EUROPEAN UROLOGY ONCOLOGY 2 (2019) 277–285

available at www.sciencedirect.com
journal homepage: euoncology.europeanurology.com

European Association of Urology

Core Biopsies from Prostate Cancer Patients in Active Surveillance Protocols Harbor *PTEN* and *MYC* Alterations

Paolo Gandellini[a,1], *Nicola Casiraghi*[b,1], *Tiziana Rancati*[c], *Matteo Benelli*[b,d], *Valentina Doldi*[a], *Alessandro Romanel*[b,e], *Maurizio Colecchia*[f], *Cristina Marenghi*[c], *Riccardo Valdagni*[c,g,h], *Francesca Demichelis*[b,2,*], *Nadia Zaffaroni*[a,2,*]

[a] *Molecular Pharmacology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy;* [b] *Laboratory of Computational and Functional Oncology, Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy;* [c] *Prostate Cancer Program, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy;* [d] *Bioinformatics Unit, Hospital of Prato, Prato, Italy;* [e] *Laboratory of Bioinformatics and Computational Genomics, Centre for Integrative Biology (CIBIO), University of Trento, Trento, Italy;* [f] *Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy;* [g] *Department of Radiation Oncology 1, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy;* [h] *Department of Oncology and Hemato-oncology, Università degli Studi di Milano, Milan, Italy*

Article info

Article history:
Accepted August 16, 2018

Associate Editor:
Gianluca Giannarini

Keywords:
Active surveillance
Whole-exome sequencing
Biopsy

Abstract

Background: Genomic characterization of prostate cancer (PCa) biopsies may improve criteria for the selection of patients suitable for active surveillance (AS).
Objective: To identify somatic genomic aberrations associated with adverse outcome as AS protocol exclusion indicators.
Design, setting and participants: Whole-exome sequencing profiles were generated for Gleason score (GS) = 3 + 3 biopsies obtained from 54 PCa patients enrolled in two AS protocols. Patients were selected as representative of a *nonindolent* population, consisting of 27 patients who dropped out from AS due to upgrading (ie, finding of GS > 3 + 3 at a follow-up biopsy) within 2 yr, and a *potentially indolent* population, consisting of 27 patients in AS for ≥4 yr without any evidence of reclassification.
Outcome measurements and statistical analysis: The genomic alteration landscape of core biopsies was analyzed using an integrated computational pipeline and correlated with patient reclassification due to upgrading.
Results and limitations: Of all the GS = 3 + 3 biopsies of the study cohort, 34% showed clear evidence of somatic copy number aberrations along the genome. Of these, 39% came from the *potentially indolent* and 61% from the *nonindolent* population. Single-nucleotide variants demonstrated low allelic fractions and included a common F133C mutation in the *SPOP* gene. The minimally altered genomic landscape of the study cohort presented a distinct set of monoallelic deletions, including on 8p, 13q, 16q, and 21q, and rare amplifications of 8q, which

[1] These authors contributed equally to this work and should be regarded as joint first authors.
[2] These authors contributed equally to this work and should be regarded as joint last authors.
* Corresponding authors. Molecular Pharmacology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Amadeo 42, 20133 Milan, Italy. Tel.: +39-02-23903260; Fax: +39-02-23902692 (N. Zaffaroni). Laboratory of Computational and Functional Oncology, Centre for Integrative Biology (CIBIO), University of Trento, Via Sommarive 9, 38123 Trento. Tel.: +39-0461-285305; Fax: +39-0461-283937 (F. Demichelis).
E-mail addresses: f.demichelis@unitn.it (F. Demichelis), nadia.zaffaroni@istitutotumori.mi.it (N. Zaffaroni).

https://doi.org/10.1016/j.euo.2018.08.010

were observed in both AS patient populations. Concerning lesions typically associated with adverse outcome, *PTEN* deletions and *MYC* amplification, though observed in a small number of cases, were detected exclusively or preferentially, respectively, in *nonindolent* patients. Such molecular findings were confirmed by immunohistochemistry on the same tissue blocks. The small sample size and the retrospective nature of the analysis represent the main study limitations.

Conclusions: Genomic features enriched in aggressive tumors can be detected in GS = 3 + 3 core biopsies of AS patients.

Patient summary: *PTEN* and *MYC* alterations at the time of diagnosis would deserve investigation in larger cohorts of AS patients to assess their potential as biomarkers for a more precise/earlier identification of patients at risk of reclassification.

1. Introduction

Prostate cancer (PCa) is the second most common tumor and the sixth leading cause of cancer-related mortality among men worldwide [1]. Screening based on prostate-specific antigen (PSA) levels resulted in a marked increase in the number of patients with newly diagnosed PCa, including a large proportion with low-grade, potentially indolent cancers that are likely to remain clinically insignificant. Overtreatment is a well-documented consequence of overdiagnosis leading to patient exposure to treatment morbidities, thus negatively affecting quality of life [2].

Active surveillance (AS) evolved as alternative to radical treatment for potentially indolent PCa with the aim to reduce overtreatment, without compromising opportunities for cure. Existing guidelines predominantly state that patients presenting with biopsy Gleason score (GS) = 3 + 3, minimal cancer volume, clinical stage T≤2, and PSA levels <10 ng/ml are suitable for AS. Patients are strictly monitored and curative treatments are avoided or deferred until evidence of more aggressive PCa is seen [3]. Unfortunately, current inclusion criteria are suboptimal and about 25% patients discontinue AS for PCa reclassification, mainly upgrading (GS > 3 + 3) at the re-biopsy [3–5]. To what extent tumor reclassification reflects progression from low- to high-grade or incomplete sampling on the initial biopsy (ie, patients with apparently low-risk disease may harbor occult higher-grade cancer) remains unclear.

