Development and validation of baseline predictive biomarkers for response to avelumab in second-line (2L) non-small cell lung cancer (NSCLC) using EpiSwitch™ epigenetic profiling

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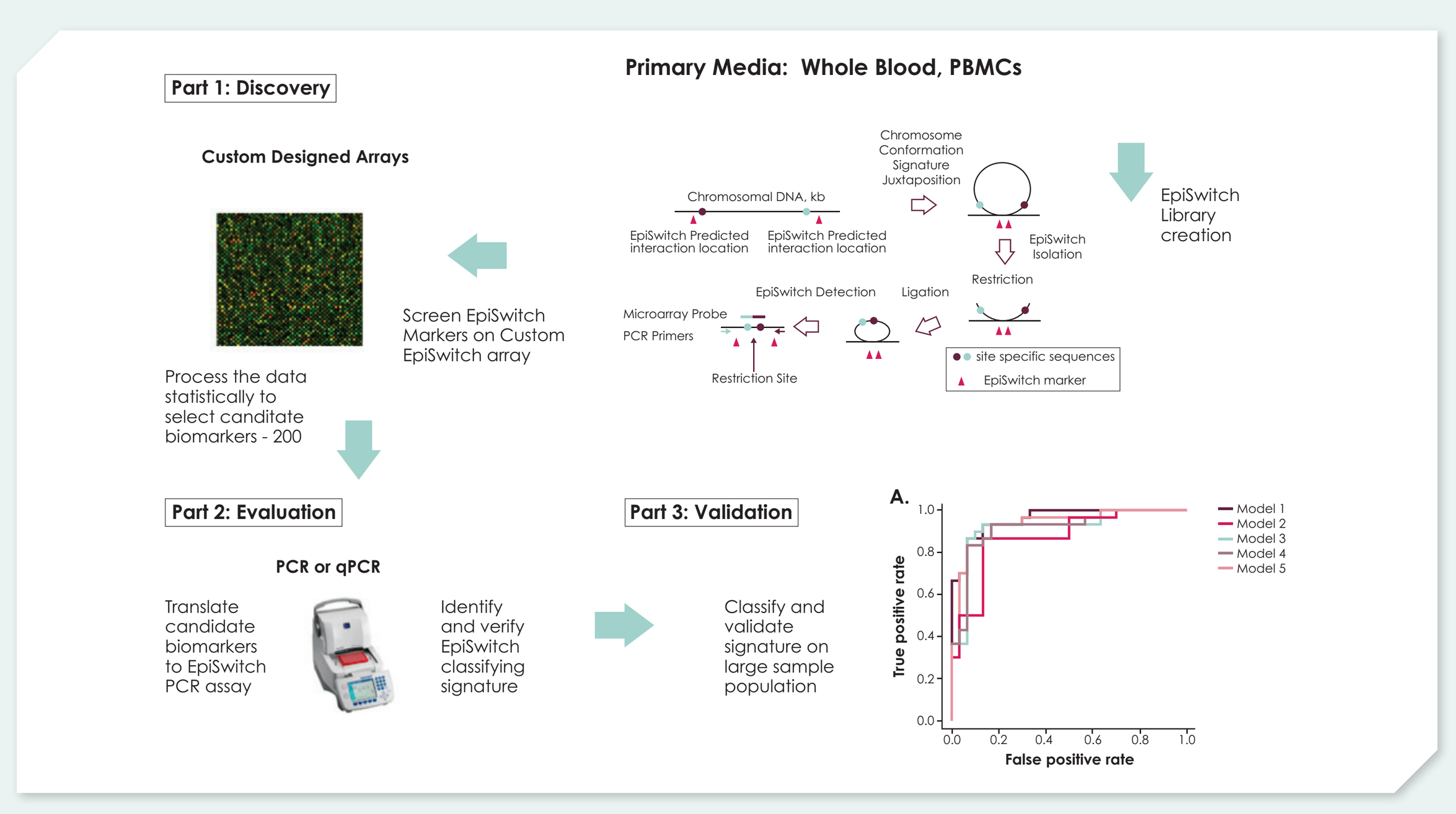
INTRODUCTION

