Fucose signatures in peripheral blood glycoproteins are associated with reduced clinical benefit of immune-checkpoint inhibitors in metastatic melanoma

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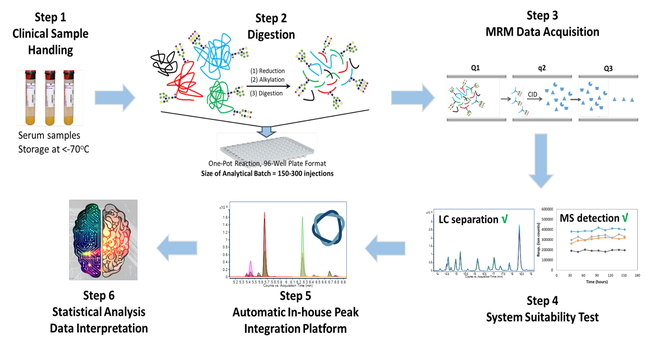
Premise: the need of methods to identify patients that do not respond to treatment with immune-checkpoint inhibitors

The clinical success of immune-checkpoint inhibition (ICI) in melanoma has confirmed the merit of therapeutic strategies that boost the immune system to counteract cancer, leading to a sea change in treatment approaches and patient outcomes. However, only about half of patients derive a long-lasting benefit[1]. While elevated PD-L1 expression and tumor mutational burden correlate with the likelihood to benefit from ICI therapy in some indications, these biomarkers have shown poor predictive performance in metastatic melanoma[2]. By applying InterVenn's glycoproteomics platform to pre-treatment plasma samples from metastatic melanoma patients receiving anti-PD-1/anti-CTLA-4 therapy, we previously identified a panel of biomarkers that differentiate patients likely to derive a benefit from those unlikely to benefit from ICI[3]. A laboratory-developed test based on these findings, DAWN™ IO Melanoma, was developed and has been recently introduced into the market.

InterVenn's glycoproteomics platform

We have developed a powerful platform that combines liquid chromatography and 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 with metastatic malignant melanoma treated with either nivolumab plus ipilimumab or pembrolizumab ICI therapy in order to build a machine learning model to predict likelihood of ICI benefit.



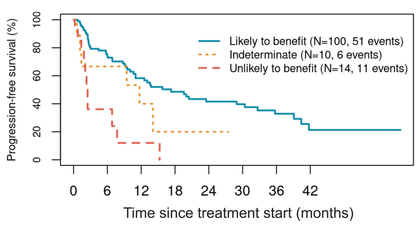
Cohort Information

	Full cohort	Training	Validation/test
Total sample size*	205	81 (39.5)	124 (60.5)
Time to death (mo.)**	13.5 (6.0, 30.3)	12.3 (5.9, 25.7)	14.3 (7.1, 32.8)
Time to progr. (mo.)**	5.8 (2.1, 15.8)	2.7 (1.7, 15.3)	6.8 (2.5, 15.9)
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)
Sex†			
Male	139 (67.8)	57 (70.4)	82 (66.1)
Female	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)

* n (row-wise %); ** median (IQR); † n (column-wise %); ‡ mean (SD)

Development of DAWN-IO Melanoma test for stratification of patients treated with immune-checkpoint inhibitors

The cohort yielded 47 biomarkers (27 glycopeptides and 20 non-glycosylated peptides) that were either strongly associated with PFS or were chosen in initial cross-validated LASSO-regularized Cox or tree-based models. Using 47 optimized biomarkers, the cohort yielded an ensemble classifier comprising 10 biomarkers.



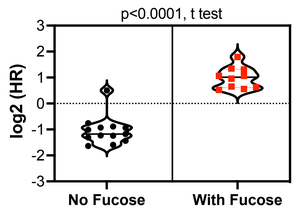
INTERVENN

We believe that no one should ever be blindsided by disease

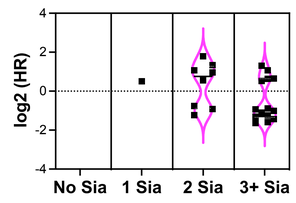
N-linked fucosylation in plasma proteins is associated with reduced clinical response to ICI therapy

Biomarkers showing a statistically significant association with PFS at FDR<0.05 were selected for analysis.

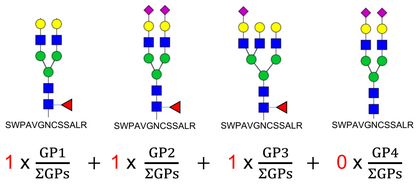
N-linked glycopeptides displayed differential associations with treatment outcome dependent on their fucosylation status. Fucosylated glycopeptides were statistically significantly associated with limited benefit of treatment.



