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

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

Methods: Baseline tumor samples from 59 nonsquamous (NSQ) and 41 squamous (SQ) NSCLC patients treated with tislelizumab 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*=0.2) 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 features, a subgroup of tislelizumab 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.