Background Multiple genomics-based biomarkers of response to immune checkpoint inhibition have been reported or proposed, including tumor mutation/neoantigen frequency, PD-L1 expression, T cell receptor repertoire clonality, interferon gene expression, HLA expression, and others. Although genomics associations of response have been reported, the primary studies have used a variety of data generation and processing techniques. There is a need for data harmonization and assessment of generalizability of potential biomarkers across multiple datasets.

Methods We acquired patient-level RNA sequencing FASTQ data files from 10 data sets reported in seven pan-cancer PD-1 and CTLA-4 immune checkpoint inhibition trials with matched clinical annotations. We applied a common bioinformatics workflow for quality control, mapping to reference (STAR), generating gene expression matrices (SALMON), T cell receptor repertoire inference (MiXCR), extraction of immune gene signatures and immune subtypes, and differential gene expression analysis (DESeq2). We analyzed i) immunogenomics features proposed as biomarkers, and ii) gene expression signatures built from each trial for association with overall survival across the set of trials using univariable Cox proportional hazards regression. In all, we assessed 9 total immunogenomics features/signatures. P-values were adjusted for multiple testing using the Benjamini-Hochberg method.

Results Of the 9 immunogenomics features assessed, cytolytic activity score and expression of the Follicular Dendritic Cell Secreted Protein gene (FDCSP) were associated with survival in two of seven studies, respectively (adjusted p < 0.05) (figure 1). No proposed biomarkers were significantly associated with survival in more than two studies. The sets of genes significantly associated with clinical benefit across the studies were highly disjoint, with only three genes significant in three studies and thirteen genes significant in two studies (figure 2). No genes were significantly associated with clinical benefit in more than three of seven studies.

Conclusions No proposed biomarkers were highly generalizable across studies. We expect that integrated modeling incorporating multiple immunogenomics features will be required to build a robust and generalizable biomarker for ICI response. Further work is needed to analyze determinants of response and clinical benefit.

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REFERENCES


**Abstracts**

**Background**

Tislelizumab, an anti-PD-1 monoclonal antibody, has demonstrated clinical benefit for patients with NSCLC. The underlying response and resistance mechanisms to tislimab treatment remain unknown.

**Methods**

Baseline tumor samples from 59 nonsquamous (NSQ) and 41 squamous (SQ) NSCLC patients treated with tislimab monotherapy (NCT02407990 and NCT04068519) were tested for gene mutations using large panel next generation sequencing and RNA expression using gene expression profiling (GEP). Precision Immuno-Oncology Panel, HTG Molecular Diagnostics. GEP analyses of NSQ and SQ NSCLC were performed separately due to different gene expression patterns.

**Results**

The ORR, mPFS, and mOS in this pooled NSCLC cohort were 15.2% (95% CI: 9.0, 23.6), 4.1 months (95% CI: 2.20, 6.11), and 15.1 months (95% CI: 11.20, NE), respectively, with a median study follow-up of 15.3 months (95% CI: 14.06, 15.90). Non-responders (NRs) exhibited distinct tumor and immune gene signature profiles and could be clustered into two subgroups: NR1 and NR2. Compared with responders, NR1 had elevated cell cycle signatures in both NSQ (P=0.02) and SQ (P=0.03) cohorts, and a trend of decreased inflamed gene signature profiles. However, NR2 showed comparable or even higher tumor inflammation (18-gene), and CD8+ T-cell signature scores in both NSQ and SQ cohorts and could be classified as immune hot. To explore the resistance mechanisms of immune hot NRs, differentially expressed gene analyses between immune hot NR2 and responders were performed. M2 macrophage and Treg signature scores were higher in NR2 in both NSQ (M2, P=0.05; Treg, P=0.03) and SQ (M2, P=0.05 [subgroup of NR2]; Treg, P=0.03) cohorts; significantly higher expression of immune regulatory genes included PIK3CD, CCR2, CD244, IRAK3, and MAP4K1 (P<0.05 in NSQ and PIK3CD, CCR2, CD40, CD163, and MMP12 (P<0.05) in SQ). Significantly higher epithelial–mesenchymal transition (EMT) and angiogenesis gene expression, including SNAI1, FAP, VEGFc, and TEK (P<0.05) genes, were also observed in SQ NR2. Moreover, gene mutation analysis identified seven immune hot NR patients harboring either driver mutations (RET fusion, ROS1 fusion, BRAF, and PIK3CA amp) or well-established resistance mutations (loss of function mutation in JAK2, STK11, and MDM2 amplification).

**Conclusions**

Despite the presence of immune hot mechanisms, a subgroup of tislimab NRs with NSCLC were identified. High levels of immune suppressive factors, such as M2 macrophage and Treg signatures, angiogenesis, and EMT genes, as well as the existence of driver/resistance mutations, may indicate mechanisms of resistance of immune hot NRs, highlighting potential novel treatment targets.

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**Trials Registration**

NCT02407990 and NCT04068519

**ELMAMARY K, DORRIS A, SHU L, BURTONS M, ZHANG Y, SHELKOH B, WU YI, WU H. POTENTIAL MECHANISMS OF RESISTANCE IDENTIFIED THROUGH ANALYSIS OF MULTIPLE BIOMARKERS IN IMMUNE HOT NON-RESPONDERS WITH NON-SMALL CELL LUNG CANCER (NSCLC) TREATED WITH TISLELIZUMAB**