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

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Background

PD-L1 positive melanoma patients, with a MEL score of 4 or 5 by immunohistochemistry (IHC), exhibited a response rate of >50% to pembrolizumab in the KEYNOTE-001 trial. Across multiple tumor types, evidence supports that response to checkpoint inhibitors (CPIs) is associated with an inflamed phenotype. Currently there is very little known about predicting response to CPIs in PD-L1 negative inflamed and non-inflamed melanomas.

Methods

86 melanomas were tested using PD-L1 IHC and a custom NGS immune gene expression assay. 72 of these patients were treated with one or more CPIs. A PD-L1 MEL score of 4 or 5 was considered positive for IHC, and over expression of a majority of 43 genes related to T-cell activation and 11 genes associated with tumor infiltrating lymphocytes were considered positive for the inflamed phenotype. RECIST v1.1 was used to assess patient response

Immune Response NGS Workflow

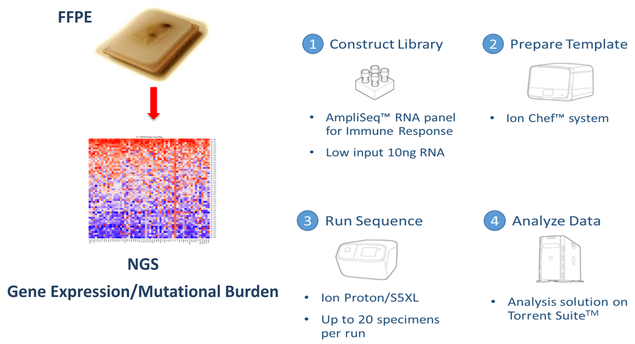


Figure 1: Immune Response NGS workflow (NYS CLEP approval pending)

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™ (Figure 1). RNA-Seq analysis was performed with the Torrent Suite™ v5.2.0, followed by data normalization.

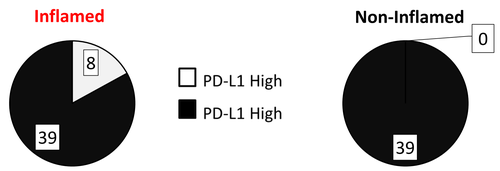
Analysis Workflow

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 (86 samples)

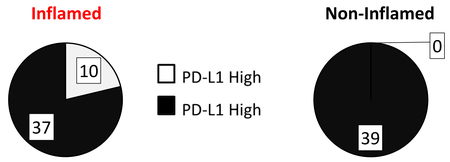
Eight (8) samples with high PD-L1 IHC for which all were inflamed. For the 78 samples without high PD-L1, 39 were inflamed and 39 non-inflamed.



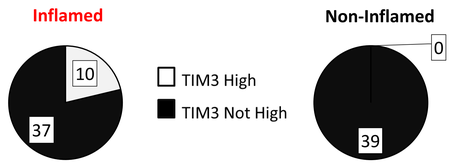
Eighteen (18) samples with high MuB for which 10 were inflamed and 8 non-inflamed. For the 68 samples without high MuB, 37 were inflamed and 31 non-inflamed.

Additional Checkpoint Blockade Biomarkers

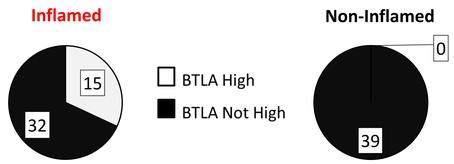
Ten (10) samples with high PD-1 GEX for which all 10 were inflamed. For the 76 samples without high PD-1, 37 were inflamed and 39 non-inflamed.



Ten (10) samples with high TIM3 GEX for which all 10 were inflamed. For the 76 samples without high TIM3, 37 were inflamed and 39 non-inflamed.



Fifteen (15) samples with high BTLA GEX for which all 15 were inflamed. For the 71 samples without high BTLA, 32 were inflamed and 39 non-inflamed.



Six (6) samples with high VISTA GEX for which all 6 were inflamed. For the 80 samples without high VISTA, 41 were inflamed and 39 non-inflamed.

Sixteen (16) samples with high LAG3 GEX for which all 16 were inflamed. For the 70 samples without high LAG3, 31 were inflamed and 39 non-inflamed.

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

Response and Single Biomarkers (48 patients)

Single Biomarker	CR	PR	SD	PD
Inflamed	5	7	6	8
Non-inflamed	3	4	2	13
PD-L1 High	0	0	0	1
PD-L1 Not high	8	11	8	20
MuB High	1	4	2	2
MuB Not high	7	7	6	19
PD-1 High	0	1	0	1
PD-1 Not high	8	10	8	20
TIM3 High	0	0	0	2
TIM3 Not high	8	11	8	19
BTLA High	1	1	1	2
BTLA Not high	7	10	7	19
VISTA High	0	0	0	2
VISTA Not high	8	11	8	19
LAG3 High	1	1	1	1
LAG3 Not high	7	10	7	20

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

Response and Combined Biomarkers (48 patients)

PD-L1	TILS or MuB	CR	PR	SD	PD
PD-L1 High	Inflamed	0	0	0	1
PD-L1 Not high	Inflamed	5	7	6	7
PD-L1 High	Non-inflamed	0	0	0	0
PD-L1 Not high	Non-inflamed	3	4	2	13
MuB High	Inflamed	1	3	1	0
MuB Not high	Inflamed	4	4	5	8
MuB High	Non-inflamed	0	1	1	2
MuB Not high	Non-inflamed	3	3	1	11

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

Conclusions

- **High MuB does not define an inflamed phenotype.**
- **Checkpoint blockade is associated with the inflamed phenotype.**
- **MuB was not associated with the inflamed phenotype.**
- **MuB and single or combined markers of checkpoint blockade do not define response.**
- **Responses occur in non-inflamed tumors with a low MuB.**
- **Response to checkpoint inhibitors is more complex than analysis of single biomarkers such as PD-L1 expression or mutational burden.**