# PREDICTION OF CLINICAL RESPONSE

## PREDICTION ALGORITHM DEVELOPMENT

Patients in the study cohort were first classified as responders or non-responders according to clinical benefit (for the 3- and 6-month time points) and durable clinical benefit (for the 12-month time point), as assessed by RECIST 1.1 or other validated methods for response evaluation. Specifically:

- Responders were defined as patients displaying complete response (CR), partial response (PR) or stable disease (SD); Non-responders were defined as patients displaying progressive disease (PD).
- Responders were defined as patients displaying 12-months progression-free survival (PFS) with continued treatment; Patients who stopped treatment before the 12 months mark due to adverse events but displayed no signs of progression at the 12 months mark were also defined as responders. All other patients were classified as non-responders.

The study cohort (n=339) was randomly divided into development (n=254) and validation (n=85) sets while maintaining an equal distribution of main clinical features between the two sets (see Fig. 1). The validation set was set aside, and the algorithm was developed using the development set data only. After completion of algorithm development, the performance of the prediction algorithm was assessed in a blinded manner on the independent validation set. This is a stringent practice, that was adopted to ensure reliable assessment of performance and to avoid common sources of over estimation of computational algorithms, such as overfitting and data leakage.

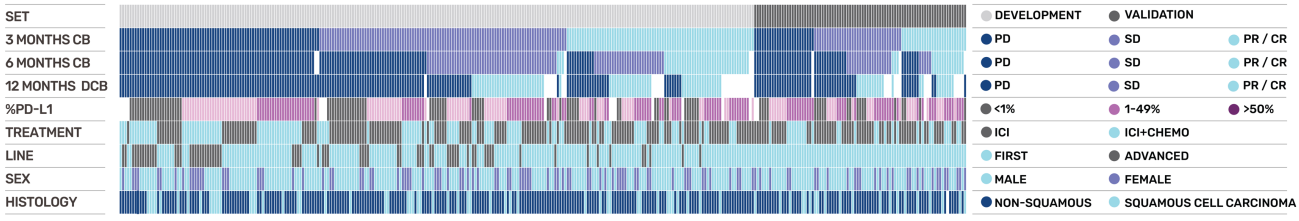


*Figure 1: Visual representation of the study cohort. Each patient is represented by a bar, where in each row a different demographic or clinical parameter is shown according to the color code indicated in the legend on the right. CB, Clinical benefit. DCB, Durable Clinical Benefit.*

*PD, Progressive Disease. SD, Stable Disease. PR, Partial Response. CR, Complete Response. ICI, Immune Checkpoint Inhibitor*

A prediction algorithm was constructed for each time point (3, 6 and 12 months). The 7,000 proteins measured by the SomaScan assay were first filtered to narrow down the dataset to 1,578 proteins with analytical reliability. Proteins displaying differential plasma levels in responder and non-responder populations were identified by the Kolmogorov-Smirnov statistical test. These proteins, termed Response Associated Proteins (RAPs), serve as potential indicators of treatment response, depending on their plasma level in the individual patient; Each protein identified as a RAP is a potential signal sign, which is set to an "off" or "on" state in a given patient. The "off/on" status is based on the similarity of that protein level in the given patient to the statistics of the responder population (in which case, the signal will be set to "on" state), or to the statistics of the non-responder population (in which case the signal will be set to "off" state). The number of signals in an "on" state is indicative of the response probability, where a patient with many response signals is expected to respond, and a patient with few response signals is expected to develop resistance. Finally, the number of response signals together with clinical parameters, are taken as predictive features by a machine learning algorithm. The final output of the algorithm is a response probability– a clinically oriented metric reflecting the patient's likelihood of responding to treatment at a specific time point during treatment (see Fig. 2).

