. Discussion

In a cohort of men with low-grade PCa on AS, we used an MRI/US fusion biopsy platform with electronic tracking to resample the same focus of cancer over time. Consistent with our prior report [17], we observed highly concordant (95%) IHC-based ERG fusion status between paired specimens obtained at initial and repeat biopsies. Using a conservative approach leveraging targeted DNA and RNA sequencing to assess clonality in a subset of patients with paired data available, we estimated clonal concordance in nearly 70%, supporting successful resampling of the same tumor focus in these cases. Among cases of upgrading with complete sequencing data, at least one-third demonstrated evidence of a clonal relationship. Although initial undersampling is one potential explanation, these data are consistent with clonal grade progression. These findings have significant implications on the management of low-grade PCa. The precise mechanism of upgrading in men with low-grade PCa is poorly understood. To a large extent, most cases of upgrading are thought to be due to previously undersampled or unsampled high-grade disease (Fig. 1) [13–16]. The uncertain etiology of PCa upgrading is a manifestation of its underlying biology, characterized by multifocality and intra- and interfocal heterogeneity [26,28]. Our cohort included 15 cases with complete molecular data that upgraded on repeat sampling of a previous GG1 cancer. Four (31%) of these cases exhibited evidence that a clonally distinct tumor was sampled on repeat biopsy, suggesting undersampling as a likely etiology of upgrading in these cases. However, at least five (33%) cases had genomic and transcriptomic data supporting shared clonality with the initial GG1 tumor. While the limited interval between biopsies supports the likelihood of initial undersampling, it is notable that three of the five tumors were mpMRI visible and underwent extensive sampling (every 3 mm) at initial biopsy, decreasing the likelihood that higher-grade cancer present at diagnosis would have gone unsampled. Thus, the possibility that these cases represent true clonal grade progression cannot be excluded (Fig. 1). The notion that high-grade PCa can, over time, evolve from low-grade disease is controversial. On the one hand, based on population-level data before and after the PSA screening era, some have suggested that grade is established early in pathogenesis and that grade progression is uncommon [13,14]. One the other hand, using biopsy misclassification rates and subsequent prostatectomy pathology, Inoue et al [29] concluded that tumor grade progression does in fact occur, estimated at 12–24% over 10 yr. Since the discovery of *ETS* gene fusions, it has been known than PCa is frequently multifocal, comprising multiple spatially and clonally distinct tumor foci of varying histologic grade [30], and early oncogenic driver events (eg, *ETS* gene fusions) in a primary tumor are retained in clonally disseminated metastatic tumor foci [31]. More recently, advanced molecular approaches have provided support for the hypothesis that clonal molecular evolution may accompany histologic grade progression within clinically localized tumors [12,16]. To our knowledge, the current study is the first to use longitudinal sampling of specific cancer foci to inform the possibility of incident temporal grade progression during AS.

An ideal approach to AS would minimize both the frequency of invasive testing and the risk of progression. While increasing use of diagnostic mpMRI and targeted biopsy appear to reduce the frequency of upgrading—presumably by reducing initial undersampling—upgrading at or following confirmatory biopsy remains common [3]. Thus, better tools are needed to distinguish between indolent low-grade cancers and those with more aggressive potential. While tissue-based gene expression tests have been proposed in this setting, previous efforts have shown that the multifocal and heterogeneous nature of PCa could preclude the capacity of a single cancer focus to inform overall biology. An exploratory component of the current analysis asked whether molecular testing of a specific tumor focus could predict upgrading of the same focus over time. In addition to sample size, limited variation from GG1 to GG2 restricted our ability to draw definitive conclusions. Acknowledging these limitations, derived prognostic scores and single gene expression showed no evidence of association with upgrading. While exploratory in nature, these data are in line with recent findings that the OncotypeDX GPS test was not significantly associated with biopsy upgrading or adverse surgical pathology in patients on AS [32]. Ultimately, there is limited evidence that tissue-based molecular assays can reduce the need for periodic tissue sampling during AS.

The current study has several clinical and research implications. Our findings highlight the importance of serial tissue assessment (ie, biopsy) as an essential component of AS, both to ensure adequate tumor sampling and to rule out the potential for temporal progression. Even considering sampling limitations of biopsy, alternative diagnostic tests have not proved to be equally reliable for detecting GG ≥2 cancer during AS. Similar to molecular testing, a growing body of evidence has revealed the limited benefit of serial surveillance mpMRI [33,34], and the risk of potential harm in using only mpMRI or clinical changes to trigger surveillance biopsy [35]. In other words, while the optimal interval for repeat biopsy is unclear, some extent of longitudinal sampling appears necessary to detect cancer upgrading. Validated tools capable of reducing the need for surveillance biopsy are needed, and liquid biomarkers (ie, blood and urine) may help overcome issues of tumor heterogeneity and multifocality.

