Pre-Clinical Proof-of-Concept of AAVLP-EpCAM Mimotope Vaccines in a Mouse Model with Human EpCAM Expressing CT26 Colon Cancer Cells

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Introduction

The transmembrane glycoprotein epithelial cell adhesion molecule (EpCAM) plays an important role in tumor development and is overexpressed in many epithelial cancers including breast, colon, lung, ovarian, cervical, prostate and head and neck cancers[1,2]. Utilizing a known anti-EpCAM monoclonal antibody (mAb), MOC31[3], we have turned this passive immunotherapy into an active immunotherapy mobilizing the patient's own immune system for a potent and long-lasting response against EpCAM positive cancer cells.

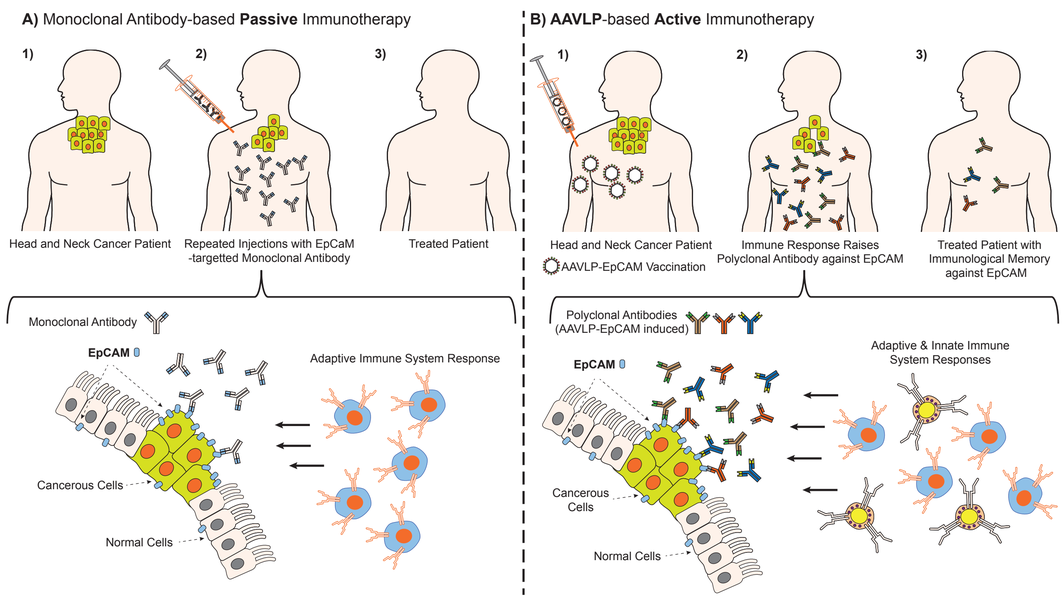


Figure 1: Active immunotherapy is superior to passive immunotherapy. Head and neck cancer used as an example. **A)** Principle of the passive immunotherapy currently used against EpCAM-positive cancer cells. This type of targeted therapy is efficient and less harsh than e.g. chemotherapy, but the huge amounts of antibody needed, in regular injections, make passive immunotherapy one of the most cost-intensive treatment options available. Moreover, the patients often become intolerant to the foreign antibodies already after 2nd or 3rd injection leading to hypersensitivity. **B)** Use of AAVLP in Active Immunotherapy against EpCAM-positive cancer cells. This is a superior option compared to passive immunotherapy as this strategy is much cheaper, eliminates side-effects caused by intolerance, confers immunological memory, and is expected to have superior efficacy.

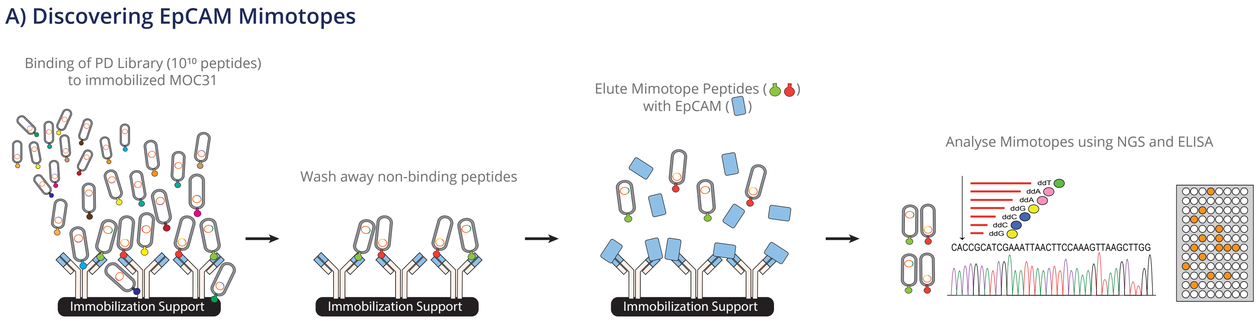
Novel use of peptide phage display in combination with next generation sequencing (PD-NGS) has been applied to retrieve peptides mimicking the original epitope to be displayed in multiple copies on a clinical approved non-proliferative adeno-associated virus-like-particle (AAVLP).

