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Prostate cancer: identification of biomarkers discriminating indolent from aggressive tumors to improve the selection of patients suitable for active surveillance

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Challenges in Data-driven Genomic Computing
Como, March 7th , 2018

ACTIVE SURVEILLANCE

PSA-based screening has resulted in a marked increase in the number of newly diagnosed prostate cancers (PCa).

Overdiagnosis (i.e., diagnosis of cancer that would never have caused any symptoms) **is estimated to occur in 30 to 75% of men with PSA screen-detected PCa**.

Treatment of these cases inevitably lead to over-treatment and its potential side effects. Ideally, **radical treatment should be restricted only to those patients who really need it.**

Over the last decade, **active surveillance (AS) has evolved as an alternative to radical treatment for low-risk, potentially indolent PCa**.

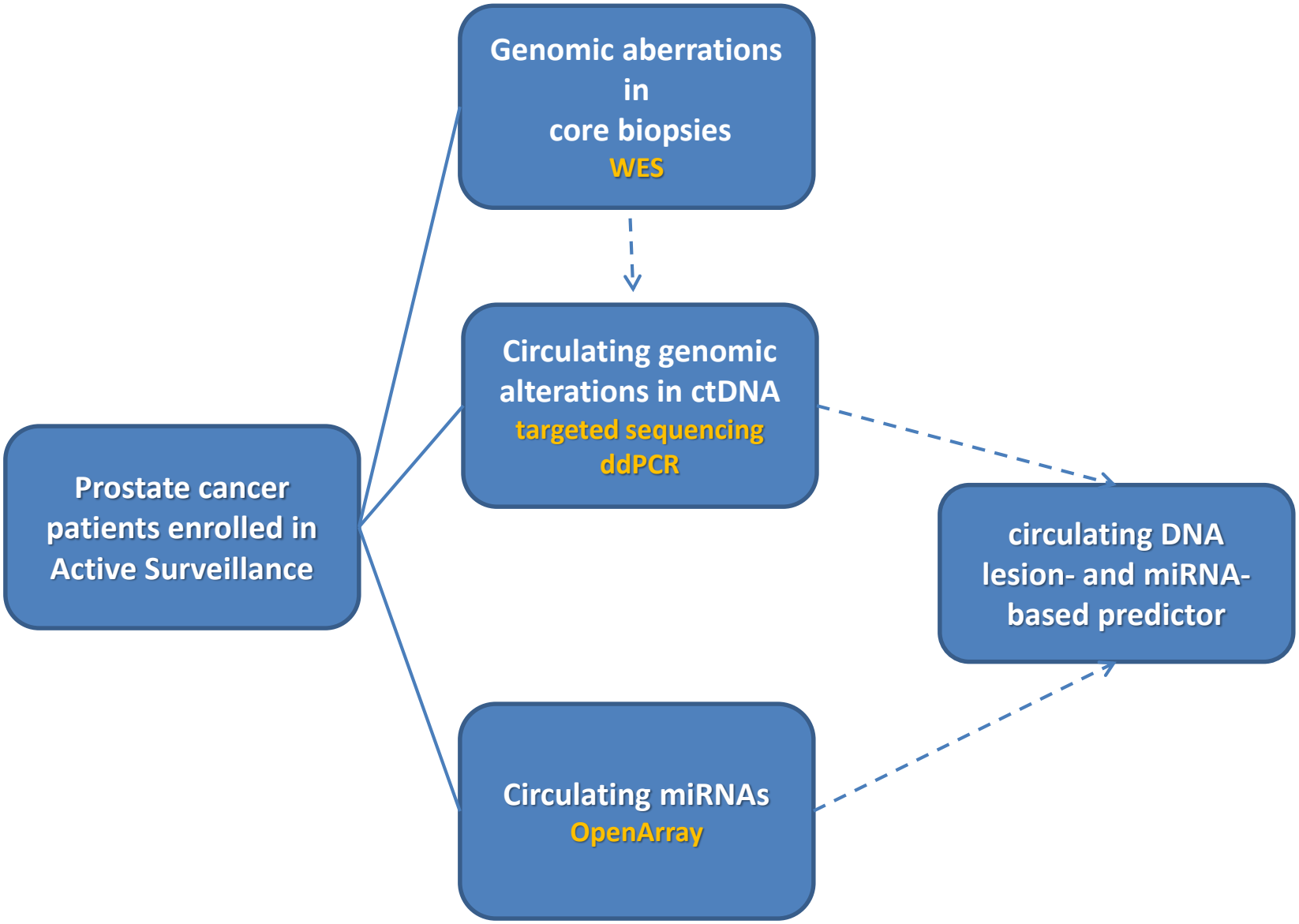
Aims:	To avoid overtreatment To delay radical treatments and side effects until needed
Pts characteristics:	Fit for radical treatments
Treatment Intent:	Radical
Treatment Timing:	Early, at disease reclassification or progression
AS Protocol:	Prostate Cancer Research International Active Surveillance (PRIAS)

