VacDesignR®: a Tool for Optimizing Recombinant Poxvirus Vaccine Production

Authors[1]: B. Grellier, N. Silvestre, JB. Marchand, Y. Schlesinger, C. Fahrner, L. Schott, AI. Moro, E. Dochy, M. Ceppi

[1] Transgene SA, Illkirch-Graffenstaden, France

ESMO AI & Digital Oncology 2025

#385P

Copies of this poster obtained through QR, AR and/or text key codes are for personal use only and may not be reproduced without written permission of the authors

BACKGROUND

Modified Vaccinia Virus of Ankara (MVA) is an attenuated vaccinia virus with a double-stranded DNA genome used to develop therapeutic vaccines. Recombinant MVAs are generated by incorporation of heterologous transgene expression cassettes by homologous recombination, as MVA is prone to homologous recombinant events. However, this genetic engineering advantage remains an issue if unwanted homologous regions are incorporated. Overexpression of hydrophobic peptides and peptides containing transmembrane (TM) domains, might impair the viability of cells and therefore the yield of virus produced. The successful incorporation of a high variety of peptides requires the control of these specific features.

OBJECTIVE

In the context of the manufacturing of an individualized MVA-based vaccine, the list of peptides are patient-specific neoantigens (eg, *from NEC's prediction platform*). The top 30 eligible peptides are combined into three protein fusions, optimized for the production and performance of the vaccine.



METHODS

VacDesignR® is a software selecting and assembling up to 30 predicted immunogenic peptides (IPs) within the MVA genome. The 3 main steps are the peptides selection, the inter-cassette repartition and the intra-cassette ordering.

The IPs are filtered for homologous regions and sorted based on TM and Hydropathy (H) scores, defining 3 "production difficulties" classes. IPs from the 3 classes are then proportionally dispatched to each cassette. The fusions are then performed for all guided combinations for each cassette. Only the fusions with the lowest H and TM scores are kept for final plasmid production.

ACKNOWLEDGEMENTS

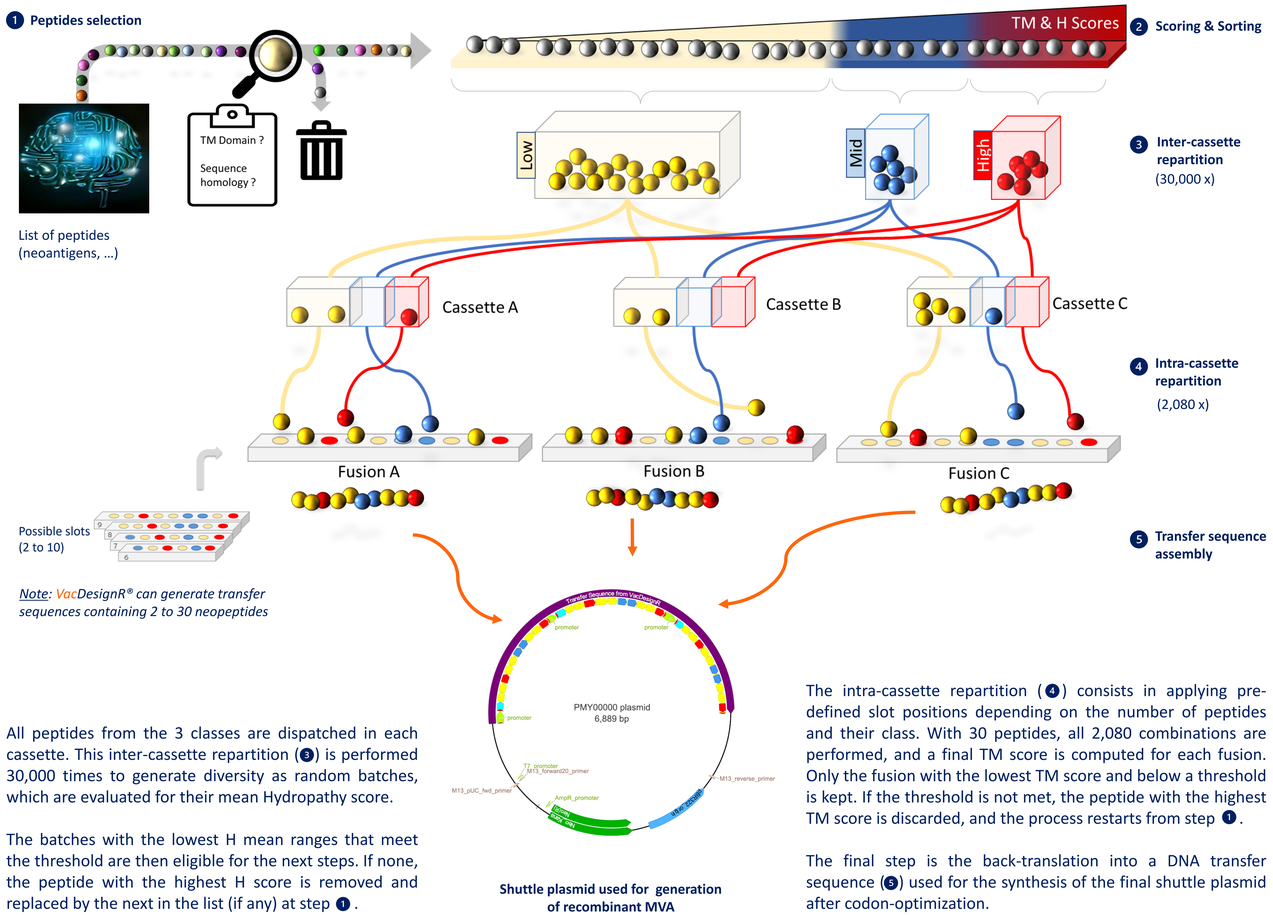
The study was industry co-funded by NEC Corporation and Transgene SA.