Our study has several limitations. First, we have a relatively small sample size and limited interval between biopsies. In addition, tumor specimens were obtained in the course of clinical care and were subject to practical limitations. While our approach to sampling MRI-visible lesions is thorough (ie, every 3 mm along the longest axis), larger studies with high-density sampling are needed to better rule out initial undersampling. Future studies should aim to pair high-density initial sampling (t_0) with surgical specimens amenable to in-depth pathologic and molecular assessment (t_1) to confirm true clonal progression. Given the difficulty of sequencing minute, low-volume FFPE samples, approximately 20% of specimens were insufficient for sequencing or did not pass QC despite meticulous preparation. In light of this, we used a targeted sequencing approach, which has limitations for assessing clonality. Furthermore, it is notable that upgrading to GG2 cancer is an imperfect endpoint and will not prove to be clinically significant in all cases. Finally, our assessment of prognostic classifiers was exploratory, and derived markers are not equivalent to commercial assays. Combined, these factors limit our ability to draw definitive

conclusions based on these data. Nevertheless, our study is the largest longitudinal molecular assessment of low-grade PCa to date.

5. Conclusions

We applied an MRI/US fusion biopsy platform to longitudinally assess the same PCa focus over time. Using a targeted molecular profiling approach, we demonstrate the potential for clonal grade progression in some cases, although the possibility of initial undersampling cannot be excluded. While additional studies are needed to validate these findings, our data highlight the importance of repeat tissue sampling to detect high-grade PCa in men on surveillance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Take Home Message

Targeted rebiopsy and molecular assessment of low-grade prostate cancers often revealed clonally related tumors. These findings highlight the importance of serial tumor sampling during active surveillance and suggest that clonal progression could be one mechanism of upgrading.

