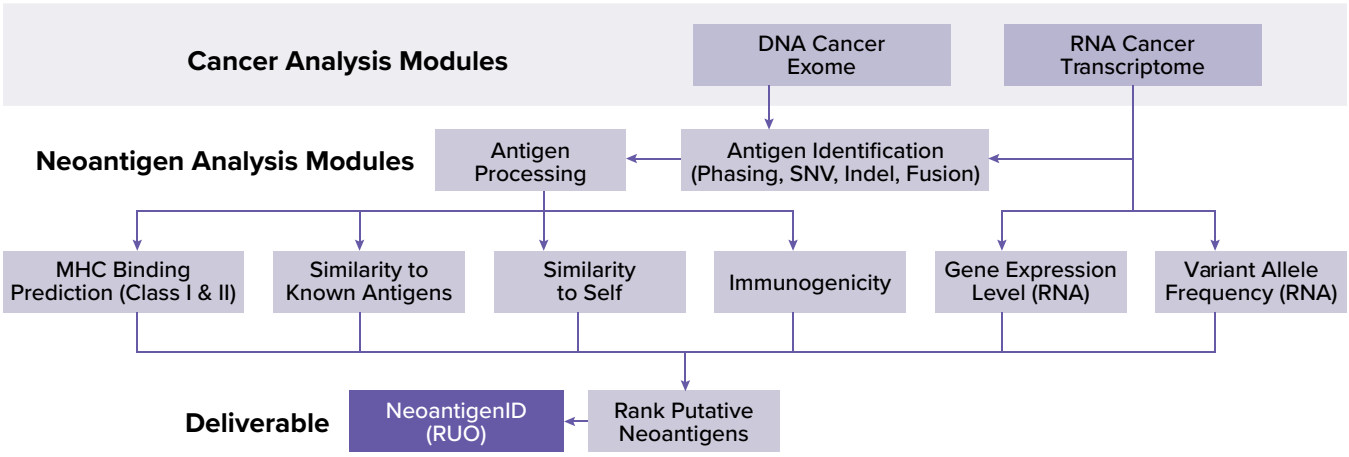
NeoantigenID™

Comprehensive identification of putative neoantigens

Neoantigens are mutated peptides that are expressed by tumor cells, but not by normal tissue. As a result, they can be recognized as foreign antigens by cells of the immune system — making them promising targets for personalized cancer immunotherapies such as vaccines and adoptive cell therapies. However, because neoantigens can arise from any gene in the genome, neoantigen identification requires exome and transcriptome sequencing.

ACE ImmunoID™, together with NeoantigenID™, helps inform neoantigen selection by integrating exome and transcriptome data into useful analytics, as well as providing tumor mutational burden (TMB) and neoantigen load. NeoantigenID is powered by Personalis' in-house Neoantigen Analytics Engine which consists of several modules with specific utility. Our Analytics Engine uses a combination of best-in-class *in silico* prediction algorithms, as well as internally developed methods, to generate NeoantigenID analytics.

Neoantigen Analytics Engine



Personalis®

It starts with a superior exome

To improve neoantigen identification, we extended standard sequencing and informatics methods. We developed an Accuracy and Content Enhanced (ACE) exome sequenced at 200X to increase sensitivity to SNPs and indels used for neoantigen identification as well as HLA performance.

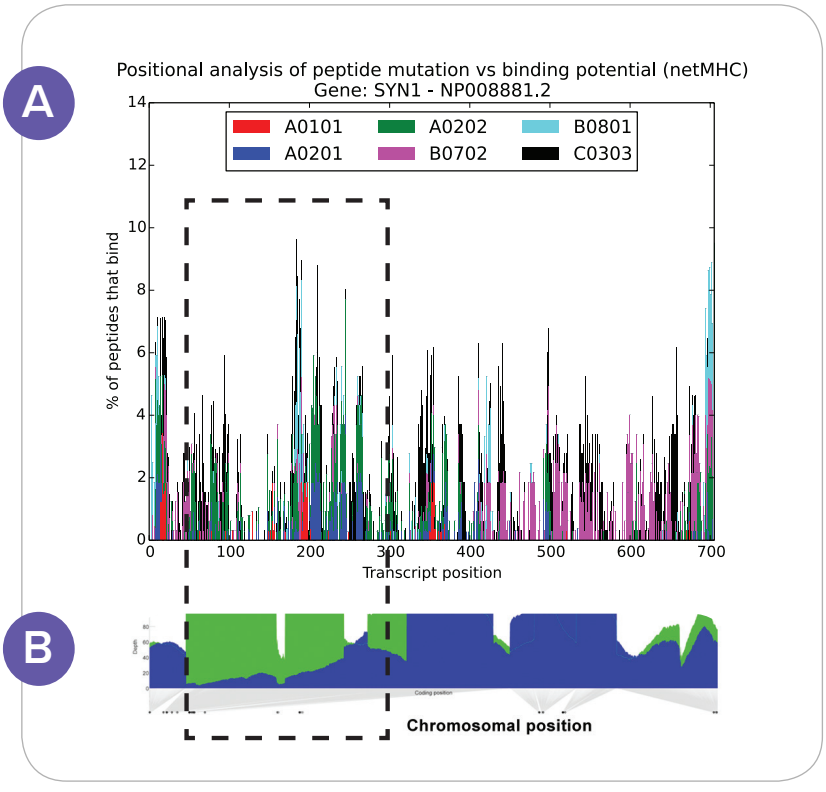
In the example below (**Figure 1**), gene-wide analysis of SYN1 (Panel B) shows the sequencing coverage provided by the standard exome (blue regions) as well as the ACE-augmented coverage (green regions). ACE Cancer Exome coverage encompasses both the standard blue and the augmented green regions.