Table 1 – Inclusion criteria, follow-up schedule, and indications for discontinuation of the two active surveillance protocols at Istituto Nazionale Tumori, Milan

	SAINT	PRIAS
Inclusion criteria		
PSA	≤10 ng/ml	<10 ng/ml
Clinical stage	T1c or T2a; T1b if cancer ≤0.5 cm^3 and negative biopsies of the peripheral zone	≤T2c
GS	3 + 3	3 + 3 or 3 + 4 in age >70 yr, tumor involving <10% core length
Number of positive cores	≤20% of all cores until December 2012; ≤25% of all cores since December 2012	≤2 or ≤15% of all cores in case of saturation biopsy (>20 cores) with a maximum of 4
PSA density	–	<0.2 ng/ml/cm^3
Core involvement	≤50% of core involvement	–
Follow-up schedule		
PSA measurement	Every 3 mo	Every 3 mo
Digital rectal examination (DRE)	Every 6 mo	Every 6 mo
Re-biopsy	Year 1 Year 2 Then every subsequent 2 yr	1, 4, and 7 yr after the diagnostic biopsy
Indications for AS discontinuation	PSA doubling time (DT) <3 yr; clinical stage >T2c as determined by DRE; disease upgrading (GS > 6) or upsizing (number of positive cores or tumor involvement per biopsy core exceeding the criteria for AS) at re-biopsy	PSA DT <3 yr; clinical stage >T2c at DRE; >2 positive cores (upsizing), or GS > 6 (upgrading) at re-biopsy

SAINT = Sorveglianza Attiva Istituto Nazionale Tumori; PRIAS = Prostate Cancer Research International: Active Surveillance; GS = Gleason score; AS = active surveillance.

Prostatectomy-based studies analyzing somatic genomic lesions and relevant breakpoints from intrapatient grade pattern 3 (G3) and G4 tumors suggested that a subset of G3 tumors may progress to G4 or emerge from a common precursor, and may be molecularly distinct from isolated G3 tumors not associated with higher grades [6]. PTEN protein loss and chromosome 8 alterations, including 8q (*MYC*) gain and 8p (*LPL*) loss, in G3 cancers were reported as associated to the presence of unsampled G4 lesions [7]. Further, tumors morphologically appearing as G3 in biopsy but characterized by PTEN protein loss demonstrated an increased rate of upgrading at prostatectomy than those retaining PTEN [8]. Recently, whole-exome sequencing (WES) of adjacent G3 and G4 tumors in radical prostatectomies revealed that the two entities shared multiple mutations and somatic copy number alterations, with G3 tumors harboring oncogenic lesions (eg, losses of *PTEN* or *NKX3-1* tumor suppressors) [9]. Remarkably, the finding that G4-associated G3 tumors retain their indolent-appearing morphology despite the acquisition of multiple genomic alterations highlights the need for validated molecular markers to be detected in diagnostic biopsies to improve the selection of patients suitable for AS. So far, few studies queried AS patients' diagnostic biopsies for selected protein expression. ERG expression identified patients with an increased risk of reclassification during AS [10] and PTEN protein loss, but not ERG expression, was significantly associated with upgrading at the re-biopsy and adverse histopathology in radical prostatectomy [11]. Altogether, these studies support the molecular investigation of diagnostic biopsies to improve criteria of AS enrollment.

Here, we performed a comprehensive genomic exercise by retrospectively analyzing the exome of GS = 3 + 3 core biopsies prospectively and consecutively collected from low-risk PCa patients enrolled on AS at Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy.

