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Evaluating biomarkers of immunotherapy response in a real-world metastatic NSCLC cohort

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BACKGROUND

Current therapeutic developments have shifted first-line therapy in metastatic non-small cell lung cancer (NSCLC) to immunotherapy (IO)-based regimens, albeit with some heterogeneity in response.

Patients harboring KEAP1 and STK11 mutations have been reported to exhibit both poorer outcomes and IO treatment resistance[1-5]. Recent work suggests gene expression signatures which characterize mutant phenotypes may better identify those with compromised IO response[6].

Leveraging a molecular real-world dataset of metastatic NSCLC patients treated with first-line IO, this study aimed to evaluate gene expression signatures representing KEAP1 and STK11-mutated phenotypes and their impact on real-world outcomes.

METHODS

Cohort selection

Metastatic NSCLC patients treated with first-line (1L) anti-PD(L)1 were identified from the multimodal real-world database of Tempus AI.

All patients in the analytical cohort had tissue biopsies sequenced on Tempus targeted DNA and whole transcriptome RNA assays within 90 days prior to the 1L IO initiation. Patients were negative for actionable mutations with record duration of at least 30 days from 1L initiation.

Mutant identification

KEAP1 and STK11 mutants were identified with likely pathogenic and/or pathogenic mutations as annotated by Tempus bioinformatic pipelines.

Gene expression signatures related to these mutations were obtained from published literature[7-8]. Signature scores are defined by mean $\log_2$TPM expression values across all genes within each signature.

Evaluation of mutations or signatures as genomic markers for IO non-response were conducted using real-world progression-free survival (rwPFS) and overall survival (rwOS). Progression events were defined as first instance of progressive disease, start of next treatment, and death.

RESULTS

Patient characteristics

Among those identified for study, a total of 332 patients were included in this analytical cohort. The most used frontline IO agent was pembrolizumab (>95%). Among regimen types, 55% of the cohort received IO combination therapies (**Table 1A**).

Patient Characteristics	N (% of 332)	Patient Characteristics	N (% of 332)
Age at IO initiation (median, range)	67 (25-86)	Smoking status	
Gender		Former smoker	193 (58%)
Female	182 (55%)	Current smoker	94 (28%)
Male	150 (45%)	Never smoked	28 (8%)
Race		Unknown	17 (5%)
White	200 (60%)	Regimen	
Black and African American	27 (8%)	IO monotherapy	148 (45%)
Asian	6 (2%)	IO combination therapy	184 (55%)
Other race	14 (4%)		
Unknown	85 (26%)		
ECOG status closest to IO initiation			
0-1	228 (69%)		
2-3	51 (15%)		
Unknown	53 (16%)		

Table 1A. Counts of analytical cohort for baseline patient characteristics.

Baseline tumor characteristics

Most of the biopsied tissues collected were from metastatic sites, and most commonly of adenocarcinoma histology (**Table 1B**).

Positive PD-L1 status was observed for 71% of cohort with the majority of tumors (85%) classified as low tumor mutational burden (TMB; <10 mut/Mb) (**Table 1B**).

TP53 was the most frequently mutated (74%) among these metastatic patients followed by KRAS mutations (29%). Other alterations in genes such as CDKN2A, CDKN2B, and SMARCA4 are also observed (**Figure 1**).

STK11 mutations were identified in 10% of the cohort with a higher proportion in non-squamous tumors compared to squamous tumors (12% vs. 3%). In addition, 9% of the cohort were KEAP1 mutants with a similar histology distribution (**Figure 1**).

Table 1B. Counts of analytical cohort for baseline tumor characteristics.

Baseline Tumor Characteristics	N (% of 332)
Biopsied tissue	
Primary lung tissue	133 (40%)
Metastatic tissue	199 (60%)
Tempus DNA assay	
xT	6 (2%)
xT.v2	82 (25%)
xT.v3	76 (23%)
xT.v4	166 (50%)
xE	2 (1%)
Tempus RNA assay	
RS	195 (59%)
RS.v2	137 (41%)
Histology	
Adenocarcinoma	224 (67%)
Squamous	72 (22%)
Other	36 (11%)
PD-L1 status	
High (TPS ≥50%)	140 (41%)
Low (TPS 1-49%)	104 (30%)
Negative (TPS <1%)	83 (24%)
Unknown	5 (2%)
TMB status	
High	49 (15%)
Low	281 (85%)
Excluded	2 (<1%)

Figure 1. Heatmap displaying the mutational profile of patients within the analytical cohort. Tumor characteristics for each patient are shown in the top panels. Observed frequencies of genes with mutations of at least 5% of cohort are displayed in the bottom panel.

