Methods

The PROphet® test was developed based on clinical, demographic, and proteomic data of 625 patients within the framework of an ongoing clinical study conducted by OncoHost (PROPHETIC; NCT04056247). Plasma was isolated from the blood and profiled via the SomaScan® Assay v4.1[1-2], and the resulting proteomic profiles were analyzed in conjunction with patient clinical data. The clinical benefit prediction algorithm was developed to identify patients who are more likely to benefit from PD-1/PD-L1 inhibitor treatment. Patient clinical benefit probability is predicted and linearly transformed to a PROphet® Score between 0 to 10. The PROphet® model development and validation are described in more detail below and in Christopoulos et. al.[3]. The analytical validity of the PROphet® test is described in detail in Yellin et. al.[4].

Prediction of Clinical Response

Study Cohort

Out of the entire study cohort of n=625, and based on patients’ clinical parameters, monitoring duration and treatment modality, specific subcohorts were analyzed for Part I and Part III of the report. Specifically, for development and validation of the clinical benefit model, a subcohort of n=500 patients was examined, undergoing PD-1/PD-L1 inhibitor treatment with or without chemotherapy, for which progression was monitored for at least 12 months. Model performances were validated and present only on first line patients. For the Clinical Evidence survival analysis presented in Part III, a subcohort of n=470 patients was examined, including 444 first-line patients undergoing PD-1/PD-L1 inhibitor treatment and 26 advanced-line patients with PD-L1 1-49% undergoing PD-1/PD-L1 inhibitor treatment as a single agent. In addition, an independent cohort of n=85 patients, including first-line patients treated with chemotherapy as a single agent, was included as a comparator.

PROphet® Model

Patients in the model cohort were first classified as patients with Clinical Benefit (CB) or patients with No Clinical Benefit (NCB) according to their progression-free survival (PFS) status at 12 months, as assessed by RECIST 1.1 or other validated methods for response evaluation. To account for variations in a patient’s 12-month clinical evaluation, we set a range of 330-400 days. Specifically, we categorized patients as experiencing CB if progressive disease (PD) was observed beyond 400 days or if no progression occurred up to 330 days. Conversely, patients were classified as receiving NCB if disease progression occurred within 400 days of treatment initiation. Patients without an event and <330 days of follow-up were excluded rather than censored for the purpose of model training. The study cohort (n=500) was divided into development (n=228) and validation (n=272) sets (Fig. 1). The validation set was put aside, and the PROphet® model was developed using the development data set only. After development completion, performance of the PROphet® model 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 overestimation of computational algorithm performance, such as overfitting and data leakage[5].

FIGURE 1.

Distribution of main clinical parameters of the PD-1/PD-L1 inhibitors cohort. The distribution of patient sex, ECOG performance status, tumor histology, PD-L1 expression, treatment modality, clinical benefit at 12 months and patient age are displayed.
Methods (continued)

To develop the PROphet® model, 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 CB and NCB populations were identified by the Kolmogorov-Smirnov statistical test. These proteins, termed Resistance Associated Proteins (RAPs), serve as the basis for the PROphet® model (Fig. 2).

A prediction model was trained using RAP expression levels and patient sex as input. The model output is a predicted CB probability, which is then linearly transformed into the PROphet® Score – a number which ranges between 0 (lowest clinical benefit probability) to 10 (highest clinical benefit probability). The PROphet® Score is a clinically validated metric reflecting the patient’s likelihood of CB and prolonged overall survival when treated with PD-1/PD-L1 inhibitors as a single agent or in combination with chemotherapy (see blinded validation results below).

Based on the PROphet® Score, a PROphet® result of POSITIVE (for PROphet® Score ≥5) or NEGATIVE (PROphet® Score <5) is assigned to the test, and treatment considerations that are based on the PROphet® result and the patient’s PD-L1 tumor proportion scores are provided.

FIGURE 2.

**The PROphet® Score algorithm - from protein levels to PROphet Score®.** A. The development set is represented by a matrix, where each column represents a measured protein, and each row represents a patient. For RAP selection, the Kolmogorov-Smirnov test was performed on the development set to identify proteins displaying statistically significant differences between plasma level distributions in CB and NCB populations. The highlighted column represents a RAP. B. Shown is the plasma level distribution of a single RAP in CB and NCB populations. RAP expression levels in two representative patients are indicated by vertical dashed lines. In ‘Patient I’ (light blue), the RAP expression level follows the CB population statistics, whereas in ‘Patient II’ (dark blue), the RAP expression level follows the NCB population statistics. Accordingly, the algorithm infers a prediction of CB and NCB for Patient I and Patient II, respectively. Predictions are inferred per RAP. As such, patients are assigned a collection of predictions based on their RAP profile. C. Shown here are the RAP profiles for Patient I and Patient II. RAPs are presented as nodes. A filled node indicates an NCB prediction, and an empty node indicates a CB prediction. The circled node represents the RAP described in B that yields a prediction of CB for Patient I and NCB for Patient II. The functional association between RAPs is represented by an edge. D. The collection of all predictions across the entire RAP set is taken as input for the PROphet® algorithm to infer the PROphet® Score and subsequent PROphet® NEGATIVE or PROphet® POSITIVE classifications.

