# A novel dendritic cell targeting platform vector Multigenome ZVex® induces robust multi-antigen T-cell immune responses without inhibitory antigenic competition

#P127

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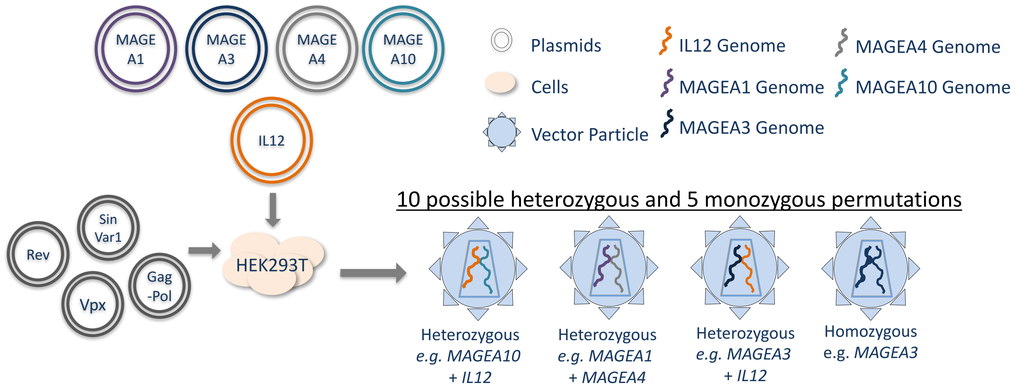
## Abstract

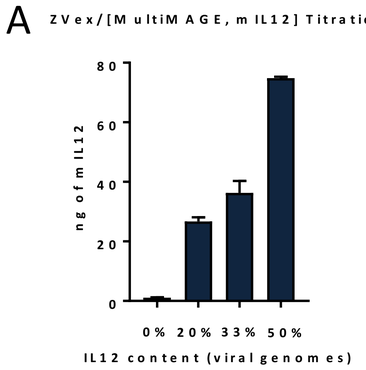
**Background:** Expression of multiple antigens in tandem from viral vaccine vectors often results in antigenic competition due to immune dominance, which limits the generation of immune responses to one or more target antigens. To address this issue, we have developed a novel technology that allows for multiple antigens and/or immune modulators to be expressed *in vivo* using the integration-deficient, dendritic-cell targeted lentiviral vector platform ZVex®. We have designed a Multigenome ZVex vector product expressing the human cancer testis antigens MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10 and Interleukin 12 (ZVex/[MultiMAGE, IL12]), in order to generate robust T cell responses specific for all four antigens simultaneously.
**ZVex/[MultiMAGE, IL12]:** In addition to the backbone plasmids encoding for essential vector components, five identical vector genomes each encoding the full-length *MAGEA1, -A3, -A4, -A10* or murine *IL12* genes were co-transfected into a producer cell line for viral vector production. Because lentiviral vectors package two RNA molecules each, this approach results in the generation of potentially 15 different homozygous or heterozygous vector genotypes. A single subcutaneous injection generated specific T cell responses against all four of the encoded MAGE-A antigens. Furthermore, IL-12 was shown to enhance immunogenicity, without being detected systemically.
**Conclusions:** Expressing multiple MAGE-A proteins and the immune enhancing cytokine IL-12 from a Multigenome ZVex vector resulted in robust and balanced antigen specific CD8 and CD4 T cell responses in mice. No evidence of inhibition due to antigenic competition was observed in these experiments. Because MAGE-A1, A3, A4, and A10 proteins are expressed by a large number of solid tumors individually or in combination, this is a potentially broadly applicable, off-the-shelf-cancer vaccine.

## ZVex is a versatile vector platform, enabling efficient multigenome delivery to generate antigen-specific T cell responses *in vivo*

