Tumor mutational burden corrected for human leukocyte antigen somatic defects predicts response to checkpoint blockade in advanced non-small cell lung cancer

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INTRODUCTION

Tumor mutational burden (TMB) has been proposed as a biomarker for predicting response to immune checkpoint blockade (ICB) in non-small cell lung cancer (NSCLC). However, a subset of patients with high TMB tumors do not have a long-term response to ICB. A potential mechanism of ICB resistance is the development of somatic defects in antigen processing machinery, and/or loss of heterozygosity in HLA genes (HLA-LOH). These defects may contribute to functionally reducing the number of neoantigens available for presentation to T cells and disrupting the hypothesized correlation between TMB and tumor neoantigen count.

Here, we compared TMB with HLA defects to TMB alone as predictors of real-world patient outcomes following ICB treatment.

METHODS

Cohort generation

Using the Tempus Database, we selected 264 de-identified records of patients with metastatic, non-squamous NSCLC treated with an FDA-approved, first-line ICB regimen. Biopsies were collected prior to ICB initiation and profiled with Tempus xT targeted-panel DNA sequencing and, when available, whole-transcriptome RNA sequencing (n=180).

HLA somatic defect feature generation

HLA-LOH in HLA-I genes (HLA-A, HLA-B, HLA-C) were identified using the Tempus HLA-LOH algorithm. TMB was adjusted to account for the fraction of neoantigens presented by the remaining HLA alleles after an HLA somatic event, using the formula described by Shim *et al*. (2020):

$$\text{HLA} - \text{corrected TMB} = \text{TMB} \times \frac{(\text{NeoAg} - \text{NeoAgL} + \text{NeoAgc})}{\text{NeoAg}}$$

- NeoAg: total number of neoantigens presented.
- NeoAgL: number of neoantigens predicted to bind to the lost HLA.
- NegAgc: number of neoantigens predicted to bind to both the lost and retained HLA.

Analysis

Cox proportional hazards (Cox PH) models were fitted to determine the relationship between HLA-adjusted TMB and time to progression (TTP), with hazard ratios (HR) and confidence intervals (CI) reported. In addition, immune inflammation signatures were calculated for the 180 patients for whom paired RNA data were available.

RESULTS

HLA-LOH is a common somatic defect in NSCLC

Molecular Feature	*Cohort Totals (N=264)*
HLA LOH, type: n (%)	HLA-A: 117 (44%), HLA-B: 119 (45%), HLA-C: 114 (43%), All: 71 (27%), Any: 156 (59%)
***B2M* loss of function**, n (%)	3 (1%)
TMB, median [IQR]	6.3 [2.9, 11.2]
Tumor purity, median [IQR]	52% [40%, 64%]

Table 1. HLA-LOH prevalence and other molecular features in the cohort.

HLA-LOH is associated with shorter TTP in TMB-high tumors

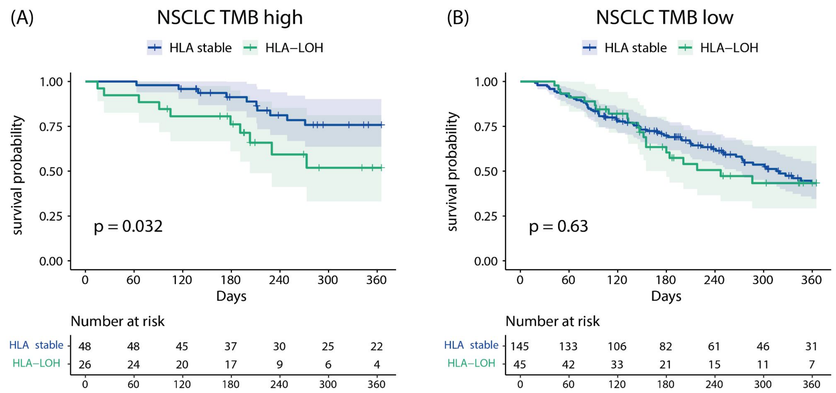

Figure 1. Kaplan-Meier (KM) analysis of TTP by HLA-LOH and TMB status. The presence of HLA-LOH at all HLA-I genes was significantly associated with shorter TTP versus HLA-stable in **(A)** TMB-high (≥10mut/Mb) tumors (HR [CI]=2.99 [1.39-6.40]), but not **(B)** TMB-low tumors (p-values, log-rank test).

