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

ImmunoID NeXT Platform™

Richard Chen, Gabor Bartha, Shujun Luo, Sean Michael Boyle, Jason Harris, Eric Levy, Pamela Milani, Zeid Rusan, Robin Li, Nick Phillips, Robert Power, Christian Croisetiere, Manjula Chinnappa, Alfred Haddad, Paul McNitt, Rena McClory, Massimo Morra, Sebastian Saldivar, Michael Clark, Christian Haudenschild, John West
Personalis, Inc. | 1330 O'Brien Dr., Menlo Park, CA 94025

Personalis®

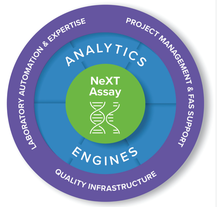
Contact:
robert.power@personalis.com

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. We believe this is necessary 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 such a process also introduces complexities associated with the integration and interpretation of disparate reporting formats and can be prohibitively costly.

Here, we present our solution to these barriers, 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. The platform represents an end-to-end solution for immuno-oncology and supports precision oncology biomarker discovery applications. It combines the pioneering NeXT assay (exome and transcriptome), sophisticated analytics engines (which are presented here), and quality support to provide researchers with comprehensive immunogenomic data to drive their drug development programs.

ImmunoID NeXT



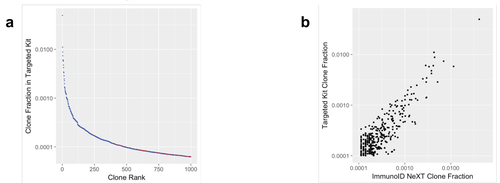
ImmunoID NeXT Analytics Modules

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, TMB and MSI characterization, oncoviral detection, immunocellular deconvolution, and more.

Immune Repertoire Profiling

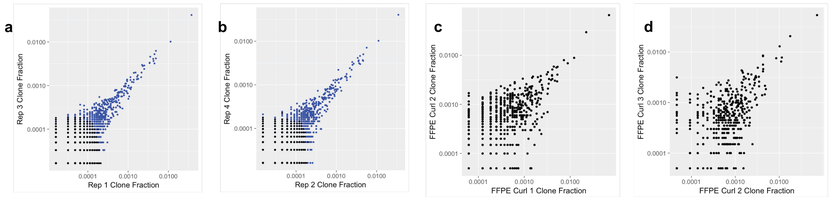
For the first time, ImmunoID NeXT enables the comprehensive characterization of all chains of the TCR and BCR (BCR reporting is currently in development) repertoires using a transcriptome-scale assay. By combining the assay's innovative probe design with an advanced analytical framework, ImmunoID NeXT 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 clonotypes, and (b) the estimated abundances of these clonotypes were highly concordant between methods (R^2=0.94).

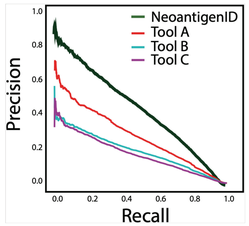
Reproducibility in PBMC and FFPE tumor samples



To test 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 and b; R^2=0.98 for both), and a difficult sample type like FFPE (c and d; R^2=0.95 and 0.91, respectively), our TCRβ profiling provides reproducible results.

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 HLA typing prediction 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.

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

HPV, HBV, HCV and EBV viruses are causally linked to >11% of cancers worldwide while KSHV and HTLV are linked to an additional ~1%. As use of immunotherapy expands to a broader variety of cancers, it's important to understand how these oncoviruses may impact immune responses in patients as part of the tumor and its TME. Unlike typical cancer genomic biomarker assays, ImmunoID NeXT's multidimensional design enables the detection of these oncoviruses from both DNA and RNA data with a high degree of sensitivity and specificity.

To test this capability, we obtained 22 cell lines from ATCC in which oncoviruses of various types were known to be present in the tumors from which the cell lines were derived. We detected 23 out of 23 expected oncoviruses (two viruses were present in one of the samples) 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.

Oncoviruses Known to be Present	Oncoviruses Detected	DNA Read Count		RNA Read Count	
		Mean	Median	Mean	Median
23	23	4185	192	3754	1240

Immunocellular Deconvolution

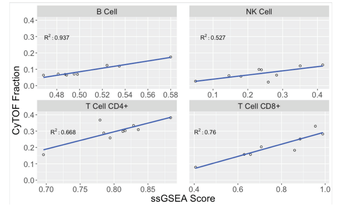
We have leveraged the high-quality gene expression data derived from the ImmunoID NeXT RNA-Seq assay to create reference signatures of immune cell type-specific genes, enabling the quantification of their cellular abundances in various sample types. This analytics module is currently in development.

Testing on *in silico* immune cell mixtures

To develop the methodology to create reference expression profiles, we obtained and sequenced purified immune cells from four healthy donors each for eight immune cell types and we then used an *in silico* mixture testing approach. For the test mixtures, 1 sample for each cell type was randomly selected, and a subset of reads from each sample was combined at set fractions. These mixtures were then processed by our pipeline to quantify their expression. The remaining 3 samples for each cell type were then used to generate the reference of marker genes. By using our identified gene sets, we were able to accurately track the actual fractions of immune cells in the mixtures using ssGSEA scores.

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.



Conclusion

- 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 state-of-the-art analytics engines, we have developed 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.