A novel, highly accurate liquid biopsy-based glycoproteomic predictor of checkpoint inhibitor treatment benefit in advanced non-small cell lung cancer

Chad Pickering, Apoorva Srinivasan, Alan Mitchell, Gege Xu, Xin Cong, Daniel Serie, Klaus Lindpaintner
InterVenn Biosciences, South San Francisco, CA

Premise

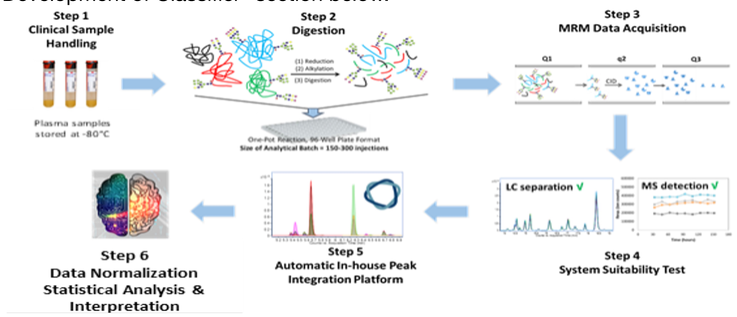
Over the last decade, the availability of immune checkpoint inhibitor (ICI) therapeutics has significantly advanced the clinical management and outcome of patients with a range of malignancies, including metastatic non-small-cell lung cancer (NSCLC). Yet, only about 30-40% of patients obtain sustained clinical benefit from single-agent ICI therapy, and neither Programmed death-ligand 1 (PDL-1) expression levels nor tumor mutational burden or microsatellite instability measures have, by and large, been found helpful as indicators of durable clinical benefit.

As an alternative domain of biomarkers, we leverage protein glycosylation, the most abundant and complex form of post-translational modification that profoundly affects protein structure and function. We have developed a powerful platform that combines liquid chromatography/mass spectrometry (LC-MS) with a proprietary AI-based high-throughput data processing engine (Peak-Integration Platform, PIP) that allows, for the first time, scalable high-resolution, glycan-conjugation-site-, and protein-specific interrogation of the glycoproteome.

We interrogated 532 glycopeptides (GPs) derived from 75 serum proteins in pre-treatment blood samples from a cohort of 123 individuals (54 females, 69 males, age range 30 to 88 years). Inclusion criteria were as follows: a diagnosis of unresectable stage 3 or 4 NSCLC, treatment with pembrolizumab monotherapy (26 patients), or treatment with combination pembrolizumab-chemotherapy (97 patients) as either first- or later-line therapy. Overall survival (OS) data were available for all patients.

Methods

Peptides and GPs from peripheral blood were identified in a pilot study by data-independent acquisition MS and translated into multiple reaction monitoring (MRM) panels to be run on high-throughput triple quadrupole MS instruments. MRMs can be viewed as hypothesis-driven instructions for the MS to scan for specific GPs. Among them, a panel of 532 GPs was selected for the targeted quantitative analysis of trypsin-digested Streck®-tube plasma samples using the workflow illustrated below. OS was used as the clinical endpoint against which the predictive power of differential abundance of GPs as well as some non-glycosylated peptides was assessed using the analytical methods outlined in the "Development of Classifier" section below.



Development of Classifier

Initial pilot cohort (n=42) yielded leave-one-out cross-validated Cox model-based classifier that used 6 GPs to predict ICI-therapy response based on OS (HR=7.4, P=0.008)

Full cohort (n=123) yielded 72 biomarkers (49 GPs + 23 non-glycosylated peptides) that were either strongly associated with OS or were chosen in initial cross-validated least absolute shrinkage and selection operator (LASSO)-regularized Cox- or tree-based models

After optimization of the 72 biomarkers, the full cohort (n=123) yielded a LASSO-regularized Cox-based generalized additive model (GAM) using 7 biomarkers (results at right)