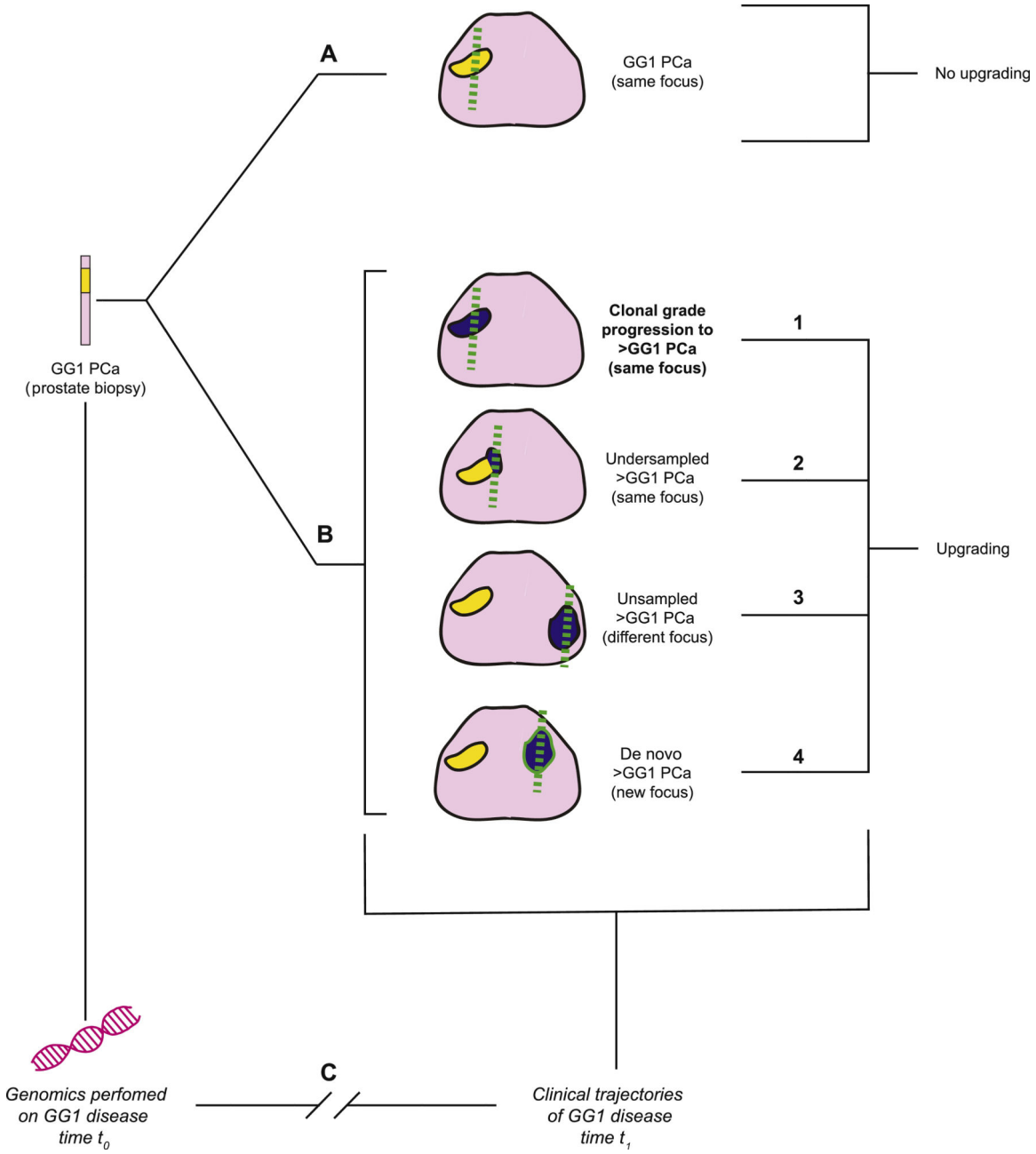

Fig. 1 –.

Potential clinical trajectories of grade group 1 (GG1) prostate cancer following diagnosis. In men on active surveillance for GG1 prostate cancer (yellow area), follow-up biopsies may reveal (A) stable GG1 disease, that is, no upgrading or (B) cancer upgrading (>GG1 disease; blue area). Cancer upgrading detected on follow-up biopsy could be explained by: (B1) clonal grade progression of the initially sampled cancer focus; (B2) initial undersampling of a high-grade component of the same cancer focus; (B3) an initially unsampled, anatomically distinct, high-grade cancer focus; and/or (B4) de novo development of high-grade cancer. Our findings support the occurrence of scenario B1 in at least some cases of upgrading. (C)

Molecular analysis of GG1 cancer from initial biopsy was not predictive of cancer upgrading on follow-up biopsy. PCa = prostate cancer.