- The EpiSwitch biomarker discovery platform detects systemic regulatory changes in the cellular genomic architecture using a microarray platform and translates useful biomarkers to a PCR-based platform (**Figure 1**).[1] EpiSwitch identifies alterations in chromosome conformation signatures (CCSs), key regulatory processes that integrate environmental cues and genetic alterations into the regulatory machinery
  - CCSs are also known as gene loops, chromosome domains, and long-range chromosomal interactions
- CCSs are the primary step in a cascade of changes in the genomic regulatory architecture and are directly linked with cellular phenotype. Because CCSs have low off rates, they are only expected to change when a physiologically signaled transition to a new phenotype occurs or due to external intervention. In addition, the measurement of these events is binary and can be measured by other cellular entities, such as changes in DNA methylation, and changes in RNA and protein expression
- We profiled patients with NSCLC who received avelumab (anti-PD-L1 drug) or pembrolizumab (anti-PD-1 drug) (**Table 1**) and generated a model to seprate responders from nonresponders using machine-learning methods

Figure 1. EpiSwitch™ biomarkers methodology: from discovery to clinical quality assay



METHODS

- In the JAVELIN Solid Tumor trial (NCT01772004), 156 first-line (1L) and 184 second-line (2L) patients with NSCLC were enrolled; PD-L1 status was determined using the PD-L1 IHC 73-10 pharmDx assay
- Overall response rates of 19.23% (1L) and 14.13% (2L) were observed in the full analysis set (FAS)
- At the original data cut-off (February 27, 2017), 10 patients had stable disease (SD); however, the number increased to 18 patients with SD in the most recent data cut-off (May 9, 2018)
- A total of 99 patients were profiled using the EpiSwitch platform, in 2 batches
- The first batch contained 12 patients each from both 1L and 2L populations and contained an equal number of responders (complete response [CR] and partial response [PR]) and nonresponders (progressive disease [PD] and not evaluable [NE])
- Fisher exact test (tables with counts <5) or χ^2 test of association was used for calculating differences between response groups
- Microarray probes associated with response (PR/CR) or nonresponse (PD/NE) in batch 1 were filtered using univariate association against a publicly available pembrolizumab melanoma data-set (NCT02083484)
- Of these, 62 probes were used in a PCR-based assay and used for classifier building
- A classifier to predict response was generated in 2 steps using 10-fold cross-validation:
  - **Step 1:** identifying univariate association of biomarkers to response using Fisher exact test
  - **Step 2:** generation of a multivariate model using XGBoost or regularized regression
- A multivariable Cox proportional hazards model was built using R package survival and plotted with Survminer
- A multivariable logistic regression model was built with R glm function
- For building multivariable models, clinical co-variates were selected based on analysis of clinical trial data from JAVELIN Lung 200 trial (NCT02395172)[2]

RESULTS

- Responses were similar in the 1L and 2L data sets (**Table 1**)
- The profiled patients set did not show any significant differences in baseline characteristics, including PD-L1 positivity (**Table 2**)
- Survival of patients with PD was comparable for both 1L and 2L data sets; 2L patients with CR/PR tended to survive longer (**Figure 2**)
- In the patients included in the analysis (n=99), PD-L1 expression was neither predictive nor prognostic of outcome (**Figure 3**)
- A 14-marker model for predicting response was built with a training set of 24 patients profiled in batch 1 and was used to predict response for independently generated profiles of 65 1L and 2L patients with NSCLC in batch 2 (**Figure 4A**)
- The classifier achieved high accuracy and positive predictive value (PPV) for 2L NSCLC in the test set (**Figure 4A**)

