Personalized Neoantigen Vaccination with Synthetic Long Peptides

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Mutations in cancer may give rise to novel antigenic peptides, known as tumor neoantigens, that are promising targets for immunotherapy [1, 2]. However, due to the complexity and heterogeneity of most cancers, a single antigenic target is often insufficient for achieving a durable response or remission [3, 4]. Vaccines using single, short synthetic peptides are vulnerable to immune evasion [5] or may even promote immune tolerance and/or anergy, leading to increased tumor growth [6]. Cancer vaccination has moved in two promising directions: using more peptides and using longer peptides. Vaccines incorporating multiple peptides have been shown to be immunogenic [7] and may reduce the risk of immune evasion [8]. Separately, studies have demonstrated that vaccines which use synthetic long peptides do not appear to induce tolerance [9] and may be more immunogenic than short peptide vaccines [10]. We are developing an early-stage personalized cancer immunotherapy trial at Mount Sinai Hospital that combines these two research findings by treating each patient with up to twenty synthetic long peptides in a single vaccine.

In order to select the contents of this highly multiplexed personalized vaccine, we have developed a computational pipeline to identify and prioritize candidate vaccine peptides. Our computational pipeline takes as input a patient's tumor sequence data and HLA type, generating a set of therapeutic vaccine peptides. The first step in the pipeline is the processing of whole-exome DNA sequence data to determine somatic coding mutations. These mutations lead to candidate antigenic peptides that are filtered by tumor expression level using RNA sequence data. Candidate peptides are then ranked based on a scoring system that incorporates in-silico peptide-MHC binding affinity prediction and a novel immunogenicity predictor. This predictor estimates T cell recognition by computing the similarity of a candidate peptide to peptides in the self-ligandome of each HLA allele.

Key Features

Synthetic Long Peptides
Rather than using minimal epitopes, our vaccine consists of 20 "long" peptides (31 residues in length). Synthetic long peptides have been demonstrated to elicit efficient cross-presentation in dendritic cells and strong responses from T-cells. The size of each vaccine peptide allows us to include multiple epitopes across different HLA alleles.

MHC Binding Prediction
Prediction of epitope binding to MHC Class I molecules is performed using NetMHCcons.
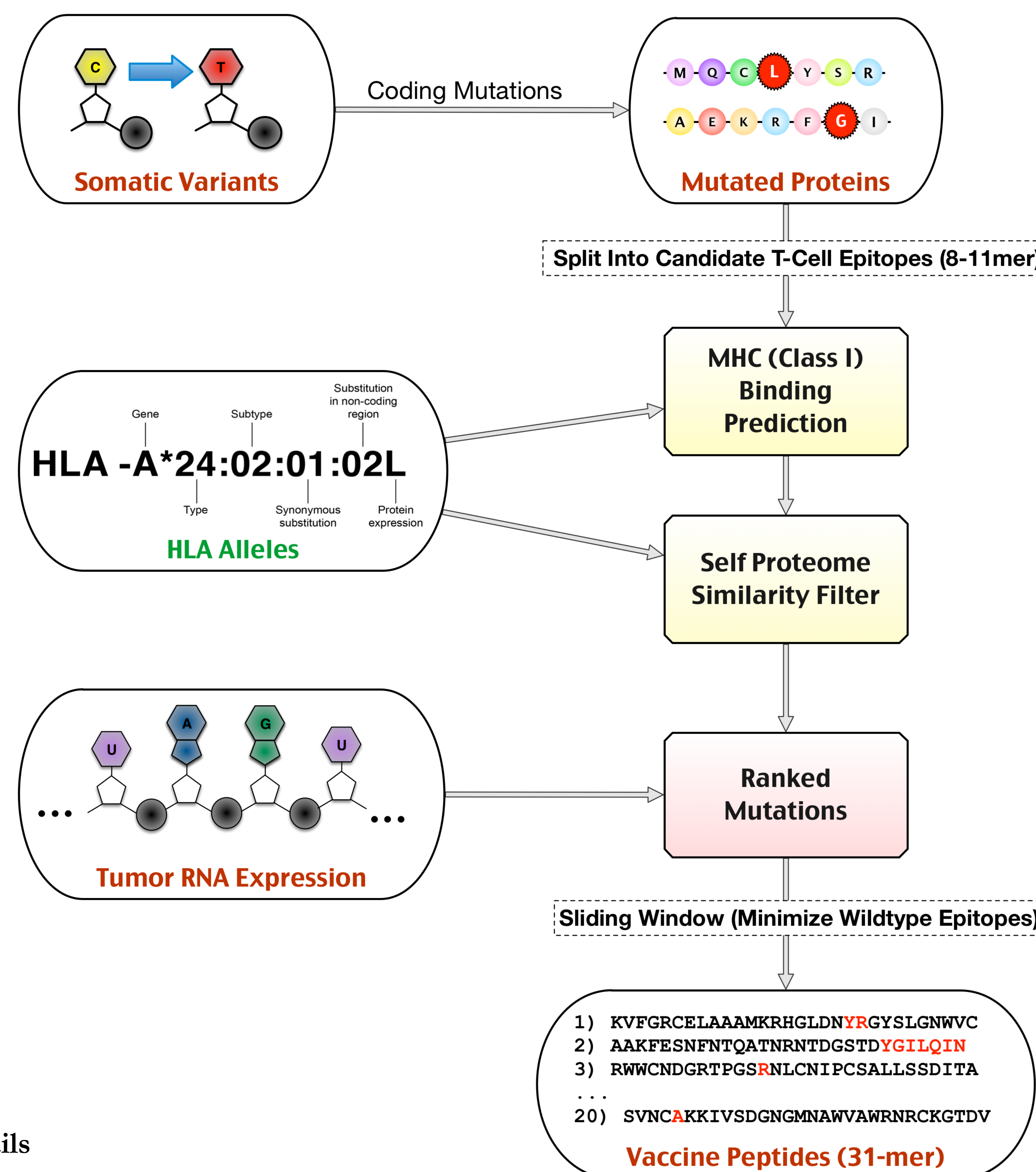
Self-Proteome Filter
When scoring candidate epitopes, we exclude any sequences which resemble MHC ligands from the reference proteome. This is done both to decrease the chances of autoimmunity and to increase the chance that selected epitopes will find circulating high-affinity T-cell receptors.

