**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 H2b 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 mm³) 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 H2b-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.

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

**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.
- **B.** Tumor cells were harvested and stained for CAIX expression ex vivo.

**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 downregulated expression of CAIX, suggesting immunization with ZVex™/hCAIX puts a selective pressure toward tumor evasion.

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

- **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.

- **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.

- **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.
- **Day 8:** Mice were immunized with recombinant hCAIX + GLA-SE. High dose 5µg, low dose 0.5µg.
- **Day 10:** C57BL/6 mice were challenged with 4x10^5 B16/F10-CAIX cells, s.c. in the flank.
- **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.