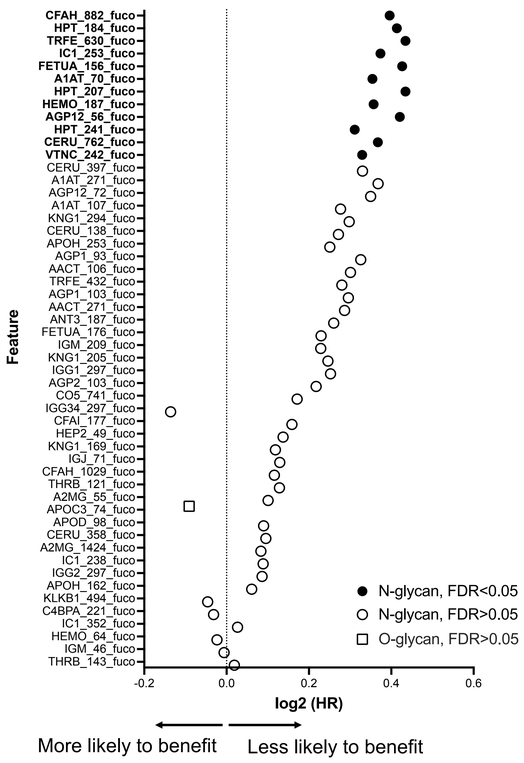
We analyzed sialic acid content in GPs, as alterations in sialic acid density in tumor cells have been extensively described in connection with immune evasion. However, there was no correlation between sialic acid levels in GPs N-glycans and benefit of ICI treatment



To test the validity of this observation, we engineered site-specific glycosylation features that represent the average number of specific monosaccharides at a given site, weighted by glycopeptide occupancy.

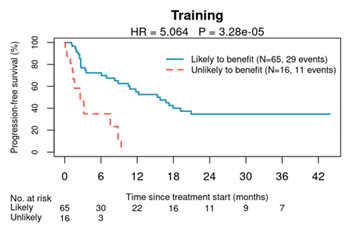


Of 52 fucose-dependent features across our full research assay, 12 were associated with benefit from ICI therapy based on univariate Cox regression analysis (FDR <0.05).

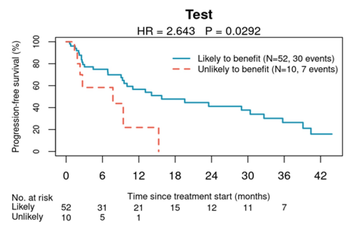


Site-specific fucose feature model stratifies patients for response to ICI treatment

Two features were ultimately retained in a repeated cross-validated LASSO-regularized Cox regression model on a training set consisting of 40% of the cohort, yielding a hazard ratio of 5.1 (P=3e-05).



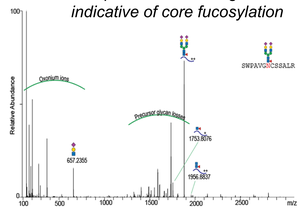
A validation set consisting of 30% of the cohort was used to tune model hyperparameters. When applied to the remaining 30% of the cohort, this tuned model resulted in a hazard ration of 2.6 (P=3e-02), indicating that fucose-dependent features stratified patients in groups that differ in the likelihood of response to ICI therapy (patients with a risk score exceeding the selected threshold were nearly three times less likely to respond).



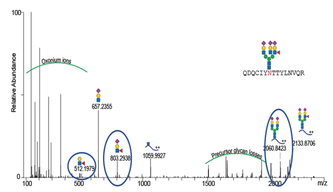
Characterization of fucose linkage in differentially expressed glycopeptides

Fragmentation analysis of glycopeptides from samples submitted to high resolution MS allowed assignment of specific fucose linkages.

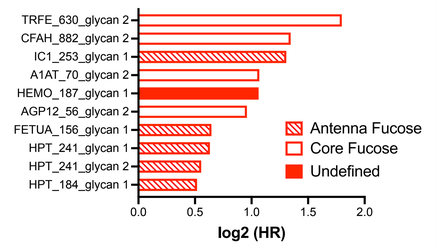
Pep+GlcNAcFuc fragment indicative of core fucosylation



Signature oxonium ions indicate antennary fucosylation

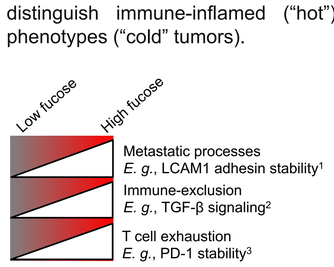


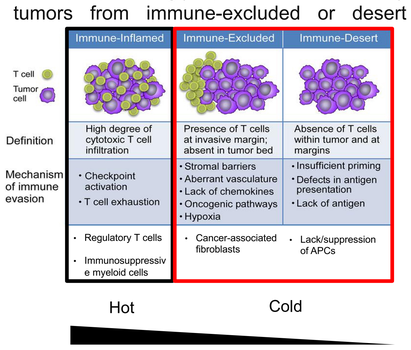
Fucose can be found linked to the GlcNAc in the core pentasaccharide structure ("core fucose") as the product of the glycosyltransferase Fut8. Other enzyme, including Fut7, Fut9 and Fut10, can modify the GlcNAc residue in the antenna glycan with a fucose moiety.



Correlation of serum signature with tumor immune state

We hypothesize that mechanisms that generate fucosylation signatures in circulating proteins might also lead to an increase of fucosylation in the tumor that may affect responses to ICI treatment. Fucosylation may alter efficacy of ICI treatment by modulating metastatic potential of cancer cells, access of lymphocyte to the tumor and T cell exhaustion. We propose that peripheral glycoprotein markers may distinguish immune-inflamed ("hot") tumors from immune-excluded or desert phenotypes ("cold" tumors).





Conclusions

- InterVenn's glycoproteomics platform identified differentially expressed markers in plasma samples from ICI pre-treatment melanoma patients. Biomarker analyses discovered fucosylation signatures in biomarkers of patients less likely to respond to treatment.
- Using engineered features that capture the presence of particular monosaccharides at specific glycosylation sites, we independently validated the observation that protein fucosylation is an indicator for determining whether patients are likely to derive benefit from ICI treatment.
- Initial structural analysis revealed that glycosylation signatures included both core and antenna fucose.

References

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