A diagnostic platform for precision cancer therapy enabling composite biomarkers by combining tumor and immune features from an enhanced exome and transcriptome

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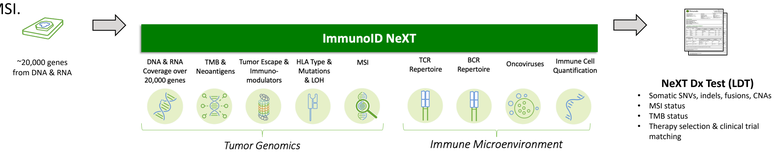
Introduction

While the success of immune checkpoint blockade (ICB) has been hugely promising, it's increasingly apparent that predicting response to immunotherapies requires a more comprehensive approach to tumor immunogenomic profiling. There is a need for the development of more advanced, composite biomarkers that can model the complex systems biology driving response and resistance to modern cancer therapies. Existing targeted diagnostic cancer panels provide limited data which is not sufficient to support the discovery and development of integrative, composite biomarkers; multivariable models that may better predict immuno- and combination therapy response than single analyte alternatives.

To address this need, we developed the ImmunoID NeXT Platform®, an enhanced exome/transcriptome-based platform that can simultaneously profile the tumor and its immune microenvironment from a single FFPE sample. We CLIA-validated this platform, demonstrating high sensitivity and specificity to somatic alterations across ~20,000 genes. As part of our NeXT Dx Test, we provide clinical diagnostic reporting on actionable mutations (SNVs, indels, CNAs, fusions) in 248 cancer-driver genes – genes that benefit from *boosted* coverage to >1,000X for enhanced performance – as well as the immunotherapy biomarkers, TMB (exome-derived) and MSI.

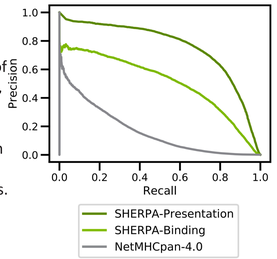


NeXT Advanced Analytics

In addition to more established markers like TMB and MSI, the platform's assays and analytics have been co-optimized to provide comprehensive tumor- and immune-related molecular information relating to additional emerging and investigational biomarkers such as neoantigen burden, HLA genotype diversity, HLA loss of heterozygosity (LOH), immune repertoire profiles, immunocellular deconvolution, oncoviruses, and more.

Neoantigen Prediction

Recent advances in immuno-affinity purification and mass spectrometry technology have made it possible to identify processed cell-surface MHC-bound peptides in an *in vivo* setting, and have enabled the development of our proprietary, machine-learning-based neoantigen prediction algorithm, SHERPA. We trained neural networks to predict MHC Class I peptide presentation using immunopeptidomics data from >60 HLA Class I alleles. SHERPA consistently achieves higher overall sensitivity and specificity than other tools based on either *in vitro* MHC-binding or immunopeptidomics-derived peptide presentation data, when tested on the same Class I alleles.

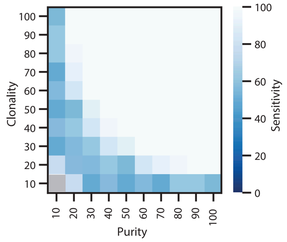


Central to the prediction of potentially-immunogenic neoantigens is our ability to accurately genotype the HLA Class I and Class II genes. To assess our performance, we conducted an extensive validation study on 15 proficiency samples (sourced from ASHI and CAP) in which >300 HLA alleles were genotyped via orthogonal clinical tests. As demonstrated in the results table, our platform was almost 100% concordant with the orthogonal calls for both Class I and Class II loci.

HLA Loci	# Calls	# Agree	Concordance
All Class I	90	90	100%
All Class II	240	237	98.8%
All Class I + II	330	327	99.1%

HLA LOH

HLA LOH has emerged as a major mechanism of immune escape and an increasingly-significant biomarker of response to ICB in many solid tumor types. However, few methods currently exist to accurately assess this phenomenon, and the sensitivity of those that do is not well understood. To this end, we developed a proprietary machine-learning tool, DASH, for evaluation of LOH events in these genes. We assessed the limit of detection (LOD) of DASH in both low clonality and low tumor purity settings using tumor-normal paired lymphoblast cell lines with known HLA LOH. After deep sequencing, we mixed the reads across a range of ratios to simulate the spectrum of purities and clonalities, successfully demonstrating the accuracy and low LOD of DASH.