PRIAS

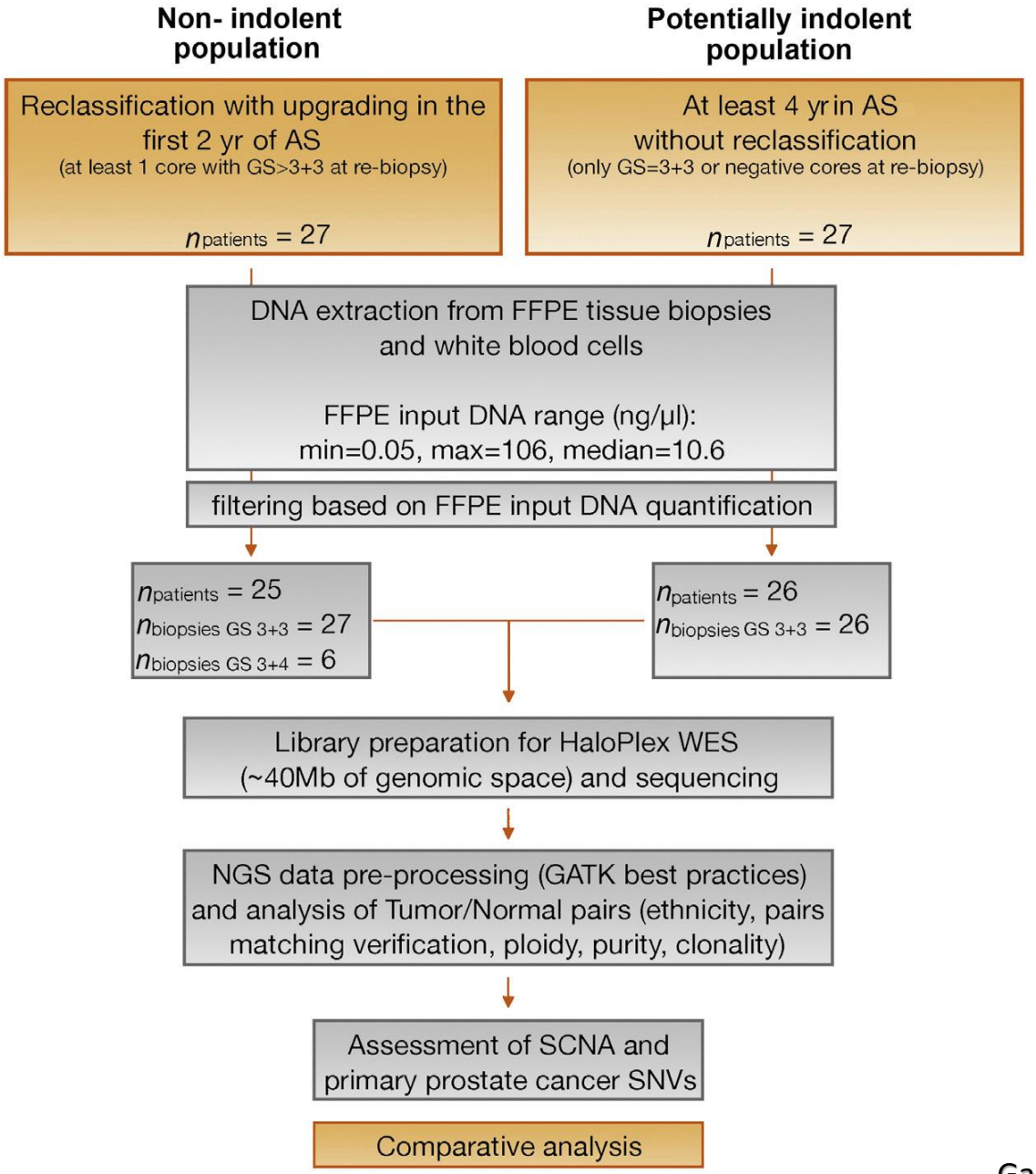
Inclusion criteria:	cT1-2; GPS ≤ 3+3; PSA ≤ 10 ng/ml; pos cores < 3; PSA density < 0.2 ng/ml/ml
Monitoring:	PSA every 3 mos Digital rectal Examination (DRE) every 6 mos Re-biopsies at 12 mos and then every 3 yrs Extra-biopsy if PSA DT: 3-10 yrs
Indications for AS drop out:	Short PSA DT (< 3 yrs) Upgrading (GPS> 3+3) Upsizing (>2 positive cores at re-biopsy)

"**An urgent need is related to the improvement of selection criteria for AS** -which are currently suboptimal and rely exclusively on clinical and pathological parameters- **with the addition of novel biological markers** for an early identification of occult high-risk PCa"

Study flow-chart



WES on core biopsies: study design



Gandellini et al., EUO 2019

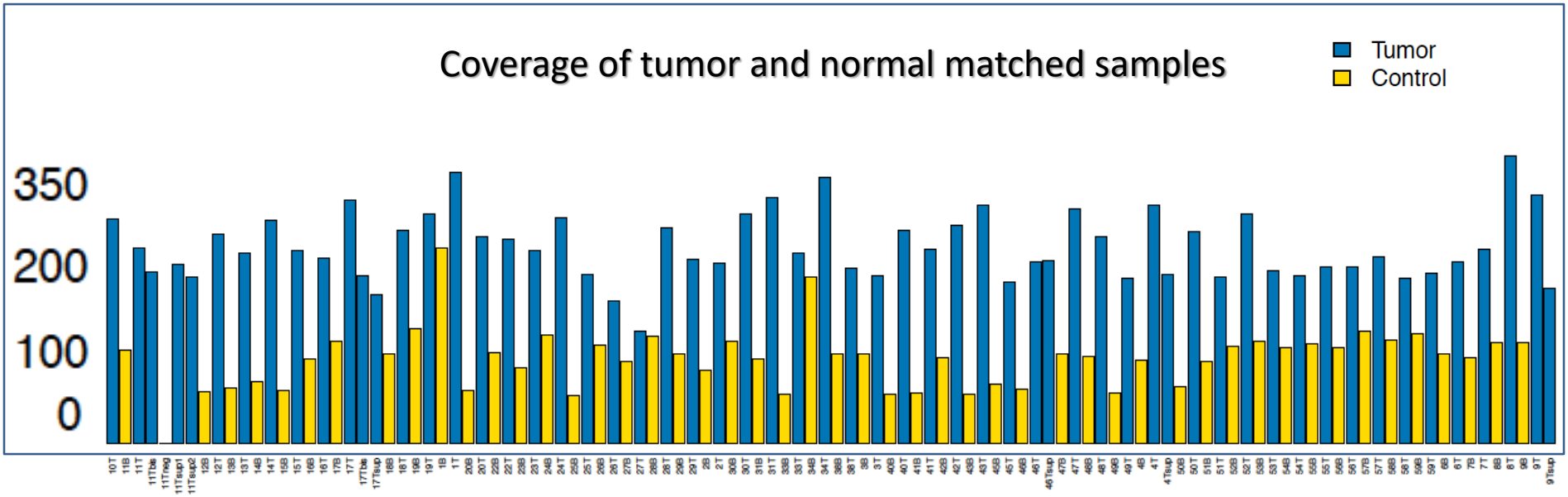
WES: Methods

DNA has been extracted from **matched tissue ad blood** samples with Maxwell 16 IVD (Promega) and the Maxwell® 16 FFPE Plus LEV DNA Purification Kit