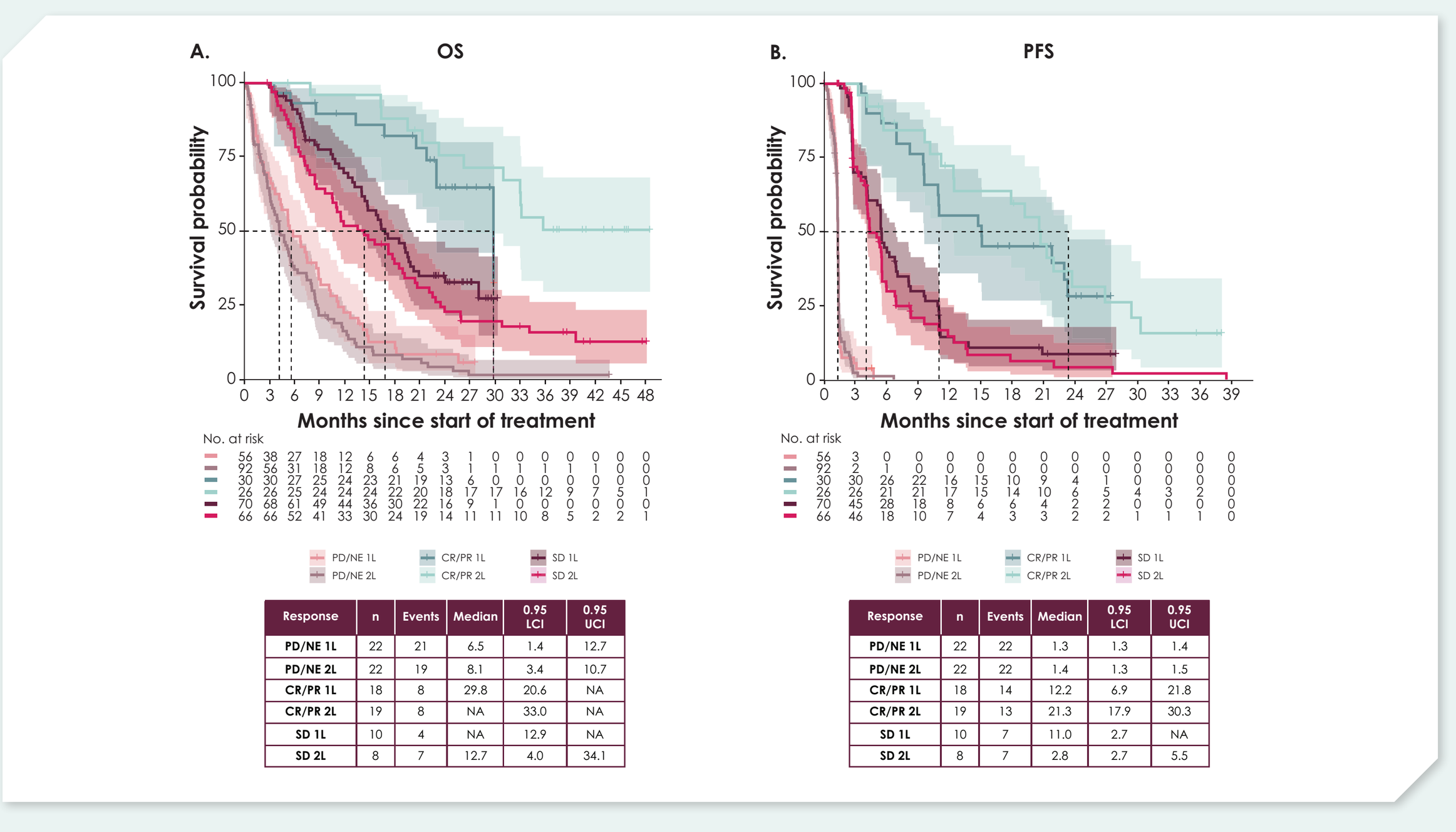
Table 1. Summary of responses per RECIST 1.1 in profiled patients (and FAS)

Treatment Line	CR	PR	SD	PD	NE	Total
1L	1 (3)	17 (27)	10 (70)	16 (39)	6 (17)	50 (156)
2L	2 (2)	17 (24)	8 (66)	21 (69)	1 (23)	49 (184)

Clinical data cut-off: May 9, 2018

Classifier generation was completed by Oxford Biodynamics using different data cut-offs

Figure 2. Efficacy in 1L and 2L profiled patients



LCI, lower confidence interval; **OS**, overall survival; **PFS**, progression-free survival; **UCI**, upper confidence interval