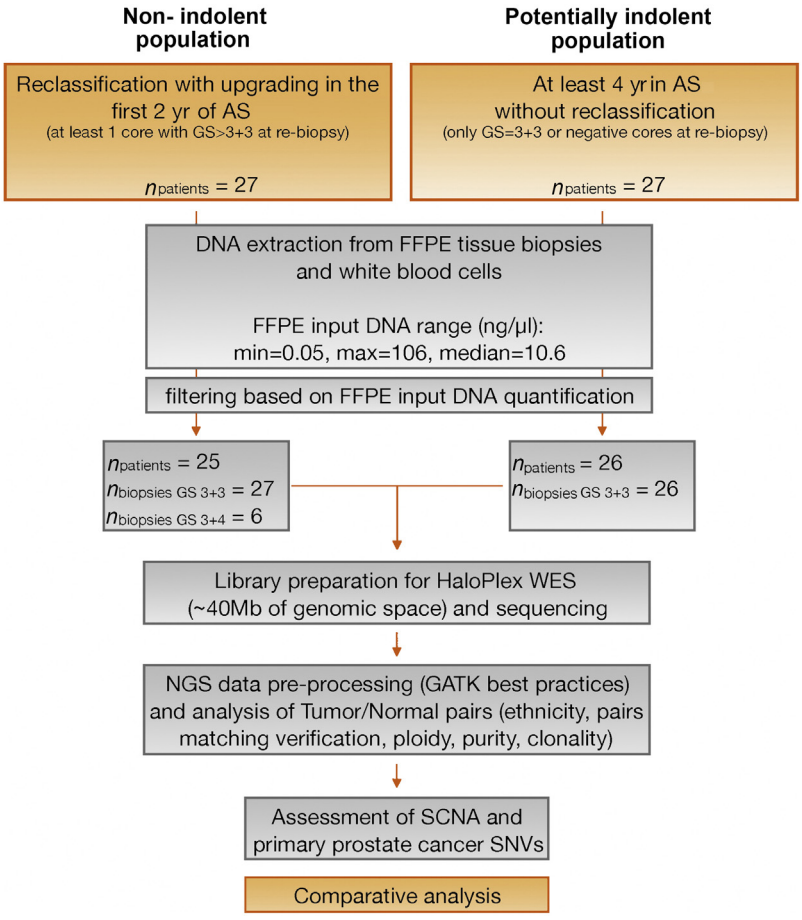

Fig. 1 – Schematic summary of study design. A total of 54 PCa patients prospectively enrolled in AS protocols were selected as representative of either a *nonindolent* ($n = 27$) or of a *potentially indolent* ($n = 27$) populations. Fourteen of 54 patients were enrolled in the SAINT protocol, while 40 of 54 were enrolled in PRIAS, with no statistically significant differences in the rate of indolent/nonindolent patients ($p = 0.063$). GS = 3 + 3 cores were obtained from all patients. For the *nonindolent* population, an additional GS = 3 + 3 core was obtained from two patients, and six GS = 3 + 4 cores were obtained from dropout biopsies of five patients. DNA extracted from formalin-fixed paraffin-embedded (FFPE) biopsies and white blood cells was quantified and evaluated prior to preparation of sequencing libraries via HaloPlex Exome Target Enrichment System. As a result, high-quality DNA material was available for 27 GS = 3 + 3 biopsies and 6 GS = 3 + 4 biopsies from 25 *nonindolent* patients and for 26 GS = 3 + 3 biopsies from 26 *potentially indolent* patients. Generated WES data were preprocessed by following well-established computational pipelines and analyzed by applying strict quality criteria in order to detect high-confidence somatic copy number alterations (SCNAs) and somatic single nucleotide variants (SNVs).

2. Patients and methods

At INT, low-risk PCa patients are proposed for AS provided they fulfill the enrolment criteria defined by either the single-center SAINT (Sorveglianza Attiva Istituto Nazionale Tumori) protocol, established in March 2005 [12], or the international PRIAS (Prostate Cancer Research International: Active Surveillance) protocol, coordinated by Erasmus University Medical Center (Rotterdam, The Netherlands) [4] and joined in November 2007. Details of the inclusion criteria, follow-up schedules, and indications for discontinuation are reported in Table 1. In this study, two sets of AS patients were considered. The first set, referred to as the *nonindolent* population, includes patients who dropped out from AS due to upgrading (ie, finding of GS > 3 + 3 at a re-biopsy) within 2 yr from inclusion. Patients who dropped out for other reasons (eg, upsizing, PSA doubling time <3 yr) were not included. The second set, referred to as *potentially indolent* population, includes patients on AS for at least 4 yr (ie, no evidence of tumor reclassification). Figure 1 reports the design of the study, which was approved by the Institutional Ethical Committee. All patients gave written informed consent to donate biological material for research purposes. Original grading of study biopsies was assigned using the ISUP 2005 guidelines. A recent revision by the study pathologist (M.C.) showed that all GS = 3 + 3 cores can be attributed to new prognostic grade group 1 (PGG1) and GS = 3 + 4 cores to new PGG2, according to the Consensus Conference ISUP 2014 criteria.

Details on sample processing, WES, computational analyses, and immunohistochemistry are reported in the **Supplementary methods**.

3. Results

3.1. Clinical characteristics of AS patients amenable to WES

Fifty-four patients were included in the study cohort including 27 from the *nonindolent* and 27 from the *potentially indolent* population. Table 2 reports the distribution of clinical–pathological features at diagnosis. No statistically significant differences were found between the two patient populations except for prostate volume, in accordance with previously published results on association between volume and the probability of upgrading at re-biopsies [12]. No statistically significant differences were observed between patients from the two AS protocols, except for PSA and PSA density, which were higher in SAINT due to lack of criteria on PSA density.

Initial quantification tests showed that nearly one whole formalin-fixed paraffin-embedded (FFPE) GS = 3 + 3 biopsy was necessary to obtain sufficient DNA for WES library preparation. In compliance with the ethical and legal issues of preserving informative diagnostic material for each patient, we could select only patients with at least two positive cores at the elected biopsy (note that approx. 70% of patients have only one positive core at diagnosis). Therefore, for the *nonindolent* population, GS = 3 + 3/PGG1 cores were obtained from the dropout biopsy in 25/27 cases; the diagnostic biopsy or 1-yr re-biopsy were used for one patient each. Six GS = 3 + 4/PGG2 cores (referred to with _H) were obtained from dropout biopsies of five patients and analyzed for comparative purposes. An additional GS = 3 + 3/PGG1 core (referred to with _sec) was analyzed for two patients. For the *potentially indolent* population, GS = 3 + 3 cores from the 1-yr re-biopsy were used for 13 patients, from the 2-yr biopsy for 6 patients, from the 3-yr biopsy for 3 patients, and from the 4-yr biopsy for 5 patients (Supplementary Fig. 1A). To maximize the chance to obtain informative data from such challenging specimens, the core with maximum length and percentage of cancer cells was selected for genomic characterization. High-quality DNA suitable for WES library preparation was obtained from 53 of 56 (94.6%) GS = 3 + 3/PGG1 and from 6 of 6 (100%) GS = 3 + 4/PGG2 FFPE biopsies (and from all matched blood samples) (Fig. 1).

3.2. Overall genomics of GS = 3 + 3/PGG1 biopsies from AS patients

Exome sequencing data were successfully generated on a 40-Mb target with >180 M reads per tumor sample and 85 M per matched normal sample, resulting in >240× and ~100× depth of coverage, respectively (Supplementary Fig. 2A and 2B and Supplementary Table 1). Genetic-based tests confirmed tumor/normal pairs [13] (Supplementary

Table 2 – Clinical and pathological characteristics for the whole population and for the two separated cohorts of "*nonindolent*" population and "*potentially indolent* (indolent)" population

