myNEO Therapeutics

myCONSTRUCT

Next-generation String-of-Beads construct optimization for multi-epitope immunotherapeutics

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myCONSTRUCT - Next-generation String-of-Beads construct optimization

Executive summary

"Will this multi-epitope nucleic acid construct generate immune responses against the targets you selected — or against unintended junctional peptides?"

Multi-epitope nucleic acid (NA) therapeutics are a powerful strategy to broaden T-cell responses, increase tumor coverage, and reduce immune escape. However, when individual epitopes are concatenated into a single open reading frame (like beads on the string of a necklace, hence the commonly used name of "string-of-beads" for the approach), antigen processing no longer occurs in isolation. Instead, the proteasome processes the entire construct, creating new peptide fragments at epitope boundaries. These so-called junctional epitopes can be efficiently presented and, in some cases, dominate the immune response.

Thus, a list of high-quality epitopes is necessary but insufficient for ensuring a functional multi-epitope vaccine. **Poor construct design can divert immune responses away** from intended targets, reduce therapeutic potency, and introduce avoidable risk.

myCONSTRUCT is a next-generation, in-silico construct optimization tool that systematically **evaluates and minimizes junctional epitope risk as well as optimizes processing of target epitopes**. By integrating proteasomal processing, MHC presentation, immunogenicity prediction, and population-level exposure into a unified risk framework, myCONSTRUCT enables proactive, data-driven design of multi-epitope NA therapeutics.

myCONSTRUCT shifts poly-epitope nucleic acid therapy development from heuristic assembly and combinatorial trial-and-error to predictive, biologically grounded engineering.

Why junctional epitopes matter

In a multi-epitope construct, epitopes are translated as a continuous protein sequence. During intracellular processing:

- The proteasome cleaves the full construct into peptide fragments
- Trimming and transport steps shape the peptide repertoire
- Peptides are loaded onto MHC molecules and presented to T-cells

Crucially, this process generates not only the intended epitopes but also **novel, unwanted epitopes,** i.e. junctional epitopes. Selecting highly immunogenic epitopes is necessary — but insufficient — without systematic construct-level optimization.

Indeed, if junctional peptides are (1) efficiently processed, (2) bind common MHC alleles, and (3) are immunogenic, they may compete with, or even dominate over, the intended epitopes.

The consequences are significant:

- **Efficacy risk:** Immunodominant junctional epitopes can divert responses away from therapeutic targets.
- **Reduced tumor coverage:** Intended epitopes may fail to elicit sufficient T-cell activation.
- **Payload inefficiency:** Limited epitope slots are effectively "wasted."
- **Potential safety risk (rare):** Unintended peptides may resemble self-peptides.

Limitations of current construct design approaches

Despite the increasing clinical relevance of multi-epitope vaccines, construct design remains largely heuristic. Common limitations include:

- **Empirical epitope ordering** without quantitative evaluation of boundary effects.
- **Spacer selection based on convention**, rather than predictive modeling.
- **Combinatorial explosion**: For N epitopes, the number of possible order × spacer combinations grows factorially, rendering exhaustive evaluation infeasible.
- **Late discovery of processing biases**, often during preclinical or clinical evaluation.

Experimental validation alone cannot realistically screen the vast design space of possible string-of-beads constructs. Hence, there is a clear unmet need for a systematic, biologically grounded framework that quantifies junctional epitope risk before construct finalization.

myCONSTRUCT for junction risk control

myCONSTRUCT is an **end-to-end computational platform designed to evaluate and optimize multi-epitope constructs at the sequence level**. It integrates four core layers of biological modeling:

Junctional peptide generation

For every epitope boundary within a candidate construct, myCONSTRUCT enumerates all peptide fragments that could arise across the junction following intracellular processing. This ensures that no potentially relevant boundary-derived peptide is overlooked. Constructs that could generate junctional self-epitopes can therefore also be identified early on during design, avoiding safety risks early on in the development process.

Processing and presentation modeling

Each junctional peptide is evaluated for (1) proteasomal processing likelihood, (2) MHC class I (and optionally class II) presentation probability, and (3) allele-specific binding and presentation features. Predictions are computed across relevant HLA panels, enabling population-level evaluation.

Immunogenicity scoring

Presentation alone does not guarantee T-cell activation. myCONSTRUCT integrates advanced immunogenicity modeling to estimate the probability that a presented peptide will elicit a T-cell response. This allows differentiation between peptides likely to be presented but immunologically silent versus peptides with high immunogenic potential.

Integrated junction risk aggregation

Processing, presentation, and immunogenicity signals are combined into a unified junction risk score. Risk scores can be (1) aggregated across alleles, (2) weighted by allele frequency, and (3) summarized at the construct level. This enables direct apples-to-apples comparison of candidate designs and provides a quantitative construct-level penalty metric.

Design optimization: order and spacer selection

Because exhaustive enumeration of all epitope order and spacer combinations is impossible, myCONSTRCT employs optimization algorithms to explore the design space efficiently. Through iterative refinement epitope order is adjusted, spacer sequences are evaluated, and construct-level junction penalties are minimized.