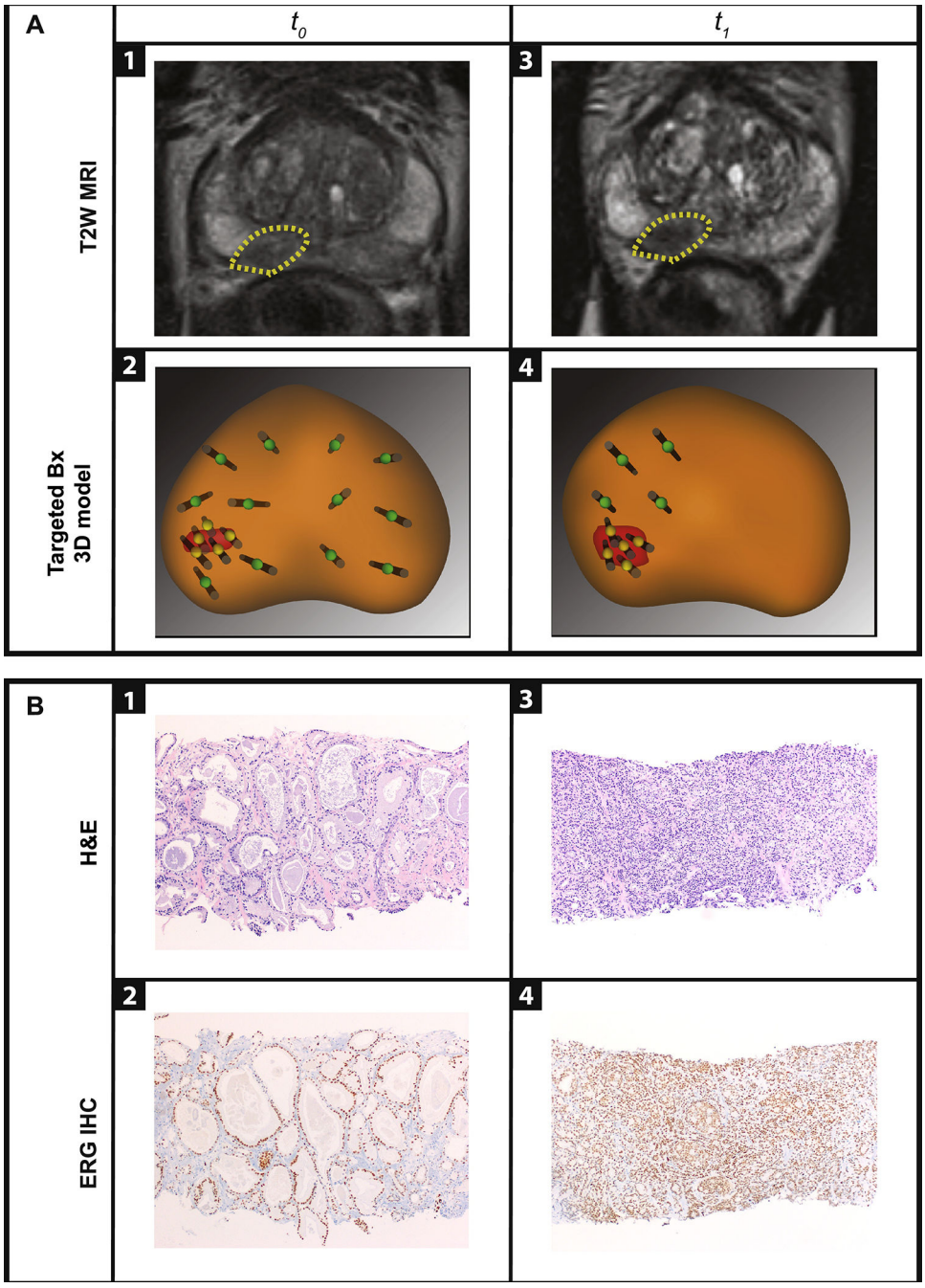

Fig. 2 –.

Tracking low-grade prostate cancer with multiparametric magnetic resonance imaging/ultrasound (mpMRI/US) fusion biopsy platform. (A1) mpMRI (T2W MRI, axial cut shown) acquired prior to initial (t_0) prostate biopsy shows a PIRADS 5 region of interest (ROI; yellow enclosure) in the right posterior prostate peripheral zone. (A2) mpMRI/US fusion biopsy of the ROI and a systematic 12-core biopsy were performed. All biopsy sites were recorded and stored in a 3D reconstruction model to facilitate repeat sampling during surveillance (ROI—red, targeted [ROI] cores—yellow, and systematic cores—green). (A3)

mpMRI prior to repeat biopsy (t_1, 11 mo later) shows the same ROI meeting PIRADS 4 criteria. (A4) All positive biopsy cores (ROI and systematic) were resampled, tracked, and stored in a 3D reconstruction model, as shown. (B1 and B2) H&E stain (10×) of ROI biopsy core and corresponding ERG IHC (10×) are shown, demonstrating GG1 cancer exhibiting positive ERG expression at t_0. (B3 and B4) At t_1, GG5 (Gleason score 4 + 5) cancer was sampled from the ROI with concordant positive ERG expression on IHC. The corresponding molecular data (from targeted DNA and RNA sequencing) at both time points are described in Supplementary Figure 2. Taken together, it is unclear whether upgrading in this case indicates undersampling due to intratumoral heterogeneity at initial biopsy or temporal clonal grade progression. Bx = biopsy; 3D = three dimensional; GG = grade group; H&E = hematoxylin and eosin; IHC = immunohistochemistry; PIRADS = Prostate Imaging Reporting and Data System; T2W = T2 weighted.

