Therapeutic Efficacy of ZVex™ and GLAAS™ Immunotherapy Platforms in a B16-F10/hCAIX Melanoma Mouse Model

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Introduction

ZVex™ and GLAAS™ are two dendritic cell (DC) targeting platform technologies designed to enhance immune responses through the *in vivo* induction of antigen specific CD8⁺ and CD4⁺ T cells, respectively. ZVex™ is a lentiviral vector pseudotyped with a modified Sindbis virus envelope engineered to deliver antigen-encoding nucleic acids to dendritic cells *in vivo*. GLAAS™ (Glucopyranosyl Lipid A Adjuvant System) activates DC by binding to the TLR-4 receptor and inducing strong Th1 type CD4 responses against co-delivered recombinant proteins. Currently both platform technologies are being investigated in Phase I clinical trials in cancer patients.

Human carbonic anhydrase 9 (hCAIX) is a tumor-associated transmembrane antigen that is overexpressed on various cancer cell types. We mapped hCAIX specific, multi-functional CD8⁺ and CD4⁺ T cell epitopes within the extracellular and transmembrane regions of the protein for the mouse haplotype $H2^b$ by intracellular cytokine staining. Mice lethally challenged *s.c.* on the flank with a B16-F10 tumor cell line expressing the hCAIX protein fully controlled large tumors (>100 mm^2) when therapeutically immunized (*s.c.* at the base of tail) with ZVex™ encoding hCAIX or recombinant hCAIX protein with GLA-SE, a formulation within the GLAAS™ platform (either *s.c.* or *i.m.)*. In both models, tumor control was dose-dependent. Additionally, the presence of a strong transmembrane $H2^b$-restricted CD8 T cell epitope was required for tumor control and regression. hCAIX-specific CD8 T-cell responses were detectable as far out as day 67 post-challenge in mice displaying full tumor regression. These results demonstrate proof of concept for ZVex™ and GLAAS™ platform technologies in an aggressive murine melanoma model.

Conclusions

Therapeutic immunization with ZVex™ encoding hCAIX or GLA-SE with recombinant hCAIX generate antigen-specific CD8⁺ and CD4⁺ T cell responses that control B16-F10/CAIX tumor growth in mice.

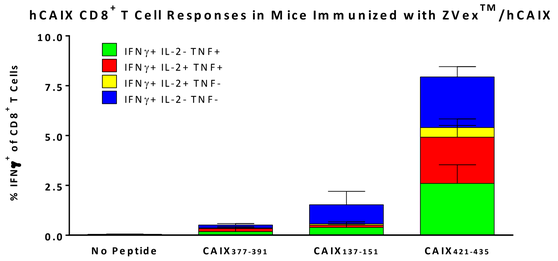
- *ZVex™ encoding hCAIX and recombinant hCAIX with GLA-SE both generate Ag-specific T cell responses.*
- *Therapeutic efficacy against tumor growth is demonstrated with both ZVex™ encoding hCAIX and recombinant hCAIX with GLA-SE.*
- *CAIX-expressing tumors initially regressed upon ZVex™/hCAIX treatment before growing out. These tumors down-regulated expression of CAIX, suggesting immunization with ZVex™/hCAIX puts a selective pressure toward tumor evasion.*

IMMUNE DESIGN

Results

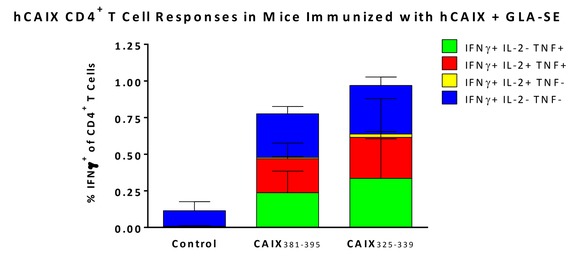
ZVex™/hCAIX and Recombinant hCAIX protein with GLA-SE induce Ag-specific T cell responses.

A. ZVex™/hCAIX induces Ag-specific CD8⁺ T cells.



- Day 0: Immunized C57BL/6 mice with ZVex™/hCAIX.
- Day 10: Cells isolated from spleens were stimulated with control or CAIX peptides as indicated for 5h and stained for flow cytometry analysis.

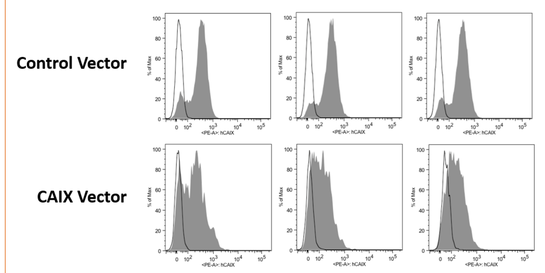
B. Recombinant hCAIX with GLA-SE induces Ag-specific CD4⁺ T cells.



- Day 0: Immunized C57BL/6 mice with hCAIX plus GLA-SE.
- Day 21: Mice were boosted with hCAIX + GLA-SE.
- Day 26: Cells isolated from spleens were stimulated with control or CAIX peptides as indicated for 5h and stained for flow cytometry analysis.