The result is a ranked set of optimized designs with reduced junctional epitope risk while preserving processing of intended targets.

Retrospective assessment of myCONSTRUCT on HBV multi-epitope constructs

To evaluate the predictive validity of myCONSTRUCT, we retrospectively analyzed experimentally characterized multi-epitope HBV DNA constructs reported by Livingston *et al.* (Vaccine, 2001). We first use myCONSTRUCT to compare alternative orderings of the same HBV epitope set in score space, and then test whether myCONSTRUCT -derived junctional risk estimates align with experimentally observed immunogenicity differences in the published construct variants.

In-silico benchmarking of epitope-ordering strategies

As an initial computational benchmark, we compared myCONSTRUCT-derived junctional epitope risk scores across three epitope-ordering strategies for the same HBV epitope set: (i) randomized epitope orders (background distribution), (ii) the published epitope order, and (iii) a myCONSTRUCT-optimized epitope order.

Random scores were generated by repeatedly sampling random epitope orders and computing the resulting junctional risk scores using myCONSTRUCT. In contrast, the published and myCONSTRUCT-optimized designs each represent deliberate ordering strategies rather than random arrangements.

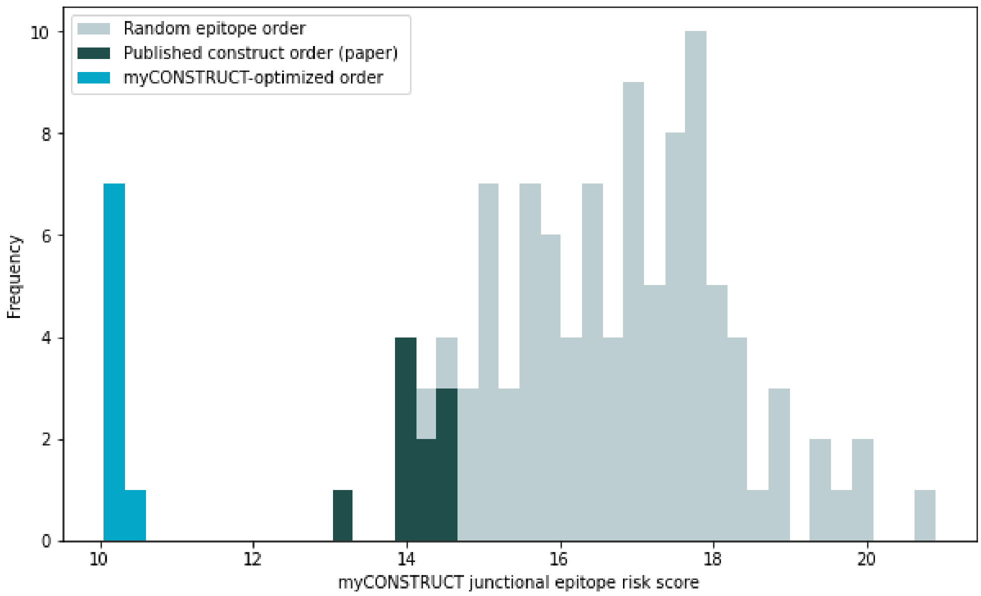

Figure 1. **Retrospective in-silico comparison of junctional epitope risk scores across epitope-ordering strategies for an HBV multi-epitope construct**. Histogram of myCONSTRUCT-computed junctional epitope risk scores for randomized epitope orders (background distribution), with the corresponding scores for the published construct order and a myCONSTRUCT-optimized order shown for comparison.

This in-silico benchmark indicates that the published construct order is consistent with deliberate reduction of junctional epitope risk relative to random epitope arrangements (**Figure 1**). At the same time, the substantially lower score obtained for the myCONSTRUCT-optimized order suggests that, under the myCONSTRUCT scoring framework, an even more favorable ordering can be identified for this epitope set.

Retrospective validation against experimental immunogenicity

We next evaluated whether the myCONSTRUCT-derived junctional risk scores align with experimentally observed immunogenicity differences in the Livingston et al. HBV construct variants. In this study, the String-of-Beads design consisted of a fixed set of concatenated CD8^{+} T-cell epitopes arranged in the same order across all construct versions. Only the immediate sequence context at a single junction (following HBV Core18 epitope) was altered: (1) either by inserting an additional epitope (HBV_1 vs HBV_2) or (2) by inserting a single amino acid (HBV_1X variants e.g., W/Y/L vs K/R/C/N/G) as shown in **Figure 2**.

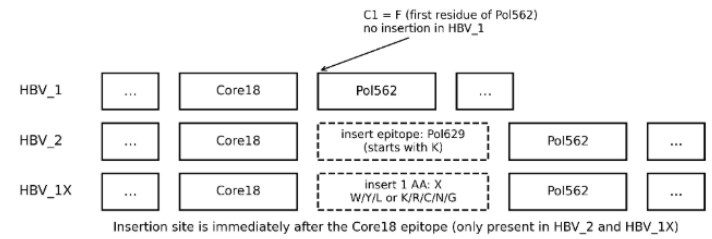

Figure 2. HBV construct layouts used as proof of concept: a single junction position is modified either by adding an epitope (HBV_2) or inserting a single amino acid (HBV_1X variants).

