# Development and validation of baseline predictive biomarkers for response to immuno-checkpoint treatments in the context of multiline and multitherapy cohorts using EpiSwitch™ epigenetic profiling

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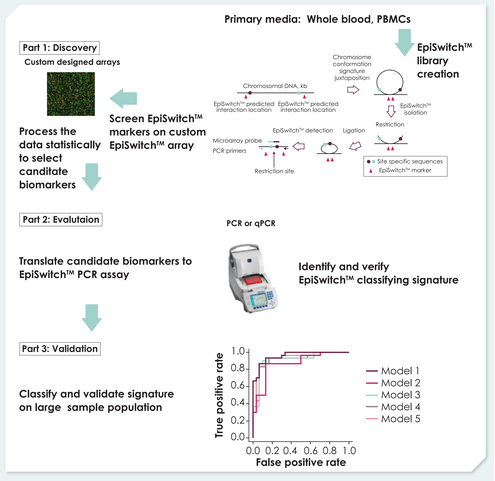
## INTRODUCTION

- The EpiSwitch biomarker discovery platform detects systemic changes in the cellular genomic architecture using a microarray and PCR-based biomarker platform (**Figure 1**).[1] It identifies and monitors chromosome conformation signatures (CCSs), key regulatory processes that integrate environmental cues and genetic alterations into the genomic regulatory machinery
  - CCSs are also known as gene loops, chromosome domains, or long-range chromosomal interactions
- CCSs are the primary step in a cascade of genomic regulatory events and are directly linked with cellular phenotype. CCSs have low off-rates and only change when a physiologically signaled transition to a new phenotype occurs or because of external intervention. As these events are binary, they can be measured by changes in other cellular entities, such as DNA methylation and RNA and protein expression
- We profiled patients with non-small cell lung cancer (NSCLC) and melanoma treated with avelumab (an anti–PD-L1 antibody), pembrolizumab (an anti–PD-1 antibody), or pembrolizumab in combination with the chemotherapeutic agent azacitidine (**Table 1**) and generated models to differentiate responders from nonresponders using machine learning methods

### Figure 1. EpiSwitch biomarker methodology: from discovery to clinical assay




### Table 1. Data sets analyzed using EpiSwitch

| Clinical response per RECIST 1.1 | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatment, n** | **Disease** | **CR** | **PR** | **SD** | **PD** | **NE** | **Total** |
| Avelumab | 1L NSCLC | 1 | 17 | 10 | 16 | 6 | 50 |
| Avelumab | 2L NSCLC | 2 | 17 | 8 | 21 | 1 | 49 |
| Pembrolizumab | 2L NSCLC | 0 | 7 | 12 | 25 | 0 | 44 |
| Pembrolizumab | Melanoma | 4 | 0 | 0 | 4 | 0 | 8 |
| Pembrolizumab + azacitidine | 2L NSCLC | 0 | 9 | 2 | 29 | 0 | 40 |

**1L**, first-line; **2L**, second-line; **CR**, complete response; **NE**, not evaluable; **PD**, progressive disease; **PR**, partial response; **SD**, stable disease

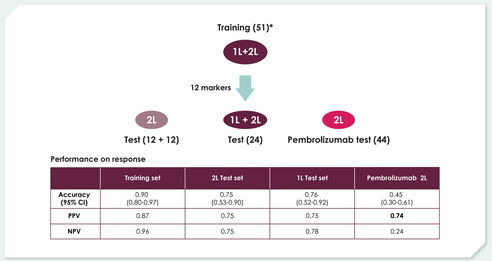
## METHODS

- The classifiers were generated in 2 steps using n-fold cross-validation:
  - Step 1: identification of univariate association between biomarkers and response using Fisher exact test
  - Step 2: generation of multivariate model using XGBoost or regularized regression
- A multivariable Cox proportional hazards model was built using R package survival and plotted using Survminer
- For building multivariate models, appropriate clinical covariates were selected based on analysis of clinical trial data from the JAVELIN Lung 200 trial (avelumab vs docetaxel in patients with platinum-treated advanced NSCLC; NCT02395172)[2]
- A multivariable logistic regression model was built with glm function