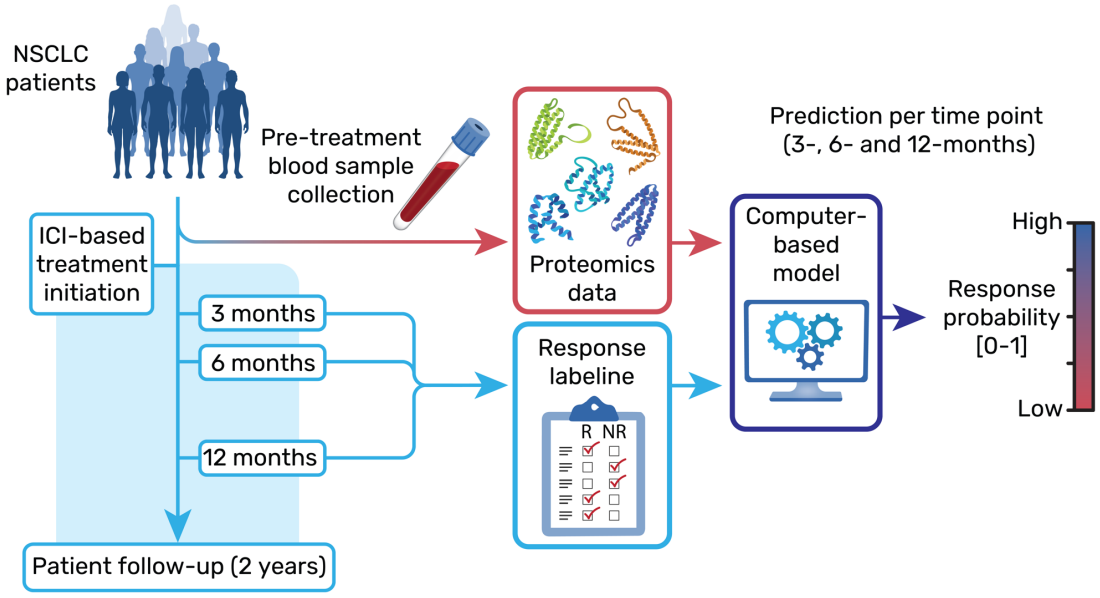

*Figure 2. Illustration of the PROphet® prediction algorithm. A cohort of advanced NSCLC patients receiving ICI-based therapy was assembled. Pre-treatment blood samples were obtained, and plasma proteomes were profiled using SomaScan technology. Response to treatment was assessed at 3, 6 and 12 months after starting treatment, and patients were followed up for 2 years. A predictive model for ICI response was developed for each response assessment time point using machine learning, resulting in response probability for 3, 6 and 12 months.*

## PREDICTION ALGORITHM VALIDATION

The performance of the prediction algorithm was examined in the independent validation set. To test the accuracy of the prediction algorithm, the predicted response probability was compared to the observed response rate. The latter is given by the fraction of responders among a group of patients that were assigned a similar response probability by the prediction algorithm. Specifically, the observed response rate was calculated for a range of $\pm 0.15$ around the corresponding predicted response probability. For example, the observed response rate for predicted response probability of 0.8, is given by the fraction of responders among the group of patients that were assigned predicted response probability between 0.65 and 0.95. The agreement between the predicted response probability and the observed response reaches a goodness of fit of $R^2=0.94$ with respect to the diagonal y=x, as shown in Fig. 3.

