Evaluation of immune-related markers in the circulating proteome and their association with atezolizumab efficacy in patients with 2L+ NSCLC

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Introduction

Anti-PD-L1/PD-1 therapy has become a standard of care in NSCLC. However, understanding of the biological mechanisms of treatment efficacy and resistance is still incomplete. Here we examine the role of the circulating proteome in 2L+ NSCLC patients treated with atezolizumab (anti-PD-L1).

Patients and Methods

Patient Cohorts

- Development Cohort – 77 NSCLC patients treated with atezolizumab (NCT01375842)
- Blinded Validation Cohort – 270 NSCLC patients treated with atezolizumab or docetaxel in the POPLAR study (NCT01903993)

	Development (N = 77)	Validation (N = 262*)
Age, median (range)	60 (24-84)	62 (36-84)
Gender, n (%)		
Female	31 (40)	103 (39)
Male	46 (60)	159 (61)
Line of Therapy, n (%)		
1st	14 (18)	0 (0)
2nd	16 (21)	175 (67)
3rd or higher	47 (61)	87 (33)
Performance Status		
0	NA	84 (32)
1	NA	178 (68)
Smoking Status, n (%)		
Current	10 (13)	41 (16)
Former	52 (68)	170 (65)
Never	15 (19)	51 (19)
Histology, n (%)		
Non-squamous	60 (78)	172 (66)
Squamous	17 (22)	90 (34)
PD-L1 Status, n(%)		
IC0/TC0	NA	113 (43)/160 (61)
IC1/TC1	NA	103 (39)/37 (14)
IC2/TC2	NA	31 (12)/38 (15)
IC3/TC3	NA	15 (6)/27 (10)

*262/270 patients assigned test result

Profiling of Circulating Proteome

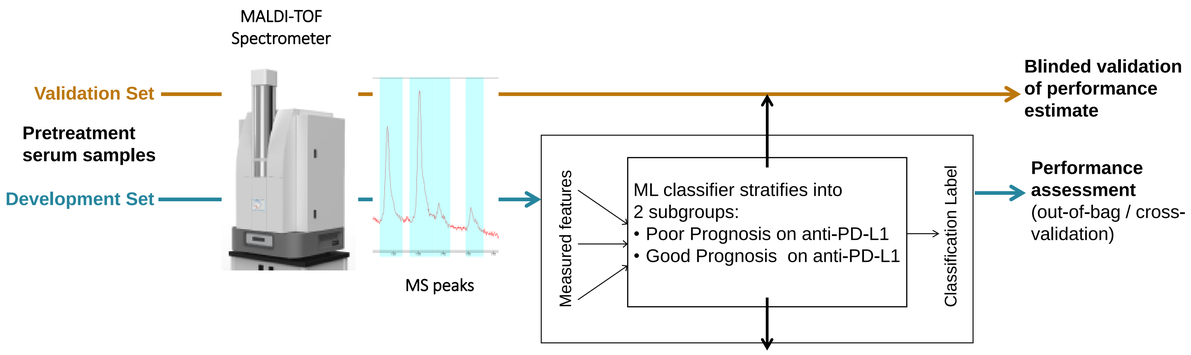
- Mass spectra generated from pretreatment serum samples using Deep MALDI® method to obtain expression data with dynamic range of 5 orders of magnitude
- Spectra processed to render them comparable; mass spectral features (peaks) defined
- Methods and parameters locked prior to running validation samples

Test Development

- Test to stratify patients into groups with better (Good) or worse (Poor) outcome on atezolizumab developed using machine learning (ML) platform designed for settings with more samples than molecular attributes
- Reliable results from development cohort generated using cross-validation-like approach
- All parameters fixed using only development cohort samples

Protein Set Enrichment Analysis (PSEA)

- SEA methods applied to an independent set of samples with both mass spectral and protein panel expression data available to assess the underlying biology of the test classification groups



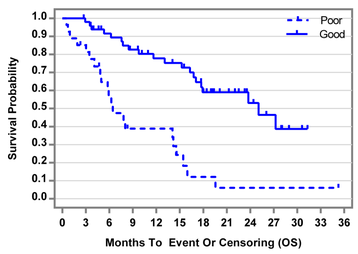
Set enrichment techniques evaluate relevance of important immune-related pathways to patient subgroups

Validation samples run blinded to all clinical and treatment data using fully locked procedure

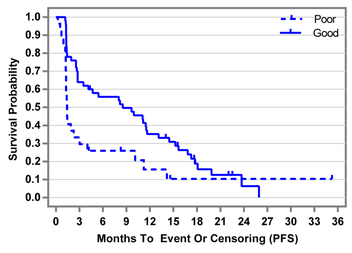
Results: Development

50 patients (65%) were assigned to the Good group and 27 (35%) to the Poor group

OS



PFS



	OS		PFS	
Median (95% CI) Poor in months	6.4 (95% CI:4.8-14.2)		1.4 (95% CI:1.2-3.0)	
Median (95% CI) Good in months	25.1 (95% CI: 17.1-undefined)		8.5 (95% CI:2.8-11.6)	
	p-value	HR (95% CI)	p-value	HR (95% CI)
Good vs Poor	<0.001	0.23 (0.12-0.44)	0.014	0.52 (0.31-0.88)
Good vs Poor (stratified by line of therapy)	<0.001	0.21 (0.10-0.42)	0.004	0.45 (0.26-0.78)

Response rate: 11% (Poor) vs 30% (Good) – Fisher's exact p = 0.090
Disease control rate: 29% (Poor) vs 64% (Good) – Fisher's exact p = 0.005

Results: Underlying Biology

PSEA indicated trends to association of increased complement activation, acute inflammation and immune response type 2 in the Poor classification phenotype.

