The Inflamed Phenotype in PD-L1 Negative NSCLC and Response to Checkpoint Inhibitors

Immunology 2017 - P763
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Background

NSCLC with a PD-L1 tumor proportion score (TPS) by immunohistochemistry (IHC) of greater than 50% has a positive predictive value (PPV) of 42% for response to pembrolizumab. Across all tumor types and different checkpoint inhibitors (CPIs), the evidence supports that the inflamed phenotype is associated with response. Currently very little is known about predicting response to CPIs in PD-L1 negative NSCLC. Additionally, the association of the PD-L1 negative NSCLC with the inflamed phenotype has not been well described. In this study we evaluated the expression of PD-L1 in the context of CD8 expression using a DNA and RNA-seq panel of 400 genes applicable to next generation sequencing (NGS).

Methods

PD-L1 (22C3) IHC and a custom NGS cancer immune gene expression (GEX) assay were used to interrogate 50 NSCLC samples of which 21 were treated with one or more CPIs. RNA-seq analysis had been previously validated such that high PD-L1 GEX coincided with a TPS =>30% by IHC. Over expression of CD8 was considered positive for the inflamed phenotype. RECIST v1.1 was used to assess patient response.

Immune Response NGS Workflow

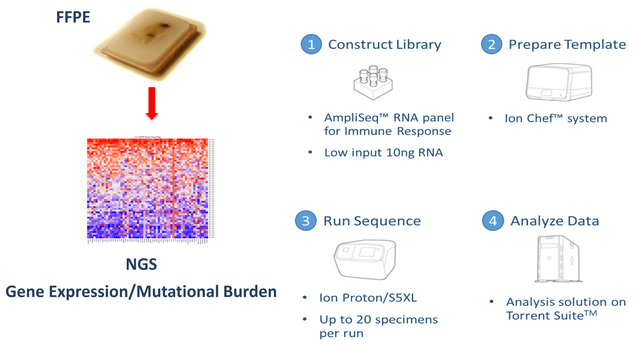


Figure 1: Immune Response NGS workflow (NYS CLEP approval pending).

Formalin-fixed paraffin embedded (FFPE) cancer samples were evaluated by RNA-Seq with the Oncomine™ Immune Response Research Assay and DNA-seq with AmpliSeq capture of 409 cancer related genes with Comprehensive Cancer Panel™ using the Ion Chef™ and S5XL™. RNA-Seq analysis was performed with the Torrent Suite™ v5.2.0, followed by data normalization (Figure 1).

Analysis Workflow

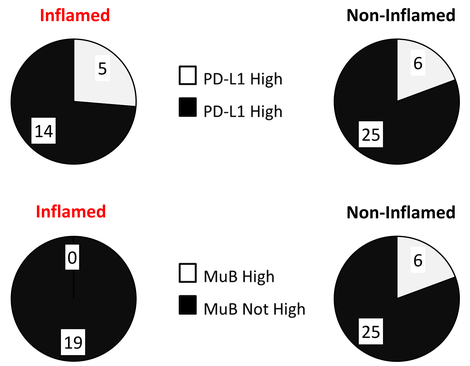
Figure 2: Immune Response analysis workflow

An immune response gene expression (GEX) panel was used to measure normalized reads per million (nRPM) for 64 validated genes. nRPM values were then ranked from 0 to 100 based on a reference population of 167 patients to derive GEX interpretation of High and Low. These interpretations were then used to visualize the immune GEX landscape of inflamed vs non-inflamed tumors. DNA Seq was used to estimate mutational burden (MuB), defined as number of nonsynonymous somatic mutations per million exonic bases (Figure 2).

Traditional Biomarkers (50 samples)

Eleven (11) samples with high PD-L1 IHC for which 5 were inflamed and 6 non-inflamed. For the 39 samples without high PD-L1, 14 were inflamed and 25 non-inflamed.

Six (6) samples with a high MuB for which all were non-inflamed.



Additional Checkpoint Blockade Biomarkers

Five (5) samples with high PD-1 GEX for which 4 were inflamed and 1 non-inflamed. For the 45 samples without high PD-1, 15 were inflamed and 30 non-inflamed.

Six (6) samples with high TIM3 GEX for which 5 were inflamed and 1 non-inflamed. For the 44 samples without high TIM3, 14 were inflamed and 30 non-inflamed.