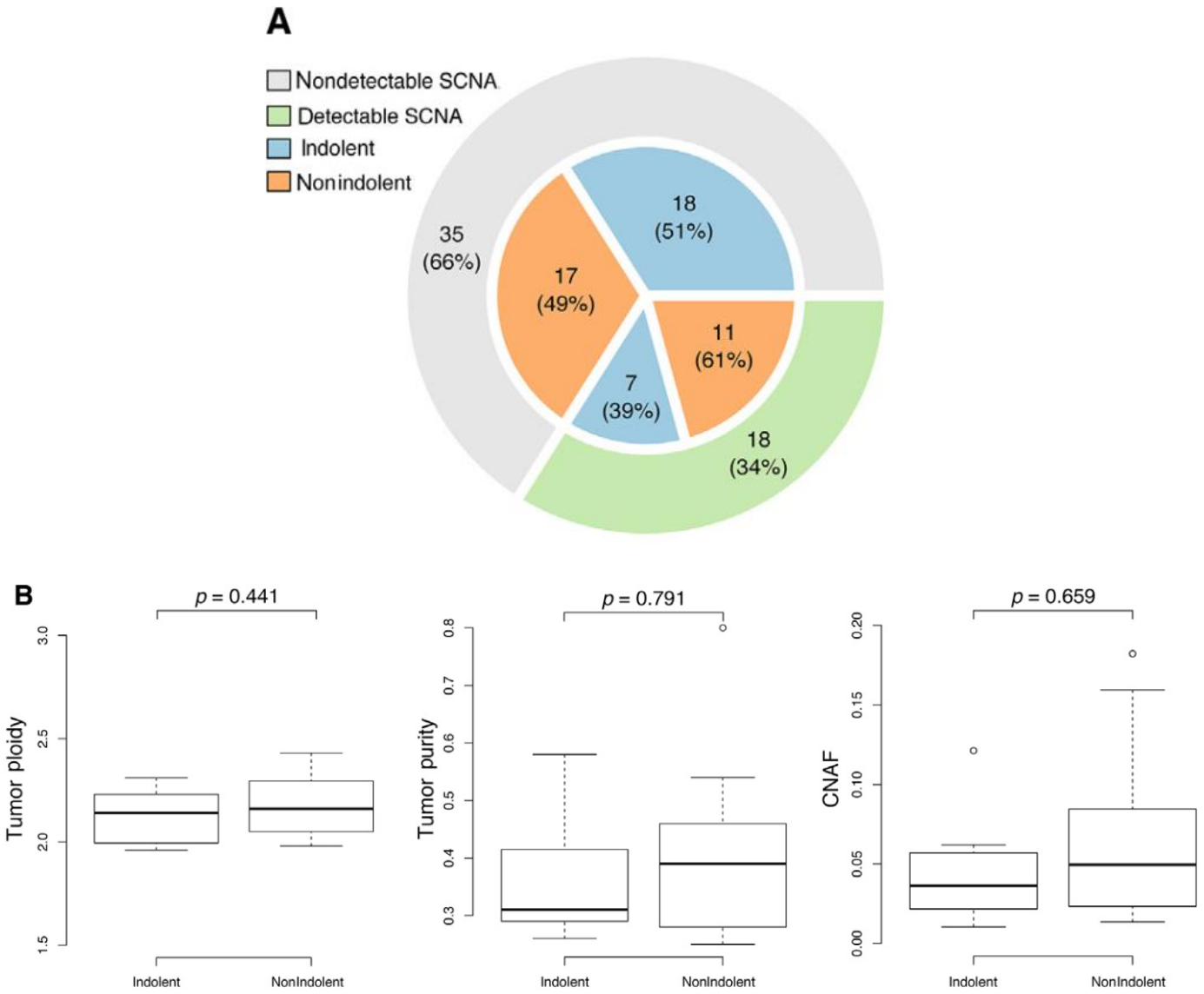
Libraries have been generated with the HaloPlex ExomeTarget Enrichment system (> 40Mb of genomic sequence)

WES has been carried out on Illumina HiSeq2000 platform (NGS Facility, University of Trento)

Data have been processed by Prof. Demichelis (Computational Oncology Laboratory, University of Trento) as in: Baca *et al*. Cell 2013; Prandi *et al*, Genome Biology 2014 (CLONET), Romanel *et al,* BMC Med Genomics 2015 (ASEQ)



Whole exome characterization of core biopsies of AS patients (I)



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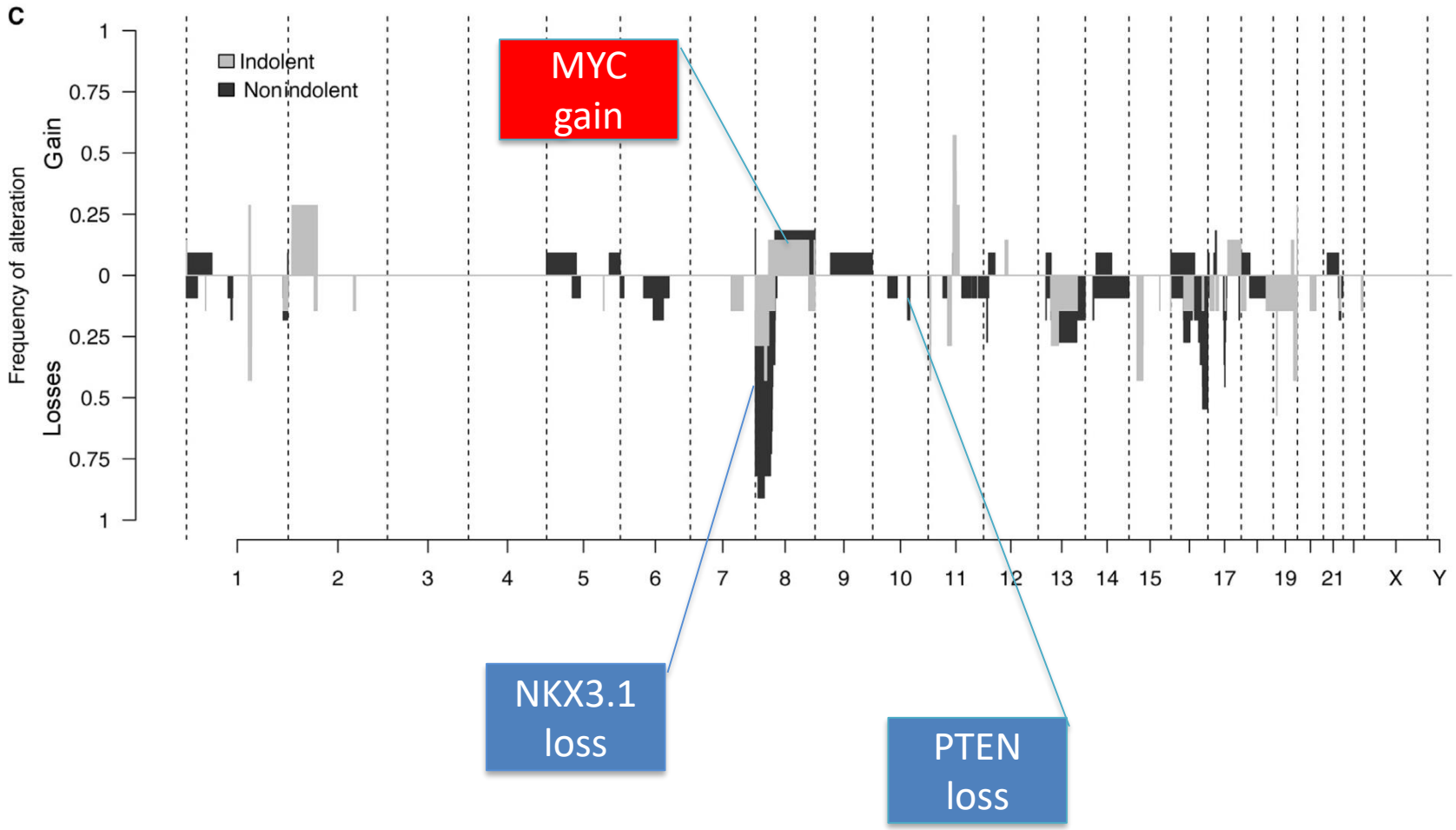
Whole exome characterization of core biopsies of AS patients (II)