Objective

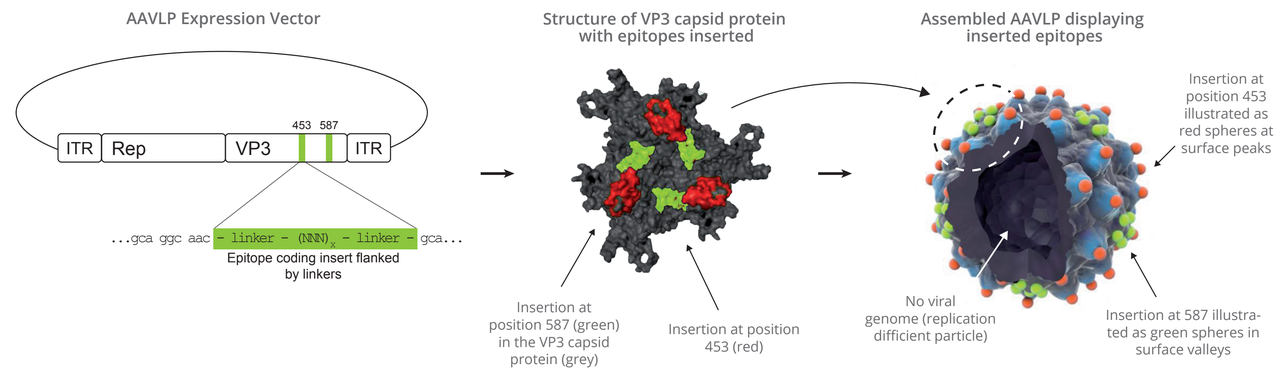
To develop an active AAVLP anti-EpCAM cancer immunotherapy engineered to display mimotopes mimicking the function of an approved anti-EpCAM antibody identified by using PD-NGS-guided molecular evolution.

Methods

A) Discovering EpCAM Mimotopes



B) Transfer of Mimotopes to AAVLP



Engineered **epitopes** are displayed **numerously** across the AAVLP surface, which also is inherently **immunogenic**.