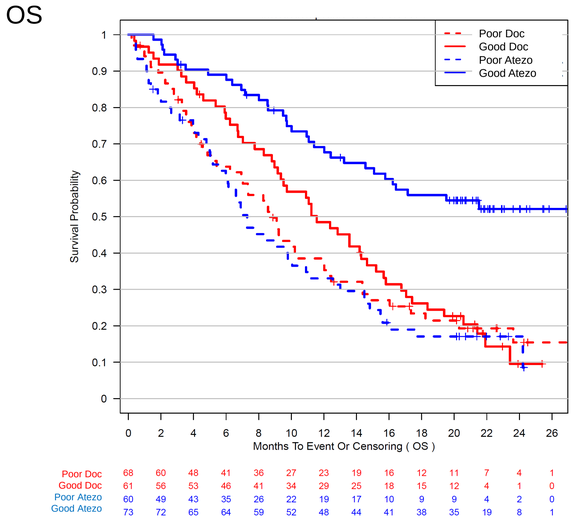
Biological Process	Enrichment Score	p value
Acute inflammation	0.372	0.075
Immune Response Type 2	0.730	0.077
Complement	0.450	0.089
Acute phase	0.458	0.110
Acute response	0.521	0.123
Interleukin-10	0.250	0.253
Immune response	0.235	0.260
Mesenchymal transition	0.386	0.480
Cancer Biomarkers	-0.197	0.488
Cell adhesion	-0.226	0.500
Immune B-cells	0.317	0.507
Adaptive immune response	-0.343	0.536
Cell cycle	-0.245	0.540
Hypoxia	0.256	0.602
Wound healing	-0.235	0.663
Extracellular matrix	-0.263	0.672
Angiogenesis	0.216	0.702
Interferon	0.222	0.731
Immune Response Type 1	-0.336	0.756
Innate Immune Response	0.301	0.807
Glycolytic Process	-0.317	0.824
GFR* signaling	-0.157	0.968
Immune T-cells	-0.153	0.981
NK regulation	0.176	0.984
Cytokine activity	0.151	0.987

Results: Blinded Validation

Predictive and prognostic properties of the test were evaluated in the setting of this randomized study

- 262/270 samples passed QC and could be classified
- 134 patients (51%) were assigned to the good outcome group

OS



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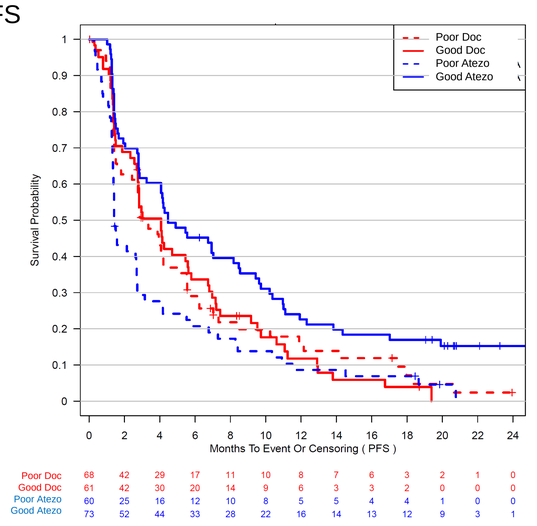
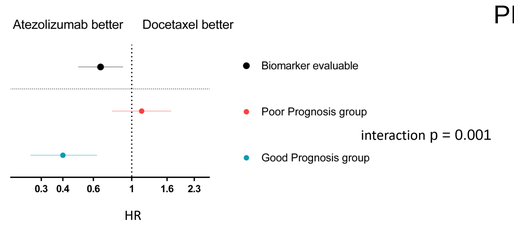


	N	OS			PFS		
	Doc/Atezo	Median Doc/Atezo	HR (95% CI) doc vs atezo	p-value	Median Doc/Atezo	HR (95% CI) doc vs atezo	p-value
Poor Group	68/60	8.6/7.3	1.14 (0.77-1.69)	0.500	3.0/1.4	1.38 (0.96-1.98)	0.086
Good Group	61/73	11.6/ not reaches	0.40 (0.26-0.63)	<0.001	4.1/4.5	0.65(0.45-0.94)	0.022

OS



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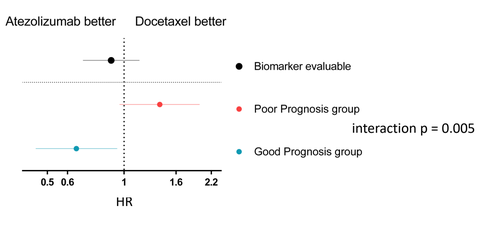
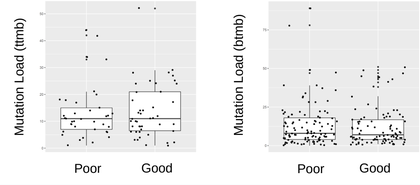


Significant classification-treatment interactions demonstrate that the test is predictive for OS and PFS between atezolizumab and docetaxel.

Test classification was not associated with PD-L1 expression (IC p =0.88, TC p=0.98), or TMB (right)



Conclusions

The data suggest that a circulating-proteome-defined phenotype characterized by complement activation, acute inflammation and immune response type 2 can provide predictive information on benefit from checkpoint inhibition.