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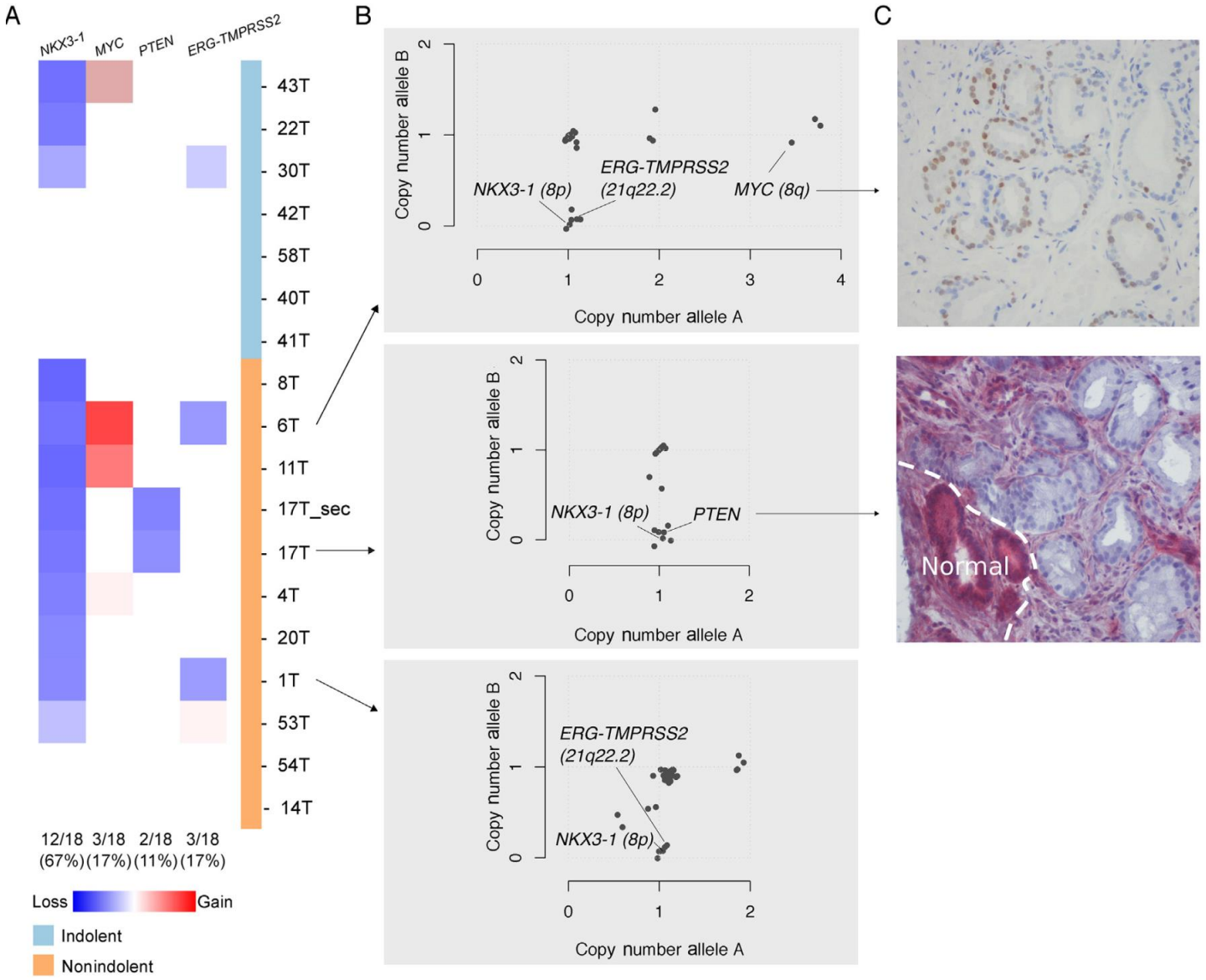
Assessment of key lesions in GS=3+3 biopsies