Figure 3. Efficacy in profiled patients by PD-L1 status

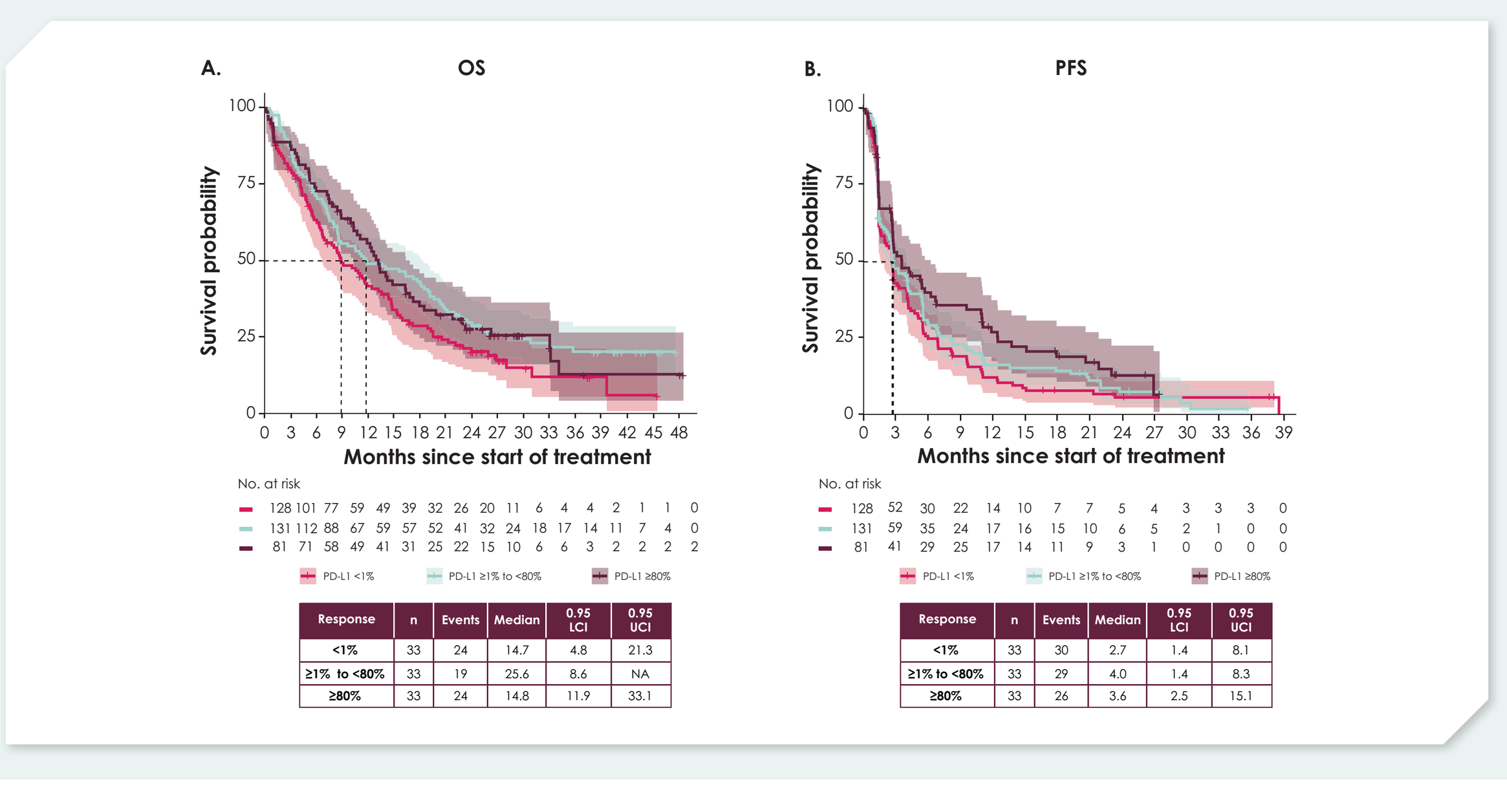


Table 2. Baseline characteristics of profiled patients

Covariate	NR (n=48)	R (n=51)	p value*
Patient age, years			
N	48	51	
Missing	0	0	
Mean	66.15	66.49	
SD	11.77	9.07	
Q1	62.25	61.5	
Median	68	67	
Q3	74	72.5	
Pooled age, n (%)			
<65 years	16 (33.3)	22 (43.1)	0.43
≥65 years	32 (66.7)	29 (56.9)	
Sex, n (%)			
Male	30 (62.5)	28 (54.9)	0.57
Female	18 (37.5)	23 (45.1)	
Race, n (%)			
Black or African American	2 (4.2)	5 (9.8)	0.58
Native Hawaiian or Pacific Islander	0	1 (2.0)	
Other	3 (6.3)	3 (5.9)	
White	43 (89.6)	42 (82.4)	
Geographic region, n (%)			
America	46 (95.8)	46 (90.2)	0.44
Europe	2 (4.2)	5 (9.8)	
Pooled race, n (%)			
Others	5 (10.4)	9 (17.6)	0.39