Variable (at diagnosis)	Median	Interquartile range	*p* value (Mann–Whitney test)
Positive cores (*n*)			
Nonindolent	1	1–2	0.06
Indolent	1	1–1	
All patients	1	1–2	
Total cores (*n*)			
Nonindolent	12	10–14	0.53
Indolent	12	10–15	
All patients	12	10–14	
Positive cores (%)			
Nonindolent	13	8–16	0.06
Indolent	10	7–12	
All patients	10	8–14	
Cores with Pca (max %)			
Nonindolent	18	6–34	0.17
Indolent	10	5–25	
All patients	10	5–30	
Age (yr)			
Nonindolent	69	70–72	0.76
Indolent	67	64–72	
All patients	67.5	63–72	
PSA (ng/ml)			
Nonindolent	5.4	4.5–6.7	0.83
Indolent	5.6	4.4–6.9	
All patients	5.4	4.5–6.7	
Prostate volume (cm^3)			
Nonindolent	36	30–46	0.01
Indolent	57	40–102	
All patients	41	35–50	
PSA density ($ng/ml/cm^3$)			
Nonindolent	0.15	0.13–0.18	0.06
Indolent	0.11	0.086–0.17	
All patients	0.13	0.094–0.17	
Clinical stage			
Nonindolent	T1c, 85.2%	T2a, 14.8%	0.38[a]
Indolent	T1c, 92.6%	T2a, 7.4%	
All patients	T1c, 88.9%	T2a, 11.1%	

[a] *p* value from *z*-test for proportions.

Fig. 1B) and classified all individuals as of Caucasian origin (Supplementary Fig. 1C) [14]. Upon data segmentation, we computationally assessed tumor ploidy and purity using CLONET [15], which exploits informative SNPs and copy number data. Of all GS = 3 + 3/PGG1 biopsies, 34% (n = 18/53) showed clear evidence of somatic copy number aberrations (SCNAs) along the genome, 39% of which came from *potentially indolent* (n = 7/18) and 61% from *nonindolent* (n = 11/18) patients (Fig. 2A). The tumors showed diploid features and median cellularity (tumor purity) equal to 31% and 39%, respectively, with an outlier sample with 0.8 tumor purity (Fig. 2B and Supplementary Table 2). The fraction of the genome affected by SCNAs (copy number altered fraction, CNAF) did not differ significantly between the two populations with median values of 4% and a slightly longer upper tail in the *nonindolent* group (Fig. 2B and Supplementary Table 2). Detected single-nucleotide variants (SNVs) included a common F133C mutation in *SPOP* (Supplementary Fig. 3) and demonstrated low allelic fractions in agreement with previous studies of localized PCa [15]. No significant differences were detected between the two groups (Supplementary Fig. 4). No statistically significant differences were observed when testing distributions of tumor content, ploidy, CNAF, and mutational load in *potentially indolent* and *nonindolent* populations stratified by AS protocol.

In line with low-grade PCa genomic profiles from prostatectomy series [16,17], the minimally altered genomic landscape of our cohort presents a distinct set of monoallelic deletions, including on 8p, 13q, 16q, and 21q, and rare amplifications of 8q (Fig. 2C and Supplementary Fig. 5). Interestingly, those lesions are observed in both populations. Overall, the most common lesion across the whole dataset spans *NKX3-1* (8p) and was present in >50% of the analyzed tumors (Fig. 3A).

3.3. Key lesions detected in GS = 3 + 3/PGG1 biopsies

To specifically assess whether the genomics of tumor biopsies from AS patients could inform on lesions typically associated with adverse outcome, we focused on genomic events in *PTEN* and *MYC* and on two common genomic events as loss of *NKX3-1* and interstitial deletion between *TMPRSS2* and *ERG* [16] (Fig. 3A). Whereas no *PTEN* deletions were evident in the *potentially indolent* set, one patient from the *nonindolent* population (n = 1/10, 10%) harbored a deletion of *PTEN* in both GS = 3 + 3 biopsies. Of note, three patients (one *potentially indolent* and