Remarkably, minor junction modifications were sufficient to switch Core18 from strongly immunogenic to weakly or non-immunogenic — despite the epitope sequence itself remaining unchanged, making it an ideal dataset for junction-aware design.

We re-analyzed these constructs using myCONSTRUCT's junctional risk scoring framework and made the following key observations:

- Constructs predicted to have **lower junctional risk** aligned with **stronger Core18 immune responses**.
- Constructs predicted to have **higher junctional risk** corresponded more frequently to weaker outcomes.
- Aromatic/bulky residue insertions (W/Y/L) behaved distinctly from non-aromatic insertions (K/R/C/N/G), illustrating how minimal boundary changes can reshape antigen processing hierarchies.

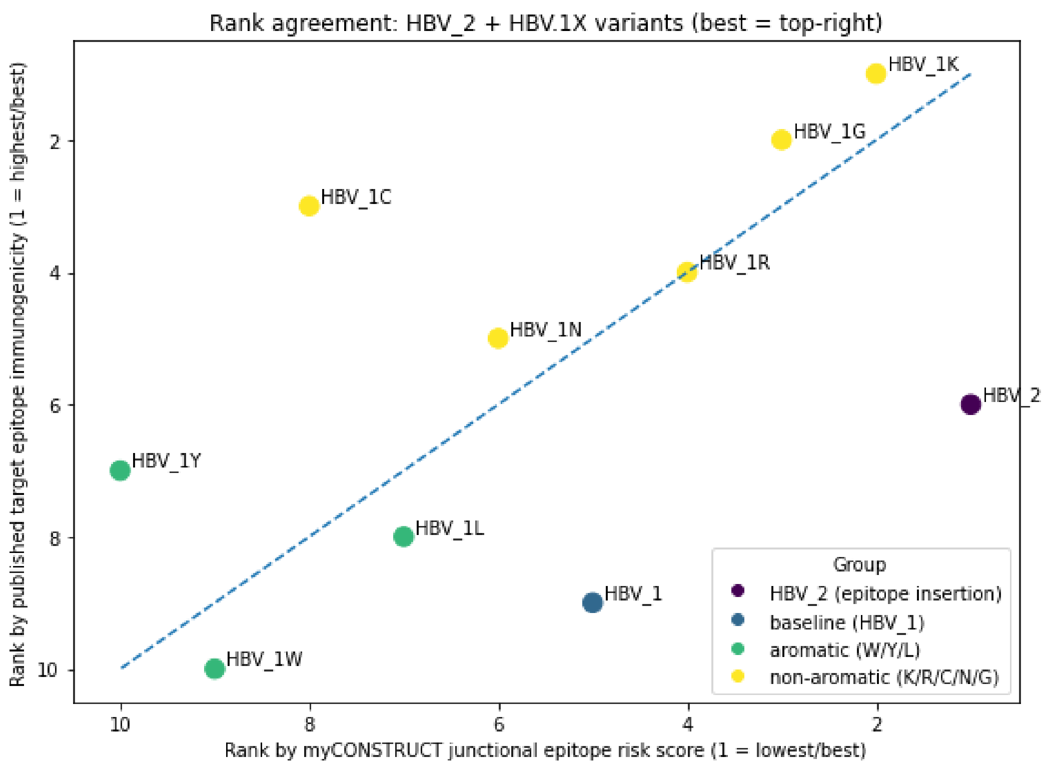

Figure 3. Retrospective validation of myCONSTRUCT using HBV multi-epitope constructs (Livingston et al., Vaccine 2001). Constructs are ranked by myCONSTRUCT-predicted junctional epitope risk (x-axis) and experimentally observed Core18 immunogenicity (y-axis).

Alignment of predicted junctional risk with experimentally observed immunogenic hierarchy demonstrates that myCONSTRUCT captures biologically meaningful construct-level effects and can discriminate between designs that preserve versus impair target epitope immunogenicity.

Conclusion

myCONSTRUCT enables rational, early-stage optimization of multi-epitope nucleic acid constructs by proactively controlling junctional epitope formation.

By integrating proteasomal processing, MHC presentation, immunogenicity prediction, and population-aware aggregation into a unified risk framework, myCONSTRUCT transforms string-of-beads design from heuristic assembly to predictive engineering.

Integration of myCONSTRUCT into NA therapeutic development pipelines:

- Reduces construct-level uncertainty and unwanted junctional epitopes
- Minimizes unintended immunodominance
- Preserves immune focus on intended therapeutic targets
- Increases the probability of robust and clinically relevant T-cell responses

myCONSTRUCT delivers what conventional construct design cannot: scalable, biologically grounded, data-driven control over multi-epitope NA therapeutics architecture.

References

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