A comprehensive, highly accurate genomics platform for precision immunotherapy: Simultaneously characterize tumors and the TME from a single FFPE sample

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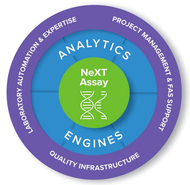
Introduction

While the success of checkpoint blockade has been hugely promising, it's increasingly apparent that predicting response to immunotherapies and developing new ones requires a more comprehensive approach to tumor immunogenomic profiling. This is critical for identifying new biomarker signatures that can effectively stratify responders and non-responders, in addition to understanding mechanisms of tumor resistance and guiding the rational design of personalized cancer therapeutics. Traditionally, generating information relating to multiple, potential biomarkers of interest has necessitated the use of several assay technologies from various sources. This is not only impractical given the often limited quantity of precious patient samples, but it also introduces complexities associated with the integration and interpretation of disparate reporting formats and is prohibitively costly.

Here, we present our solution to these issues, the ImmunoID NeXT Platform™. ImmunoID NeXT is a universal cancer immunogenomics platform that consolidates multiple biomarker assays into one; providing a multidimensional view of the tumor and its TME from a single sample. It combines the pioneering NeXT assay (exome and transcriptome), sophisticated analytics engines, and quality support to provide researchers with the comprehensive immunogenomic data they need to drive their drug development programs.

ImmunoID NeXT



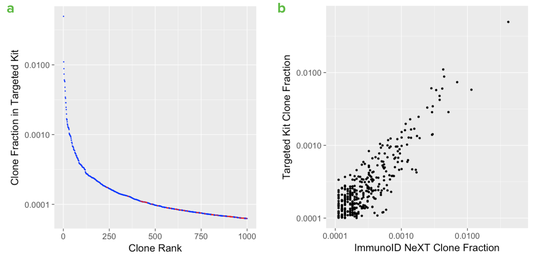
ImmunoID NeXT Analytics Modules

With ImmunoID NeXT, we optimized the design of our sequencing assay and analytics engines to increase performance for: immune repertoire profiling, somatic variant/neoantigen detection across ~20,000 genes, HLA typing and HLA LOH, TMB and MSI characterization, oncoviral detection, immunocellular deconvolution, and more.

Immune Repertoire Profiling

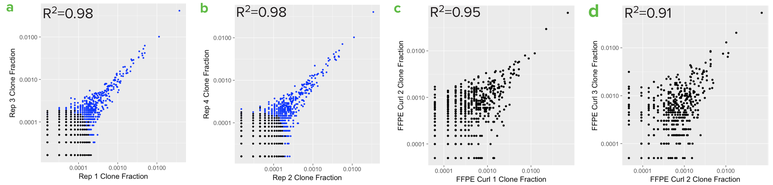
ImmunoID NeXT enables the comprehensive characterization of all chains of the TCR and BCR (BCR reporting is currently in development) repertoires using a transcriptome-scale assay. By combining the assay's innovative probe design with an advanced analytical framework, ImmunoID NeXT provides a sensitive and accurate readout on the clonality, top clonotypes, and aggregate metrics of the immune repertoire found in the TME of FFPE tumor samples.

Identification of top clonotypes



To evaluate the capability of ImmunoID NeXT to identify the highest-abundance TCRβ clonotypes in healthy donor PBMCs, we compared our results to the top 1,000 clonotypes identified in a commercially-available targeted TCR kit. (a) We identified 95.3% of the top 1,000 clonotypess, and (b) the estimated abundances of these clonotypes were highly concordant between methods (R2=0.94).

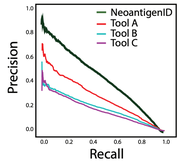
Reproducibility in PBMC and FFPE tumor samples



To test the reproducibility of ImmunoID NeXT, we sequenced replicates of RNA from the same healthy donor PBMC sample, as well as serial curls from an FFPE tumor block. Abundances for common clonotypes showed high concordance, demonstrating that even with a highly-complex repertoire in healthy PBMCs (a & b), and a difficult sample type like FFPE (c & d), our TCRβ profiling provides reproducible results. This analysis was replicated to evaluate our TCRα profiling performance, producing similar levels of concordance and reproducibility.

Neoantigen Prediction

Recent advances in immuno-affinity purification and mass spectrometry technology now make it possible to identify processed cell-surface MHC-bound peptides in an *in vivo* setting, enabling the development of improved neoantigen prediction pipelines, such as our own NeoantigenID. We have trained neural networks to predict MHC Class I peptide presentation using immunopeptidomics data from 30 HLA Class I alleles. NeoantigenID consistently achieves higher overall sensitivity and specificity than other tools based on either *in vitro* MHC-binding or immunopeptidomics-derived data, when tested on the same Class I alleles.