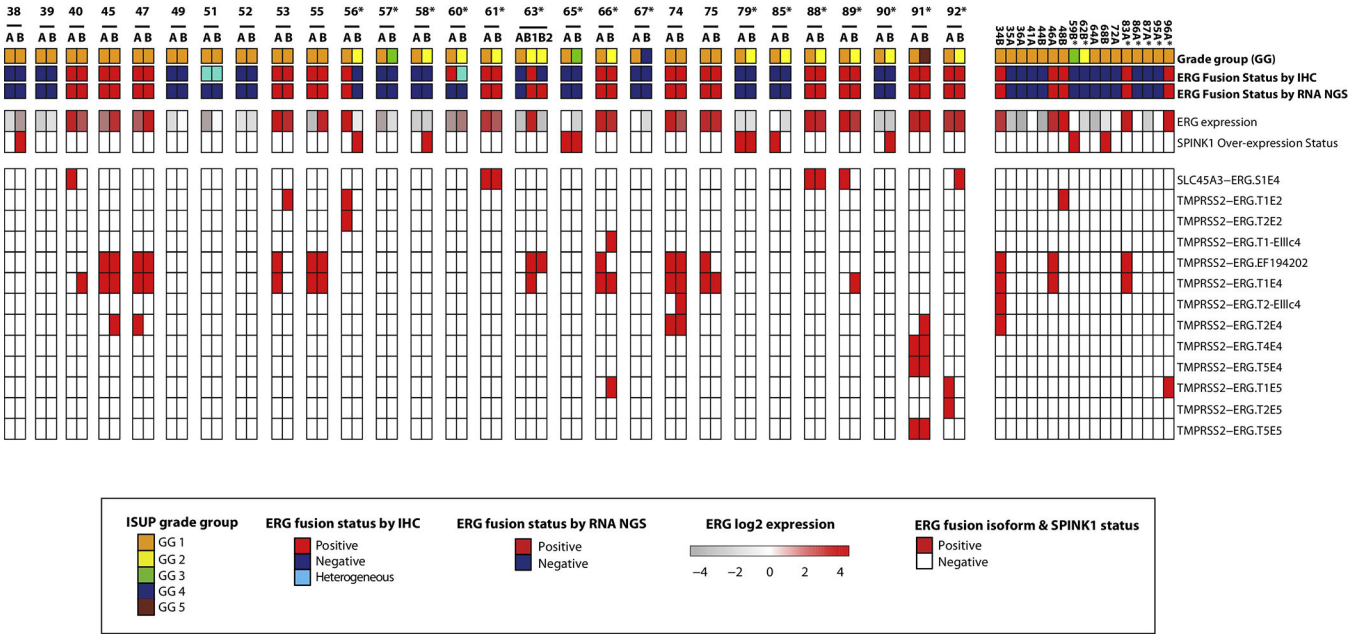

Fig. 3 –.

Integrative targeted genomic and transcriptomic profiling of tracked prostate cancer foci. Each column represents a patient sample ordered by patient number and by time points (t_0 and t_1). Paired samples from the same patient are indicated with “A” and “B” to represent biopsy time points t_0 and t_1, respectively. The panel to the left includes patients with evaluable samples at both time points, while the panel to the right includes patients with data available at one time point only. Patients who progressed to higher-grade disease at time t_1 are denoted by an asterisk (*). Overall, 63 (95%) patients showed concordant ERG immunohistochemistry (IHC) status between paired t_0 and t_1 biopsies, supporting frequent sampling of the same clonal focus overtime. Among the 74 specimens passing NGS quality control, three (51A, 51B, and 60B) demonstrated heterogeneous staining on IHC, and thus could not be classified as concordant or discordant with the NGS-based ERG fusion assessment (positive vs negative). Among the remaining 71 samples, ERG fusion status by IHC and targeted RNA NGS were concordant in 69 (97%). All 11 samples with SPINK1 overexpression were ERG fusion negative. GG = grade group; ISUP = International Society of Urological Pathology; NGS = next-generation sequencing.

Table 1 –

Demographic and clinical characteristics of the study cohort (N = 66 unless indicated)

