Comprehensive profiling of the the tumor-immune microenvironment using an augmented transcriptome

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Introduction

Comprehensive profiling of both the tumor and tumor microenvironment (TME) can help further our understanding of tumor progression and response to treatment. Many immune features can be extracted from transcriptomic data, including characterization of the immune infiltrate and profiling of the diversity of immune receptors. To address this, we have developed multiple TME profiling features as part of the ImmunoID NeXT Platform®, an augmented, immuno-oncology-optimized exome/transcriptome platform designed to provide comprehensive information regarding the tumor and TME from a single FFPE tumor sample. These features include quantification of immune cell infiltration and profiling of the T-cell receptor (TCR) and B-cell receptor (BCR).

Comprehensive tumor and immune profiling with the ImmunoID NeXT Platform®

To address the challenge of providing characterization of both the tumor and TME, we have developed the ImmunoID NeXT Platform, an augmented, immuno-oncology-optimized exome/transcriptome platform designed to provide comprehensive information from a single FFPE tumor sample.

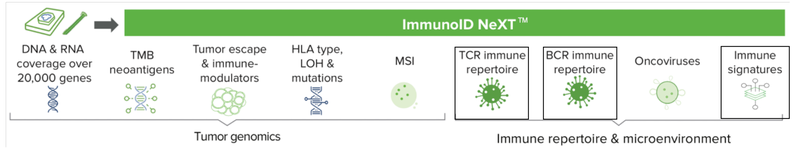


Figure 1: Overview of results provided in ImmunoID NeXT.

Development of an immune infiltrate quantification methodology

Profiling of reference immune cell samples

We first profiled the transcriptomes of eight purified immune cell types using the ImmunoID NeXT Platform to generate a reference dataset. We confirmed the utility of the transcriptome profiles by comparing them to published gene sets[1]. We then utilized these profiles to develop platform-specific gene expression signatures for each cell type. We also evaluated published gene sets and selected between our in-house and published gene sets using internally-developed criteria. Finally, we implemented a scoring method to quantify the abundance of each cell type using ssGSEA[2], which provides a semi-quantitative score that can be compared across samples for the same cell type.

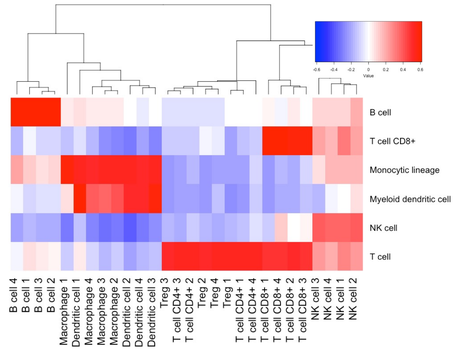


Figure 2: Heatmap of ssGSEA scores for purified immune cells using published gene sets.

Immune infiltrate quantification concordance with tumor FFPE samples

We performed both transcriptome sequencing and immunohistochemistry (IHC) on a set of FFPE samples to demonstrate accuracy in tumor samples. We observe significant concordances between the cell fractions by IHC and our transcriptome-based scores, suggesting that our method accurately quantifies tumor-infiltrating immune cells.

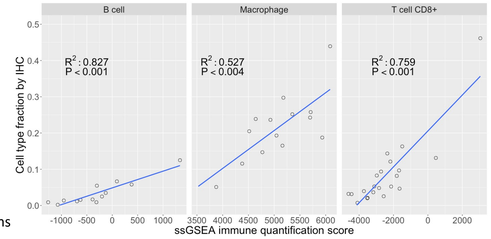
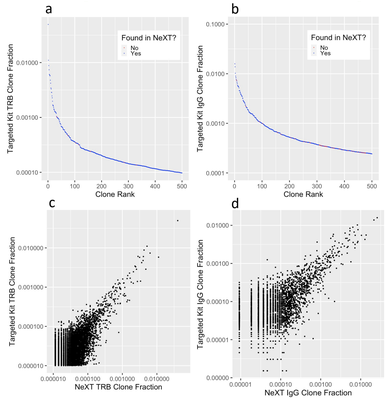


Figure 3: Comparison of ssGSEA scores using the ImmunoID NeXT Platform with immune cell fractions evaluated with IHC for tumor FFPE samples.