Corresponding author email address: grellier@transgene.fr

The first author is an employee of Transgene which sponsored this work. The content of this poster has been reviewed in accordance with Transgene policies. The first author declares no additional conflicts of interest.

SOFTWARE OPERATIONS

The input is a ranked list of peptides (*eg* from neoantigen prediction platform) that are first filtered based on their transmembrane (TM) predicted score and their sequence homology; all but one peptides sharing identical domains of 6 amino acids or more are temporarily discarded (❶).
The top 30 are then sorted based on TM and Hydropathy (H) scores to define 3 classes of "production difficulty": Low, Mid and High (❷)



All peptides from the 3 classes are dispatched in each cassette. This inter-cassette repartition (❸) is performed 30,000 times to generate diversity as random batches, which are evaluated for their mean Hydropathy score.

The batches with the lowest H mean ranges that meet the threshold are then eligible for the next steps. If none, the peptide with the highest H score is removed and replaced by the next in the list (if any) at step ❶ .

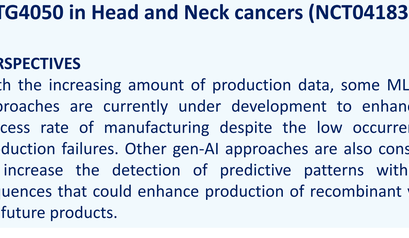
Shuttle plasmid used for generation of recombinant MVA

The intra-cassette repartition (❹) consists in applying pre-defined slot positions depending on the number of peptides and their class. With 30 peptides, all 2,080 combinations are performed, and a final TM score is computed for each fusion. Only the fusion with the lowest TM score and below a threshold is kept. If the threshold is not met, the peptide with the highest TM score is discarded, and the process restarts from step ❶ .

The final step is the back-translation into a DNA transfer sequence (❺) used for the synthesis of the final shuttle plasmid after codon-optimization.

EXPERIMENTAL ASSESSMENT

Production of recombinant MVA-based personalized neoantigens vectors before the clinical trials.



Transfer sequences were designed with or without VacDesignR® and used for the generation of recombinant MVAs.

The sequence analysis of the 32 constructs generated without vaccine design tools led to the determination of key parameters (TM, H scores,...) to setup the optimization tool.
The result is an increase in success rate from less than 35% to over 80% thanks to VacDesignR®.

KEY MESSAGES

- **VacDesignR® is efficient and effective to optimize transfer sequence design**
- **Avoiding unwanted homologous recombination and production issues by keeping the TM and H scores low**
- **It is currently used for the therapeutic vaccine production in the Phase I/II clinical trial evaluating TG4050 in Head and Neck cancers (NCT04183166)**

PERSPECTIVES

With the increasing amount of production data, some ML-based approaches are currently under development to enhance the success rate of manufacturing despite the low occurrence of production failures. Other gen-AI approaches are also considered to increase the detection of predictive patterns within the sequences that could enhance production of recombinant viruses for future products.