## RESULTS

- The 2L NSCLC classifier, built using baseline profiles of avelumab-treated patients, achieved good generalization performance for predicting response in a 2L NSCLC test set; however, this classifier did not have acceptable classification performance for predicting response in 1L NSCLC (see poster P142)
- This was also the case when the baseline 1L NSCLC classifier was used to predict response in a 2L NSCLC test set (see poster P142)
- However, the composite classifier trained using baseline profiles of both 1L and 2L patients achieved good generalized performance for both treatment lines (**Figure 2**)
- This classifier also had good positive-predictive value (PPV) when identifying responders to pembrolizumab from baseline profiles (**Figure 2**)
  - Pembrolizumab, like avelumab, inhibits the PD-L1/PD-1 pathway
- However, the negative predictive value (NPV) was reduced, suggesting that responders to both avelumab and pembrolizumab may have similar profiles but the profiles of non-responders to these drugs could be different

### Figure 2. Generation of indication-agnostic NSCLC classifier



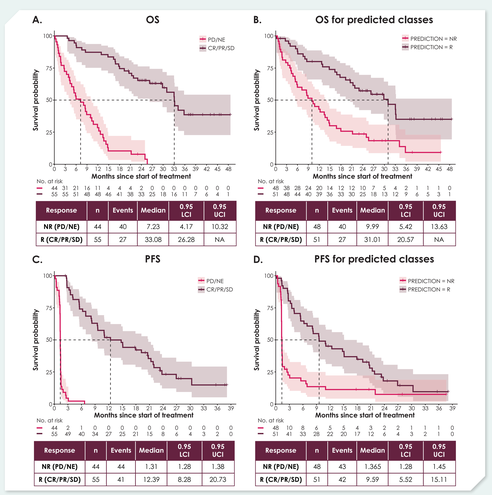

**Performance on response**

| | Training set | 2L Test set | 1L Test set | Pembrolizumab 2L |
|---|---|---|---|---|
| **Accuracy (95% CI)** | 0.90 (0.80-0.97) | 0.75 (0.53-0.90) | 0.76 (0.52-0.92) | 0.45 (0.30-0.61) |
| **PPV** | 0.87 | 0.75 | 0.75 | **0.74** |
| **NPV** | 0.96 | 0.75 | 0.78 | 0.24 |

* Patients with SD were taken as responders and used in training/test

- There was a significant difference (log-rank p<0.001) in progression-free survival (PFS) and overall survival (OS) between the predicted response groups (**Figure 3**)
- This survival difference was not observed with PD-L1 when patients were stratified at different thresholds of PD-L1 positivity (**Figure 4**)

### Figure 3. OS plots for classifier predictions




| Response | n | Events | Median | 0.95 LCI | 0.95 UCI |
|---|---|---|---|---|---|
| NR (PD/NE) | 44 | 40 | 7.23 | 4.17 | 10.32 |
| R (CR/PR/SD) | 55 | 27 | 33.08 | 26.28 | NA |

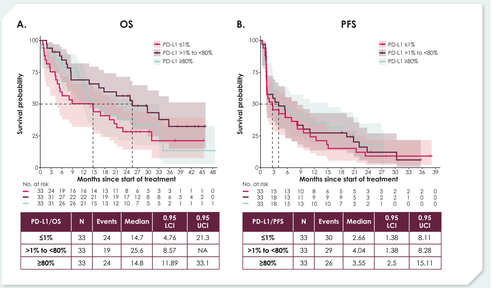
| Response | n | Events | Median | 0.95 LCI | 0.95 UCI |
|---|---|---|---|---|---|
| NR (PD/NE) | 48 | 40 | 9.99 | 5.42 | 13.63 |
| R (CR/PR/SD) | 51 | 27 | 31.01 | 20.57 | NA |

| Response | n | Events | Median | 0.95 LCI | 0.95 UCI |
|---|---|---|---|---|---|
| NR (PD/NE) | 44 | 44 | 1.31 | 1.28 | 1.38 |
| R (CR/PR/SD) | 55 | 41 | 12.39 | 8.28 | 20.73 |

| Response | n | Events | Median | 0.95 LCI | 0.95 UCI |
|---|---|---|---|---|---|
| NR (PD/NE) | 48 | 43 | 1.365 | 1.28 | 1.45 |
| R (CR/PR/SD) | 51 | 42 | 9.59 | 5.52 | 15.11 |

**LCI**, lower confidence interval; **NA**, not available; **NR**, no response; **R**, response; **UCI**, upper confidence interval