Validation of clinical biomarkers

Previously validated biomarkers, including TMB, T-cell inflamed GEP signature[9], and PD-(L)1 expression, were associated with prolonged rwPFS (**Figure 2**).

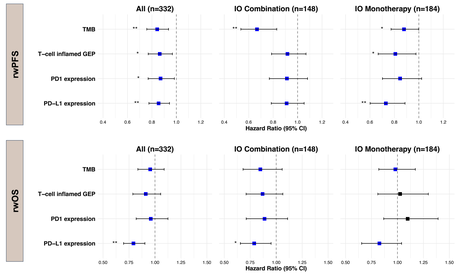
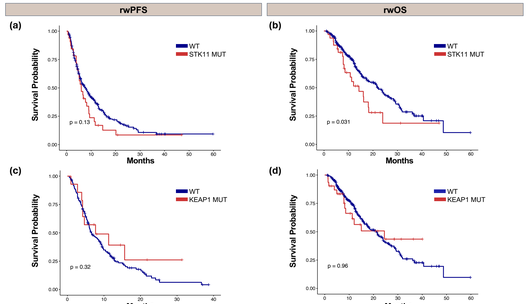


Figure 2. Forest plot summarizing cox regression analysis for validated biomarkers with real-world survival outcomes. Significant associations of continuous scores or expression values with rwPFS and rwOS are indicated (*P-value <0.05, **P-value<0.01).

Association of STK11 and KEAP1 mutations with outcomes

STK11 mutants trended towards worse real-world survival outcomes, while no significant association with rwPFS or rwOS was observed for KEAP1 mutants compared to wild-type (**Figure 3**).

Figure 3. Kaplan-Meier analysis of anti-PD(L)1 rwPFS and rwOS by mutational status. Comparisons between patients harboring STK11 mutations (a-b) and KEAP1 mutations (c-d) are shown. P-values from the log-rank test comparing the survival curves between mutant and wild-type groups are displayed.



Association of STK11 and KEAP1 gene signatures with outcomes

STK11 mutants have higher expression of the STK11 gene signature compared to wild-type tumors (**Figure 4**). High expressors of STK11 gene signature significantly associate with worse real-world outcomes (**Figure 5**).

High expression of KEAP1 gene signature are observed for KEAP1 mutants; however, do not associate with real-world outcomes (**Figures 4-5**).

Figure 4. Distribution of scores for each evaluated gene signature separated by wild-type and STK11 or KEAP1 mutants. P-values from Wilcoxon two-sided tests comparing the signature scores between wild-type and mutant groups are shown.

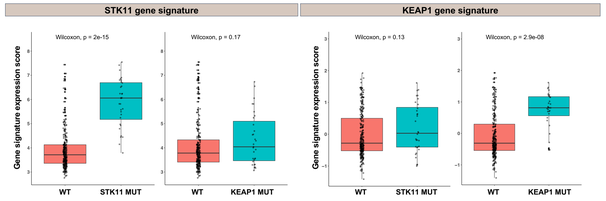
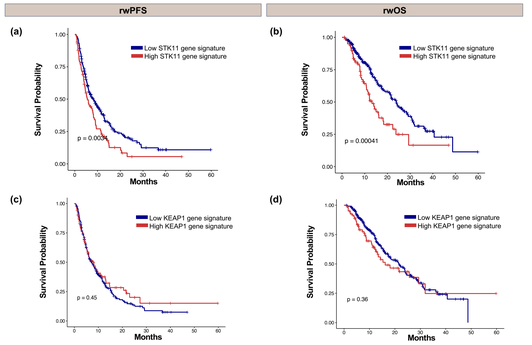


Figure 5. Kaplan-Meier analysis of anti-PD(L)1 rwPFS and rwOS by STK11 (a-b) and KEAP1 (c-d) gene signature levels using 3rd quartile as threshold for high vs. low expressors of signature. P-values from the log-rank test comparing the survival curves between high and low signature groups are displayed.



CONCLUSIONS

STK11 inactivation phenotype described by a gene expression signature was able to distinguish patients with significantly poorer survival outcomes. This finding would need validation in independent cohorts. This real-world cohort study adds supporting evidence for the utility of gene expression signatures encompassing mutant phenotypes for patient stratification.

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