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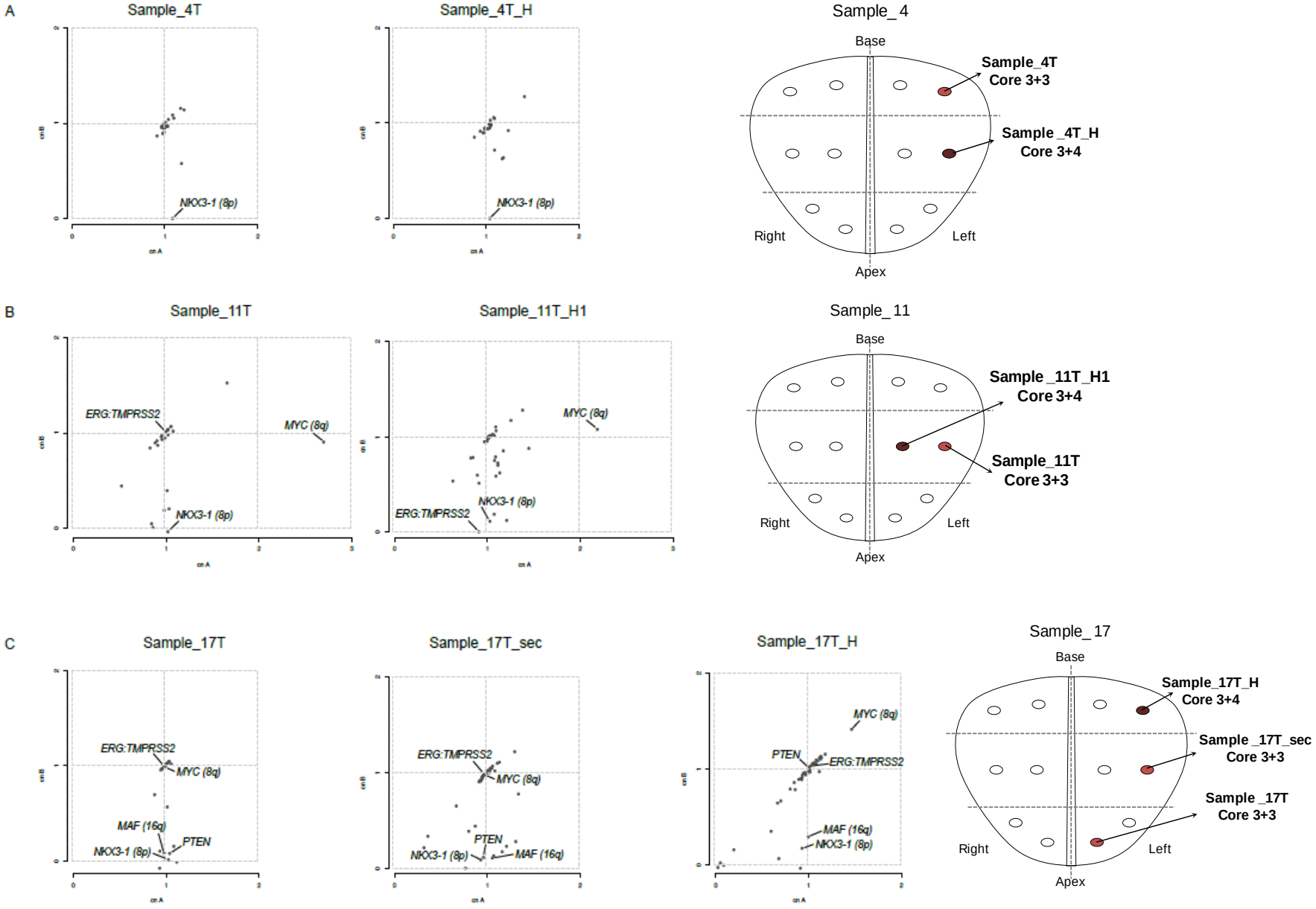
Comparison between GS=3+3 and GS=3+4 biopsies in non-indolent patients



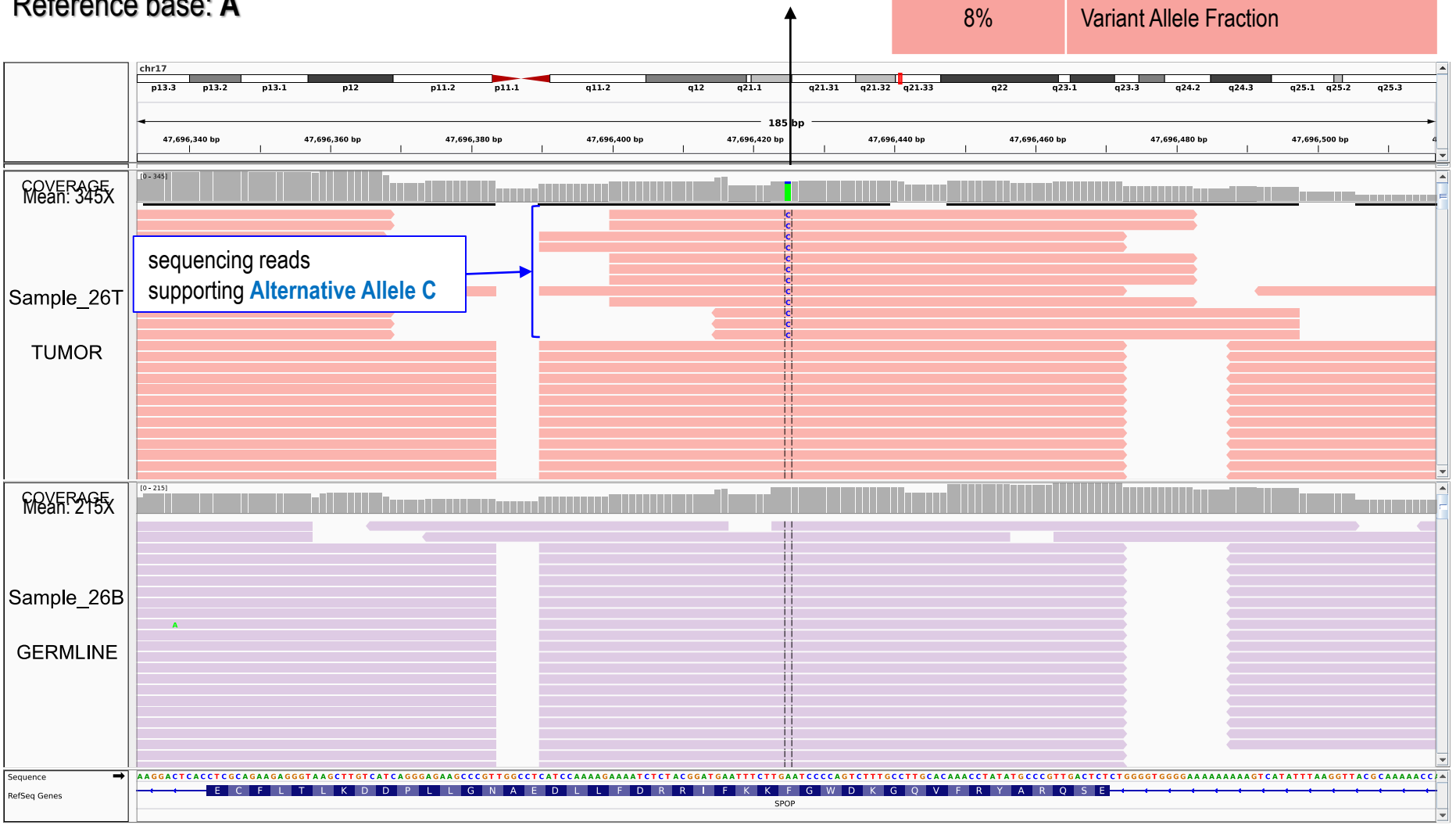
Gandellini et al., EUO 2019

SPOP p.F133C mutation

chr17:47696425 (GRCh37/hg19)

Reference base: **A**

Sample_26T chr17:47696425 (GRCh37/hg19)	
206	Reads supporting **allele A**
18	Reads supporting **allele C**
8%	Variant Allele Fraction