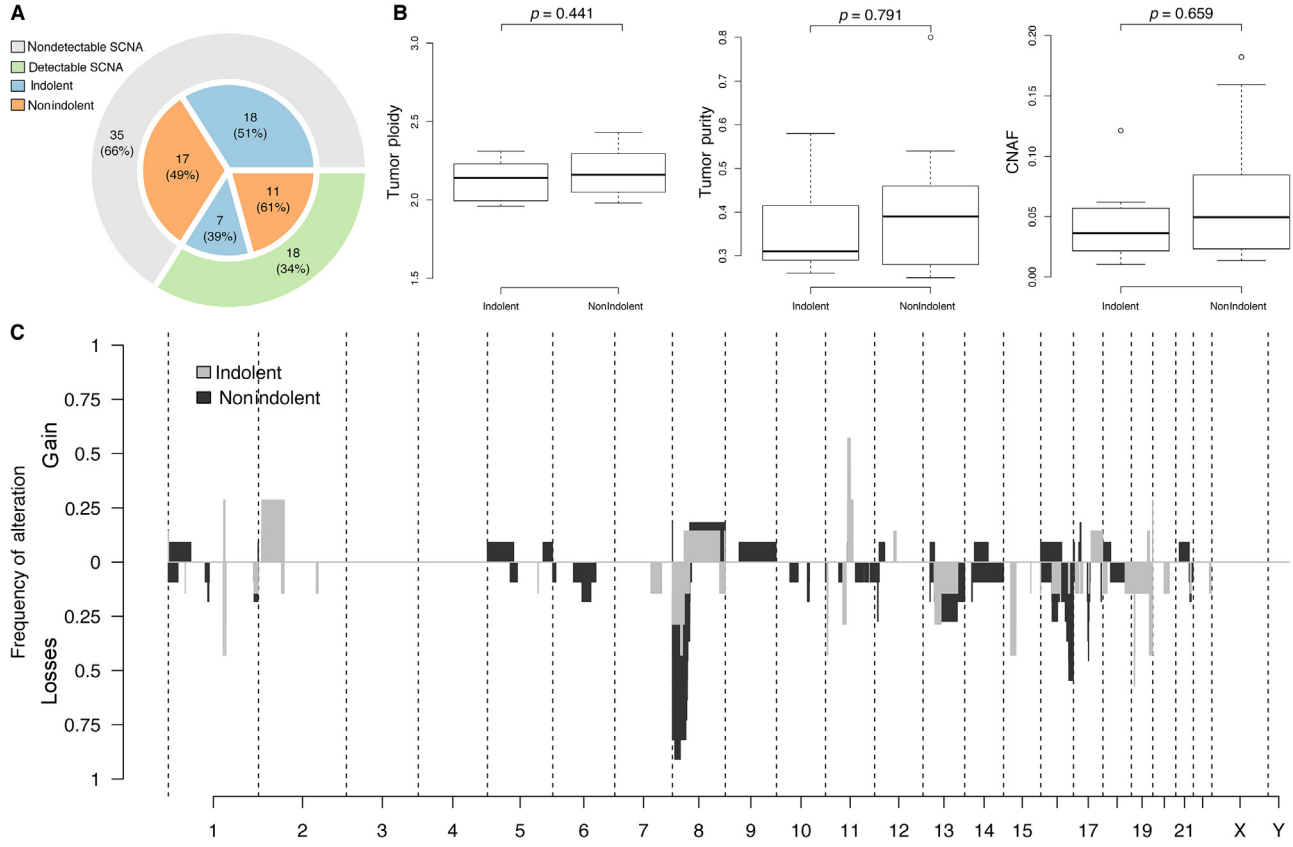

Fig. 2 – Whole-exome characterization of clinical biopsies of AS patients. (A) Pie chart reporting the number of samples with detectable and nondetectable somatic copy number alterations (SCNAs) and stratified by *potentially indolent* and *nonindolent* patients. (B) From left to right, distributions of tumor ploidy, tumor purity, and fraction of genome affected by SCNAs are compared between *potentially indolent* and *nonindolent* patients; *p* values from Wilcoxon tests. (C) Frequency of gains and losses in queried genomes, color coded by population, which shows SCNAs in the study cohort.

