Lysosomal-associated membrane protein-1-targeting of a poly-neoepitope DNA vaccine elicits potent immune responses and inhibits tumor growth

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Abstract

Cancer vaccines have traditionally succeeded in preventing viral-induced malignancies, such as papilloma virus-associated cervical cancer and hepatitis B-associated hepatocellular carcinoma. Conceptually, tumor-specific protein coding mutations (neoantigens) are ideal targets for cancer immunotherapy. Neoantigens are not expressed in healthy tissues and therefore are exempt from central tolerance and can potentially be recognized by T cells to facilitate tumor rejection. However, every patient's tumor possesses a unique set of mutations that must first be screened for optimal target selection, presenting a challenge for neoantigen vaccine development. We present here the merging of two innovative and complementary approaches for identifying neoantigens and delivering personalized cancer vaccines.

The CT26 mutanome was evaluated with Ancer™, an innovative and automated neoepitope prediction platform that combines proprietary machine learning-based MHC class I and MHC class II neoepitope identification tools with removal of inhibitory regulatory T cell (Treg) epitopes for optimal personalized cancer vaccine design. MHC class I- and MHC class II-restricted CT26 neoepitopes, devoid of putative Treg epitopes, were ranked according to their immunogenic potential and tumor expression level. The twenty most highly Ancer-ranked neoepitopes were subsequently introduced as a "string of beads" DNA vaccine into the UNITE (UNiversal Intracellular Targeted Expression) platform (poly-neoepitope UNITE vaccine). The UNITE platform is based in part on lysosomal targeting technology which results in enhanced antigen presentation and a balanced T cell response.

We report that prophylactic vaccination with poly-neoepitope UNITE vaccine successfully induced IFNγ-producing Th1 cells, with complete rejection of CT26 tumors observed in 50% of mice. Mice rejecting tumors were also protected from rechallenge with CT26, demonstrating that effective antigen-specific memory was induced in these animals. In therapeutic vaccination studies, we observed CT26 tumor growth inhibition in 46% of animals immunized with the poly-neoepitope UNITE vaccine, as well as significantly prolonged survival, as compared to animals immunized with the control vector.

Therefore, targeting multiple mutations encoding the best set of CD4 and CD8 neoepitopes, as predicted by Ancer™, and using the UNITE platform may solve critical problems in current cancer immunotherapy development. Follow-up studies will include evaluation of the poly-neoepitope UNITE vaccine in combination with checkpoint inhibitors.

Introduction

Goal: Design a string-of-beads DNA vaccine targeting mutations in the CT26 syngeneic tumor model and evaluate the efficacy in vivo in tumor prevention and therapy studies.

How: Use Ancer™ to process CT26 variants, identify and rank vaccine candidate peptides, and design string-of-beads DNA vaccine using the UNITE platform (poly-neoepitope UNITE vaccine).

Source Mutanome: Charles River Laboratories provided a list of mutations identified in their CT26 model as well as the results of their transcriptome analysis of the CT26 line. An additional published CT26 mutanome and transcriptome was retrieved from Castle et al. BMC Genomics 2014 15:190.

In silico design of a neoantigen vaccine

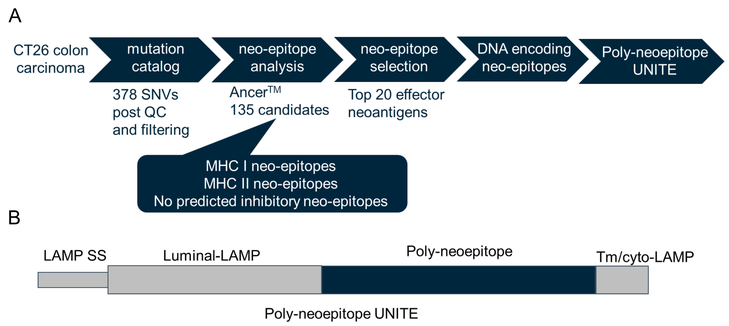
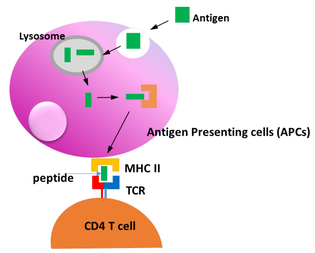