Results

Cohort Information

	All	Training	Test
Total sample size	123	88 (72%)	35 (28%)
Female sex	54 (44%)	39 (44%)	15 (43%)
Age (yrs.; mean, SD)	67.7 (10.5)	67.8 (9.9)	67.5 (11.9)
Ancestry			
Caucasian/White	81 (66%)	58 (66%)	23 (66%)
African American	15 (12%)	12 (14%)	3 (9%)
Other	5 (4%)	3 (3%)	2 (6%)
Missing	22 (18%)	15 (17%)	7 (20%)
Death events	73 (59%)	53 (60%)	20 (57%)
OS (months; median, 95% CI)	11.6 (8.9, 18.9)	11.2 (8.4, 16.4)	13.9 (8.5, NR)
Treatment			
ICI+Biologic+ChemoRx	1 (1%)	1 (1%)	0 (0%)
ICI+Chemo	91 (74%)	65 (74%)	26 (74%)
ICI+ChemoRx+TKI	5 (4%)	4 (5%)	1 (3%)
ICI monotherapy	26 (21%)	18 (20%)	8 (23%)
First-line therapy	104 (85%)	77 (88%)	27 (77%)
Histology			
Non-squamous	96 (78%)	69 (78%)	27 (77%)
Squamous	20 (16%)	14 (16%)	6 (17%)
Unknown	7 (6%)	5 (6%)	2 (6%)
Mutation status			
EGFR mutant/ALK variant	12 (10%)	8 (9%)	4 (11%)
EGFR wild type	111 (90%)	80 (91%)	31 (89%)

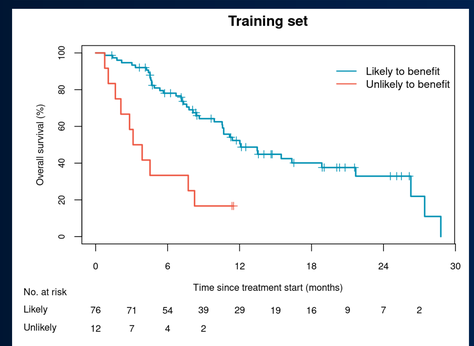
Classifier prediction	Events/N	Median OS (95% CI)	HR (95% CI)	P-value
Training set (n=88)				
Likely to benefit	43/76	12.1 (10.6, NR)	Reference	
Unlikely to benefit	10/12	3.5 (2.1, NR)	4.0 (1.9, 8.6)	3.5×10^{-4}
Test set (n=35)				
Likely to benefit	13/27	23.2 (13.9, NR)	Reference	
Unlikely to benefit	7/8	5.9 (2.9, NR)	10.8 (2.9, 41.0)	4.6×10^{-4}
Full cohort (n=123)				
Likely to benefit	56/103	13.9 (11.2, 26.3)	Reference	
Unlikely to benefit	17/20	4.2 (2.8, 8.5)	4.1 (2.3, 7.4)	3.6×10^{-6}
First-line & non-squamous+squamous, training set (n=72)				
Likely to benefit	38/64	11.2 (8.6, 21.7)	Reference	
Unlikely to benefit	7/8	4.2 (3.1, NR)	2.8 (1.2, 6.8)	0.021
First-line & non-squamous+squamous, test set (n=26)				
Likely to benefit	7/19	23.2 (19.3, NR)	Reference	
Unlikely to benefit	6/7	4.7 (2.2, NR)	13.1 (3.0, 57.3)	6.1×10^{-4}
First-line & non-squamous+squamous, full cohort (n=98)				
Likely to benefit	45/83	13.5 (10.7, NR)	Reference	
Unlikely to benefit	13/15	4.5 (2.9, 8.9)	3.9 (2.0, 7.7)	7.1×10^{-5}

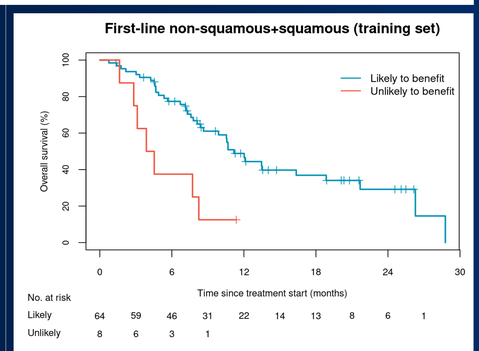
	Full cohort (n=123)		First-line & non-sq.+squamous (n=98)	
Variable	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Classifier prediction	3.6 (2.1, 6.3)	6.7×10^{-6}	3.5 (1.8, 6.6)	1.2×10^{-4}
Age*	1.0 (1.0, 1.0)	0.638	1.0 (1.0, 1.0)	0.825
Sex (male ref.)*	0.8 (0.5, 1.3)	0.335	0.8 (0.5, 1.4)	0.404
Monotherapy (ref.) vs not*	1.2 (0.7, 2.2)	0.551	1.5 (0.7, 3.0)	0.263
First-line (ref.) vs not*	1.5 (0.8, 2.8)	0.189	Not applicable	
Non-sq. (ref.) vs not*	1.4 (0.8, 2.3)	0.259	Not applicable	
Wild type (ref.) vs not*	0.8 (0.3, 1.7)	0.498	1.9 (0.3, 14.2)	0.514