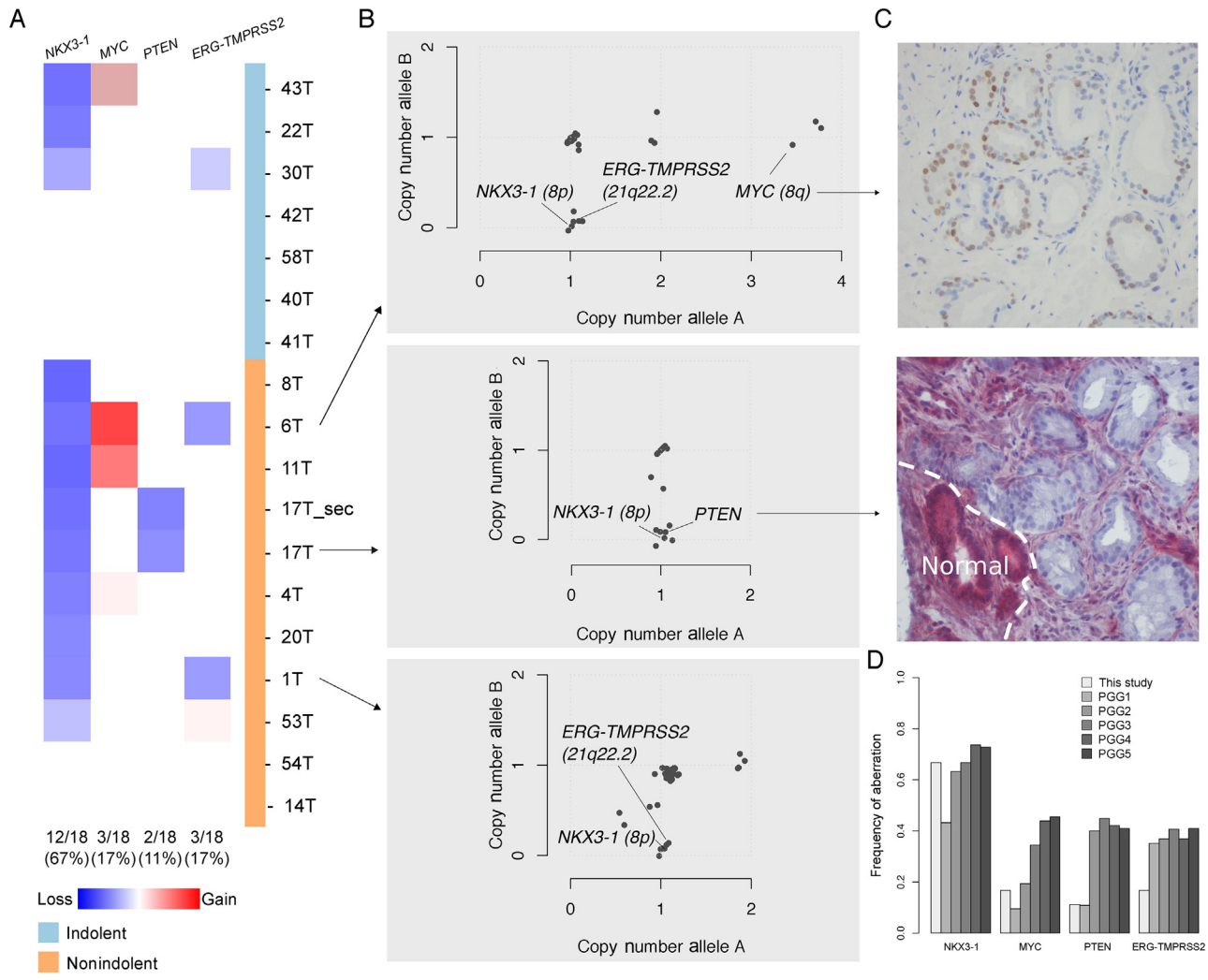

Fig. 3 – Assessment of key lesions in GS = 3 + 3 biopsies. (A) Heatmap of somatic copy number alteration (SCNA) status of *NKX3-1* (8p), *MYC* (8q), *PTEN*, and interstitial deletion between *TMPRSS2* and *ERG* (21q22.2, *ERG-TMPRSS2*) in *potentially indolent* ($n = 7$) and *nonindolent* ($n = 11$) samples. (B) Allele-specific copy number analysis by CLONET of three nonindolent patient samples, 1T, 6T, and 17T, shows distinct clusters of nonaberrant diploid segments (allele A = 1, allele B = 1) together with clonal hemizygous deletions (allele A = 1, allele B = 0). Allele-specific copy number gain (two to three extra copies of allele A) for a set of genes including *MYC* (8q) is observed for patient 6T. (C) Immunohistochemistry of GS = 3 + 3 biopsies from two patients. Top, sample 6T: photomicrograph (×200 magnification) shows positive c-Myc staining in most tumor cells. Bottom, sample 17T: photomicrograph (×200 magnification) demonstrates PTEN protein loss in tumor cells with preservation of PTEN staining in adjacent normal tissue. (D) Frequency of genomic aberration of *NKX3-1*, *MYC*, *PTEN*, and ERG-*TMPRSS2* genes in this study and in a collection of 426 clinically localized PCas treated by radical prostatectomy and stratified into five prognostic grading groups (PGGs) ranging from 1 to 5 defined as Gleason grades $\leq$ 6, 3 + 4, 4 + 3, 8, and $>$ 8, respectively.

two *nonindolent*) ($n = 3/18$, 17%) demonstrated clear amplification of *MYC*. Allele-specific copy number analysis (Supplementary Fig. 6) indicated up to four DNA copies of *MYC* in sample 6T (Fig. 3B), which was confirmed by immunohistochemistry, showing high although heterogeneous c-Myc staining, with some tumor cells completely negative and some with high expression within the same gland (Fig. 3C). The same analysis confirmed segregation of *PTEN* deletion signal in clonal monoallelic clusters with *NKX3-1* deletions in sample 17T (Fig. 3B). This finding was paralleled by in situ evidence of PTEN protein loss in tumor glands, with preservation of positive staining in adjacent normal cells (Fig. 3C).

Next, we compared the study incidence of these key lesions with what was previously observed in prostatectomy series stratifying the data by PGG [18]. Despite the relatively small size of the current study, observed frequencies are comparable to those of PGG1 and/or PGG2, which group patients with GS = 3 + 3 and 3 + 4, respectively (Fig. 3D).

3.4. *Comparative analysis of upgrading biopsies*

Upgrading biopsies (GS = 3 + 4/PGG2) were considered for comparative analysis for a subset of *nonindolent* patients. Of those, 67% ($n = 4/6$) demonstrated clear somatic copy number signal and data were retained for allele-specific SCNA profiles. Figure 4 reports biopsy sites and allele-specific results for the three individuals. One patient (patient 4) showed fairly identical profiles between the