Four (4) samples with high BTLA GEX for which 3 were inflamed and 1 non-inflamed. For the 46 samples without high BTLA, 16 were inflamed and 30 non-inflamed.

Ten (10) samples with high VISTA GEX for which 4 were inflamed and 6 non-inflamed. For the 40 samples without high VISTA, 15 were inflamed and 25 non-inflamed.

One (1) sample with high LAG3 GEX which was inflamed. For the 49 samples without high LAG3, 18 were inflamed and 31 non-inflamed.

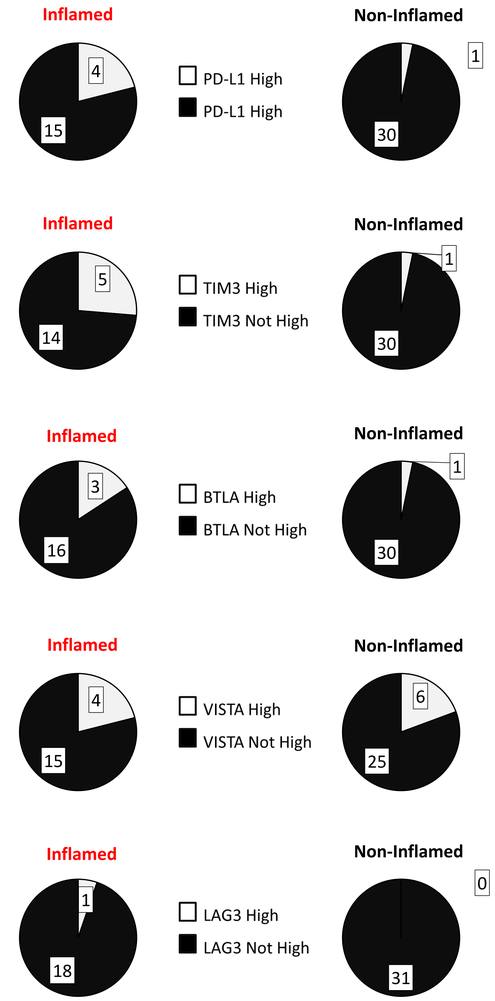


Figure 3: NSCLC expression profiles of traditional (top) and additional (bottom) checkpoint blockade biomarkers and their association with the inflamed phenotype.

Response and Single Biomarkers (21 patients)

Single Biomarker	CR	PR	SD	PD
Inflamed	1	1	1	8
Non-inflamed	0	1	4	5
PD-L1 High	1	1	1	1
PD-L1 Not high	0	1	4	12
MuB High	0	0	0	0
MuB Not high	1	2	5	13
PD-1 High	0	0	0	2
PD-1 Not high	1	2	5	11
TIM3 High	0	0	0	3
TIM3 Not high	1	2	5	10
BTLA High	0	0	0	3
BTLA Not high	1	2	5	10
VISTA High	0	1	0	6
VISTA Not high	1	1	5	7
LAG3 High	0	0	0	0
LAG3 Not high	1	2	5	13

While the number of patients evaluated are quite limited, there is no one single biomarker that predicts response.

Response and Combined Biomarkers (21 patients)

PD-L1	TILS or MuB	CR	PR	SD	PD
PD-L1 High	Inflamed	1	0	0	1
PD-L1 Not high	Inflamed	0	1	1	7
PD-L1 High	Non-inflamed	0	1	1	0
PD-L1 Not high	Non-inflamed	0	0	3	5
PD-L1 High	MuB High	0	0	0	0
PD-L1 Not high	MuB High	0	0	0	0
PD-L1 High	MuB Not high	1	1	1	1
PD-L1 Not high	MuB Not high	0	1	4	12

While the number of patients evaluated are quite limited, there is no combination of single biomarkers that predicts response.

Conclusions

- **High PD-L1 does not define an inflamed phenotype.**
- **MuB was more common in a non-inflamed phenotype.**
- **High expression of other checkpoint blockade targets does not define an inflamed phenotype.**
- **MuB and single or combined markers of checkpoint blockade do not define response.**
- **Response to checkpoint inhibitors is more complex than analysis of single biomarkers such as PD-L1 expression or mutational burden.**