Immunization with ZVex™/hCAIX puts a selective pressure toward tumor evasion.

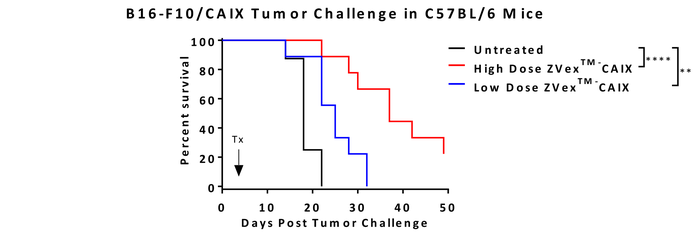
A. CAIX expression is reduced *in vivo* in tumor cells from mice treated with ZVex™/hCAIX but not with control vector.

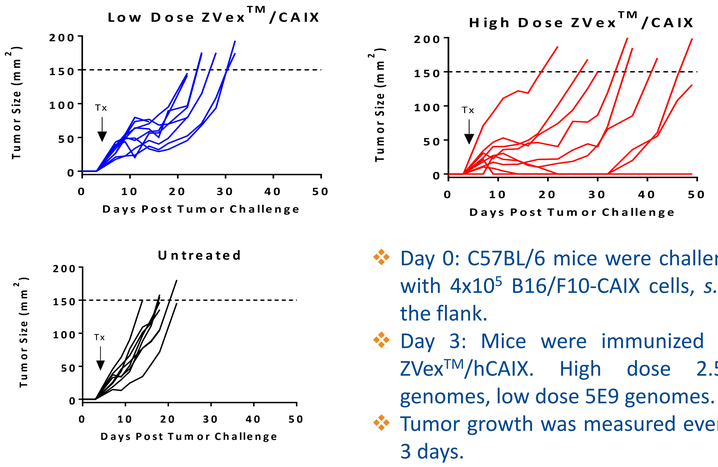


- Day 0: C57BL/6 mice were challenged with $4x10^5$ B16/F10-CAIX cells, *s.c.* in the flank.
- Day 3: Mice were therapeutically immunized with ZVex™/hCAIX or with a control vector.
- Tumor cells were harvested and stained for CAIX expression *ex vivo*.

ZVex™/hCAIX and recombinant hCAIX protein with GLA-SE demonstrate therapeutic efficacy against aggressive B16 melanoma.

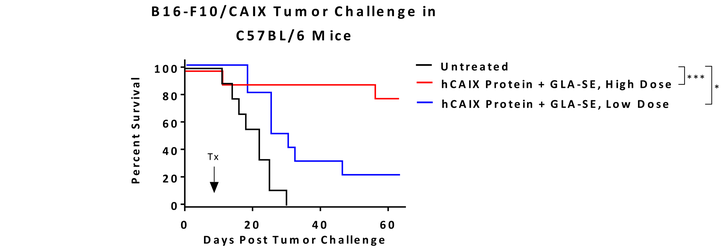
A. ZVex™/hCAIX mediates therapeutic protection against lethal tumor growth.

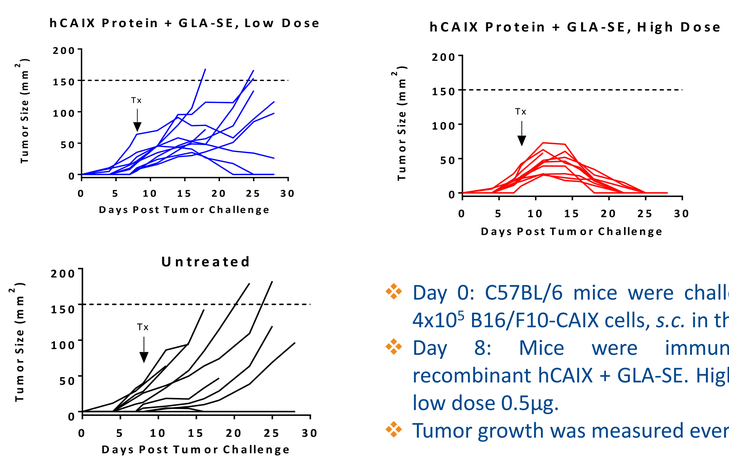




- Day 0: C57BL/6 mice were challenged with $4x10^5$ B16/F10-CAIX cells, *s.c.* in the flank.
- Day 3: Mice were immunized with ZVex™/hCAIX. High dose 2.5E10 genomes, low dose 5E9 genomes.
- Tumor growth was measured every 2-3 days.

B. Recombinant hCAIX with GLA-SE mediates therapeutic protection against lethal tumor growth.





- Day 0: C57BL/6 mice were challenged with $4x10^5$ B16/F10-CAIX cells, *s.c.* in the flank.
- Day 8: Mice were immunized with recombinant hCAIX + GLA-SE. High dose 5µg, low dose 0.5µg.
- Tumor growth was measured every 2-3 days.