Vaxrank: A computational tool for designing personalized cancer vaccines

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Abstract

Therapeutic vaccines targeting mutant tumor antigens ("neoantigens") are an increasingly popular form of personalized cancer immunotherapy. Vaxrank is a computational tool for selecting neoantigen vaccine peptides from tumor mutations, tumor RNA data, and patient HLA type. Vaxrank is freely available at www.github.com/hammerlab/vaxrank under the Apache 2.0 open source license and can also be installed from the Python Package Index.

1 Introduction

Mutated cancer proteins recognized by T-cells have become known as "neoantigens" and are considered an essential component of a tumor-specific immune response (Finnigan *et al.*, 2015; Gubin *et al.*, 2015; Schumacher and Schreiber, 2015). Therapeutic vaccination against neoantigens is an emerging experimental cancer therapy that attempts to mobilize an antigen-specific immune response against mutated tumor proteins (Türeci *et al.*, 2016; Zhang *et al.*, 2017). Since few tumor mutations are shared between patients, neoantigen vaccines must be personalized therapies. A common approach for achieving personalization is high-throughput sequencing of tumor and normal patient samples followed by in-silico prioritization of mutated peptides that are likely to be presented on the surface of tumor cells by MHC (major histocompatibility complex) molecules.

Vaxrank is a tool for selecting mutated peptides for personalized therapeutic cancer vaccination. Vaxrank determines which peptides should be used in a vaccine from tumor-specific somatic mutations, tumor RNA sequencing data, and a patient's HLA type. These peptides can then be synthesized and combined with an adjuvant to attempt to elicit an anti-tumor T-cell response in a patient.

The sequence of each mutated protein is determined by assembling variant RNA reads. Mutant protein sequences are ranked using a scoring system which seeks to satisfy two objectives: choosing mutations that are abundant in the tumor and choosing those whose translated amino acid sequences contain likely MHC ligands. Additionally, Vaxrank considers surrounding non-mutated residues in a peptide to prioritize vaccine peptide candidates and to improve the odds of successful synthesis.

Vaxrank was designed for and is currently being used in the Personalized Genomic Vaccine Phase I clinical trial at the Icahn School of Medicine at Mount Sinai (NCT02721043) (Rubinsteyn *et al.*, 2016a).

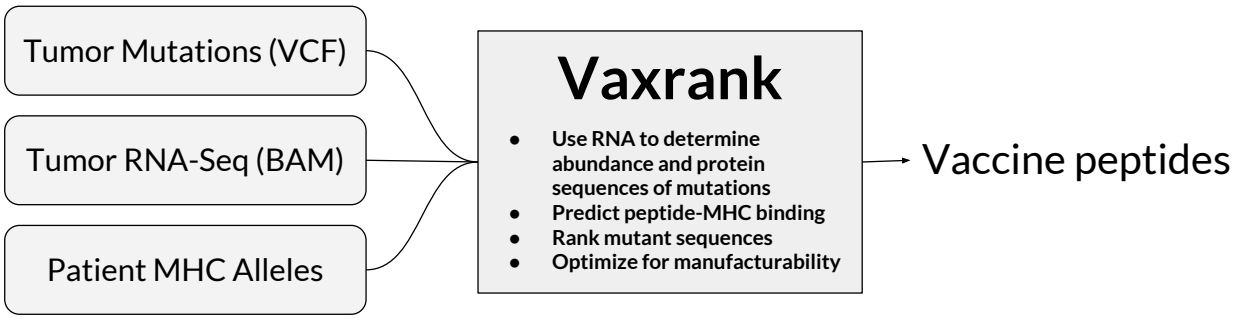

Figure 1: Users provide tumor mutations, tumor RNA sequence data, and patient HLA type. These are used to determine mutant protein sequences and rank them according to expression and predicted MHC affinity.

2 Running Vaxrank

To generate a Vaxrank vaccine report, the user must provide one or more files containing somatic variants (in VCF, MAF, or JSON format), aligned tumor RNA-seq reads (as an indexed BAM), and the HLA alleles to be used for MHC binding prediction:

```
vaxrank
  --vcf somatic-variants.vcf
  --bam tumor-rna.bam
  --mhc-predictor netmhc
  --mhc-alleles H2-Kb,H2-Db
  --mhc-peptide-lengths 8-10
  --vaccine-peptide-length 21
  --min-alt-rna-reads 3
  --output-pdf-report vaccine-peptides.pdf
```

The `--mhc-predictor` argument controls which program is used to predict the affinity between a peptide-MHC pair. Vaxrank supports the use of locally installed instances of NetMHC (Andreatta and Nielsen, 2016), NetMHCpan (Nielsen *et al.*, 2007), NetMHCcons (Karosiene *et al.*, 2012), MHCflurry (Rubinsteyn *et al.*, 2016b), or a variety of web-based predictors through IEDB (Vita *et al.*, 2015). The `--min-alt-rna-reads` argument controls the minimum number of RNA reads supporting a variant required to include that variant in the output report. In addition to quantifying tumor expression of a mutations, the RNA reads are used to phase adjacent variants when reconstructing the mutated coding sequence. A more complete list of options for input data, filtering, and output formats can be seen by running `vaxrank --help`. Vaxrank's output can be formatted as PDF, plain-text, HTML, or an Excel spreadsheet. The output lists variants in ranked order along with vaccine peptide(s) containing that variant, predicted MHC ligands, number of supporting RNA reads, and sequence properties that affect manufacturability.

3 Ranking Mutations

A patient's coding mutations are ranked according to a score that combines each mutation's degree of expression and aggregate affinity of overlapping mutant peptides for that patient's MHC alleles.

$$RankingScore = ExpressionScore \cdot TotalBindingScore$$
$$ExpressionScore = \sqrt{\#\ RNA\ reads\ supporting\ variant\ allele}$$
$$TotalBindingScore = \sum_{s \in subsequences} \sum_{a \in alleles} BindingScore(s, a)$$

The *BindingScore* function is, by default, a logistic transformation of the peptide-MHC binding affinity that loosely approximates the probability of T-cell response (Sette *et al.*, 1994). Alternatively, binding predictions can be scored using an affinity threshold (commonly $\leq$ 500nM) or a threshold on the percentile rank of the affinity. Only subsequences which overlap mutant residues and do not occur in the reference proteome are considered as part of the *TotalBindingScore*.

4 Manufacturability

Vaxrank was designed under the assumption that its output will be used to make long peptides, due to their favorable immunological properties (Rosalia *et al.*, 2013). Unfortunately, long peptides are also more difficult to synthesize using traditional solid phase chemistry (Bodanszky, 1988). To avoid known difficulties in synthesis, Vaxrank selects a window of amino acids around each mutation that minimizes the following undesirable properties:

1. total number of cysteine residues
2. max(0, mean hydrophobicity of 7 residues at C-terminus)
3. max(0, mean hydrophobicity of any 7 amino acid window)
4. glutamine, glutamic acid, or cysteine at N-terminus
5. cysteine at C-terminus
6. proline at C-terminus
7. asparagine at N-terminus
8. total number of asparagine-proline bonds

Manufacturability optimization does not affect the ranking of mutations but is only used for selecting which surrounding residues should be included. In cases where a mutation spans a "difficult" sequence (e.g. long hydrophobic stretch), minimizing these criteria may fail to salvage manufacturability.

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