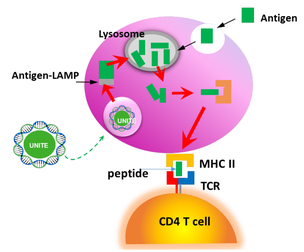
Figure 1: A. CT26 mutations were filtered based on genomic and transcriptomic features to yield 378 candidate single nucleotide variants (SNVs) for neo-epitope analysis. Corresponding mutated and wild-type sequences were analyzed with the Ancer™ platform to identify MHC I- and MHC II-restricted neo-epitopes and to evaluate their potential to induce inhibitory (regulatory) T cell responses due to high homology with other self-sequences. Neoantigen sequences containing high-quality neo-epitopes were subsequently ranked by Ancer™ based on immunogenicity, homology, genomic, and transcriptomic factors. The 20 highest ranking neoantigen sequences were concatenated into a poly-neoepitope string of bead design and introduced into the UNITE™ platform for vaccine studies. **B**. The neoepitope sequence was cloned into the NTC8382-VA1-LAMP plasmids.

UNITE™ platform: Unique Mechanism of Action

- UNITE™-VAX utilizes plasmid DNA expressing poly-neoepitope and LAMP to deliver poly-neoepitope to the MHC II compartment, enhancing antibody generation and CD4+ T cell responses.
- Based on our previous studies, we hypothesize that UNITE™-VAX can rapidly prime and activate antigen-specific CD4⁺ T cells and these activated CD4⁺ T cells play an essential role for the function and infiltration of CD8⁺ T cells into tumor sites.



Classical activation of CD4⁺ T cells is initiated by antigen-presenting cells that process extracellular antigen and present it through MHC class II



poly-neoepitope DNA-mediated activation of CD4⁺ T cells is achieved through processing of endogenous antigen by antigen-presenting cells

Poly-neoepitope UNITE vaccine induces robust neoantigen-specific IFNγ production

Figure 2: A. Vaccination schedule. BALB/c (AnNCrl) female mice, 6-7 weeks (Charles River) were immunized with either control vector or poly-neoepitope UNITE vaccine by i.d. delivery in ear and electroporation on days 0, 14, and 28. Mice were euthanized on day 40 and spleens were collected. Cellular responses of BALB/c mice were measured using IFNγ (**B**) or IL-10 (**C**) ELISPOT. Splenocytes from A were stimulated with EO peptides (2ug/ml) in T cell media for 48h. Spots of experimental – media for each mouse is shown. N=10 mice per group. One way ANOVA and Tukey's multiple comparison test using Prism software showed p<0.0001*

Poly-neoepitope UNITE vaccine prevents 50% of CT26 colon tumor growth and delays tumor growth in the other 50% of mice

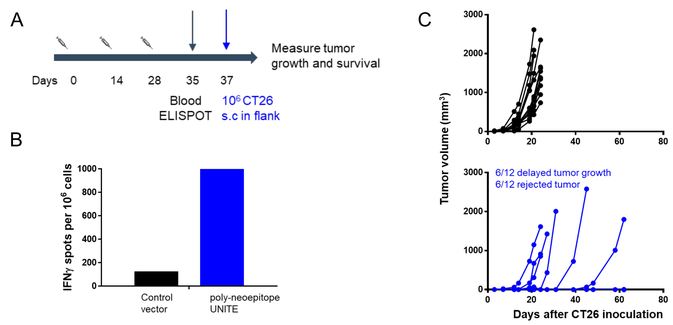


Figure 3: A. Vaccination schedule. BALB/c mice (12 per group) were immunized with either control vector (black) or poly-neoepitope UNITE vaccine (blue) by ID+EP with three doses at 2 weeks intervals. **B**. Seven days after the last immunization, blood samples from each group were pooled, and cellular responses were measured using IFNγ ELISPOT. Cells were cultured with 10μg/ml of pooled peptides. Data represent spots forming cells from pooled samples for each group. **C**. Nine days after the last immunization, mice were inoculated with 10^6 CT26 tumor cells (ATCC). Tumor growth was recorded. Individual tumor volume of n=12 mice per group; 6/12 mice had delayed tumor growth and 6/12 mice rejected tumor with the vaccine.