### Figure 4. Survival plots for PD-L1 subgroups




| PD-L1/OS | N | Events | Median | 0.95 LCI | 0.95 UCI |
|---|---|---|---|---|---|
| ≤1% | 33 | 24 | 14.7 | 4.76 | 21.3 |
| >1% to <80% | 33 | 19 | 25.6 | 8.57 | NA |
| ≥80% | 33 | 24 | 14.8 | 11.89 | 33.1 |

| PD-L1/PFS | N | Events | Median | 0.95 LCI | 0.95 UCI |
|---|---|---|---|---|---|
| ≤1% | 33 | 30 | 2.66 | 1.38 | 8.11 |
| >1% to <80% | 33 | 29 | 4.04 | 1.38 | 8.28 |
| ≥80% | 33 | 26 | 3.55 | 2.5 | 15.11 |

- The composite classifier had independent predictive power when assessed in the context of a multivariable Cox proportional hazards model (**Table 2**) and a multivariable logistic regression model (data not shown)

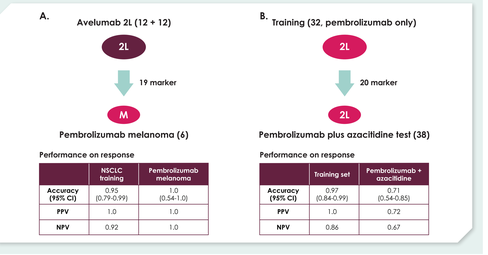
### Table 2. Multivariable survival model suggesting independent power of classifier

| Term | Level* | Exp (coef) | 95% LCI | 95% UCI | p value |
|---|---|---|---|---|---|
| **(Intercept)** | | 1.27 | 0.19 | 8.36 | 0.8 |
| **Prediction** | (NR) R | 45.07 | 12.86 | 212.99 | 0 |
| **Treatment line** | (1L) 2L | 0.93 | 0.26 | 3.29 | 0.92 |
| **Sex** | (F) M | 0.15 | 0.03 | 0.55 | 0.01 |
| **ECOG PS** | (0) ≥1 | 0.54 | 0.15 | 1.89 | 0.35 |
| **Histology** | (Adenocarcinoma) Squamous cell carcinoma | 4.25 | 0.89 | 24.14 | 0.08 |
| **Histology** | (Adenocarcinoma) Other | 1.6 | 0.12 | 29.07 | 0.74 |
| **Tumor volume at baseline** | Tumor volume at baseline | 0.99 | 0.97 | 1 | 0.09 |
| **PD-L1 (1%)** | (Nonevaluable) Negative | 0.63 | 0.06 | 5.66 | 0.68 |
| **PD-L1 (1%)** | (Nonevaluable) Positive | 1.51 | 0.32 | 7.25 | 0.59 |

**ECOG PS**, Eastern Cooperative Oncology Group performance status
* Level in brackets suggests baseline category

- We also demonstrated that classifiers could be built with power to predict response across indications (**Figure 5A**) and combination treatment settings (**Figure 5B**)
- As observed in the avelumab and pembrolizumab 2L NSCLC analysis, the pembrolizumab monotherapy and combination classifiers had lower NPV, suggesting potential differences in non-responder profiles (**Figure 5**)

### Figure 5. Application of monotherapy signatures to predict response across indications and for treatment combinations




**Performance on response**

| | NSCLC training | Pembrolizumab melanoma |
|---|---|---|
| **Accuracy (95% CI)** | 0.95 (0.79-0.99) | 1.0 (0.54-1.0) |
| **PPV** | 1.0 | 1.0 |
| **NPV** | 0.92 | 1.0 |

**Performance on response**

| | Training set | Pembrolizumab + azacitidine |
|---|---|---|
| **Accuracy (95% CI)** | 0.97 (0.84-0.99) | 0.71 (0.54-0.85) |
| **PPV** | 1.0 | 0.72 |
| **NPV** | 0.86 | 0.67 |

## CONCLUSIONS

- **Profiling patients who received avelumab or pembrolizumab with EpiSwitch demonstrated that:**
  - **It is possible to derive predictive signatures from baseline patient profiles that are applicable across agents with similar modalities, treatment lines, and indications**
  - **The biomarkers assayed using the EpiSwitch platform had high effect size because the training process requires a small number of samples to train**
  - **The signatures were small in size, provided good generalization on test sets (eg, PPV>0.7), and had independent predictive power**
- **Limitations**
  - **The single-arm design did not allow differentiation between the prognostic and predictive values of the classifiers**
- **Future work**
  - **Apply the classifier(s) to a blinded/independent test set that reflects real-world populations of a clinical trial and that also has a comparator arm**

## REFERENCES

1. Tordini F, et al. Front Genet. 2016;7:194.
2. Barlesi F, et al. Lancet Oncol. 2018;19:1468-79.

## ACKNOWLEDGMENTS

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