Positional analysis of peptide mutations and HLA binding potential across the SYN1 genes (Panel A, highlighted region) indicates the number of predicted binding peptides that are captured in the augmented green region only (ACE supplementation) — these peptides would have been missed by the standard similarity-to-self offering.

DNA Cancer Exome and RNA Cancer Transcriptome Modules

The DNA Cancer Exome and RNA Cancer Transcriptome modules use best-of-breed third-party tools and internally-developed proprietary algorithms in a robust, analytically-validated workflow. Together with ACE ImmunoID, these modules provide highly accurate alignments and variant outputs.

Figure 1



Antigen identification

Somatic mutations, including single nucleotide variants (SNVs), indels, and fusions, each produce different protein products. While SNVs result in single amino acid changes, indels and fusions can create multiple frame-shift protein products through alternative splicing. As genes also have many transcripts, a single somatic mutation can result in numerous protein products. Collectively, accurate peptide usage relies on inclusion of frame-shift events, proper transcript selection, application of variant phasing, and consideration of variant expression.

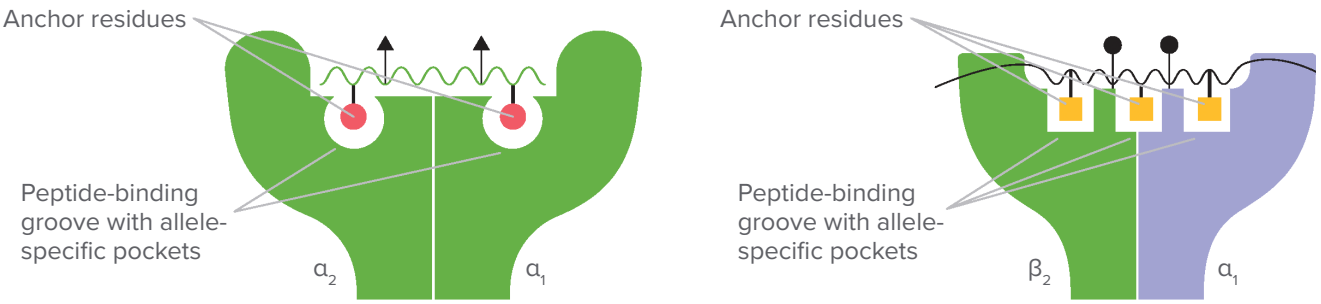
The Personalis antigen identification module incorporates information from different variant types beyond SNVs, to also include indels and fusions in antigen considerations. This is critical as frame-shift indels could prove to be a rich source of immunogenic neoantigens as has been recently shown in the literature[1].

Antigen processing

MHC Binding Prediction & HLA Typing Module

For MHC binding prediction, our module utilizes NetMHC, NetMHCpan, and NetMHCIIpan software. For peptide sequence determination, this analysis is performed using our in-house bioinformatics somatic analysis module. The peptide sequence listed in the report is performed from our tumor/normal exome data comparison. We then take the somatic variant calls and create flanking amino acids to generate the peptide sequence. This module also provides *in silico* HLA typing and binding prediction metrics for both MHC Class I and Class II (see **Figure 2**). HLA typing is performed using output data derived from tertiary analysis of exome data from the matched normal sample. HLA types include Class I (A, B, C) and select Class II (DRB1, DPA1, DPB1, DQA1, DQB1, DRB3, DRB4, DRB5).

Figure 2: HLA Class I and Class II Structures



Similarity-to-known antigens module

This module measures how similar a peptide is to a database of known antigens. The more negative score, the less the peptide looks similar to known antigens. Peptides that are similar to known antigens are more likely to be recognized by T cells. We use the full epitope database from IEDB[2], excluding human epitopes, thus these are mainly epitopes from viruses and other pathogens. This is an internally developed tool at Personalis.

