Abstract 9545

Glycoproteomics as a powerful liquid biopsy-based predictor of checkpoint-inhibitor treatment benefit in metastatic malignant melanoma

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Premise

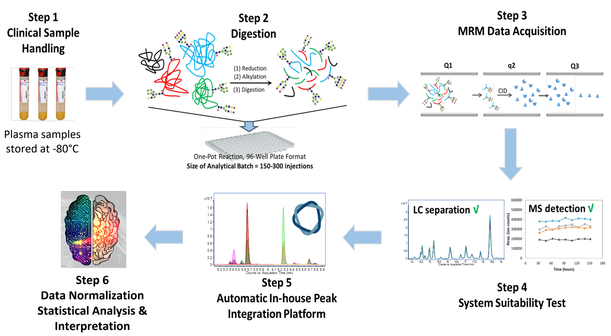
Over the last decade, the availability of immune checkpoint inhibitor (ICI) therapeutics has significantly advanced the clinical management and outcome of patients with a range of malignancies, including metastatic melanoma. Yet, only about 30-40% of patients obtain sustained clinical benefit from single-agent ICI therapy, and neither PDL-1 expression levels nor tumor mutational burden have been found helpful as indicators of durable clinical benefit.

As an alternative slate 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 that allows, for the first time, scalable high-resolution, site-specific interrogation of the glycoproteome.

We interrogated 532 glycopeptides (GPs) derived from 75 serum proteins in pre-treatment blood samples from a cohort of 205 individuals sourced from Massachusetts General Hospital (MGH) with metastatic melanoma treated with either nivolumab plus ipilimumab or pembrolizumab ICI therapy in order to build a machine learning-based classifier to predict likelihood of ICI benefit.

Methods

Peptides and GPs from peripheral blood were identified in a prior study by data-independent acquisition. Among them, a panel of >500 GPs was selected for the targeted quantitative analysis of trypsin-digested plasma samples using the workflow illustrated below. Progression-free survival (PFS) was used as the clinical endpoint against which the predictive power of differential abundance of GPs and non-glycosylated peptides was assessed using the analytical methods outlined in the "Development of Classifier" subsection at right.



Development of Classifier

MGH-sourced initial pilot cohort (n=36) yielded leave-one-out cross-validated classifier that used 5 GPs to predict ICI therapy response (HR=7.438, P=0.007)

MGH-sourced full cohort (n=205) yielded 47 biomarkers (27 GPs + 20 non-glycosylated peptides) that were either strongly associated with PFS via univariate Cox regression or were chosen in initial cross-validated LASSO-regularized Cox Proportional Hazards or tree-based models

After biomarker optimization, training set in MGH-sourced cohort (n=81) yielded an ensemble classifier for PFS containing 5 GPs and 5 non-glycosylated peptides by LASSO shrinkage

Classifier was validated in the remaining MGH-sourced samples (n=124)

Preliminary external validation from University of South Australia (n=27) further validates final classifier (HR=10.291, P=0.007)

