Profiling tumor-infiltrating immune cells using an augmented transcriptome

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Introduction

Due to the complexity of tumor-immune interactions, comprehensive profiling of both the tumor and tumor microenvironment (TME) can help further our understanding of tumor progression and response to treatment. One significant application is quantification of the immune infiltrate, which has the potential to characterize response to checkpoint blockade therapy[1]. While common experimental approaches exist to profile tumor-infiltrating immune cells, they can have significant practical limitations. Instead, RNA sequencing can be used to comprehensively profile the immune composition of the TME in a scalable way. To address this, we have developed an approach to quantify eight immune cell types in tumor samples, and compare it to quantification by orthogonal methods.

Methods

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.

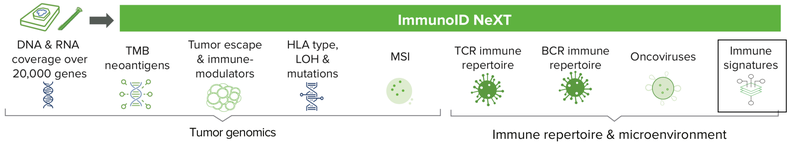


Figure 1: Overview of results provided in ImmunoID NeXT.

Development of an immune infiltration quantification methodology

We generated a reference dataset by profiling the transcriptomes of eight purified immune cell types using the ImmunoID NeXT Platform. We confirmed the utility of the the transcriptome profiling results by comparing them to published gene sets[2]. Then, we utilized these profiles to develop platform-specific expression signatures for each cell type. We also evaluated a selection of published gene sets, and utilized internally-developed criteria to select between our in-house and published gene sets. Finally, we implemented a method to score the abundance of each cell type using ssGSEA[3], which provides a semi-quantitative score that can be compared across samples for the same cell type.

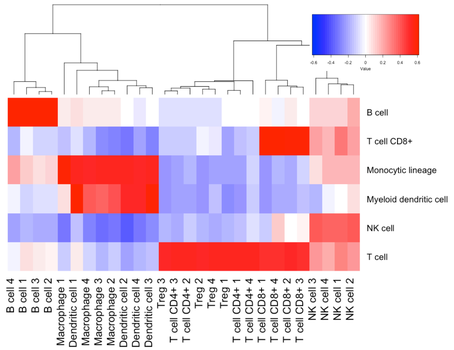


Figure 2: Heatmap of ssGSEA scores for purified immune cells using published gene sets.

Results

Quantification concordance with immune cell mixtures

To assess the accuracy of our quantification methodology, we first created a set of mixtures combining four purified immune cell populations. We then quantified the abundances of each immune cell type with flow cytometry, and compared to our transcriptome-based approach. The strong concordance suggests that our scores accurately reflect the underlying immune cell composition.

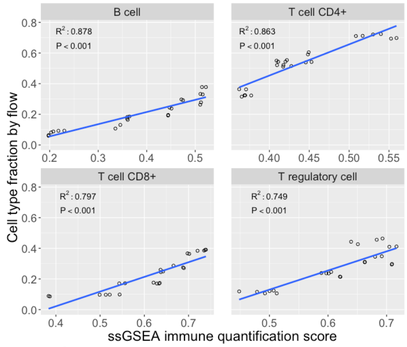
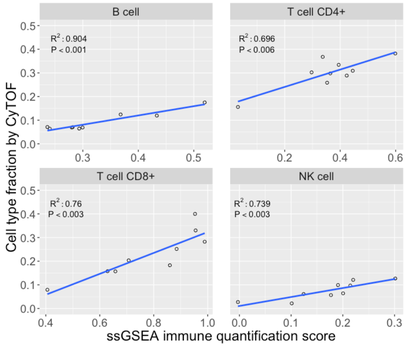


Figure 3: Comparison of ssGSEA scores using the ImmunoID NeXT Platform with immune cell fractions evaluated with flow cytometry for immune cell mixtures.

Quantification concordance with healthy PBMC samples



Next, we profiled the immune cell composition in a set of healthy donor PBMCs with cytometry by time of flight (CyTOF), and compared to our transcriptome-based approach. We observe a significant relationship between our immune quantification scores and the abundances from CyTOF. This suggests accuracy at immune profiling in real samples with diverse immune populations.

Figure 4: Comparison of ssGSEA scores using the ImmunoID NeXT Platform with immune cell fractions evaluated with CyTOF for healthy donor PBMCs.

Quantification concordance with tumor FFPE samples

To demonstrate accuracy in tumor samples, we performed both transcriptome sequencing and immunohistochemistry (IHC) on a set of FFPE samples. The significant concordance between the cell fractions by IHC and our transcriptome-based scores suggests that our method is able to accurately quantify tumor-infiltrating immune cells.

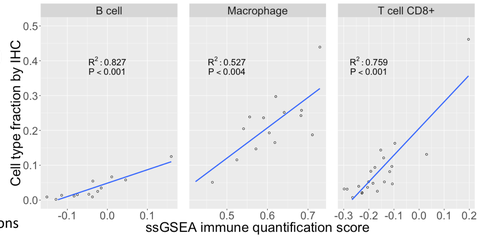


Figure 5: Comparison of ssGSEA scores using the ImmunoID NeXT Platform with immune cell fractions evaluated with IHC for tumor FFPE samples.

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Profiling of immune infiltration across tumor types

Finally, we highlight the diversity of immune populations across cancer types by applying the ImmunoID NeXT Platform to 708 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, including quantification of immune cell types.

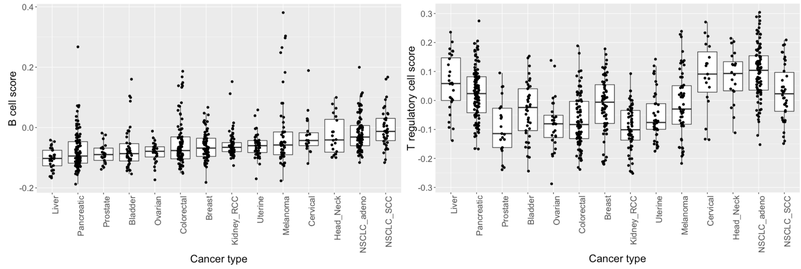

Figure 6: Comparison of ssGSEA scores representing B cells and T regulatory cells across 708 tumor samples from 14 tumor types.

Conclusion

RNA sequencing can be used as a scalable approach to profile the immune infiltrate in tumors. Such analysis can add to our understanding of the tumor-immune interaction, including studies of response to immunotherapy. Here, we test the accuracy of our approach using multiple sample sets with orthogonal profiling. We demonstrate that the ImmunoID NeXT Platform can accurately evaluate the composition of infiltrating immune cells in tumor samples.

References

1. Finotello F, Trajanoski Z. Quantifying tumor-infiltrating immune cells from transcriptomics data. *Cancer Immunol Immunother*. 2018;67(7):1031-1040. doi:10.1007/s00262-018-2150-z
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