String-of-Beads Pipeline

Construct design for multi-epitope-based vaccines

INTRODUCTION

In an effort to evade the immune system, cancer cells undergo a constant transformation, posing a significant challenge for single-epitope-targeting approaches to achieve a sustained and effective immune response. It is therefore crucial to develop **multi-epitope vaccines** not only to ensure broader protection against the evolving tumor mutanome but also to guarantee the mutual expression and presentation of these epitopes by dendritic cells (DCs). Indeed, it has been reported that strongly immunogenic epitopes can enhance the immune response of less immunogenic epitopes when co-presented by DCs.

While several in-silico approaches have been developed for epitope identification and selection, the challenge remains **how to best "package"** multiple different epitopes together in one construct**.** There is a vast permutation search space to be evaluated when combining individual epitopes in specific orders cross-linked with junction-optimized linkers into one single mRNA strand. Each combination can result in different levels of translation efficiency, immunogenicity specific to the construct and target, and the potential creation of junctional epitopes. The term "**junctional epitope**" refers to an antigenic region that is formed at the interface of two epitope sequences, similar to a gene fusion epitope. If these epitope sequences result in highly immunogenic epitopes, they could diminish the vaccine's effectiveness by promoting **immune dominance of irrelevant epitopes**. In rare cases, the junctional epitopes could even resemble epitopes that naturally occur in the human proteome and cause unwanted immune responses in healthy tissue. This could compromise the safety of the vaccine by causing serious adverse events.

To address this, **myNEO Therapeutics** has developed a **String-Of-Beads (SOB) pipeline**, a comprehensive platform to support construct design of multi-epitope-based vaccines. This pipeline optimizes the nucleotide sequence of multi-epitope constructs to avoid the occurrence of junctional epitopes and to ensure a sustained and effective immune response against the targets of interest. Furthermore, the pipeline enables the selection of a set of diverse candidates for efficient validation experiments.

Advantages of SOB Pipeline:

- ✓ Effective combination of multiple epitopes into a single sequence
- ✓ Sustained and effective immune response
- ✓ Avoidance of unwanted immune responses and potential side-effects
- ✓ Increased vaccine efficacy
- ✓ Reduced time & costs of validation

CASE STUDY

For a US-partnered project in cancer vaccines, we applied the SOB pipeline to design two multi-epitope constructs. Each construct was designed so that it could provide a **targeted and maximal T-cell immune response**, by minimizing junctional epitopes as well as by estimating proteasome cleavage patterns (Figure 1).

1. Proteasomal Cleavage Prediction of Desired Epitopes and Pre-Selection of Junctions

To ensure optimal immunogenic potential of the target epitopes, we evaluated each possible junction between two epitopes for proteasomal cleavage before optimizing their sequence order. We filtered out junctions that were likely to hinder optimal proteasomal cleavage, as such junctions could make the target epitopes less accessible and less efficiently processed.

An **ensemble approach** was used for proteasomal cleavage prediction, where confidence was increased by using multiple predictor tools. To identify the best junctions, a **cleavage score**, which is based on the combined confidence of predicted cleavage from all callers, was assigned to each junction (Figure 2). Junctions with higher cleavage scores were considered more likely to correctly process the target epitopes.

Furthermore, we conducted two rounds of optimization: the first focused on optimizing the epitope order without spacers, and the second involved inserting spacers into junctions provided they offered further improvement.

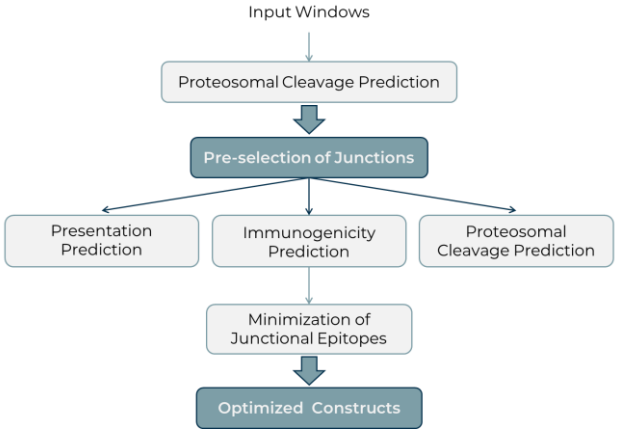

Figure 1: Overview of the steps in the strings-of-beads construct design

2. Proteasomal Cleavage Prediction of Junctional Epitopes

After pre-filtering junctions to ensure the selected set of epitopes was not affected, we assessed junctions based on the number of undesired junctional epitopes they

could produce. Hence, the proteasomal cleavage of junctional epitopes was predicted. For each pre-selected candidate junction, all possible junctional epitopes were scored based on their likelihood of being efficiently cleaved.