Genomic lesions in ctDNA

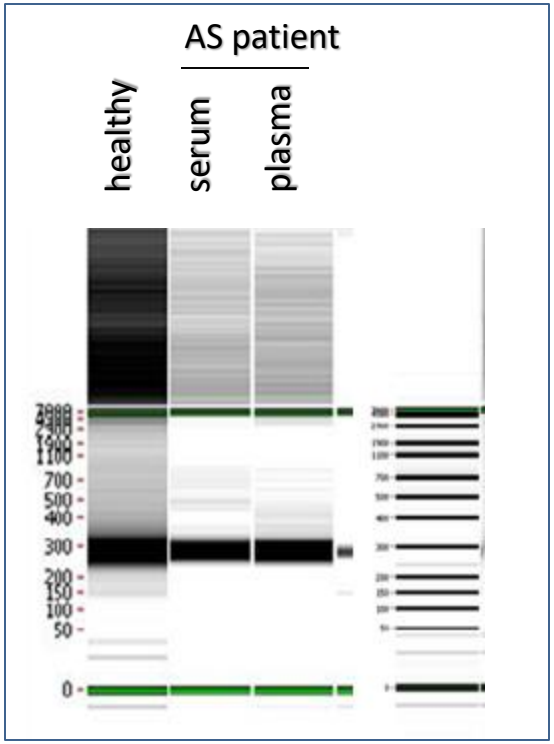
AIM: To search genomic aberrations found in core biopsies in plasma/serum samples of the same patients by targeted sequencing

- DNA extraction from plasma and serum of 5 AS patients — OK
- Library preparation using TruSeq® Custom Amplicon Low Input Library Prep kit (TSCAv2, Illumina) — OK
- Sequencing on Illumina MiSeq (CIBIO) and data analysis — For 3 patients: non OK

Genomic aberrations in core biopsies

- SPOP mutation p. F133c
- MYC copy number gain
- PTEN deletion

	serum	plasma
volume	1.35 ml	1.1 ml
DNA yield	44.8 ng	6.85 ng
library concentration	31.2 ng/ul	20.4 ng/ul
coverage	3,148X	2,383X
properly mapped reads	78%	74%



Circulating miRNAs

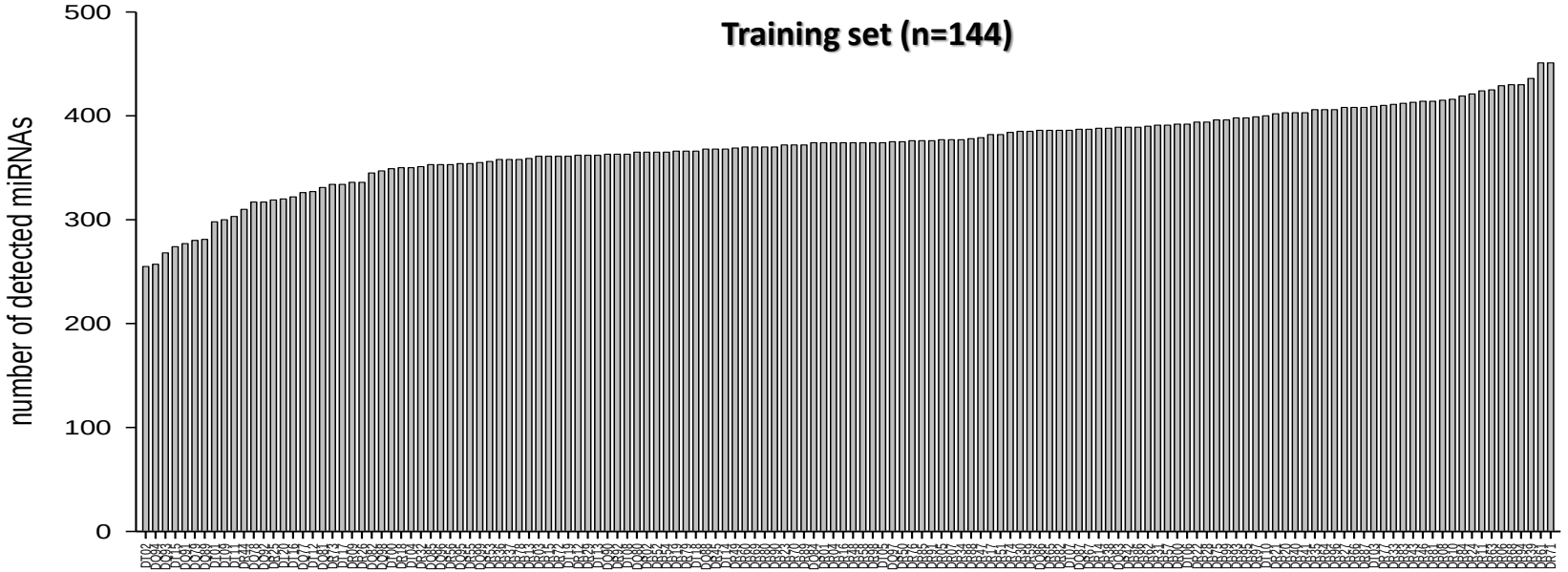
mRNA expression profiling (qRT-PCR based OpenArray Technology, 754 miRNAs and 4 control RNAs in replicates) on *baseline plasma samples* from patients prospectively followed at INT in the context of PRIAS AS protocol

