Methods

The PROphet® model 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 inhibitors. Patient clinical benefit probability is predicted and transformed to a PROphet® score between 0 to 10.

This test was conducted using V2.1 of PROphetNSCLC®. The PROphet® model development and validation are described in more detail below and in Christopoulos et. al.[3] The analytical validity of the PROphetNSCLC® test is described in detail in Yellin et al.[4]. The protocol adaptation for shipping blood samples using Streck cfDNA BCT® tubes has been supported by an internal validation study (data not shown).

Prediction of Clinical Benefit

Study Cohort

Out of the entire study cohort of n=625, and based on patient clinical parameters, monitoring duration, and treatment modality, specific sub-cohorts were analyzed for various sections of the report. Specifically, for development and validation of the clinical benefit model, a sub-cohort of n=500 patients undergoing PD-1/PD-L1 inhibitor treatment with or without chemotherapy was examined, for which progression was monitored for at least 330 days. Model performance was validated and presented only on first-line patients. For the survival analyses presented in the PROphetNSCLC® report, a sub-cohort of n=444 patients undergoing PD-1/PD-L1 inhibitor treatment was examined and compared with an independent cohort of n=85 first-line patients treated with chemotherapy as a single agent.

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 in 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 was done to ensure reliable assessment of performance and to avoid an overestimation of computational algorithm performance, such as overfitting and data leakage.[5]

FIGURE 1.