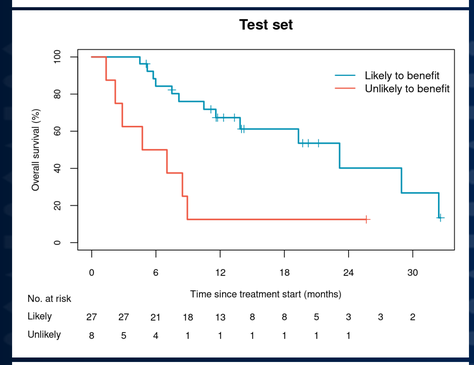
TKI: tyrosine kinase inhibitor; EGFR: epidermal growth factor receptor; ALK: anaplastic lymphoma kinase; SD: standard deviation; HR: hazard ratio; CI: confidence interval; NR: not reached; * indicates results are adjusted for classifier prediction

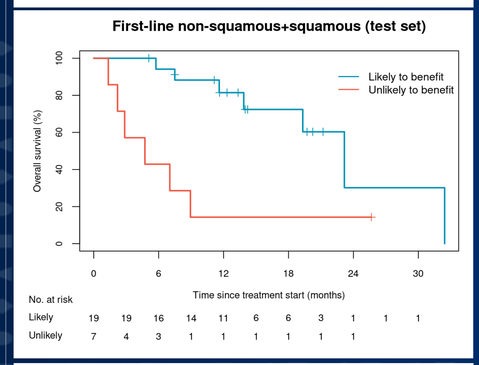
Predictive Cox Modeling of ICI Benefit Likelihood

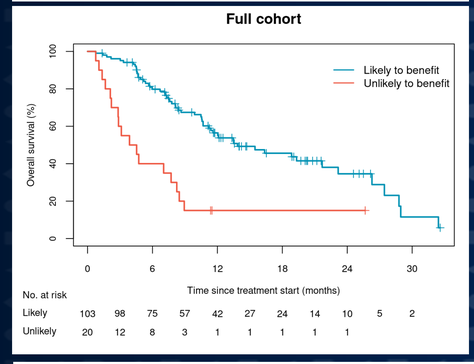
The ensemble glycoproteomic classifier was developed using a majority of the full cohort (n=88) and validated in the remainder of patients (n=35), yielding similar statistical significance in Cox regression analysis for separating patients who are likely to benefit from ICI therapy and those who are not, with a sensitivity of >95% to accurately predict likely ICI benefit while performing at a specificity of 33% to predict those who are unlikely to benefit. Results were further analyzed in patients with either non-squamous or squamous NSCLC with first-line therapy (n=98). HRs corresponding to the Kaplan-Meier plots below are adjusted for age, sex, ICI therapy category, and, for results involving the full cohort, first-line therapy, histology, and mutation status. Tick marks on plots represent censoring times.

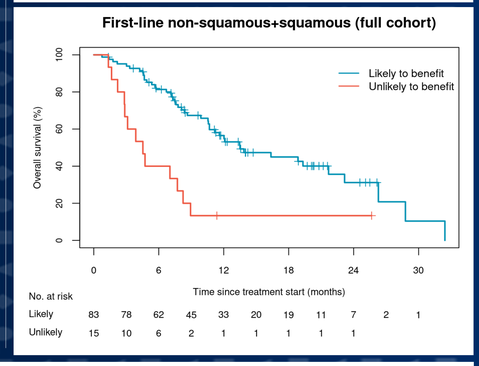












Conclusions

The glycoproteomic classifier described here predicts with high sensitivity which patients are likely to benefit from ICI therapy. In addition to potentially reducing the use of ICIs in a safe manner in patients who would be unnecessarily subjected to possible adverse drug reactions, our classifier has the potential of safely reducing the burden of health care expenditure. Our results indicate that glycoproteomics holds a strong promise as a predictor for ICI treatment benefit, outperforming other currently available biomarkers.