

## **The landscape and genomic correlates of immune infiltration in 200 metastatic prostate cancers**

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**Background:** The prognostic value of tumor infiltrating lymphocytes (TILs) has been extensively reported in several primary cancers. The broad prognostic utility of TIL profiling in primary solid tumors was further demonstrated by a number of retrospective large-scale meta-analyses. These studies were enabled by in silico methods to assess leukocyte infiltration and T-cell clonality from transcriptome data. However, in contrast to primary cancers, the landscape of immune infiltration within metastases is largely unknown. In particular, it is unknown to what extent immune infiltration plays a role in metastatic castration resistant prostate cancer (mCRPC). This represents a significant gap in knowledge as immune evasion is widely recognized as a hallmark of metastatic cancer. To identify genetic and functional mechanisms that enable immune escape of individual tumors and to better understand tumor-host immune interactions in mCRPC we characterize immune infiltration across 200 mCRPC.

**Methods:** We obtained integrative molecular data, comprising whole-exome and transcriptome sequencing, and clinical data for a subset of patients enrolled under the PCF/SU2C integrative genomic sequencing program. We utilized existing and novel computational methods to quantify the level of immune infiltration (TIL-score) and the predicted composition of the infiltrate within each sample. Further, to identify facets of tumor-host immune interactions we characterized the joint expression profiles of immune receptors, ligands, cytokines, and HLA molecules. Finally, we correlated those phenotypic readouts with genomic and clinical data.

**Results:** Application of TIL-scores revealed a large heterogeneity of lymphocyte infiltration levels both within and across metastatic sites (liver, lymph node, bone marrow). Multiple lines of evidence suggest that over 50% of tumors lack significant T-cell infiltration and that HLA expression is almost completely lost in lymph node metastases for mCRPC. This is corroborated in others solid tumors. Based on the breakdown of immune cells, cancers were most strongly typified by the ratios of M2 to M0 macrophages and CD8+ to CD4+ T-cells. Unsupervised analysis revealed coherent expression of immune ligands and their cognate receptors (CD80/CD86 and CD28), but anticorrelation between stimulatory (CD80/CD86) and suppressive (PD-L1/PD-L2) ligands.

**Conclusions:** To develop the most effective immunotherapeutic strategies for metastatic cancer, it is essential to dissect the specifics of immune evasion in each tumor, determine the regulatory network that links these mechanisms, and identify biomarkers that predict sensitivity to specific immune therapies. This study highlights the heterogeneity of metastatic immune infiltration and represents the first step in the development of immunophenotypic biomarkers in metastatic prostate cancer.

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