Results

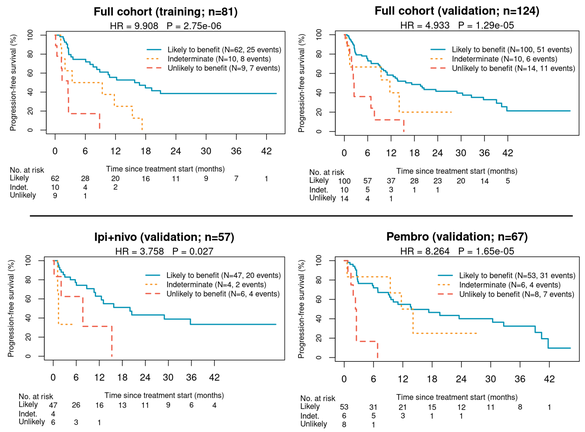
Cohort Information

	Full cohort	Training	Validation
Total sample size*	205	81 (39.5)	124 (60.5)
Med. follow-up (mo.)**	30.8 (24.8, 37.3)	25.1 (21.7, 37.7)	32.7 (26.9, 37.8)
Time to progr. (mo.)**	11.8 (9.4, 16.8)	10.5 (7.6, 17.9)	13.1 (9.6, 20.5)
Female sex†	66 (32.2)	24 (29.6)	42 (33.9)
Age at trt. start (yr.)‡	63.9 (13.6)	64.7 (12.2)	63.6 (13.0)
Had prior ICI†	74 (36.1)	31 (38.3)	43 (34.7)
Current ICI therapy†			
Ipilimumab+nivolumab	95 (46.3)	38 (46.9)	57 (46.0)
Pembrolizumab	110 (53.7)	43 (53.1)	67 (54.0)
BRAF mutation status†			
Wild type	107 (64.1)	46 (68.7)	61 (61.0)
V600	51 (30.5)	18 (26.9)	33 (33.0)
Non-V600	9 (5.4)	3 (4.5)	6 (6.0)
Unknown	38	14	24
LDH (units/L)†			
≤ULN	98 (56.6)	38 (55.1)	60 (57.7)
>ULN	75 (43.4)	31 (44.9)	44 (42.3)
>2×ULN	18 (10.4)	6 (8.7)	12 (11.5)
Unknown	32	12	20
Metastasis staging†			
M0	15 (8.3)	8 (11.1)	7 (6.5)
M1	165 (91.7)	64 (88.9)	101 (93.5)
Unknown	25	9	16
Melanoma subtype†			
Acral	5 (2.8)	3 (4.2)	2 (1.9)
Cutaneous	112 (62.2)	43 (59.7)	69 (63.9)
Mucosal	18 (10.0)	11 (15.3)	7 (6.5)
Uveal	15 (8.3)	5 (6.9)	10 (9.3)
Unknown primary	30 (16.7)	10 (13.9)	20 (18.5)
Missing/unknown	25	9	16
ECOG performance status†			
0	109 (61.2)	35 (50.0)	74 (68.5)
1	56 (31.5)	25 (35.7)	31 (28.7)
≥2	13 (7.3)	10 (14.3)	3 (2.8)
Unknown	27	11	16

* n (row-wise %); ** median (95% CI); † n (column-wise % excluding unknown group); ‡ mean (SD)

Predictive Cox Modeling of ICI Benefit Likelihood

The ensemble classifier was developed using 40% of the full cohort (n=81) and validated in the remaining 60% of patients (n=124), yielding similar statistical significance for separating patients who are likely to benefit from ICI therapy and those who are not, with a sensitivity of >99% to accurately predict likely ICI benefit while still performing at a specificity of 26% to accurately predict those who are unlikely to benefit. Results were validated in each ICI therapy group (see figures at right), as well as in patients with prior ICI therapy and those without. Hazard ratios at right and below are adjusted for age, sex, and, for those describing the full cohort, baseline ICI therapy.



All adjusted HRs above are with respect to the contrast likely to benefit (reference) vs unlikely to benefit

Performance in Predicted Categories in Validation Set

	Events/N (%)	Median PFS (95% CI)	HR (95% CI)	P-value
Overall	68/124 (54.8)	6.77 (2.47, 15.89)		
Likely to benefit	51/100 (51.0)	17.34 (11.02, 32.07)	reference	
Indeterminate	6/10 (60.0)	11.71 (1.38, NR)	1.70 (0.68, 4.26)	0.255
Unlikely to benefit	11/14 (78.6)	2.50 (1.84, NR)	4.93 (2.41, 10.11)	1.29×10^{-5}

HR: Hazard Ratio; NR: Not Reached; HRs are adjusted for age, sex, and baseline ICI therapy

Conclusions

- Our glycoproteomic-based blood test classifies patients with advanced melanoma as either likely or unlikely to derive PFS benefit from either pembrolizumab monotherapy or ipilimumab and nivolumab combination therapy. The test can help clinicians make shared decisions with patients in terms of expectations for benefit from standard-of-care immunotherapy treatment options, and considerations for treatment alternatives. Our test may also be useful for selecting patients for clinical trials of novel therapies by enriching for patients most likely to derive benefit.
- InterVenn's proprietary perspectIV™ platform, based on a high-throughput LC-MS/MS-AI/ML approach, interrogates the blood glycoproteome at an unprecedented depth and scale, revealing a new domain of biology for the identification of novel biomarkers and diagnostic tools in a broad range of applications.