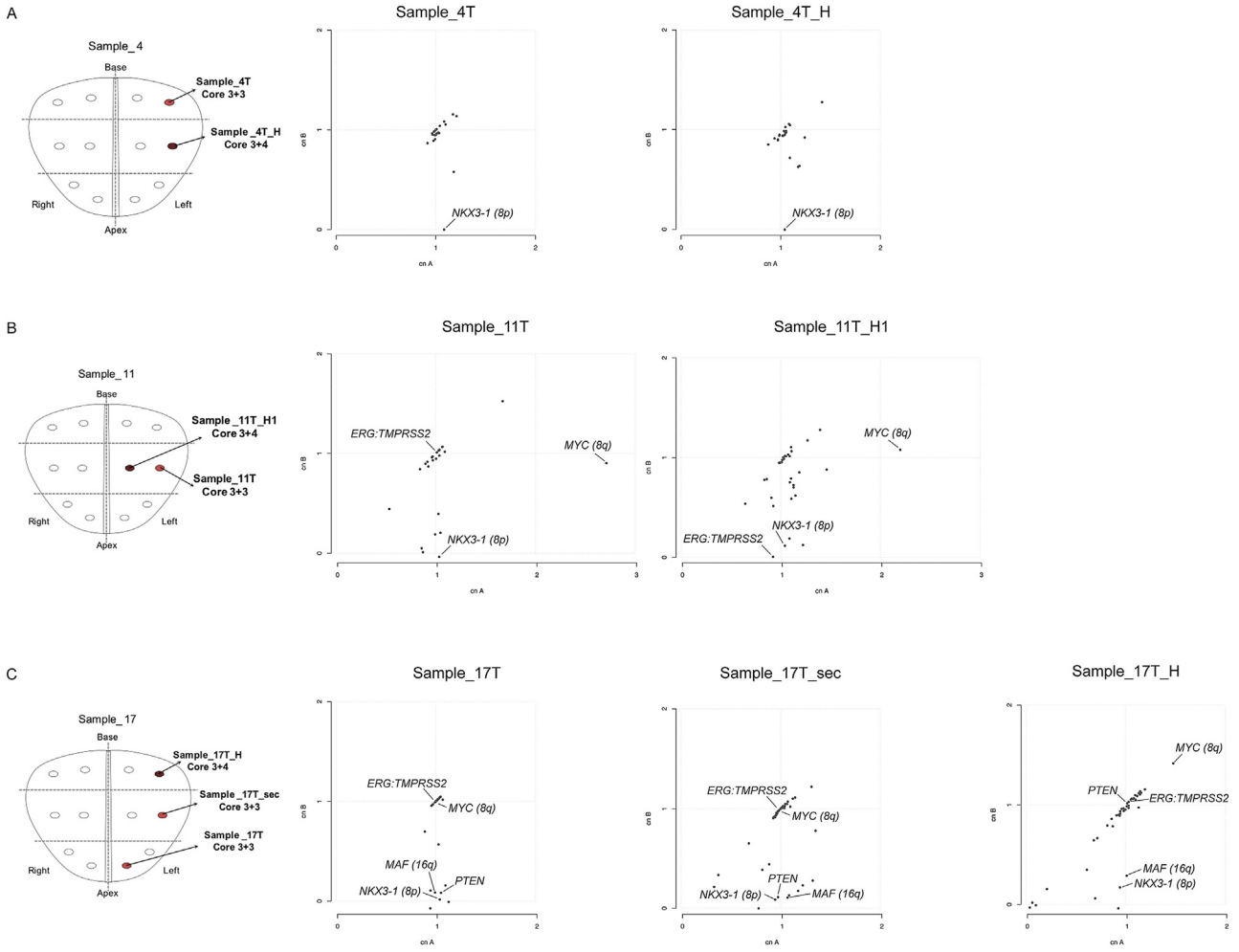

Fig. 4 – Comparison between GS = 3 + 3 (_T and _Tsec) and GS = 3 + 4 (_H) biopsies in 3 nonindolent patients. (A) Overall concordant copy number status between the two biopsies does not provide evidence of distinct founding clones. GS = 3 + 4 core has pattern 4 with fused and cribriform morphology. (B) Different genomic copy number status of the genomic region encompassing *ERG* to *TMPRSS2* genes (21q22.2), both clonal, is observed, suggesting the presence of two distinct clones. GS = 3 + 4 core has pattern 4 cribriform morphology. (C) Both GS = 3 + 3 cores available for patient 17 show concordant genomic profiles suggesting the same origin. Conversely, core GS = 3 + 4 (having pattern 4 fused and cribriform morphology) differs from both GS = 3 + 3 cores in the genomic status of *PTEN* (loss in GS = 3 + 3 cores, copy number normal in GS = 3 + 4 core) and *MYC* (copy number normal in GS = 3 + 3 cores, gain in GS = 3 + 4 core). This suggests the presence of a different clone.

GS = 3 + 3 and the upgrading biopsy with overall modest somatic changes but clear evidence of a few aberrations, including *NKX3-1* deletion. Given the incidence of such copy number loss in localized disease, we can neither suggest nor exclude distinct founding clones between the GS = 3 + 3 and 3 + 4 cores. In contrast, patient 11 demonstrated different and highly clonal genomic copy number status of the region encompassing *ERG* to *TMPRSS2* genes (21q22.2), suggesting two distinct clones between the cores [16].

Finally, both GS = 3 + 3 cores available for patient 17 showed concordant genomic profiles with multiple aberrations, whereas the GS = 3 + 4 core differed from both GS = 3 + 3 foci in the genomic status of *PTEN* (loss in GS = 3 + 3 cores, normal copy number in GS = 3 + 4 core) and *MYC* (normal copy number in GS = 3 + 3 cores, gain in GS = 3 + 4 core). This latter patient's data are suggestive of distinct clonal origin of the GS = 3 + 4 clone with respect to GS = 3 + 3.

4. Discussion

Management options for men with low-grade PCa include AS as a noninvasive alternative to radical treatment. AS enrollment criteria rely primarily on biopsy pathological characteristics; however, due to biopsy sampling, subjectivity of grading or tumor progression, upgrading, or upstaging is observed in 25% of cases [3,4]. Molecular biomarkers to be eventually assessed on bioptic cores might aid enrollment criteria overall. Genomic features enriched in higher GS tumors or associated with worse prognosis and disease progression in large cohorts of PCa patients and prostatectomy series could serve as AS protocol exclusion indicators and potentially spot lethal clones [19].

Prostatectomy-based studies focused on the characterization of somatic genomic lesions along the exome showed that G3 tumors can retain their indolent-appearing morphology despite the acquisition of multiple genomic alterations [7–

9]. Whether GS = 3 + 3 cores of patients who upgrade to GS > 3 + 3 during follow-up harbor a distinct genomic profile to men who continue on AS has not yet been investigated, partly due to the ethical and legal hurdles inherent in the analysis of these tumors and the technical challenges related to the scant available material. To the best of our knowledge, our study is indeed the first unbiased characterization of the genomic landscape of low-grade PCa patients in AS. To analyze a collection of tumors that is representative of the clinical routine, we included samples that span a wide range of pathology-based cellularity, from 5% to 90% (Supplementary Table 2). WES data successfully generated from *nonindolent* and *potentially indolent* biopsies demonstrated that >1/3 of GS = 3 + 3/PGG1 tumors have non-flat genomes, irrespective of the class. Given the experimental design including high sequencing coverage (>200× for tumors) and full exome (40 Mb), we exclude that the observed flat genomes are entirely ascribable to low cellularity of bioptic cores, but rather represent true biologically flat genomes. Cases with comparable pathological cellularity assessment, below 40%, indeed led to detectable lesions. Overall, the fraction of GS = 3 + 3 cores with no evidence of SCNAs (66%) is slightly higher than what is observed in Gleason 6 tumors from The Cancer Genome Atlas (TCGA) prostatectomy collection [16].

When focusing on genomic lesions enriched in metastatic disease [20] and associated with disease recurrence upon prostatectomy, including *MYC* amplification and *PTEN* loss, all but one were detected in the GS = 3 + 3 core biopsies of *nonindolent* patients. The allele-specific multisample analyses performed on selected patients to compare GS = 3 + 3/PGG1 with the upgrading GS = 3 + 4/PGG2 core included evidence both for common (patient 11) and distinct clonal origin (patient 17), concordant with the observations from Boutrous and colleagues [21] reporting on spatial genomic heterogeneity of localized multifocal PCa.