White	43 (89.6)	42 (82.4)	
Prior anticancer therapies for metastatic or locally advanced disease, n (%)			
≤1	38 (79.2)	41 (80.4)	0.93
2	8 (16.7)	7 (13.7)	
≥3	2 (4.2)	3 (5.9)	
Sum of longest lesion diameter by investigator per RECIST, mm			
N	48	51	
Missing	0	0	
Mean	78.71	66.37	
SD	48.71	40.43	
Q1	4.3	38.05	
Median	63.5	58	
Q3	101.25	86	
Histology, n (%)			
Adenocarcinoma	34 (70.8)	32 (62.7)	0.73
Others	3 (6.3)	5 (9.8)	
Squamous cell carcinoma	11 (22.9)	14 (27.5)	

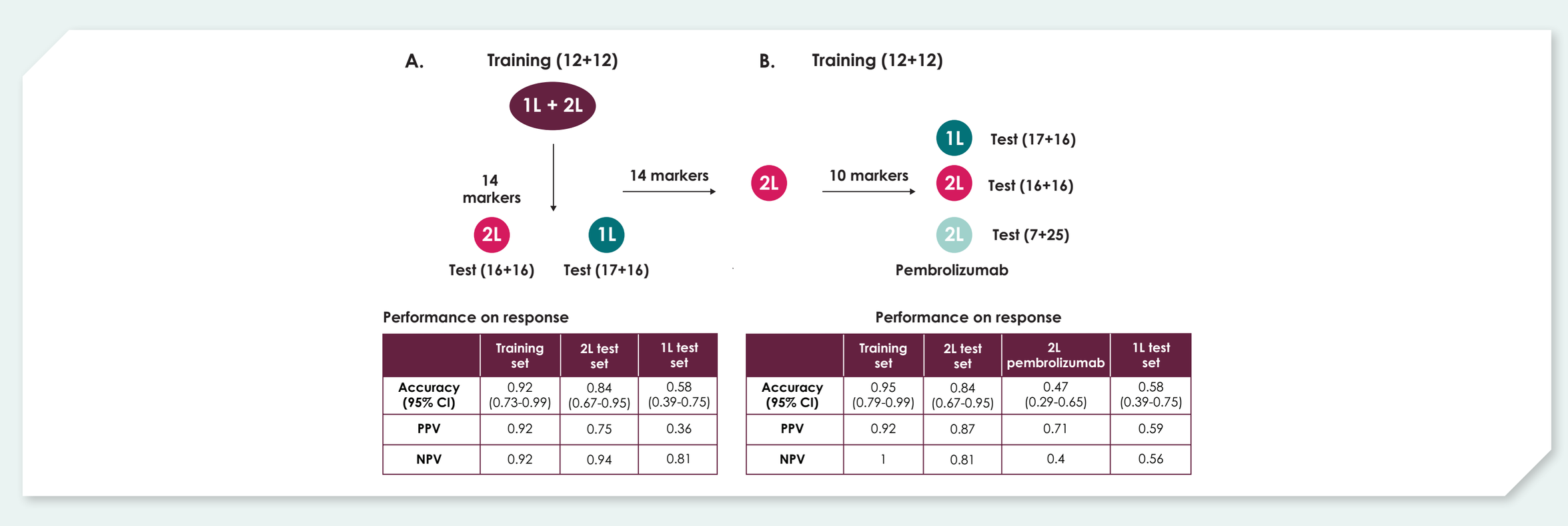
Covariate	NR (n=48)	R (n=51)	p value*
Baseline weight, kg			
N	48	51	
Missing	0	0	
Mean	80	75.49	
SD	20.14	19.38	
Q1	65.75	60.95	
Median	76.5	73.6	
Q3	94.55	84	
Baseline ECOG PS, n (%)			
0	20 (41.7)	24 (47.1)	0.85
1	27 (56.3)	26 (51.0)	