3. Binding Prediction and Population Coverage of Junctional Epitopes

After proteasomal processing, peptides are trafficked to and presented on the cell surface by MHC receptors. To evaluate the likelihood of each junctional epitope to be processed and presented on the cell surface, we used **neoMS**, myNEO Therapeutics' proprietary presentation prediction algorithm.

Figure 2: The figure shows junctional epitopes (red) that occur at the junctions between epitopes as well as the indication (arrow) where proteasomal cleavage is considered for individual peptides in the epitope sequences, close to the junction.

To determine the impact of MHC-presented junctional epitopes in a population, the population coverage for each epitope was calculated. This calculation measures the percentage of the population that has at least one of the specific alleles needed to present the junctional epitope. By doing this, the relevance and impact of these epitopes across different population groups was assessed.

4. Immunogenicity Prediction of Junctional Epitopes

Once a peptide is processed and presented, it still needs to be recognized by the immune system to trigger a response. To assess the likelihood of junctional epitopes to elicit an immune response, we use **neoIM**, an immunogenicity predictor algorithm developed by myNEO Therapeutics. neoIM predicts peptide immunogenicity across different HLA-type populations, by scoring the MHC-presented peptides for their likelihood of eliciting a CD8 T-cell response. For each junctional epitope of which the MHC presentation was confirmed, **an immunogenicity score** was assigned via neoIM.

5. Scoring of Junctional Epitopes

For each epitope, we combined the three scores - cleavage score, presentation score, and immunogenicity score - to create an individual **penalty score** (Figure 3).

All individual penalty scores obtained for each epitope in a junction were summed up to an overall penalty score for the given junction. If the number and impact of junctional epitopes increases, so does the overall penalty score of a junction.

6. Optimization of Epitope Order

Finally, to determine the optimal order of the epitopes, we used an **optimization algorithm** because exhaustively comparing all possible options is not feasible. An initial set of different epitope orders in the search space was chosen to start from. The algorithm then fine-tuned each of these solutions by exploring 'neighbouring solutions' to minimize the overall penalty score of all junctions. This process was repeated until the algorithm found an optimum, i.e., the epitope order with the lowest overall penalty score. Ultimately, the best epitope order was selected from the optimized candidate solutions.

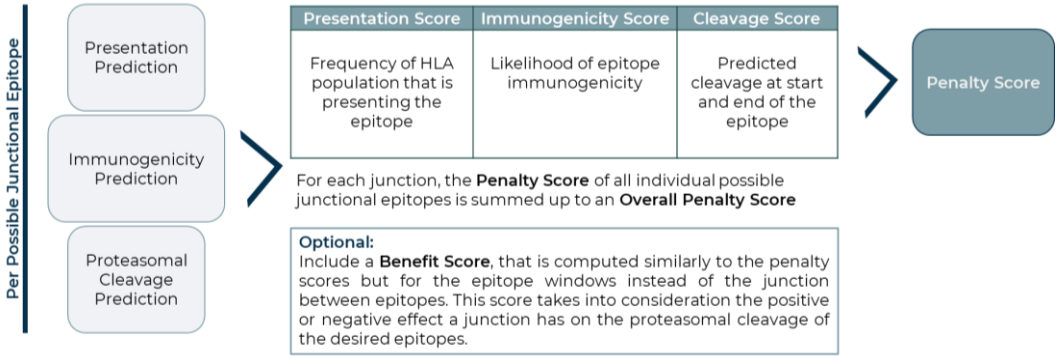

Figure 3: Scoring scheme

CONCLUSION

myNEO Therapeutics' expertise in AI and ML led to the development of a groundbreaking construct optimization pipeline for multi-epitope vaccine design. This innovative solution ensures optimal epitope order, maximizing efficacy while minimizing the potential negative impact of junctional epitopes. By leveraging the power of AI and ML, we've created a solution that streamlines the vaccine design process, saving time and resources while delivering unparalleled results.

myNEO Therapeutics

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Do you want to implement an SOB pipeline in your next vaccine design?

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