Vaccine Adjuvant & Administration
In addition to therapeutic peptides, our vaccine uses the TLR agonist Poly-ICLC as an adjuvant. The vaccine is administered via subcutaneous injection.

Vaccine Peptide Selection Details

- All non-synonymous somatic variants identified via WES translated into a 40+ character long string corresponding to the amino acid sequence of the mutated genetic region.
- Every string is broken into multiple 8-11 character long overlapping substrings, each of which is assessed for pMHC binding affinity, and immunogenicity using NetMHCcons.
- Mutated amino acid sequences are ranked based on the sum of putative epitope scores contained in their sequence, and 31-mer vaccine peptides are chosen from the twenty highest scoring sequences via sliding window optimization.
- Among all 31-mer sliding windows with equivalent mutant epitope content (candidate vaccine peptides), we select the vaccine peptide with the least number of wildtype epitopes.
- The mutation in each vaccine peptide must be at least 5 residues from either the beginning or end of the peptide sequence.

Somatic Variants → Coding Mutations → Mutated Proteins

Split Into Candidate T-Cell Epitopes (8-11mer)

HLA -A*24:02:01:02L

HLA Alleles

MHC (Class I) Binding Prediction

Self Proteome Similarity Filter

Tumor RNA Expression

Ranked Mutations

Sliding Window (Minimize Wildtype Epitopes)

1) KVFGRCELAAAMKRHGLDNYRGYSLGNWVC
2) AAKFESNFNTQATNRNTDGSTDYGILQIN
3) RWWCNDGRTPGSRNLCNIPCSALLSSDITA
...
20) SVNCAKKIVSDGNGMNAWVAWRNRCKGTDV

Vaccine Peptides (31-mer)

Vaccine Peptide Ranking

$$\textbf{VaccinePeptideScore}(p) = \textbf{EpitopeContent}(p) \cdot \textbf{ExpressionFilter}(p)$$

$$\textbf{EpitopeContent}(p) = \sum_{k=8\ldots11} \sum_{i=0}^{|p|-k} \textbf{EpitopeScore}(p[i:i+k])$$

$$\textbf{EpitopeScore}(e) = \sum_{a \in \text{HLA}} \sigma(\text{BindingAffinity}(e,a)) \cdot \textbf{SelfFilter}(e,a)$$

$$\sigma(s) = \frac{1}{1 + e^{(s-500\text{nM})/100}}$$

$$\textbf{SelfFilter}(e,a) = \begin{cases} 0, & \text{if epitope } e \text{ similar to "self" ligand of allele } a \\ 1, & \text{otherwise} \end{cases}$$

$$\textbf{ExpressionFilter}(p) = \begin{cases} 0, & \text{if expression level (RSEM) for isoform of } p < 10^{-3} \\ 1, & \text{otherwise} \end{cases}$$

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