Overall, these data demonstrate that genomic characterization of bioptic material from AS patients, despite being challenging, provides insights towards the sampling versus 'tumor evolution' explanation of upgrading. Although descriptive in nature (more qualitative than quantitative), this study represents a proof-of-concept of the feasibility of performing large-scale genomic analysis from challenging biopsies with low percentages of tumor positivity. The main limitations are the retrospective analysis (though on prospectively collected material) and the limited cohort size. As we opted for large-scale genomics (WES of biopsy DNA), the latter was decided by availability of at least two tumor biopsies, which shortlisted 54 from >300 enrolled AS patients. Our results suggest that a well-designed targeted next-generation sequencing assay—for which DNA extracted from biopsy slides could be sufficient—to profile a large collection of AS patients should help unravel the true determinants of dropouts, which could then be considered for patient exclusion from or more strict monitoring during AS..

5. Conclusions

Although exhaustive molecular characterization of positive biopsies is not feasible for all AS patients due to insufficient tumor material, here we showed that *MYC* amplification and *PTEN* deletion detected by WES were confirmed by immunohistochemistry. Due to the limited sample size, the role of *PTEN* deletion and *MYC* amplification should be further investigated in larger cohorts of AS patients to understand if they can represent biomarkers for a more precise and earlier identification of patients at risk of reclassification/progression prior to the manifestation of conventional pathological markers.

Acknowledgements: We authors would like to thank Veronica De Sanctis and Roberto Bertorelli from the Next Generation Sequencing Facility at CIBIO, University of Trento, and Elisa Campi, from Molecular Pharmacology Unit, INT, Milan, for technical support, and Davide Prandi for fruitful discussions. The work was supported by the Italian Association for Cancer Research (Special Program Innovative Tools for Cancer Risk Assessment and Early Diagnosis, 5X1000, #12162, and Investigator Grant #19221) and by the Italian Ministry of Health (5X1000 Funds 2010).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euo.2018.08.010.

References

[1] Center MM, Jemal A, Lortet-Tieulent J, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol 2012;61:1079–92.
[2] Hugosson J, Carlsson S. Overdetection in screening for prostate cancer. Curr Opin Urol 2014;24:256–63.
[3] Bruinsma SM, Bangma CH, Carroll PR, et al. Active surveillance for prostate cancer: a narrative review of clinical guidelines. Nat Rev Urol 2016;13:151–67.
[4] Bokhorst LP, Valdagni R, Rannikko A, et al. A decade of active surveillance in the PRIAS study: an update and evaluation of the criteria used to recommend a switch to active treatment. Eur Urol 2016;70:954–60.
[5] Bruinsma SM, Zhang L, Roobol MJ, et al. The Movember Foundation's GAP3 cohort: a profile of the largest global prostate cancer active surveillance database to date. BJU Int 2018;121:737–44.
[6] Sowalsky AG, Ye H, Bubley GJ, Balk SP. Clonal progression of prostate cancers from Gleason grade 3 to grade 4. Cancer Res 2013;73:1050–5.
[7] Trock BJ, Fedor H, Gurel B, et al. PTEN loss and chromosome 8 alterations in Gleason grade 3 prostate cancer cores predicts the presence of un-sampled grade 4 tumor: implications for active surveillance. Mod Pathol 2016;29:764–71.
[8] Lotan TL, Carvalho FL, Peskoe SB, et al. PTEN loss is associated with upgrading of prostate cancer from biopsy to radical prostatectomy. Mod Pathol 2015;28:128–37.
[9] Sowalsky AG, Kissick HT, Gerrin SJ, et al. Gleason score 7 prostate cancers emerge through branched evolution of clonal Gleason pattern 3 and 4. Clin Cancer Res 2017;23:3823–33.
[10] Berg KD, Vainer B, Thomsen FB, et al. ERG protein expression in diagnostic specimens is associated with increased risk of progression during active surveillance for prostate cancer. Eur Urol 2014;66:851–60.
[11] Lokman U, Erickson AM, Vasarainen H, Rannikko AS, Mirtti T. PTEN loss but not ERG expression in diagnostic biopsies is associated with increased risk of progression and adverse surgical findings in men with prostate cancer on active surveillance. Eur Urol Focus 2017. http://dx.doi.org/10.1016/j.euf.2017.03.004, In press.

[12] Marenghi C, Alvisi MF, Palorini F, et al. Eleven-year management of prostate cancer patients on active surveillance: what have we learned? Tumori 2017;103(5):464–74. http://dx.doi.org/10.5301/tj.5000649. PubMed PMID: 28623636
[13] Demichelis F, Greulich H, Macoska JA, et al. SNP panel identification assay (SPIA): a genetic-based assay for the identification of cell lines. Nucleic Acids Res 2008;36:2446–56.
[14] Romanel A, Zhang T, Elemento O, Demichelis F. EthSEQ: ethnicity annotation from whole exome sequencing data. Bioinformatics 2017;33:2402–4.
[15] Prandi D, Baca SC, Romanel A, et al. Unraveling the clonal hierarchy of somatic genomic aberrations. Genome Biol 2014;15:439.
[16] Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25.
[17] Rubin MA, Girelli G, Demichelis F. Genomic correlates to the newly proposed grading prognostic groups for prostate cancer. Eur Urol 2016;69:557–60.
[18] Epstein JI, Zelefsky MJ, Sjoberg DD, et al. A contemporary prostate cancer grading system: a validated alternative to the Gleason score. Eur Urol 2016;69:428–35.
[19] Haffner MC, Mosbruger T, Esopi DM, et al. Tracking the clonal origin of lethal prostate cancer. J Clin Invest 2013;123:4918–22.
[20] Armenia J, Wankowicz SAM, Liu D, et al. The long tail of oncogenic drivers in prostate cancer. Nat Genet 2018;50:645–51.
[21] Boutros PC, Fraser M, Harding NJ, et al. Spatial genomic heterogeneity within localized, multifocal prostate cancer. Nat Genet 2015;47:736–45.