In recent years, the field of oncology has grown to include the use of immuno-therapeutics and combinatorial therapies in the treatment of patients across multiple cancer indications. While significant responses have been observed in a subset of patients, outcomes are variable and there is still a need to identify additional predictive biomarkers. To fully enable precision medicine, a more comprehensive understanding of the immune potential within the tumor microenvironment and its impact on treatment outcomes is necessary. Due to the high multiplexing capabilities of next generation sequencing (NGS) relative to IHC, it is expected that RNA sequencing may provide a more comprehensive assessment of the tumor microenvironment than IHC. Exploratory studies such as those presented here using targeted RNA sequencing will continue to shed light on our understanding of tumor-immune cell interactions and how this information can be used to guide therapeutic decisions.

In this study, a subset of 32 FFPE tumor samples that had been previously analyzed using the MolecularMD IO IHC Panel were selected for analysis with the ThermoFisher Oncomine™ Immune Response Research Assay. This targeted RNA-based NGS panel measures the expression of 395 genes involved in tumor-immune cell interactions including the checkpoint inhibitor, Programmed death-ligand 1 (PD-L1). Presented here, Figure 1 shows the range of PD-L1 expression levels across 26 of the FFPE tumor samples tested including non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and colorectal cancer (CRC) cases.

Fig. 1 Range of PD-L1 expression

Fig. 1 The Oncomine™ Immune Response Research Assay was used to analyze a 32 FFPE tumor sample set including NSCLC, CRC and RCC cases with the majority being ≥70% tumor cell content. RecoverAll Total Nucleic Acid Isolation Kit was used for RNA extraction from each tumor tissue and 10 ng of RNA was used for library preparation. Sequencing was performed using a 540 chip on the Ion S5XL. While the reads of majority samples were above 1 million, three samples presenting with read coverage below 500K were excluded from further analysis. Three additional samples were excluded from further analysis due to poor correlation with other samples (data not shown). Using the Torrent Suite software, read counts from a set of 11 reference genes was employed for data normalization. PDL1 (CD274) expression levels are plotted on the y-axis using a mean housekeeping scaled log2 count.
PD-L1 has been identified as an immunosuppressive driver acting directly to suppress T-cell activation against a tumor. Accordingly, drug therapies targeting PD-L1 act to release the brakes on these adaptive immune responses in order to potentially increase T cell anti-tumor activity. However, PD-L1 is only one component of a complex system of immune checks and balances. The identification of additional informative biomarkers within the tumor microenvironment is needed.

In this pilot study, the NGS dataset revealed gene subsets differentially expressed in relationship to PD-L1 expression levels. A subset of 8 samples demonstrating the highest and lowest PD-L1 expression levels (highlighted in Figure 1) were next selected for differential gene expression analysis using the Affymetrix Transcriptome Analysis Console. Forty-two genes were found to be differentially overexpressed in the PD-L1 high samples (Figure 2). Upon further analysis, the 42 gene list was refined to a final set of 16 genes. The functional categories for the 16 genes included Lymphocyte infiltrate, Checkpoint pathway, TCR co-expression, Interferon signaling, Cytokine signaling, Type I interferon signaling, Innate immune response, NK cell marker and PD-1 signaling.

Datasets such as that described here detail how targeted RNA sequencing can identify patient subsets based on a multiplexed signature rather than a single marker. These types of studies will also further our understanding of the tumor microenvironment and its effect on tumor and immune cell interactions. Targeted RNA sequencing panels including the Oncomine™ Immune Response Research Assay will enable immuno-oncology research through the quantitative evaluation of the expression of markers associated with different leukocyte subsets, antigen presentation, checkpoint pathways, and tumor progression. The use of both molecular and tissue-based assays will lead to a more comprehensive understanding of tumor and immune biology that may uncover new biomarkers for optimal stratification of patients for personalized immunotherapy treatment.