

DISCOVERY OF BIOMARKERS OF RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE IN NON-SMALL-CELL LUNG CANCER (NSCLC) USING HIGH-PLEX DIGITAL SPATIAL PROFILING

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Background Despite the clinical effectiveness of Immune Checkpoint Inhibitors (ICI) in lung cancer, only around 20% remain disease free at 5 years. Predictive biomarkers for ICIs are neither sensitive nor specific. Here, we used the GeoMx Digital Spatial Profiler (DSP) (NanoString, Inc.) to analyze high-plex protein in a quantitative and spatially resolved manner from single formalin-fixed paraffin embedded tissue sections toward the goal of identification of new biomarkers with better predictive value.

Methods Pre-treatment samples from 56 patients with NSCLC treated with ICI were collected, represented in Yale tissue microarray 471 (YTMA471), and analyzed. A panel of 71 photocleavable oligonucleotide-labeled primary antibodies (NanoString Human IO panel) was used for protein detection. Protein expression was measured in 4 molecularly defined tissue compartments, defined by fluorescence co-localization (tumor [panCK+], leukocytes [CD45+/CD68-], macrophages [CD68+] and an aggregate stromal immune cell compartment, defined as the sum of leukocyte and macrophage expression [panCK-/CD45+/CD68+]) generating 284 variables representing potential predictive biomarkers. Promising candidates were orthogonally validated with Quantitative Immunofluorescence (QIF). Pre-treatment samples from 40 patients with NSCLC (YTMA404) that received ICI, and 174 non-ICI treated operable NSCLC patients (YTMA423) were analyzed to provide independent cohort validation. All statistical testing was performed using a two-sided significance level of $\alpha=0.05$ and multiple testing correction (Benjamini-Hochberg method, FDR < 0.1).

Results Initial biomarker discovery on 284 protein variables were generated by univariate analysis using continuous log-scaled data. High PD-L1 expression in tumor cells predicted longer survival (PFS; HR 0.67, $p=0.017$) and validated the training cohort. We found 4 markers associated with PFS, and 3 with OS in the stromal compartment. Of these, expression of CD66b in stromal immune cells predicted significantly shorter OS (HR 1.31, $p=0.016$) and shorter PFS (HR 1.24, $p=0.04$). Tertile analysis using QIF on all three tissue cohorts for CD66b expression, assessed by QIF, showed that CD66b was indicative but not prognostic for survival [discovery cohort, YTMA471 (OS; HR 3.02, $p=0.013$, PFS; HR 2.38, $p=0.023$), validation cohort; YTMA404 (OS; HR 2.97, $p=0.018$, PFS; HR 1.85, $p=0.1$), non-ICI treated cohort YTMA423 (OS; HR 1.02, $p>0.9$, PFS; HR 0.72, $p=0.4$)].

Conclusions Using the DSP technique, we have discovered that CD66b expressed in the stromal immune [panCK-/CD45+/CD68+] molecular compartment is associated with resistance to ICI therapy in NSCLC. This observation was validated by an orthogonal approach in an independent ICI treated NSCLC cohort. Since CD66b identifies neutrophils, further studies are warranted to characterize the role of neutrophils in ICI resistance.

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Ethics Approval All tissue samples were collected and used under the approval from the Yale Human Investigation Committee protocol #9505008219 with an assurance filed with and approved by the U.S. Department of Health and Human Services

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