Abstract # 12113

Co-expression patterns of immune checkpoint molecules in relation to PD-L1 expression

CONTRIBUTING RESEARCHERS

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BACKGROUND

- Monoclonal antibodies directed at PD-1/PD-L1 have garnered FDA approval across multiple indications
- Treatment with these agents has resulted in durable responses, but they typically occur in a minority of patients within each disease type
- Across the burgeoning number of immunotherapy trials (estimated at over 3,000), few employ molecular selection.
- Recently, efforts have been made to combine novel checkpoint inhibitors (CPIs)
- Combinations of CTLA4-directed therapies with PD-1/PD-L1 inhibitors have demonstrated impressive results across several histologies, but this strategy is frequently limited by toxicity
- Multiple novel checkpoint inhibitors targeted distinct entities such as IDO, 41BB and LAG3 (amongst others) have been combined with PD-1/PD-L1 directed therapies
- Clinical data has emerged suggesting some combinations (e.g., IDO- and PD-1 directed therapies in combination) do not demonstrate optimal synergy

METHODS

- To suggest optimal pairing of novel CPIs, we interrogated a large database of formalin-fixed, paraffin-embedded (FFPE) slides or tissue blocks from patients with advanced cancer
- A total of **1,467 unselected clinical cases** were analyzed, with histologies including breast (17.8%), colon (9.5%), lung (7.9%), pancreatic (6.5%), ovarian (5.4%), brain (4.9%) and prostate cancer (2.7%)
- Cases were **categorized as PD-L1-low, PD-L1-normal and PD-L1-high** by cutoffs defined in TCGA expression profiles
- Expression and co-expression of **6 checkpoint markers** (PD-L1, PD-L2, CTLA4, IDO1, LAG3 and TIM3) were analyzed for tissue-specific enrichment
- Expression of individual checkpoint markers was segregated by PD-L1-defined categories (high versus low)
- **Immune-cell infiltration was estimated** using RNA deconvolution based on known immune cell marker genes

RESULTS

Figure 1. Expression and correlation of PD-L1 and other checkpoint molecules and correlation in patients designated PD-L1 high (N=59).

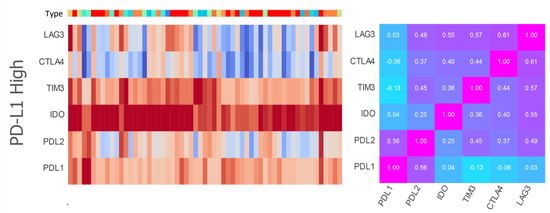


Figure 2. Expression and correlation of PD-L1 and other checkpoint molecules and correlation in patients designated PD-L1 normal or intermediate (N=1,340).

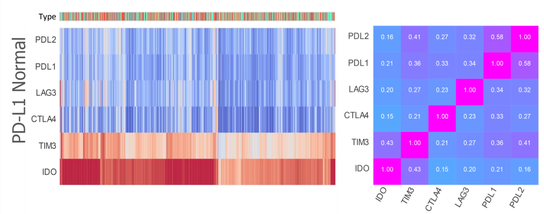


Figure 3. Expression and correlation of PD-L1 and other checkpoint molecules and correlation in patients designated PD-L1 low (N=65).

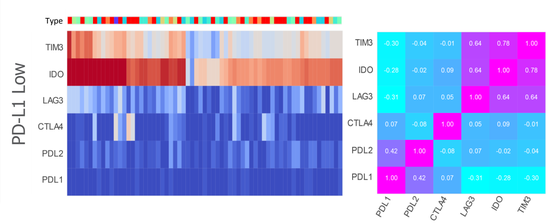


Figure 4. Proportion of times an immune-cell type was considered lower (left) or higher (right) than expected, grouped by PDL1 expression category.

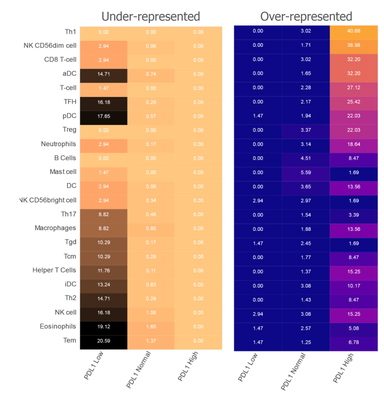
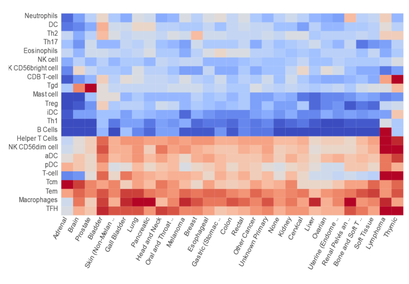


Figure 5. Immune cell category activation by tissue-type. Average expression for all genes in each immune cell category, split up in to reported cancer types.



KEY FINDINGS

- Checkpoint expression did not cluster in a tissue-dependent manner
- PD-L1 shows no significant co-expression pattern with any of the analyzed checkpoint markers aside from its ortholog PD-L2 (R = 0.77; P = 1.9x10-285)
- Within the PD-L1-low category, IDO1 and TIM3 had relatively high expression and were highly correlated with each other (R = 0. 81; P = 4.6x10-17)
- The PD-L1-low category was especially deprived of memory T cells and eosinophils
- Within the PD-L1-high category, overall expression of all checkpoint markers was higher
- Amongst PD-L1 high patients, CTLA4 expression was highly variable (mean 2.5±1.1; log2[TPM+1]) and lacked correlation with PD-L1 (R = -0.09)
- In contrast, while LAG3 also had variable expression in the PD-L1-high setting, it was strongly correlated with CTLA4 (R = 0.79, P = 7.4x10-14)
- The PD-L1-high category is enriched for multiple kinds of T-cells & T-helper cells, especially Th1, NK CD56dim, and CD8 T-cells

CONCLUSIONS

To the authors' knowledge, this is the largest report of the association between PD-L1 and other clinically relevant immune checkpoints. Key findings include a lack of strong correlation between PD-L1 and other interrogated immune checkpoints, with the exception of its ortholog PD-L2. **Amongst PD-L1** low patients, several striking findings were observed, including extraordinarily **high expression of TIM3 and IDO1**. **Variable expression of LAG3** was observed in PD-L1 high patients, but the moiety was **strongly correlated with CTLA4.**

Applying this knowledge retrospectively, **trials assessing combinations of PD-1/PD-L1 and IDO1** (recently reported to be negative) **may have employed a suboptimal design**. While the task of molecular selection does carry inherent challenges (e.g., baseline biopsy, tissue screening and so on), it is a potent enrichment strategy

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