Accurate quantification of infiltrating B cell composition and clone diversity in tumor samples

#5021

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I. Background

Tumors harbor a complex ecosystem of malignant, immune, and stromal cells. While malignant cells dictate much of the tumor biology, there is evidence that the tumor microenvironment (TME) also plays a major role in disease etiology. Given the complexity and abundance of the TME cellular composition, investigating the role of immune cell types will yield novel biomarkers for tumor progression and response to therapies.

II. Augmented exome and transcriptome capture with ImmunoID NeXT

The role of B cells as a prognostic biomarker remains elusive. For instance, infiltrating B cells in CRC has positive and negative prognostic values. Thus, a scalable approach to quantify B cells and the B-cell receptor (BCR) repertoire could yield novel insights into the role of B cells in tumor biology. To address this, we have developed immune cell quantification (InfiltrateID™ V2) and immune receptor repertoire profiling (RepertoireID™) methods as part of the ImmunoID NeXT Platform®, an augmented, immuno-oncology-optimized exome and transcriptome platform.

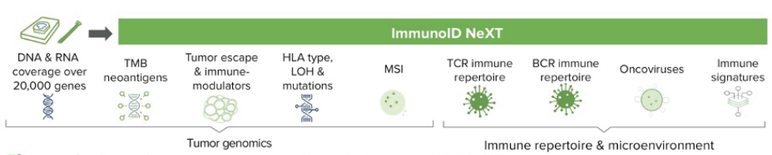

Figure 1: Overview of results provided in ImmunoID NeXT.

We estimate B cell abundance and BCR repertoire by profiling Formalin-Fixed Paraffin-Embedded (FFPE) and Peripheral Blood Mononuclear Cell (PBMC) samples using ImmunoID NeXT. In expanding upon InfiltrateID to further estimate B cell abundance, here our new method regress the bulk RNA-seq readout from a reference signature from purified immune cell types. We also generate orthogonal quantifications of B cell abundance by profiling samples with cytometry by time of flight (CyTOF), single-cell RNA-seq, flow cytometry, and immunohistochemistry (IHC). The BCR ImmunoID NeXT results are compared to a standalone sequencing approach to evaluate the concordance of top clones and the clonotype correlation between the two assays. We then utilize B-cell fraction estimation and BCR profiling from ImmunoID NeXT to analyze clonality and isotype composition in over 600 tumor samples.

III. InfiltrateID V2

We first use InfiltrateID V2 to estimate absolute B cell fractions in over 50 samples with orthogonal quantification of B-cells. Overall, we observe a high correlation between InfiltrateID V2 estimates and CyTOF, scRNA, IHC, and in-vitro cell mixture orthogonal datasets (Figure 1A - R^2=0.902). The performance was comparable in PBMC and FFPE. Respectively, we find R^2=0.89, R^2=0.85, R^2=0.86, R^2=0.62 for IHC, in-vitro mix, CyTOF and scRNA. Overall, the high correlation performance shows that InfiltrateID V2 can accurately leverage the augmented transcriptome platform to estimate absolute B-cell fraction in FFPE and PBMC samples.

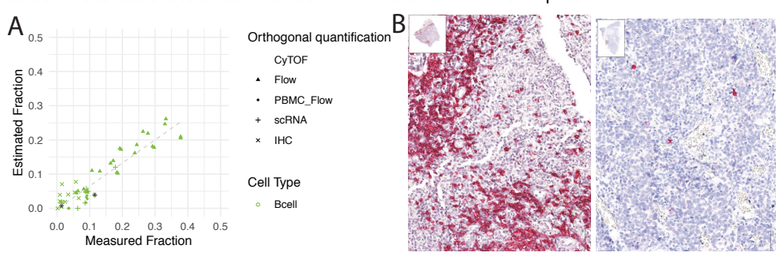

Figure 2: (A) High correlation between InfiltrateID V2 and orthogonal methods on PBMC and FFPE samples. (B) Two orthogonal samples with high (12.5%) and low (0.9%) B-cell infiltration.

IV. RepertoireID

When comparing BCR results from RepertoireID to a standalone BCR sequencing method that profiles IgM and IgG, we identify 475 and 387 of the top 500 clones in IgG and IgM, respectively, with highly concordant abundances across all clones (R^2>0.72 and R^2>0.82 in IgM and IgG, respectively). This shows that our BCR results are able to accurately profile high-abundance clones.

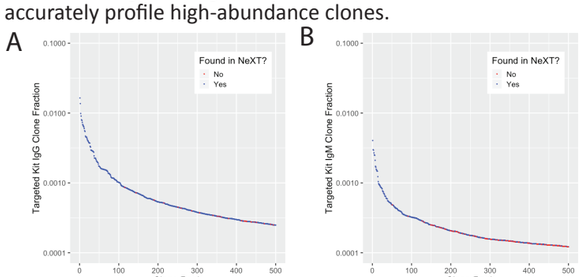

Figure 3: Clonotype estimates performance in PBMC: concordance between top 500 clones for (A) IgG and (B) IgM.

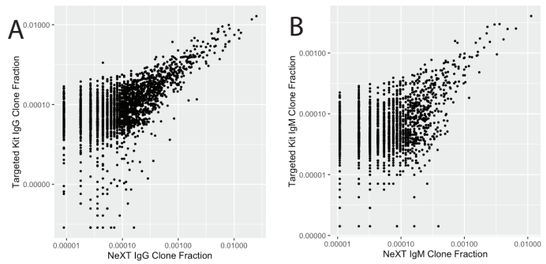

Figure 4: Clonotype estimates performance in PBMC: correlation between clonotype fraction for (A) IgG and (B) IgM.

V. Application of InfiltrateID V2 and RepertoireID a pan-cancer cohort

After assessing the performance of both InfiltrateID V2 and RepertoireID, we estimate absolute B cell fractions as well as IgH clonality in over 650 samples from 15 tumor types. On average, solid tumors, irrespective of tumor type, display low B cell fractions which agrees with both prior work by others and our internal IHC quantifications. We identify higher B cell fractions in lung, kidney, head and neck, breast, and cervical tumors. Interestingly, the fraction of B-cells infiltrated in solid tumors did not correlate with IgH clonality.

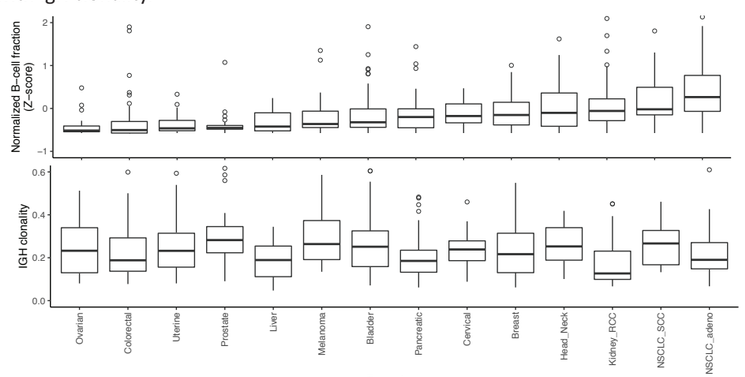

Figure 5: Distribution of normalized B cell fraction and IgH clonality across 650 solid tumor samples.

Conclusion

We show that InfiltrateID V2 and RepertoireID accurately capture the composition and clone diversity of infiltrating B cells in tumor samples.

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