2	1 (2.1)	1 (2.0)	
Smoking status, n (%)			
Never smoked	8 (16.7)	4 (7.8)	0.23
Ever smoked	40 (83.3)	47 (92.2)	
Prior anticancer therapies, n (%)			
≤1	36 (75.0)	37 (72.5)	0.78
2	8 (16.7)	11 (21.6)	
≥3	4 (8.3)	3 (5.9)	
Baseline PD-L1 status			
n	48	51	
Missing	0	0	
Mean	34.44	39.02	
SD	42.45	43.42	
Q1	0	0	
Median	3.5	10	
Q3	90	90	
PD-L1 ≥1%, n (%)			
Negative	7 (14.6)	4 (7.8)	0.44
NE	9 (18.8)	14 (27.5)	
Positive	32 (66.7)	33 (64.7)	
PD-L1 ≥5%, n (%)			
Negative	16 (33.3)	11 (21.6)	0.35
NE	9 (18.8)	14 (27.5)	
Positive	23 (47.9)	26 (51.0)	
PD-L1 ≥50%, n (%)			
Negative	23 (47.9)	16 (31.4)	0.23
NE	9 (18.8)	14 (27.5)	
Positive	16 (33.3)	21 (41.2)	
PD-L1 ≥80%, n (%)			
Negative	25 (52.1)	19 (37.3)	0.31
NE	9 (18.8)	14 (27.5)	
Positive	14 (29.2)	18 (35.3)	