HLA Typing

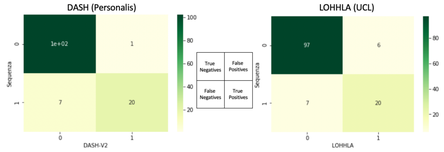
Owing to the specific targeting of not only the primary reference HLA Class I and Class II loci, but also all MHC ALT reference sequences, ImmunoID NeXT provides exceptional HLA typing performance by combining augmented DNA data with an advanced algorithm. Validation was performed on 18 samples from IHWG plus NA12878 from CEPH pedigree.

HLA Loci	# Calls	# Agree	ImmunoID NeXT Concordance
All Class I	112	111	99.1%
All Class II	222	209	95%
All Class I + II	334	320	96.4%

We can perform HLA typing using either tumor or normal DNA data – as well as somatic variant detection in HLA genes – which can help identify potential tumor escape mechanisms associated with the tumor's APM.

HLA Loss of Heterozygosity (LOH)

At Personalis, we've been pioneers in utilizing exome data and proprietary in silico methods to not only accurately genotype HLA genes, but we've now introduced the first commercially-available, machine learning tool, DASH, for evaluation of LOH events in these genes, a common mechanism of resistance to immunotherapy.



In the absence of a gold-standard methodology against which to test DASH's performance, we compared HLA LOH calls made by DASH (across 130 heterozygous HLA genes) to deletion calls made by a standard CNA caller, Sequenza, in the flanking regions of HLA loci. We assumed that genomic segments adjacent to the HLA locus often share the same copy-number profile as the HLA locus itself, except in cases of highly-focal HLA deletions. Finally, we compared DASH's performance to LOHHLA's, an academic tool developed at University College London (McGranahan et al., 2017).[1]

TMB and MSI Status

In recent years, TMB and MSI have emerged as key biomarkers of response/non-response to immunotherapy. Therefore, it was imperative for ImmunoID NeXT to have the ability to accurately report the status of both. We report three variations of TMB: 1) non-synonymous SNVs per Mb, 2) non-synonymous indels per Mb, and 3) total non-synonymous small variants per Mb (i.e. 1 + 2). For MSI, we provide the stability status of the five Updated Bethesda Consensus Panel canonical loci, as well as the proportion of all microsatellite loci (exome-wide) that are found to be unstable.

Oncoviral Detection

As the use of immunotherapy expands to a broader variety of cancers, it's important to understand how the presence of oncoviruses may impact immune responses. ImmunoID NeXT's multidimensional design enables the detection of seven known oncoviruses from both DNA and RNA data with a high degree of sensitivity and specificity.

To test this, we obtained 22 cell lines from ATCC in which oncoviruses were known to be present. We detected 22 out of 22 expected oncoviruses in both the DNA and RNA. Crucially, no reads were detected in any cell line for any oncovirus that wasn't expected to be present.

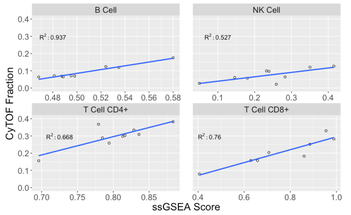
Total ATCC Cell Lines w/ Oncoviruses Present	Oncoviruses Detected	DNA Read Count		RNA Read Count	
		Mean	Median	Mean	Median
22	22	4523	153	4592	2555

Immunocellular Deconvolution

We have leveraged the high-quality gene expression data derived from the ImmunoID NeXT RNA-Seq assay to create reference signatures of immune cell type-specific genes, enabling immunocellular quantification of eight classes of immune cell in multiple sample types. This analytics module is currently in development.

Orthogonal testing on PBMC samples

For initial testing on samples with complex/diverse immune populations, we compared our expression profiling on 9 healthy PBMC samples with corresponding cellular abundances as measured by CyTOF. For cell types present in the PBMCs, our ImmunoID NeXT-based gene set enrichment approach enabled the accurate tracking of their respective abundances.



Conclusions

- With ImmunoID NeXT, we have developed a novel, universal cancer immunogenomics platform that can be used to provide a comprehensive view of both a tumor and its TME from a single sample.
- By combining our proprietary, augmented sequencing assay with our analytics engines, we have optimized this platform for the:
  - comprehensive analysis and evaluation of a broad range of immuno-oncology-relevant biomarkers,
  - discovery of novel biomarker signatures, and
  - identification of personalized therapeutic targets.
- ImmunoID NeXT can be leveraged by our biopharmaceutical customers/partners to enable the development of safer, more efficacious precision cancer immunotherapies, as well as improving response rates to existing therapies.

References

1. *McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, Birkbak NJ, Veeriah S, Van Loo P, Herrero J, Swanton C; TRACERx Consortium. Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. Cell. 2017 Nov 30;171(6):1259-1271.e11. doi: 10.1016/j.cell.2017.10.001. Epub 2017 Oct 26. PMID: 29107330; PMCID: PMC5720478.