Comprehensive Immune and Mutational Profile of Melanoma

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Introduction

The introduction of checkpoint inhibitor immunotherapy, predominantly anti-PD-1 and anti-CTLA-4 monoclonal antibodies, has provided significant clinical benefit for melanoma patients. The tumor microenvironment (TME) plays an essential role in therapeutic response and also tumor progression. The development of predictive biomarkers that measure overall tumor mutational burden, microsatellite instability and PD-L1 expression are widely used to characterize the immune TME and guide therapy selection. However, the genomic determinates that trigger an immunologically active tumor are not clear. In this study, we examined somatic mutations in metastatic melanoma samples to determine if there is an association between tumor mutational profiles and immune phenotypes, as measured by next-generation sequencing (NGS). The findings indicate that the mutational landscape effects the immune composition of the TME.

Methods

306 metastatic melanoma samples were tested by NGS using a comprehensive cancer panel for mutational status and an immune response panel which interrogates the expression profile of 54 validated immune-related genes1 (Figure 1).

![Figure 1: NGS workflow](image)

Figure 1: NGS workflow

Tumors evaluated had historically confirmed, previously untreated stage III or IV melanoma and the availability of tumor tissue from a metastatic or unresectable site for NGS assessment of somatic mutations and gene expression of immune markers. The level of gene expression was ranked against a reference population and represented as seven immune phenotypes (Figure 2).

- Checkpoint Blockade (PD-L1/CTLA4): primary inhibitory checkpoints (PD1, PD-L1, PD-L2, CTLA-4)
- Checkpoint Blockade Other: other inhibitory checkpoints (LAG3, TIM3, VISTA, HAVEM)
- Immune Escape: metabolic immunosuppression (ADORA2A, CD38, IDO1)
- Myeloid Suppression: MECRC immunosuppression (CCR2, CCR3, CD163, CD8, CD14)
- Anti-inflammatory: inhibitory molecules (IL10, TGFBR1)
- Pro-inflammatory: inflammatory signaling molecules (IL1B, STAT3, TNF, DERRK, MX1, CIIC1L, CIIC6L)
- T cell Prime: co-stimulatory mechanisms (CD40, CD27, IFNG, CD40, CD40LG, GITR, ICOS, ICOSLG)

Figure 2: Immune phenotypes

Somatic mutations were classified into four genomic subtypes: triple wild-type (WT), BRAF mutant, RAS mutant (NRAS, HRAS or KRAS), and NF1 mutant, as identified by The Cancer Genome Atlas (TCGA)2. Copy number gain and loss were limited to focal amplifications >4 copies, and homozygote loss (<1 copy), respectively.

Figure 3 shows the number of genomic mutations detected in 306 metastatic melanomas.

Figure 3: Total number of somatic mutations, frequency of mutation subtypes (Triple WT, BRAF, RAS and NF1) and other variants detected in 306 metastatic melanomas.

![Figure 3](image)

Principal component analysis (PCA) was performed to determine association of BRAF/RAS/NF1 mutations and Triple WT with immune phenotypes and immune response gene expression as measured by the NGS panels. PCA demonstrated that the first and second dimension explain 86% of the variation in the mutation profiles of the 306 melanomas.

Figure 4: The first principal component highly correlated with BRAF mutations (p < 0.001), the second highly correlated with RAS mutations (p < 0.001), and the third principal component, although not informative, highly correlated with NF1 mutations (p < 0.001).

![Figure 4](image)

Conclusions

BRAF/RAS/NF1 mutant subtypes are immunophenotypically distinct from Triple WT and do not associate with an inflamed tumor microenvironment, suggesting that these most common melanoma driver mutations are associated with “cold” tumors that may not promote an adaptive anti-tumor response. Triple WT samples present with an overall activated immune phenotype, representative of an inflamed “hot” tumor. These findings suggest that an integrated genomic and immunophenotypic approach is necessary to better understand the immune microenvironment, the effect of genomic alterations on immune response and implications to immunotherapy modalities.

References


The right drug or right trial…For Every Patient