ECOG PS, Eastern Cooperative Oncology Group performance status.

* Fisher exact test (count <5) or X2 test as appropriate

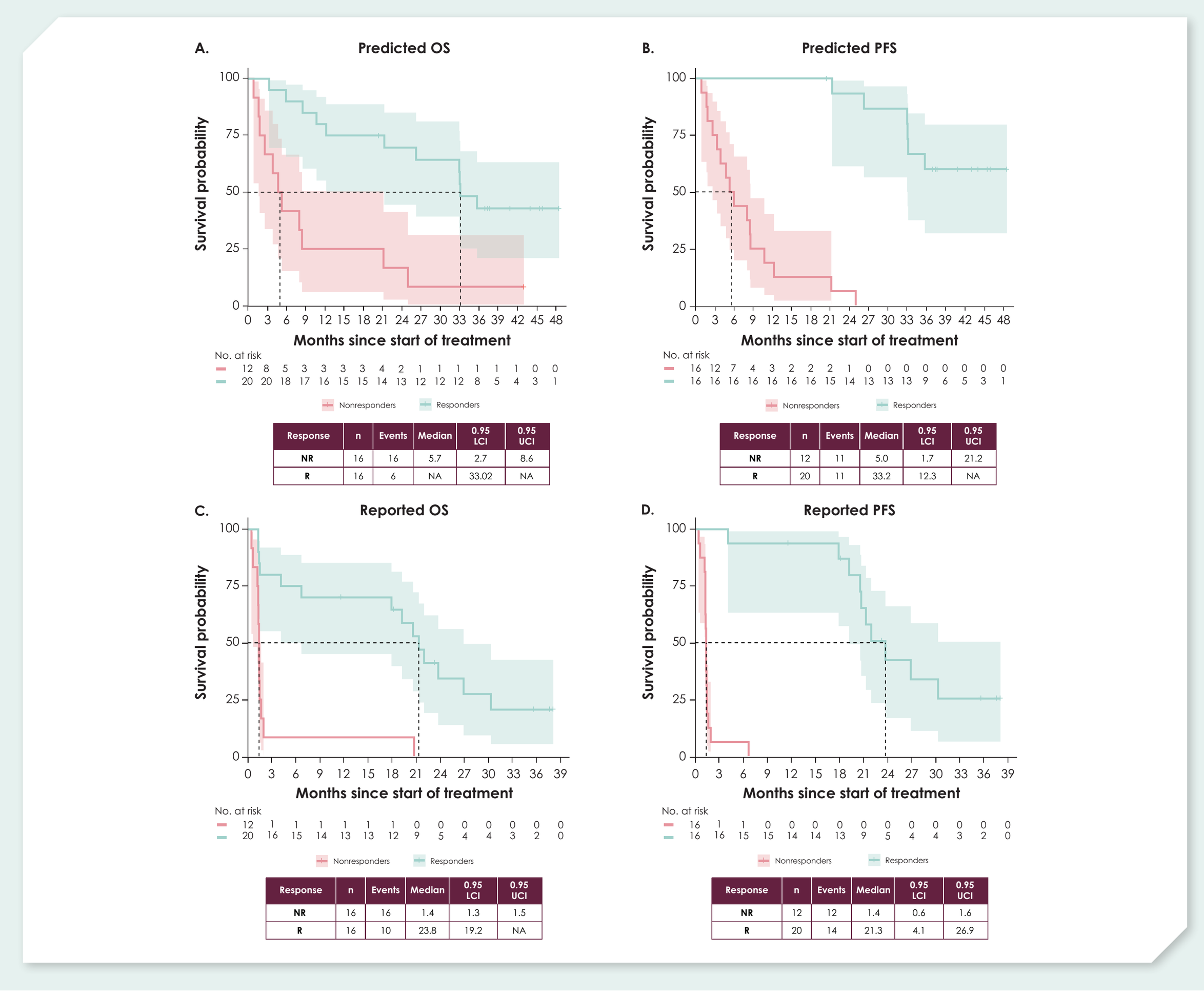
- A second classifier containing 10/14 of the original markers was trained on batch 1 samples in order to make it applicable to a 2L NSCLC pembrolizumab data set (**Figure 4B**)
- The 10-marker classifier achieved high PPV (**Figure 4B**), validating its ability to predict response in an independent data set
- The classifier performance using the pembrolizumab data set also suggested that 2L NSCLC responders to pembrolizumab have similar genomic characteristics to avelumab responders
- There were significant differences in mean OS and PFS in the response groups predicted by the 14-marker model (**Figure 5A-D**)

Figure 4. Generation and validation of 2L NSCLC signatures



All predictions are averages from 10-fold coefficient of variance

Figure 5. Reported and predicted OS/PFS for the 2L test set



- Differences in survival were more meaningful when analyzed according to PD-L1 expression (**Figure 6**)
- The classifier predictions had independent power to predict OS and PFS as well as response when multivariate models were fit. This performance was independent of multiple PD-L1 expression thresholds
- An example multivariate model for OS according to PD-L1 expression of ≥1% is shown (**Table 3**)