C) Pre-clinical Study

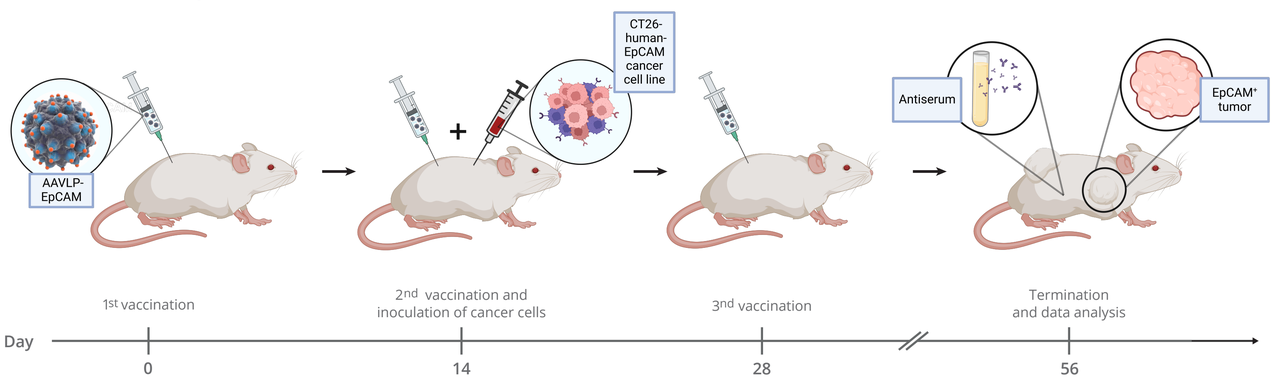


Figure 2: Demonstration of the scientific rationale for developing mimotope-based AAVLP-EpCAM vaccines. **A)** Development of MOC31-binding (EpCAM mimicking) mimotopes using phage display (PD), next generation sequencing (NGS) analysis and *in vitro* characterization using ELISA. **B)** Transfer of mimotopes to AAVLP. Mimotopes are cloned into the capsid protein VP3 for display in multiple copies on the AAVLP surface. In total, 55 vaccine candidates have been developed. **C)** Nine AAVLP-EpCAM vaccine candidates were selected and tested in a murine model inoculated with a colon cancer cell line (CT26) genetically expressing human EpCAM. Mice (*n* = 5/group) were vaccinated s.c. with 100 µl vaccine with adjuvant (Alhydrogel) on days 0, 14, and 28 and sera were collected on days 0 (pre-serum), 28, and 56. Mice were inoculated with CT26-human-EpCAM cancer cells (0.25x10⁶/mouse) after the second vaccination. An irrelevant AAVLP vaccine was used as control. Tumor progression was followed until study termination on day 56 or until humane endpoint was reached.

Results

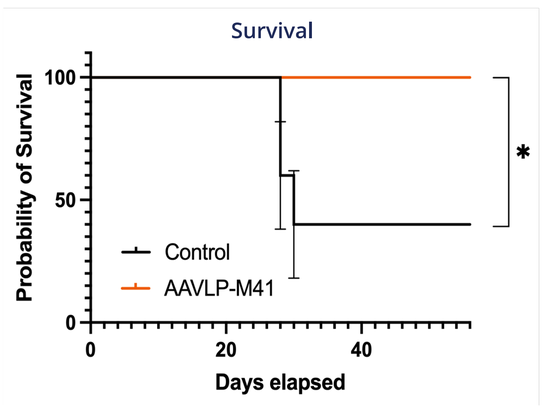


Figure 3: Kaplan-Meier survival curve representing probability of survival (%) when vaccinated with AAVLP-M41 (orange) compared to control (AAVLP-HPV, black), (n=5/group). The statistical analysis applied is a log-rank (Mantel-Cox) test. Survival was significantly higher when vaccinated with AAVLP-M41 (100%) compared to control (40%), (p = *0.0486*). Results presented with SE.

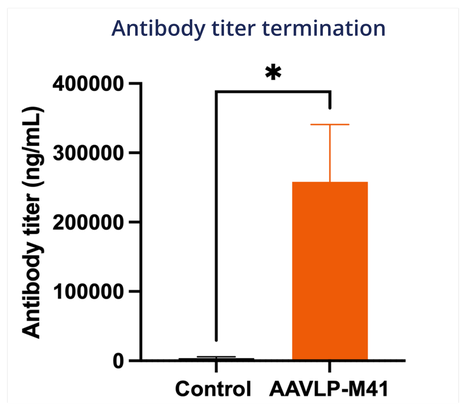


Figure 4: Anti-EpCAM antibody titer (ng/mL) measured at termination for control (black bar) and AAVLP-M41 (orange bar) vaccinated mice, (n=5/group). The statistical analysis applied is an unpaired t-test. AAVLP induced significant higher anti-EpCAM antibody titer (258041 ng/mL) than compared to control (3935 ng/mL), (p = *0.0153*). Results are presented as mean ± SEM.

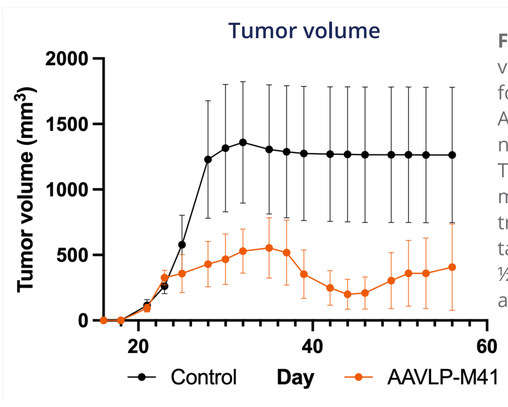


Figure 5: Measured tumor volume (mm³) over time for control (black) and AAVLP-M41 (orange) vaccinated mice, (n=5/group). Tumor volume was determined in two dimensions trice per week using a digital Vernier caliper (L x W x ½W). Results are presented as mean ± SEM.

Mice vaccinated three times with a 2-week interval demonstrated tumor regression and resulted in a significantly improved survival (100%) compared to control group.

Acknowledgements

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Conclusion

- Preliminary proof-of-concept studies demonstrated cancer regression of EpCAM positive tumors after treatment with active immunotherapy created by PD-NGS to mimic a known monoclonal antibody and integrating amino acid sequences into the capsid structure of an AAVLP for multiple display.
- Dose-concentration studies for AAVLP-M41 are currently ongoing.
- For future perspectives, incorporation of this vaccine as an adjuvant therapy together with chemotherapy and/or radiation or as a stand-alone therapy are to be investigated.

References

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