**PROphet® Model Blinded Validation**

The performance of the PROphet® model was examined on an independent validation set (n=272). To test algorithm accuracy, predicted CB probability was compared to the observed CB rate, where the latter refers to the proportion of observed CB patients within the group of patients assigned a similar CB probability by the PROphet® model. Specifically, the observed CB rate was calculated for a range of ±0.05 around the corresponding predicted CB probability. The agreement between predicted CB probability and observed CB reaches a goodness of fit of $R^2=0.98$ (Fig. 3A). Additionally, the model ability to predict benefit in overall survival was evaluated using a Kaplan-Meier survival analysis (Fig. 3B). The PROphet® POSITIVE class showed survival benefit compared to PROphet® NEGATIVE, with a Hazard Ratio (HR) of 0.51 (CI: 0.37-0.7), p-value < 0.001.
**Methods (continued)**

**FIGURE 3.**

PROphet® model performance evaluation.

A. Observed clinical benefit rate as a function of predicted clinical benefit (CB) probability. Each dot represents a patient in the validation set. The observed CB rate is plotted against the predicted CB probability. Goodness of fit, $R^2=0.98$. B. Kaplan-Meier analysis comparing overall survival of PROphet® POSITIVE and PROphet® NEGATIVE classes.

**About PROphet®**

The PROphet® platform combines bioinformatics, system biology, proteomic pattern recognition and machine learning in a cancer patient’s blood plasma to predict clinical benefit probability (probability for at least 12 months progression-free survival) in response to PD-1/PD-L1 inhibitors - as a single agent or in combination with chemotherapy.

The PROphet® NSCLC Test is a novel and robust predictive computational model that uses SomaScan® Assay V4.1 to analyze and identify proteomic profiles in pre-treatment blood plasma to predict the probability of clinical benefit when treated with PD-1/PD-L1 inhibitors. The analytical validity of the PROphet® test is described in detail in Yellin et. al.[5].

The PROphet® NSCLC Score is a clinically validated metric reflecting the patient’s likelihood of clinical benefit (defined as a progression-free survival of at least 12 months) and prolonged overall survival when treated with PD-1/PD-L1 inhibitors as a single agent or in combination with chemotherapy. The PROphet® Score ranges between 0 (lowest clinical benefit probability) to 10 (highest clinical benefit probability) and is based on identification of differentially expressed proteins using a novel and robust predictive computational model that analyzes proteomic patterns in pre-treatment plasma.

**Intellectual Property**

PROphet® is protected by trademarks and patent applications.

**Intended Use**

The PROphet® NSCLC Test is intended for use as a treatment decision tool in the management of newly diagnosed stage IV non-small cell lung cancer (NSCLC) patients being considered for treatment with PD-1/PD-L1 inhibitors as a single agent or in combination with chemotherapy in the first-line setting. The test is intended for patients aged 18 and above, with ECOG performance status of 0-2, normal hematologic, renal, and liver functions. The test is not intended for patients with any concurrent and/or other active malignancies that have required systemic treatment within two years of the first dose of therapy.

**OncHost Disclaimer**

All content herein related to the PROphet® result, PROphet® Score, treatment options and clinical evidence is provided for informational purposes only. The patient’s physician is responsible for considering all available information and options before making patient-specific management or treatment decisions. OncHost is not liable for medical judgment regarding diagnosis, prognosis, or treatment. PROphet® Test results and information contained within this report are current as of the date provided and will not be updated by OncHost, even if subsequent changes would have led to additional or conflicting results. PROphet® uses an internal data set with data gathered from various sources. The analyses may be subjected to certain biases that restrict the generalizability or applicability to individual patients. The data that comprise the PROphet® dataset may not represent patient populations as a whole, nor be relevant to this specific patient. Testing is performed by OncHost’s CLIA-certified and COLA-accredited laboratory located in the USA. The test was developed, and its performance characteristics were determined by OncHost LTD. The test has not been cleared or approved by the FDA.

**References**

3. Christopoulos, P. Plasma Proteome–Based Test for First-Line Treatment Selection in Metastatic Non–Small Cell Lung Cancer. JCO PO, 2024. 8e2300555.