Figure 6. Efficacy according to PD-L1 expression

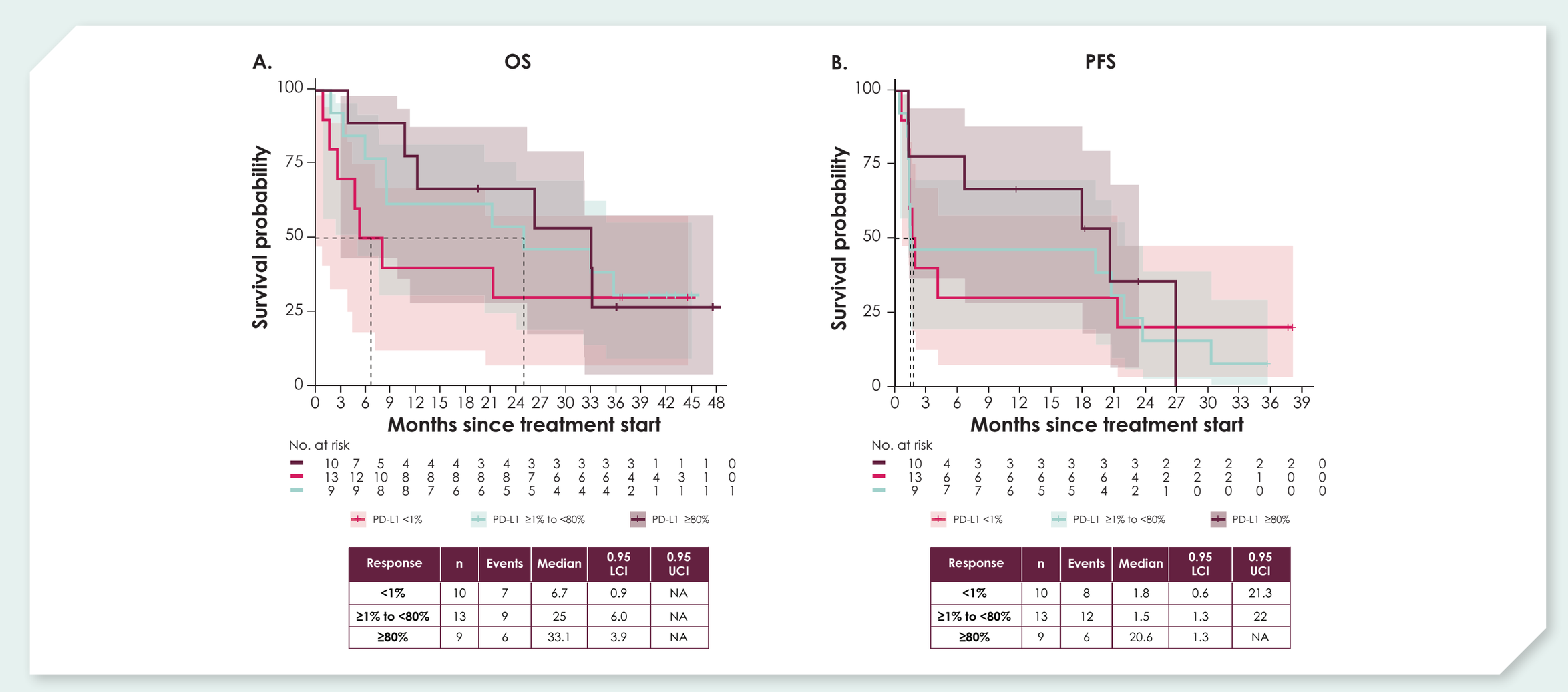


Table 3. Multivariate OS model

Predictor	Level	HR (95% CI)	p value
Predicted response	(NR) R	0.22 (0.09-0.53)	0.00075
Sex	(F) M	2.18 (0.85-5.63)	0.11
ECOG PS	(0) ≥1	2.05 (0.87-4.8)	0.1
Histology	(Adenocarcinoma) Squamous cell carcinoma	1.18 (0.45-3.13)	0.74
	(adenocarcinoma) Other	1.37 (0.39-4.86)	0.62
Baseline tumor volume		1.01 (0.99-1.02)	0.29
PD-L1 ≥1%	(NE) Negative	0.18 (0.02-1.55)	0.12
	(NE) Positive	0.42 (0.16-1.09)	0.07

CONCLUSIONS

- This work describes a 14-marker classifier, developed using the EpiSwitch platform, which performed similarly in both test and independent data sets
- The classifier uses binary information from patient epigenomes, requires few examples to learn, and generalized well on independently generated test data sets
- The classifier was generated with baseline profiles of patients with NSCLC who received avelumab and could predict response from the baseline profiles of patients who received pembrolizumab, suggesting similarities in patient epigenomic characteristics
- Classifier predictions provided independent information in multivariable models for predicting survival as well as response
- The JAVELIN Solid Tumor trial lacked a comparator arm; it is therefore not possible to understand whether these markers are predictive or prognostic
- We are in the process of validating this classifier in a blinded manner and in a data set that reflects real trial outcomes and those that have a comparator arm
- See poster P143 for a treatment line agnostic classifier developed using this data set

REFERENCES

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2. Barlesi F, et al. Lancet Oncol. 2018;19:1468-79.

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