Poly-neoepitope UNITE vaccine generates Ag-specific memory response in tumor-bearing mice

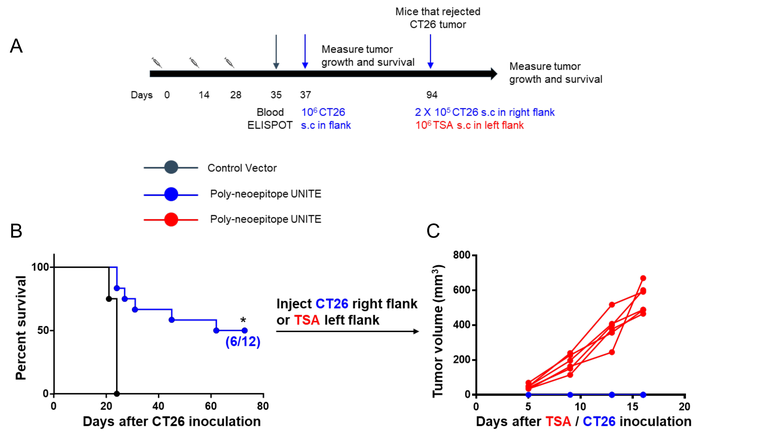


Figure 4: A. Vaccination schedule. BALB/c mice (12 per group) were immunized with either control vector or poly-neoepitope UNITE vaccine by ID+EP with three doses at 2 weeks intervals. Nine days after last immunization, mice were inoculated with 10^6 CT26 tumor cells (ATCC). Survival was recorded. **B**. Kaplan-Meier plot to show survival. The surviving mice (6/12) from **B** were re-injected with CT26 tumor cells in the right flank and TSA tumor cells in the left flank. Tumor growth was recorded (**C**). Individual tumor volume of n=6 surviving mice, there was no CT26 tumor re-growth in right flank.*p<0.0001 by Log-rank (Mantel-Cox) test and Gehan-Breslow-Wilcoxon test.

Poly-neoepitope UNITE vaccine therapy extends survival in CT26 tumor-bearing mice

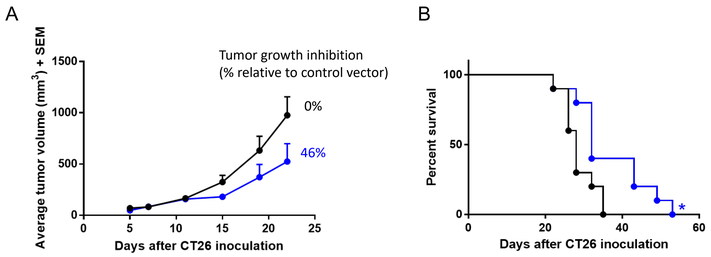


Figure 5: Female BALB/c (Charles River) were inoculated s.c in flank with $2x10^5$ CT26 (ATCC) tumor cells on day 0. Vaccine was started on day 6 when average ± SEM tumor volume for control vector, and poly-neoepitope UNITE vaccine were 69.6 ± 8.5, and 48.0 ± 4 mm^3 respectively. **A**. Data shown is average + SEM of ten mice per group. **B**. Survival was recorded. All the control mice were dead by day 35, however poly-neoepitope UNITE vaccinated mice survived until day 53. * indicates p<0.05 on Log-rank (Mantel-Cox) test and Gehan-Breslow-Wilcoxon test.

Conclusions

- CT26 variants were screened with Ancer™, a highly specialized neoantigen discovery platform, to identify MHC I- and MHC II-restricted neo-epitopes with low potential for inducing regulatory T cell responses.
- Highest ranking neoantigen sequences were concatenated into a poly-neoepitope string of bead design and introduced into the UNITE platform.
- Poly-neoepitope UNITE vaccine elicits robust neoantigen-specific IFNγ responses in vivo, which in turn have significant antitumor effects in the murine CT26 colon cancer model.
- Mice rejecting tumors were protected from re-challenge with CT26, demonstrating that antigen-specific memory was induced in these animals.
- Future experiments will test the poly-neoepitope UNITE vaccine in combination with checkpoint blockade in a therapeutic setting.

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References

- Garris CS, Arlauckas SP, Kohler RH, Trefny MP, Garren S, Piot C, Engblom C, Pfirschke C, Siwicki M, Gungabeesoon J, Freeman GJ, Warren SE, Ong S, Browning E, Twitty CG, Pierce RH, Le MH, Algazi AP, Daud AI, Pai SI, Zippelius A, Weissleder R, Pittet MJ. Successful Anti-PD-1 Cancer Immunotherapy Requires T Cell-Dendritic Cell Crosstalk Involving the Cytokines IFN-γ and IL-12. Immunity. 2018 Dec 18;49(6):1148-1161.e7.
- Ahrends T, Spanjaard A, Pilzecker B, Bąbała N, Bovens A, Xiao Y, Jacobs H, Borst J. CD4(+) T Cell Help Confers a Cytotoxic T Cell Effector Program Including Coinhibitory Receptor Downregulation and Increased Tissue Invasiveness. Immunity. 2017 Nov 21;47(5):848-861.e5.
- https://www.epivaxonco.com/select-publications

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