Similarity-to-self module

This module produces a metric for each peptide that indicates how similar the peptide is to any portion of any protein that the subject's normal tissues produce. The more negative, the less the peptide looks similar to the body. If this value is "0", this means that the peptide matches a "self" protein. This is also an internally developed tool at Personalis.

Immunogenicity

Immunogenicity is a measure of the likeliness that a given peptide MHC-complex (pMHC) will be recognized by T cells. Critical factors regarding immunogenicity prediction are 1) position of a presented peptide (P4 through P6 for Class I), and 2) type of amino acid (aromatic or non-aromatic, acidic or basic, charged or non-charged).

This module was developed internally based on published literature[3] and implements an algorithm that scores the immunogenicity of a pMHC based on an amino acid enrichment analysis of a large set of immunogenic and non-immunogenic pMHCs. Increasingly positive scores indicate higher immunogenicity. This score is independent of the similarity modules.

Gene- and variant-level expression

Gene-level expression changes are undeniably helping to shape our understanding of patient response to checkpoint inhibitors like nivolumab and pembrolizumab. Likewise, RNA variant — and gene-level — expression can be a powerful tool to filter neoantigen candidates. As witnessed in the latest first-in-human (FIH) neoantigen-based cancer vaccine clinical trials, RNA has been suggested as a criteria for helping to determine potential putative neoantigen candidates[4].

Rank putative neoantigens

Our analytics provide all detected neoantigens as well as a list of top ten neoantigens ranked based on binding. This summary includes predictions for MHC Class I binding only. While we do provide Class II data, this is not included in the ranking.

However, Personalis provides the additional modules discussed earlier (immunogenicity, similarity-to-self, etc.) as secondary metrics to further enable customers to make informed selections of potential candidate neoantigens.

Footnotes

1. Turajlic et al. Lancet Oncol. 2017 Aug; 18(8):1009-1021.
2. Immune Epitope Database and Analysis Resource http://www.iedb.org.
3. Callis et al. PLoS Comput Biol. 2013 Oct:9(10):e1003266.
4. Sahin et al. Nature. 2017 Jul 13;547(7662):222-226.

NeoantigenID Output Example

Mutational Burden

Total non-synonymous mutations 201

Non-synonymous mutations / Mb 2.96

Neoantigen Burden

Total Neoantigens 399

Neoantigens / Mb 5.87

Peptide Binders

With allelic fraction

≥ 5% in DNA and RNA 168

	Prediction Bound (<500 nM)	Observed Expressed	Affinity Range	Median Affinity
Number of peptides binding to any one HLA type	399	266	3.54 – 498.74	178.02
Number of peptides binding to any two HLA types	18	10	5.50 – 491.43	134.43
Number of peptides binding to any three HLA types	3	3	7.60 – 427.10	85.49

Top 10 Neoantigen Peptides - Based on binding

Peptide Sequence	HLA	Expressed	AF DNA	AF RNA	NetMHC Affinity	NetMHC Pan Affinity	Binding Core	Similarity to Self	Similarity to Known Antigen	Immuno-genicity
FVYHINTHR	A6801	Y	0.11	N/A	5.10	3.54	FVYHINTHR	-8	-22	0.21
YTMGHLIQR	A6801	Y	0.11	N/A	7.00	4.15	YTMGHLIQR	-4	-17	0.04
MIFKDKFFWR	A6801	N	0.11	N/A	7.10	4.70	MIFKDKFFR	-3	-23	-0.19
LTFCWELAR	A6801	Y	0.12	N/A	15.30	7.18	LTFCWELAR	-9	-15	0.31
HTPPFGVVPR	A6801	Y	0.14	0.22	12.40	7.48	HTPPFVVPR	0	-25	0.17
YVHTASSLR	A6801	N	0.08	N/A	6.50	8.08	YVHTASSLR	-7	-16	-0.21
YSRNNITLL	C1203	N	0.11	N/A	24.20	8.21	YSRNNITLL	-3	-20	0.16
WASPEAIAYR	A6801	Y	0.10	N/A	13.20	8.65	WASPAIAYR	-5	-21	0.13
TAFDGSNYL	C1203	Y	0.07	0.22	12.00	8.93	TAFDGSNYL	-8	-13	-0.07
MESFVGTLER	A6801	Y	0.10	0.23	18.80	9.15	MSFVGTLER	-6	-17	0.20

Get in touch

To learn more about how we can help accelerate your personalized cancer vaccine or neoantigen-based adoptive cell therapy development program, contact us at **info@personalis.com.**

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