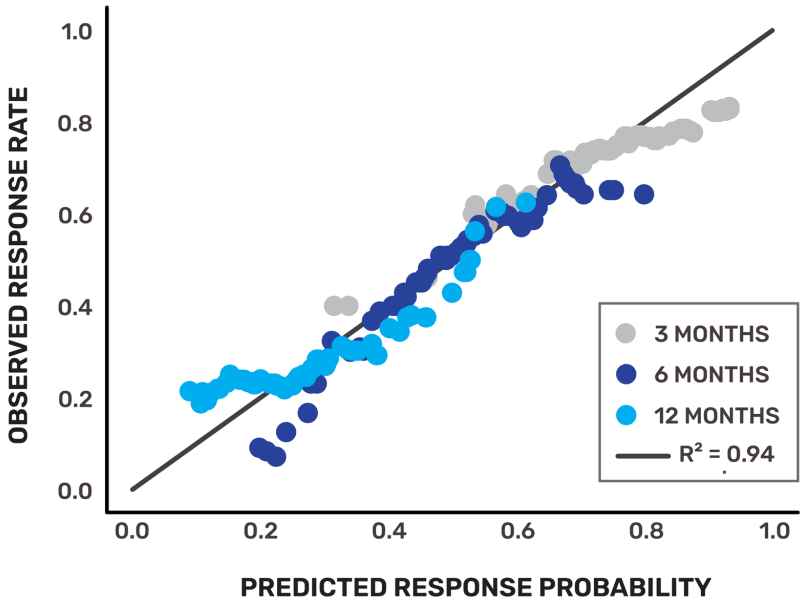


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*Figure 3: Predicted response probability as a function of observed response rate. Each dot represents a patient in the validation set. For each patient, the observed response rate is plotted against the predicted response rate for all three time points: 3, 6 and 12 months are indicated by gray, dark and light, respectively. For details regarding the calculation of the observed response rate see text. The diagonal y=x is indicated by a black line (goodness of fit $R^2$=0.94; see text).*

# OUTCOME ANALYSIS

## STRATIFICATION TO FAVORABLE OR UNFAVORABLE OUTCOME

In addition to response probability, the prediction algorithm stratifies each patient to one of two populations: (i) favorable outcome or (ii) unfavorable outcome (Fig. 4). The outcome in this context is in terms of survival: PFS and OS. A patient with a with Favorable outcome prediction is likely to follow the clinical outcome trend depicted by the green line in the survival curves (favorable outcome population). A patient with an unfavorable outcome prediction is likely to follow the clinical outcome trend depicted by the red line in the survival curves (unfavorable outcome population).

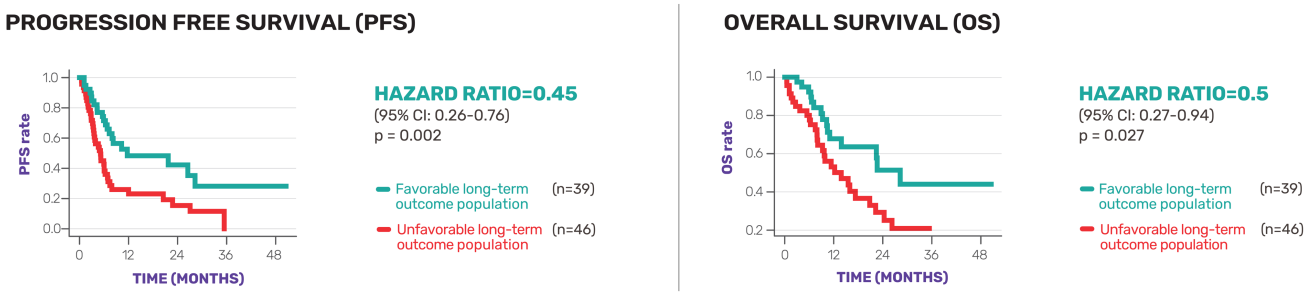


*Figure 4: The PROphet® test is predictive of clinical outcomes in NSCLC patients treated with standard of care. In a blinded study population, patients that were predicted to have favorable outcome, showed a considerably better prognosis than patients with an unfavorable outcome prediction, as demonstrated in the Kaplan-Meier curves. Patients in the favorable outcome group survived significantly longer than patients in the unfavorable outcome group.*

*The stratification quality is quantified by the hazard ratio as was calculated using Cox-regression for the validation set patients.*

## Progression-free survival (PFS)

PFS is defined as the time from treatment initiation to disease progression or death from any cause

## Overall survival (OS)

OS is defined as the time from treatment initiation to death from any cause.

## Standard of care

ICI therapy as a single agent or in combination with chemotherapy.