	Overall	Non-upgraded	Upgraded	*p* value
N (%)	66	36 (55)	30 (45)	
Age (yr)	64.1 (58.8–69.4)	62.8 (57.2–69.0)	64.4 (62.2–69.4)	0.4
Positive FH PCa (n = 50)	14 (28%)	7 (28%)	7 (28%)	>0.9
Prostate volume (cm^3)	46.1 (33.3–58.9)	53.0 (40.1–64.0)	36.7 (29.6–47.8)	0.01
Initial biopsy (t_0)				
PSA (ng/ml)	4.9 (3.3–6.4)	4.9 (2.8–7.1)	4.9 (3.4–6.0)	0.7
PSA density	0.12 (0.08–0.18)	0.11 (0.08–0.15)	0.13 (0.9–0.19)	0.15
PIRADS score				
1	10 (15%)	4 (11%)	6 (20%)	0.6
2	5 (7.6%)	3 (8.3%)	2 (6.7%)	
3	24 (36.4%)	16 (44%)	8 (27%)	
4	19 (29%)	9 (25%)	10 (33%)	
5	8 (12%)	4 (11%)	4 (13%)	
Percent positive bx cores	0.17 (0.07–0.25)	0.14 (0.06–0.20)	0.18 (0.08–0.29)	0.13
Positive core length (mm)	3.0 (1.5–5.0)	2.5 (1.4–4.0)	5.0 (2.0–6.0)	0.06
CAPRA score	1 (1–2)	1 (1–2)	1 (1–2)	0.7
Bx interval (mo)	11 (6–13)	12 6–15)	7 (6–11)	0.02
Repeat biopsy (t_1)				
PSA (ng/ml)	5.6 (3.6–7.5)	5.6 (3.2–7.3)	5.5 (3.8–7.5)	>0.9
PIRADS score (n = 48)				
1	11 (23%)	8 (27%)	3 (17%)	0.7
2	3 (6.3%)	1 (3.3%)	2 (11%)	
3	16 (33%)	10 (33%)	6 (33%)	
4	10 (21%)	7 (23%)	3 (17%)	
5	8 (17%)	4 (13%)	4 (22%)	
Grade group				<0.001
1	36 (54%)	36 (100%)	0 (0%)	
2	21 (31%)	0 (0%)	21 (70%)	
3	7 (10%)	0 (0%)	6 (20%)	
4	2 (3.0%)	0 (0%)	2 (6.7%)	
5	1 (1.5%)	0 (0%)	1 (3.3%)	
Percent positive bx cores	0.29 (0.14–0.45)	0.18 (0.13–0.31)	0.40 (0.29–0.63)	<0.001
Positive core length (mm)	4.0 (2.0–5.0)	4.0 (1.5–5.0)	4.5 (4.0–6.0)	0.011
CAPRA score	2 (1–3)	2 (1–2)	3.5 (3–5)	<0.001

Bx = biopsy; CAPRA = Cancer of the Prostate Risk Assessment (0–10); FH = family history; PCa = prostate cancer; PIRADS = Prostate Imaging Reporting and Data System; PSA = prostate-specific antigen.

Data are presented as median (interquartile range) or frequency (%).

Table 2 –

Integrated temporal clonality assessment in patients passing RNAseq and DNAseq quality control at both time points (N = 27)

Pt	Bx interval	GG t_1	IHC: ERG discordance	RNAseq: 5′ ETS fusion partner		RNAseq: ETS transcript discordance	Informative/ prioritized DNA mutations		Variant allele freq.		Clonal discordance
				t_0	t_1		Gene	Mutation	t_1	t_1	
38	34	1		–	–						
39	34	1		–	–						
40	24	1		S3	T2	×					×
45	14	1		T2	T2						
47	11	1		T2	T2						
49	13	1		–	–						
51	11	1		–	–						
52	13	1		–	–		*MGA*	R134fs	31.7%	18.5%	
53	12	1		T2	T2	× [a]					×
55	23	1		T2	T2						
56	5	2	×	T2	–	×	*SPOP*	F133I	0%	32.6%	×
57	12	3		–	–						
58	7	2		–	–						
60	14	2		–	–						
61	11	2		S3	S3						
63	5	2	×	–	T2/T2	×	*PIK3R1*	G5R	0%	18.3%	×
66	6	2		T2	T2						
67	6	4		–	–		*SF3B1*	K700E	23.8%	24.1%	
74	8	1		T2	T2						
75	18	1		T2	T2						
79	7	3		–	–						
85	12	2		–	–						
88	6	2		S3	S3						
89	6	2		S3	T2	×					×
90	6	2		–	–		*TP53*	Y236D	0%	7.4%	
91	11	5		T2	T2		*TP53*	G245S	0%	44.8%	
92	7	3		T2	S3	×					×

Bx = biopsy; GG = grade group; IHC = immunochemistry; Pt = patient; S3 = *SLC45A3*; T2 = *TMPRSS2*.

[a] Patient 53 expressed concordant 5′ ETS fusion to TMPRSS2 with differing predominant isoforms expressed across time points (eg, *TMPRSS2-ERG.T1E4/T1E5* vs *TMPRSS2-ERG.T1E2*; see Fig. 2). This could be due to sampling of a clonally distinct focus or differential fusion isoform expression of the same clonal focus [27]. Adopting a conservative interpretation, we have classified such cases as discordant.