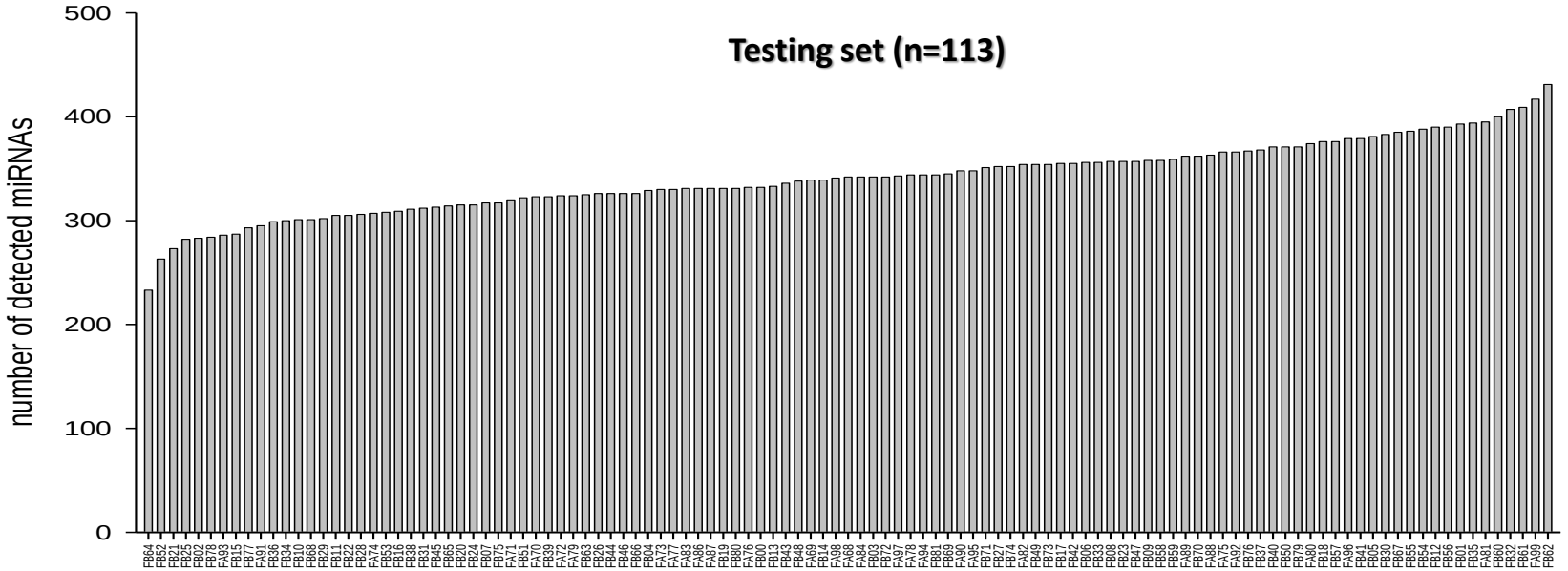
Identification of a circulating miRNA signature associated to disease reclassification during AS

Overall case series: 257 patients

TRAINING SET (TRS) : 144 (23 upgrading <1 yr; 121 indolent)

TESTING SET (TES) : 113 (20 upgrading <1 yr; 93 indolent)


Training set (n=144)
number of detected miRNAs
500
400
300
200
100
0


Testing set (n=113)
number of detected miRNAs
500
400
300
200
100
0

Circulating miRNA: study scheme

Training set (TRS)

Heamolysis-forced experiment

- One plasma sample contaminated with different percentages of RBCs
- Evaluation of haemolysis influence on miRs expression

Identification of reference miRs

- 121 samples
- 17 miRs detected in all samples
- Identification of the candidate reference miRNAs using an updated version of NqA algorithm *(Verderio P et al. Anal Biochem. 2014)*

Identification of the best combination of candidate haemolysis-free reference miRNAs

Selection and combination of candidate miRs in signatures

- Normalization with the best combination of 4 haemolysis-free reference miRNAs
- 121 samples
- Identification of 19 promising miRNAs

Identification of the most promising miR-based signatures

Testing set (TES)

Statistical validation and Identification of the best signatures

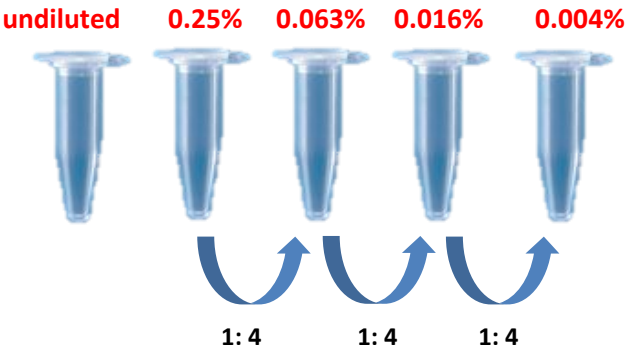
- Normalization with the best combination of 4 haemolysis-free reference miRNAs
- 113 samples
- Evaluation of TRS miR-based signatures on TES

Haemolysis forced experiment

Haemolysis-forced experiment (HFE): scheme design

Each sample was analyzed in triplicate for the entire spectrum of absorbance with Nanodrop before miRNA extraction. An indolent plasma sample of the training set was used for the implementation of the forced-haemolysis experiment

Dilution-scheme of the forced-haemolysis experiment



Percentage of contamination	n. of replicates	A414 (median value)
undiluted	3	0.177
0.004%	3	0.184
0.016%	3	0.191
0.063%	3	0.220
0.25%	3	0.329

In orange the dilution points profiled with OA

Haemolysis-forced experiment (HFE): results from OA experiment

Computation of the Simultaneous Confidence Intervals *(Pizzamiglio S et al. Oncology reports, 2010; Pizzamilglio S at al. Oncology Letters 2017)* by using the overall mean of miRs expressed in all samples (n=152) as normalization approach. Subsequently, for each miRNA (i =1,.., n), the relative quantity (RQ) was computed by subtracting the ΔCt value of each contamined sample (s =1,2) from the undiluted ones as follow:

$RQ_{is} = 2(-\Delta\Delta Ct_{is})$, where $\Delta\Delta Ct_{is} = \Delta Ct_{is} - \Delta Ct_{i\ undiluted}$

~90% of miRNAs resulted not affected by haemolysis

Circulating miRNA: study design

Training set (TRS)

Heamolysis-forced experiment

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Training set results

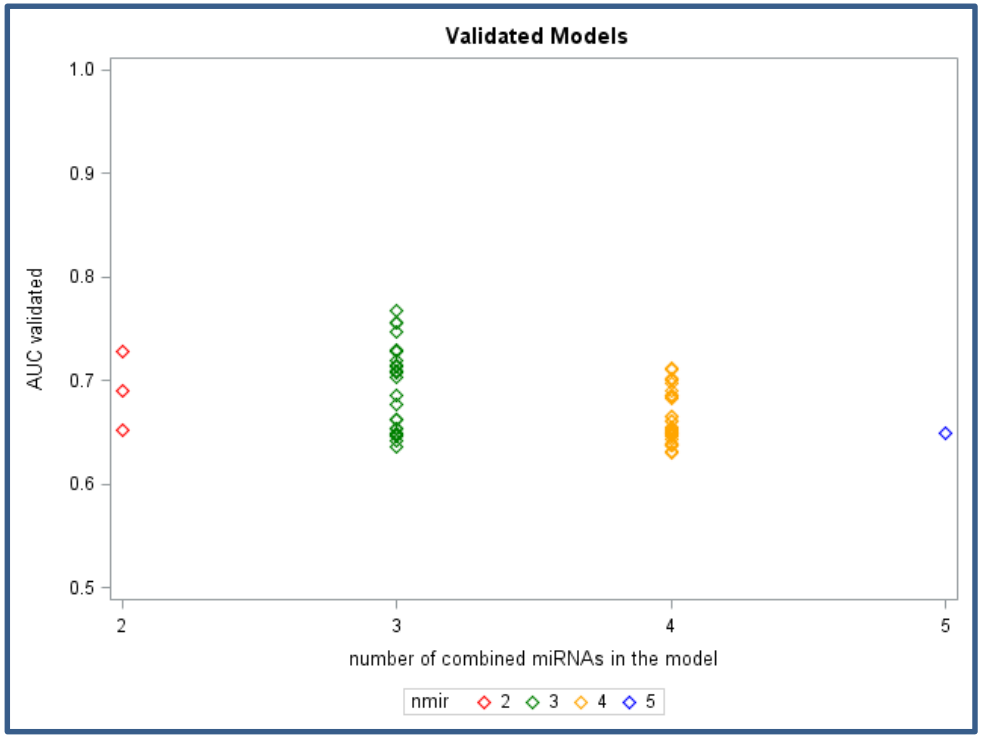
Reference miRNAs
hsa-let-7c-000379
hsa-miR-126-002228
hsa-miR-26b-000407
mmu-miR-379-001138