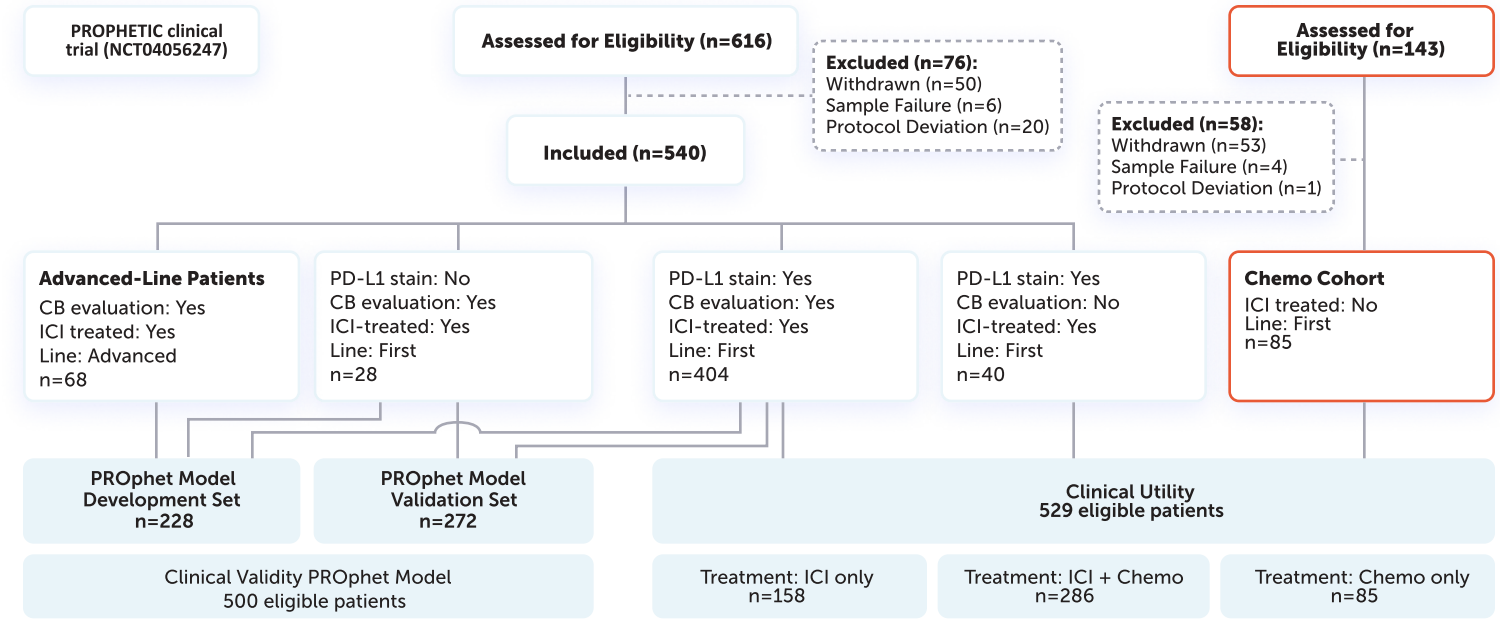

Fig. 1: PROphet® Model Development, Validation and Clinical Evidence Cohort. *The PROPHETIC study assessed patients with NSCLC who were treated with a PD-1/PD-L1 inhibitor (n=616, n=540 after exclusions). In addition, a comparative control group of chemotherapy-treated patients was included (n=143, n=85 after exclusions), resulting in a total of n=625 patients after exclusions.*

Methods (continued)

To develop the PROphet® model, the 7,596 proteins measured by the SomaScan® assay were first filtered to narrow down the dataset to 1,578 proteins with analytical reliability, based on the proteins stability across different plasma separation methods[3]. 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).

Independent prediction models were trained for each one of the RAPs, using their expression level as an input. The model output is a predicted CB probability, obtained by summing thresholded predictions for each RAP model. The predicted probability is then 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 the validation results in Fig. 3).

Based on the PROphet® score, a PROphetNSCLC® test 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 level are provided.

FIGURE 2.

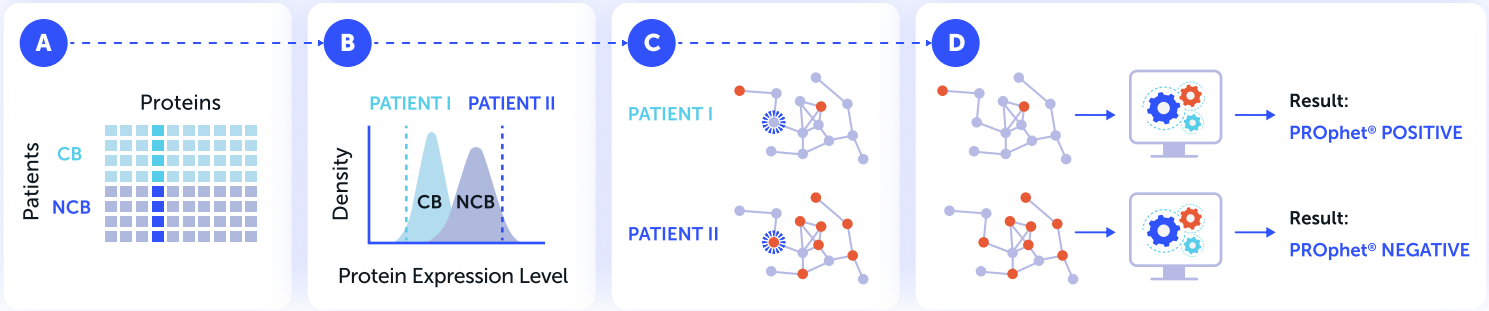

Fig. 2: The PROphet® Score Algorithm: From Protein Levels to PROphet® Score. ***A.*** *The development set is represented by a matrix, where each column represents a measured protein and each row represents a patient. For RAP selection, the Kolmogorov-Smirnov test was performed on the development set 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 Validation

The reported performance of the PROphet® model was examined on an independent set (n=272) as part of the validation of PROphetNSCLC® V2.1. 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=0.93 (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 0.47 (CI: 0.34-0.65), p-value <0.0001.

Methods (continued)

FIGURE 3.

