# Accurately Identifying Neoantigens Utilizing Both DNA and RNA Somatic Variants in an Enhanced Platform

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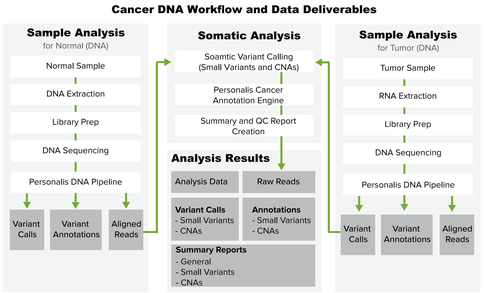
## Introduction

The identification of neoantigens is a crucial step in the development of neoantigen-based personalized cancer vaccines and other immunotherapies. Accurately predicting which neoantigens are likely to be immunogenic remains a key challenge owing to the complex processes involved in determining neoantigen immunogenicity including the antigen presenting machinery, likelihood of MHC class I and II binding, similarity to self, and ability to interact with the TCR.

## The Importance of an Augmented Exome (ACE)

We have developed a neoantigen detection pipeline built upon our analytically validated Accuracy and Content Enhanced (ACE) exome and transcriptome sequencing platform and somatic variants calling pipeline through combined DNA and RNA analysis. Our ACE platform augments difficult to sequence regions (green) that are missed by traditional exomes (blue). This is particularly important for Immuno-oncology as immunogenic antigens have the potential to arise from mutations anywhere in the exome.

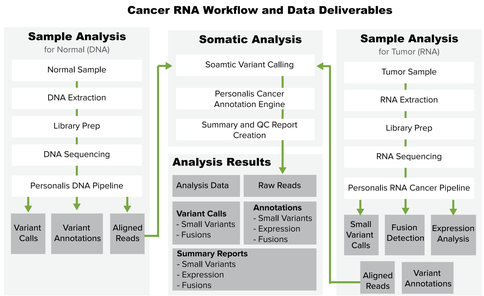

## Accurately Detecting DNA Somatic Variants for Neoantigen Assessment




| Sensitivity | LOD >10% (PPA) | LOD >10% (PPV) |
|---|---|---|
| >98% | >97% | >98% |

Each somatic mutation has the potential to produce neoantigens, including in-frame and out-of-frame SNVs and indels. While SNVs result in single amino acid changes, Indels and fusions can create frame-shift or out-of-frame protein products. As each gene can have many different transcripts, a single somatic mutation can result in a potentially very large number of protein products. For MHC class I, each of these proteins will generally be broken down into 8-11 amino acid peptides, each of which will then have a chance to be presented on the MHC for immune recognition.

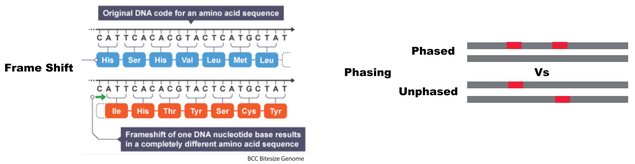
## Expressed Variants Are More Likely To Produce Neoantigens




| Small Var Sensitivity | Limits Of Detection (PPV) | Fusion Sensitivity |
|---|---|---|
| >97% | >97% (10-100% VAF) | >99% |

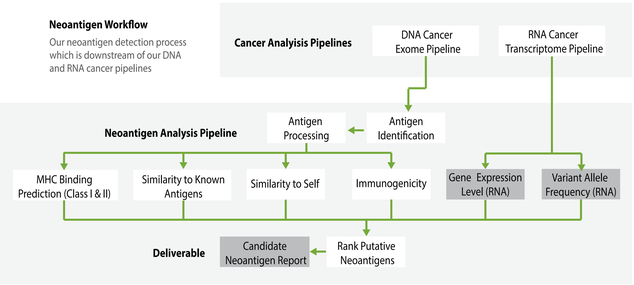
Accurately detecting variants directly from expressed transcripts is increadibly important for neoantigen prediction. Expressed variants are far more likely to be translated into proteins which are in turn cleaved, transported, and presented on MHC class I. From a technical standpoint, detection of somatic variants in the RNA represents additional challenges above and beyond those of somatic detection in DNA. The widely varying expression levels of cancer genes, alternative splicing, and RNA editing are all features that make somatic variant calling in RNA uniquely challenging. We took these challenges into consideration when developing and validating a highly accurate method for somatic variant

## Phasing and Frame-Shift Variant Peptide Creation




Frame-shift variants can result in entirely distinct downstream protein sequences. Correctly assessing these mutations at the transcript level produces a wealth of peptides. Proper phasing allows for assignment of correct peptides. As each amino acid matters when assessing MHC binding or immunogenicty, phasing can have a major impact on neoantigen predictions.

## Developing a Comprehensive Neoantigen Pipeline




Within our neoantigen pipeline, variants that are detected by our DNA and RNA cancer analysis pipelines are processed for antigen identification, including SNVs, indels, and fusion events. Importantly, both in-frame and out-of-frame events are accurately considered by transcript, allowing for detection of a wealth of candidate neoantigens. Our pipeline includes assessment of important immunologic components including HLA prediction, MHC binding (class I and II), immunogenicity, similarity to self, and similarity to known antigens. Additionally, peptides are evaluated for variant allele frequency in both the RNA and DNA of the tumor sample and gene expression level is considered. Collectively, our ImmunoID product provides a comprehensive assessment of features that may be used for identifying and ranking potentially immunogenic neoantigens.

## Accurately Detecting Known Immunogenic Neoantigens


**Neoantigen Prediction**
96% Sensitivity
(22/23 Epitopes)

To assess the effectiveness of this pipeline in predicting immunogenic neoantigens, we assembled a gold-set of 23 known, previously experimentally-validated immunogenic neoantigens from the literature. We spiked in these neoepitopes into exome data and assessed the ability of our neoantigen pipeline to find and rank these immunogenic known neoantigens. Preliminary results show our neoantigen pipeline is able to accurately identify 22 out of 23 (~96%) of the spiked in neoantigens as being potentially immunogenic.

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