List of 19 candidate miRNAs (from univariate analysis)
hsa-miR-122-002245
hsa-miR-1255B-002801
hsa-miR-128a-002216
hsa-miR-142-5p-002248
hsa-miR-16-000391*
hsa-miR-181c-000482
hsa-miR-199a-000498
hsa-miR-204-000508
hsa-miR-27b-000409
hsa-miR-324-5p-000539
hsa-miR-330-000544
hsa-miR-337-3p-002157
hsa-miR-361-000554
hsa-miR-422a-002297
hsa-miR-424z-002309
hsa-miR-502-001109
hsa-miR-511-001111
hsa-miR-572-001614
hsa-miR-598-001988

*excluded from the subsequent analysis as haemolysis related

AUC values of the 60 statistically validated signatures on TES data

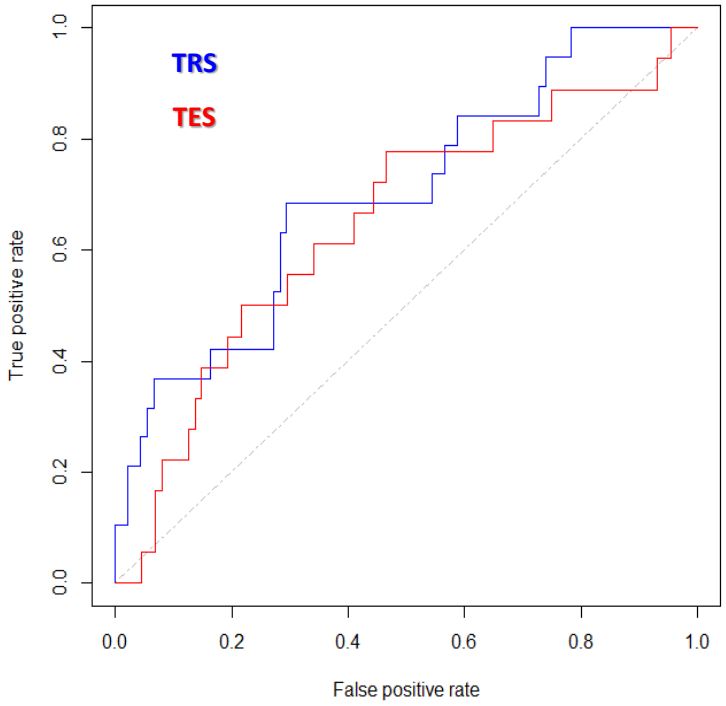
Testing set results



Training & Testing results

Top candidate model: miR-598, mir-361, miR-324-5p, mir-27b, *miR-204*

set	UPG	IND	AUC (95%CI)
TRS	19/23	92/98	0.699 (0.565; 0.833)
TES	18/19	88/92	0.649 (0.501; 0.797)



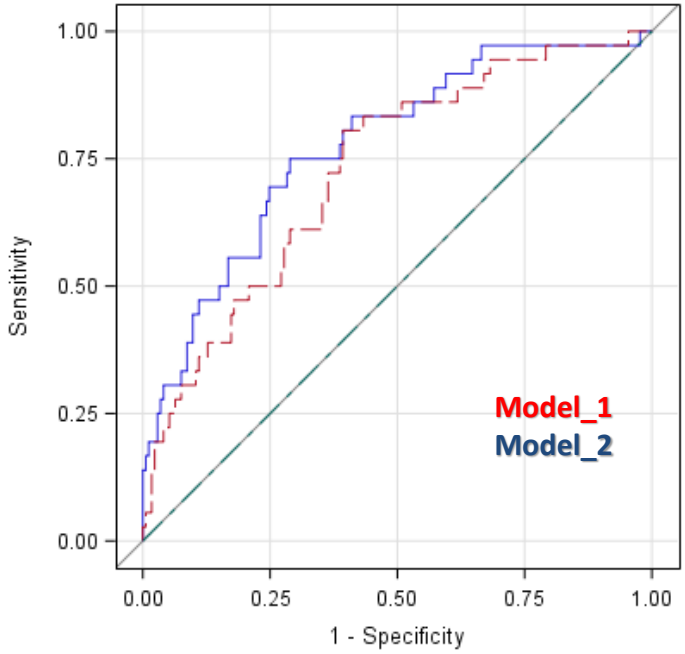
Extension with clinical data

Significant variables in univariate logistic regression analysis – overall cohort (TRS+TES)

variables	n. of UPG	n. of IND	OR	95%CI		AUC	95%CI	
Age	42	190	1.068	1.015	1.124	0.621	0.532	0.709
PSA density	42	190	2.956	1.356	6.444	0.646	0.548	0.744
Volume	42	190	0.304	0.147	0.629	0.641	0.564	0.719
CORE pos	42	190	2.455	1.242	4.851	0.605	0.522	0.688
% CORE_pos	42	190	1.080	1.022	1.141	0.637	0.542	0.733
Max positivity	41	182	1.034	1.012	1.056	0.666	0.578	0.754

Age: age at biopsy; CORE_pos: n. of positive core at diagnostic biopsy; PSA density: PSA/Volume (on logarithmic scale)
Volume: Prostatic volume dichotomized at 45cc (median value of the overall cohort); Max positivity: max % of tumor positivity; % CORE_pos: % positive core
Abbreviation: UPG: upgrading, IND: indolent, OR: odds ratio, CI: confidence intervals, AUC: Area under the ROC curve

Model	Variables included	AUC (95%CI)
Model_1	Age, PSA density, Max positivity	0.728 (0.639; 0.817)
Model_2	Age, PSA density, Max positivity + miRNA Score	0.774 (0.689; 0.859)



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