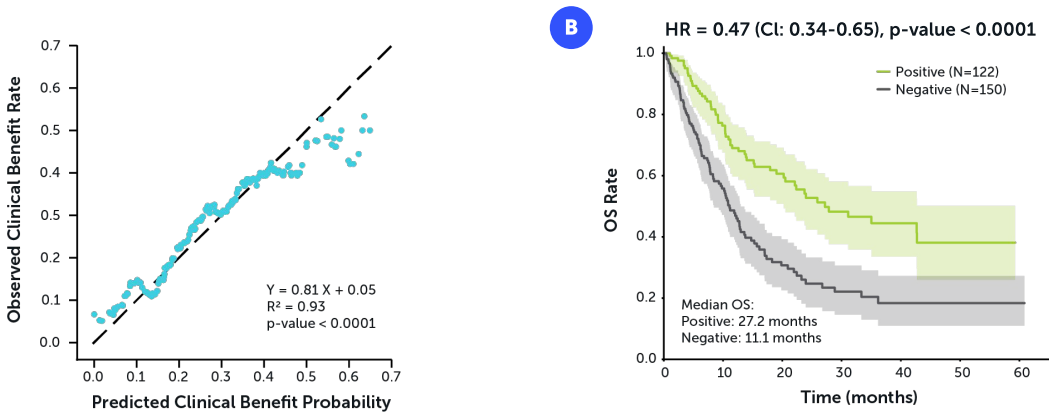

Fig. 3: PROphet® Model Performance Evaluation. ***A.*** *Observed clinical benefit rate as a function of predicted clinical benefit (CB) probability. Each dot represents a patient in the validation set. The observed CB rate is plotted against the predicted CB probability. Goodness of fit, R^2=0.93.* ***B.*** *Kaplan-Meier analysis comparing overall survival of PROphet® POSITIVE and PROphet® NEGATIVE classes.*

About PROphet®

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

The PROphetNSCLC® test is a novel and robust predictive computational model that uses SomaScan® Assay V4.1[3-4] 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 PROphetNSCLC® test is described in detail in Yellin et al.[4]

The PROphet® score is a clinically validated metric reflecting the patient's likelihood of clinical benefit (CB).[1] CB is defined as no evidence of progressive disease (PD) within 12 months post treatment initiation based on radiographic imaging according to RECIST version 1.1, or other clinical criteria consistent with PD. The PROphet® score ranges between 0 (lowest CB probability) to 10 (highest CB probability). A PROphet® result of POSITIVE (score ≥5) or NEGATIVE (score <5) is assigned, and treatment considerations based on the PROphet® result and the patient's PD-L1 tumor level are provided.

Analytical Sensitivity

The PROphet® model utilizes 388 biomarkers, each with characterized analytical attributes including limit of background, a measure of analytical sensitivity provided by the assay manufacturer. All of the PROphet® model biomarkers are measured above their limit of sensitivity.

Clinical Sensitivity

The PROphet® score is a continuous metric reflecting the patient's likelihood of CB, validated using two standard metrics for continuous predictions: the area under the receiver operating characteristic curve (ROC AUC) [6] and the correlation between predicted CB probability and observed CB rate [7]. Of note, for a continuous model result (such as probability for clinical benefit) binary (classification) performance measures (such as sensitivity, specificity, NPV and PPV) do not apply [6].

Intellectual Property

PROphet® and PROphetNSCLC® are protected by patents applications and registered trademarks.

Intended Use

The PROphetNSCLC® 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.

OncoHost 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. OncoHost 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 OncoHost, even if subsequent changes would have led to additional or conflicting results. PROphet® uses an internal dataset 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 OncoHost's CLIA-certified and CAP-accredited laboratory located in the USA (CLIA ID: 34D2250951, CAP ID: 9586418) and is authorized in all US states, including New York and California (NYS PFI: 9895, CA ID: CDS-90008562). The test was developed, and its performance characteristics were determined by, OncoHost Ltd. The test has not been cleared or approved by the FDA.

References

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