SINGLE CELL EVALUATION OF COSTIMULATORY AND COINHIBITORY RECEPTORS AND THEIR LIGANDS ACROSS SOLID TUMOR INDICATIONS

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Background Costimulatory and coinhibitory receptors on T-cells are key mediators of the overall immune response, and the clinical success of checkpoint inhibitor therapies has proven the therapeutic benefits of targeting these pathways. Viable, cryopreserved dissociated tissue provides an opportunity to understand the dynamic expression patterns of these receptor/ligand sets at the single-cell level, providing insights into the differential expression of each receptor/ligand pair across different cancer indications as a means to target new therapeutic interventions.

Methods The study compared expression patterns from two cohorts composed of indications that are clinically approved for checkpoint inhibitor therapy versus indications that are not currently approved for checkpoint therapy. We performed bulk RNA sequencing on both cohorts to understand the global expression of costimulatory and coinhibitory receptors, and their ligands, within the tumor microenvironment. Furthermore, we developed a flow cytometry panel that allows for efficient screening of these receptor/ligand sets on the major cellular subsets (tumor cells, T-cells, B-cells, myeloid cells) within the tumor microenvironment. These data sets were initially evaluated for the expression patterns of PD1/PDL1/PDL2 as the standard for immunomodulatory receptors, and to evaluate the accuracy and sensitivity of the methods for the evaluation of receptor/ligand expression. In addition, we evaluated the expression patterns of two less well-characterized immunomodulatory receptors sets: TIGIT/PVR/PVRL2/CD226 and CD160/HVEM/BTLA/LIGHT to provide new insight into the expression patterns of these potential receptor/ligand sets and to better understand their value as therapeutic targets for specific cancer indications.

Results The protocols developed for this study were able to provide a clear and detailed picture of the receptor-ligand expression patterns across different cancer indications. Consistent with previous reports, PD1 expression was high on T-cell subsets, but low on the other lymphoid subsets within the tumor microenvironment. PDL1, on the other hand, was more differentially expressed on tumor cells across patient samples and was also expressed on myeloid cell populations. The expression patterns for the TIGIT and CD160 receptors and their associated ligands demonstrate the distinct differences in expression patterns across cancer indications.

Conclusions Given the varied and distinct expression patterns observed in different cancer indications, it is clear that the successful targeting of immune modulatory therapeutics will require an understanding of the expression of individual receptors and ligands at the single-cell level. This study demonstrates the utility of dissociated tumor cells as a tool to provide detailed insight into, and an understanding of the receptor-ligand expression of different cancer indications.