Development of an immune repertoire profiling methodology



Accurate profiling of top clones

We profiled TCR and BCR repertoires using both targeted kits and ImmunoID NeXT to compare the concordance of top clones. Compared to the standalone approaches, we identify 98% of the top 500 TCRβ clones and 92% of the top 500 IgG clones, both with highly concordance abundances across all shared clones (R^2=0.95 and R^2=0.79 in TCRβ and IgG, respectively). This shows that our approach has the capability to accurately profile top clones.

Figure 4: Identification of the top 500 clones as identified in targeted kits for TCRβ (a) and IgG (b). Comparison of the abundances of clones found in the targeted kits vs. ImmunoID NeXT (clones ≥0.00001) for TCRβ (c) and IgG (d).

Reproducibility in FFPE samples

We also profiled tumor-infiltrating immune repertoires by using ImmunoID NeXT on a set of patient-derived colorectal cancer (CRC) FFPE samples. We analyzed the reproducibility of clones identified using serial sections of the FFPE samples. We observe a strong concordance of the abundances for shared clones between the sections, showing that our approach is robust to degraded FFPE samples.

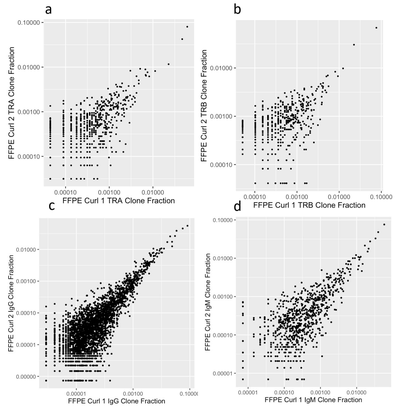


Figure 5: Comparison of abundances for clones found in common between serial sections of a tumor FFPE sample. R^2 values of 0.92 for TCRα (a), 0.94 for TCRβ (b), 0.93 for IgG (c), and 0.92 for IgM (d).

Applications of comprehensive immune infiltrate profiling

Profiling of immune infiltration across tumor types

We highlight the diversity of immune populations across cancer types by applying the ImmunoID NeXT Platform to 647 tumor samples from 14 different tumor types. This is part of an ongoing effort to profile a diverse set of tumor types with the ImmunoID NeXT Platform, which will provide a deeper understanding of the distributions of many immune features.

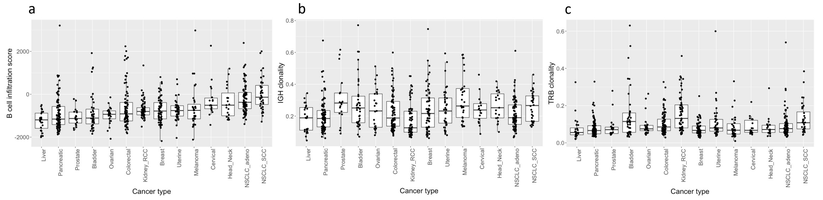


Figure 6: Comparison of B cell infiltrate scores (a), IGH clonality (b), and TCRβ clonality (c) across 647 tumor samples from 14 tumor types.

Immune features in a cohort of melanoma patients

To further analyze the utility of immune infiltrate features, we used ImmunoID NeXT to profile pre-treatment tumor samples in a cohort of melanoma patients who underwent PD-1 blockade therapy. In this cohort, we observe a significant difference in responders to checkpoint inhibition based on TCRβ clonality (p=0.028) and the CD8+ T cell infiltration score (p=0.01).

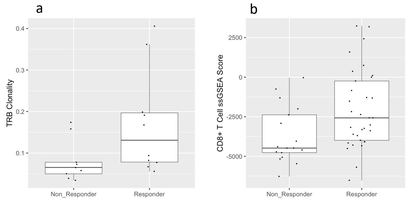


Figure 7: Comparison of responders and non-responders of checkpoint blockade therapy for TCRβ clonality (a) and CD8+ T cell infiltration score (b).

Conclusion

We apply the ImmunoID NeXT Platform to accurately and effectively evaluate the composition and diversity of tumor-infiltrating immune cells. Further, we utilize the platform to profile immune infiltration across a cohort of late-stage melanoma patients, demonstrating associations between TCRβ clonality and CD8+ T cell infiltration with response to checkpoint blockade therapy.

References

1. Becht E, Giraldo NA, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biology*. 2016;17:218. doi:10.1186/s13059-016-1070-5.
2. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*. 2013;14:7. doi:10.1186/1471-2105-14-7.

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