HLA-adjusted TMB modestly improves estimated HR in comparison to unadjusted TMB

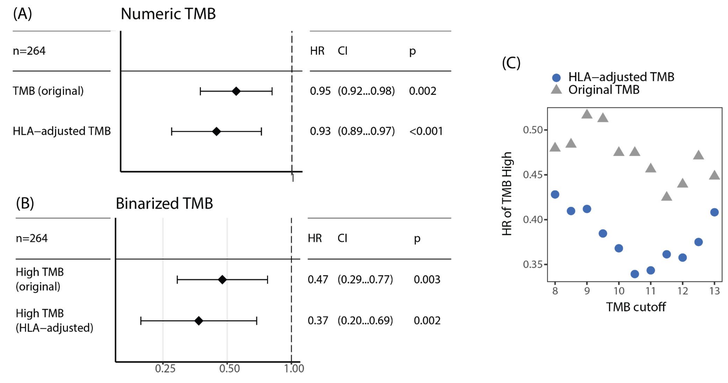

Figure 2. HR estimates of unadjusted ("original") and HLA-adjusted TMB, split into **(A)** numeric and **(B,C)** binarized TMB. **(C)** HLA-adjusted TMB-high had a lower HR compared to unadjusted TMB-high across various TMB cutoffs, suggesting its improved performance is robust to TMB cut point.

Clustering of immune-inflammatory signatures reveals a mixture of hot and cold tumors across HLA-adjusted TMB-high and -low groups

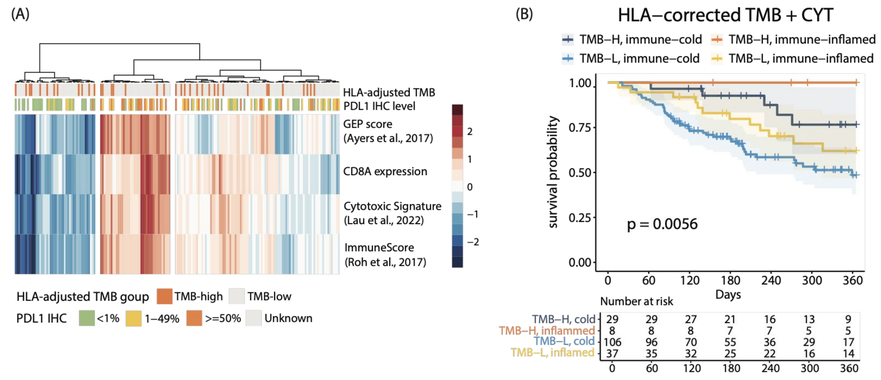

Figure 3. The immune microenvironment of NSCLC tumors. **(A)** Expression of immune-inflammatory signatures annotated by HLA-adjusted TMB categories and PD-L1 IHC status. **(B)** KM plot of TTP by HLA-adjusted TMB and immune categories. Immune status was defined by median cytotoxic gene signature (Lau *et al.*, 2022) split into inflamed (≥median) and cold (<median) categories. Patients with TMB-high (HLA-adjusted) immune-inflamed tumors exhibited longer TTP relative to all other groups.

CONCLUSIONS

- TMB-high tumors with stable HLA were significantly associated with longer TTP compared to TMB-high tumors with HLA-LOH.
- In addition, HLA-adjusted TMB outperformed unadjusted TMB in predicting ICB response.
- An inflamed immune phenotype was associated with longer TTP within HLA-adjusted TMB subgroups. Combining immune cytotoxic signature and HLA-adjusted TMB further stratified patient risk of disease progression.
- Our findings suggest that HLA-adjusted TMB can be used alone as a DNA-based biomarker or in combination with other features of immune activity to better predict ICB outcomes in NSCLC patients.

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