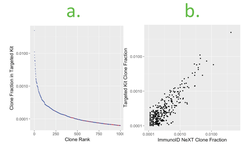


Immune Repertoire Profile

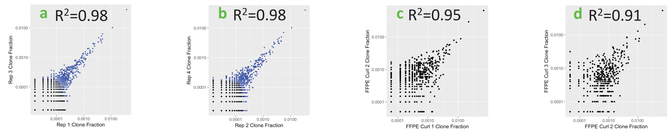
The ImmunoID NeXT Platform enables the comprehensive characterization of all chains of the TCR and BCR repertoires (BCR reporting is currently in development) using our augmented NeXT Transcriptome. By combining the assay's innovative probe design with an advanced analytical framework, the ImmunoID NeXT Platform provides a sensitive and accurate readout on the clonality and top clonotypes of the immune repertoires associated with the TME of FFPE tumor samples.

Identification of top clonotypes

To evaluate the platform's ability to identify the highest-abundance TCRβ clonotypes in healthy donor PBMCs, we compared our results to the top 1,000 clonotypes identified by a commercially-available targeted TCR kit. (a) We identified 95.3% of the top 1,000 clonotypes, and (b) the estimated abundances of these clonotypes were highly concordant between methods (R^2=0.94).



Reproducibility in PBMC and FFPE tumor samples



To test our reproducibility, we sequenced replicates of RNA from the same healthy donor PBMC sample, as well as serial curls from an FFPE tumor block. Abundances for top clonotypes showed high concordance, demonstrating that even with a highly-complex repertoire in healthy PBMCs (a & b), and difficult sample types like FFPE (c & d), our TCRβ profiling provides highly-reproducible results. Similar analyses were performed to evaluate our TCRα and BCRH chains profiling performance, producing comparable levels of concordance and reproducibility in both cases.

Immunocellular Quantification

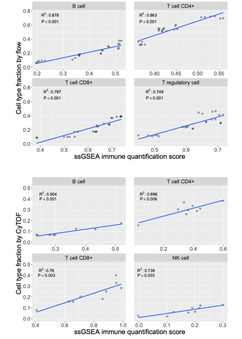
The ImmunoID NeXT Platform is also optimized for the evaluation and inter-sample comparison of the abundances of a given immune cell population across multiple samples. To enable the analysis of tumor-infiltrating leukocytes, proprietary, cell type-specific signature matrices were created for eight distinct immune cell types using NeXT Transcriptome gene expression data derived from purified immune cell populations. The expression profile of the individual signatures is translated into a single score per cell type which correlates with the abundance of the corresponding cells in the TME of the respective tumor sample. (This analytical module is currently in development.)

Correlation with FCM in immune cell mixtures

To evaluate the accuracy of our methodology, we created mixtures combining four purified immune cell populations at different ratios. We then compared the quantifications from our transcriptome-based approach to the immune cell fractions determined by flow cytometry (FCM). The strong correlation suggests that our scores accurately reflect the underlying immune cell composition.

Correlation with CyTOF in PBMC samples

To further confirm our performance, we carried out both NeXT Transcriptome sequencing and CyTOF on healthy donor PBMCs. We observed a strong linear relationship between our immune quantification scores and the CyTOF-determined abundances, illustrating the accuracy of our approach in real samples with diverse immune cell populations.

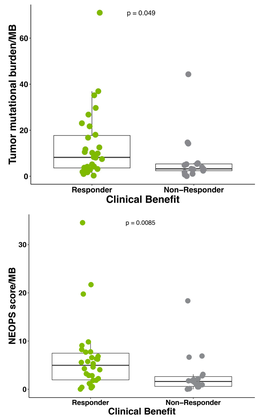


NeXT-derived Composite Biomarker

As has been demonstrated, the ImmunoID NeXT Platform can be used to investigate key tumor- and immune-related areas of cancer biology, consolidating multiple oncology biomarker assays into one and maximizing the biological information that can be generated from a single tumor specimen. The ImmunoID NeXT Platform's breadth of deliverables makes it the ideal platform for exploring the potential of multidimensional composite biomarkers that may better predict response to immunotherapies and combination therapies.

As part of an ongoing collaboration with the Inova Medical Group, we performed immunogenomic profiling with the ImmunoID NeXT Platform on pre-treatment paired tumor FFPE and normal blood samples from 55 late-stage, unresectable melanoma patients treated with anti-PD-1 therapy. These data, along with clinical outcomes, were analyzed to identify potential biomarkers of response or resistance to ICB.

We formulated a composite neoantigen presentation score (NEOPS) which incorporates resistance mechanisms such as damaging APM (e.g. HLA, B2M, etc.) mutations and HLA LOH to better predict response to ICB than either TMB or neoantigen burden alone, thus highlighting the promise of integrated, multivariable biomarker models to improve our predictive capabilities in the immuno-oncology era.



Conclusion

The ImmunoID NeXT Platform represents a novel, validated, universal cancer immunogenomics platform, purpose-built for both today's clinical applications in precision oncology as well as the discovery and development of more predictive composite biomarkers of the future. This is all possible due to the platform's ability to provide comprehensive biomarker information relating to both the tumor- and immune-related components of the TME from as little as four FFPE tissue slides.

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