Evaluation of the Expression of Immune Functional Markers in the Tumor Microenvironment

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Background
Recent clinical successes with immune checkpoint blockade have provided promising immune-based therapeutic approaches for combating malignancy. While therapeutic antibodies against CTLA-4 and PD-1/PD-L1 have resulted in potent and durable clinical responses in many patients, there still remains an urgent need to develop biomarkers to identify patients who may benefit from these approaches while avoiding toxicity and without compromising efficacy. While gene and protein expression of immune cell and cytokine/cytokine, or tumor markers.

Design
In this investigation of 60 archived formalin-fixed paraffin embedded (FFPE) non-small cell lung cancer (NSCLC) tissues, expression profiles of immune checkpoint markers and immune functional molecules were evaluated in the tissue environment by RNAscope ISH assay (Figure 1). Each of the tumors displayed distinct co-expression patterns of immune checkpoint markers as well as varying levels of cytokines (including IFN-γ and TGF-β1) and chemokines, which were measured in respect to their relative location in the tumor or stromal regions. Additionally, the detection of immune cell markers revealed the location of infiltrating lymphocytes.

Results
Detection of co-expression profiles of multiple checkpoint markers in the TME revealed a large proportion of NSCLC cases (>80%) were identified as PD-L1 positive by RNAscope ISH assay. PD-L1 positive tumors are generally highly inflamed with infiltrated immune cells expressing multiple checkpoint markers and other functional markers.

Conclusions
In this study, the expression profiles of immune checkpoint and functional markers were evaluated in the tumor microenvironment of 60 NSCLC FFPE tissues by RNAscope®. A large proportion of NSCLC cases (>80%) were identified as PD-L1 positive by RNAscope ISH assay. PD-L1 positive tumors are generally highly inflamed with infiltrated immune cells expressing multiple checkpoint markers and other functional markers.

- The presence and capacity of tumor infiltrated immune cells present potential roles of downstream immune responses such as cytokine and chemokine production within the microenvironment of the tumor.
- Detection of co-expression profiles of multiple checkpoint markers in the TME revealed a heterogeneous pattern of expression in different tumor tissues. This information may reveal potential insights into combination therapies targeted against different checkpoint pathways.

Figure 1. RNAscope® technology and workflow

Figure 2. Human NSCLC TMA schematic and HALO analysis

Figure 3. Heat map of selected TMA cores representing percent positive cells expressing respective markers: immune lineage, checkpoint-related, chemokine/cytokine, or tumor markers.

Figure 4. Evaluation of target gene expression with images: representative images with TMA expression in three different tissue cores

Figure 5. Evaluation of cytokines and chemokines expression in a tumor microenvironment (core 2F2)

Figure 6. Co-expression profiles of checkpoint markers in the tumor microenvironment (core 2E2)

Other