### Production of ZVex/[MultiMAGE,IL12]

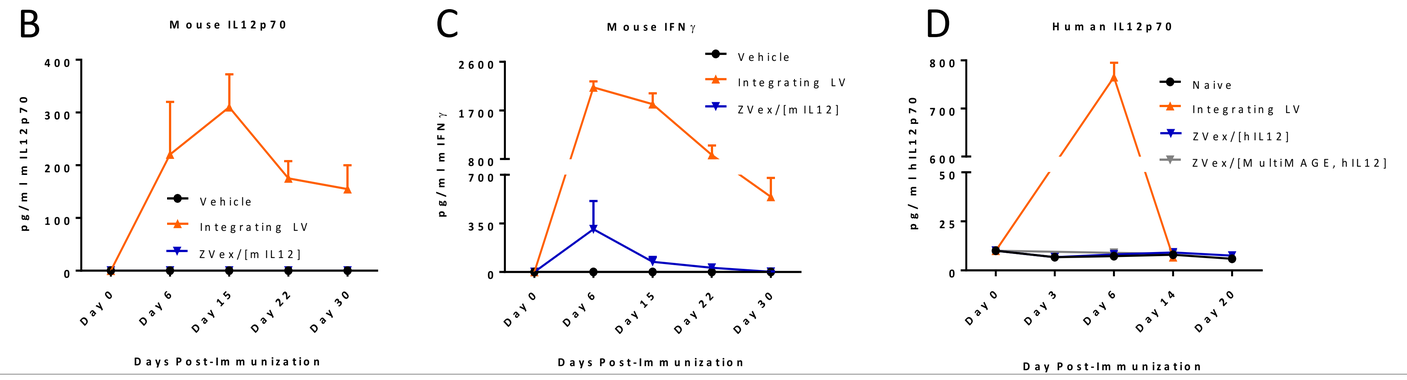



### ZVex/[MultiMAGE, IL12] can be titrated to achieve desired IL12 expression



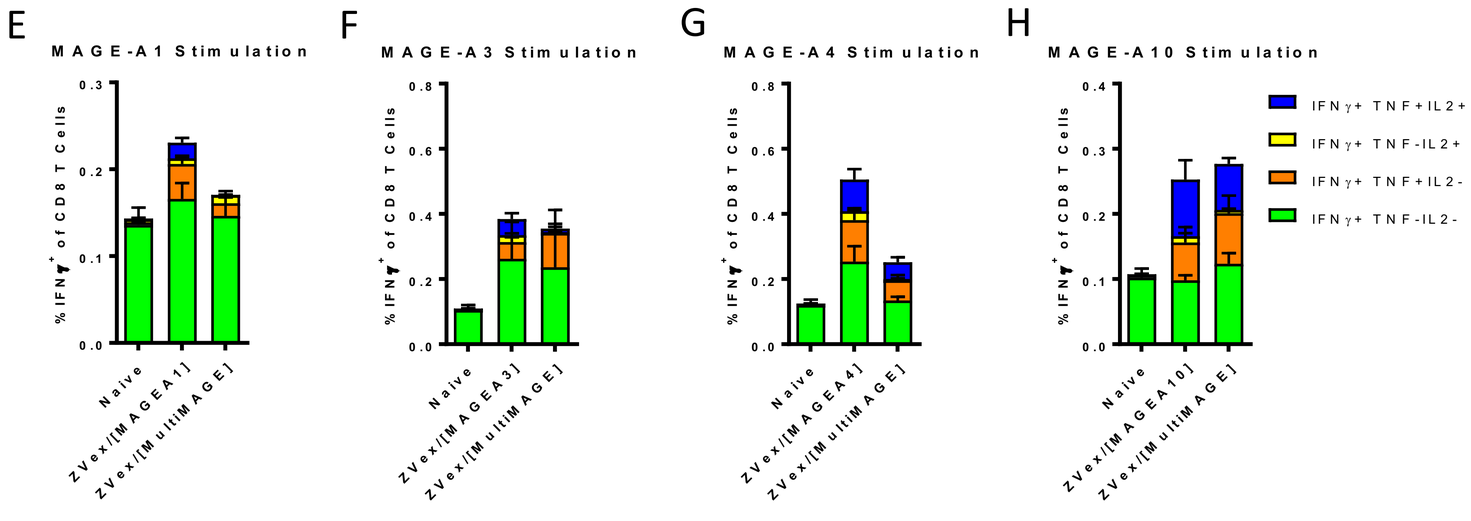

**Methods**: (Schematic) Plasmids encoding essential vector components such as Vpx, gag-pol and envelope and Rev proteins were transfected into producer cells (HEK293) alongside plasmids encoding for whole MAGE-A1, -A3, -A4 and -A10 antigens and mouse or human IL-12. Lentiviral vectors package 2 RNA molecules each, potentially resulting in 15 distinct homozygous or heterozygous vectors. IL-12 plasmid to MAGE-A plasmid ratios can be adjusted to produce vector products with controlled relative genome content. (Fig. A) DC-SIGN expressing HEK 293s were transduced with $1x10^{10}$ ZVex/[MultiMAGE, mIL12] genomes with varying ratios of *IL12* to *MAGEA* genomes. Supernatants were collected and analyzed using ELISA.

## ZVex/[IL12] induces undetectable systemic IL12p70, but is immunologically active




**Methods**: (Figs. B-D) BALB/c females were dosed with similar amounts of vectors (including integrating LV as a positive control) encoding mouse (mIL12), human IL12 (hIL12) or ZVex/[MultiMAGE, hIL12]. mIL12 mouse IFNγ (mIFNγ) and hIL12 levels were assessed in plasma collected at indicated intervals using ELISA.
**Conclusion:** ZVex/[mIL12] does not produce detectable levels of systemic IL12p70 at any time point analyzed. However, low levels of mIFNγ could be detected in the plasma of mice immunized with these vectors, indicating that IL12p70 is produced and active. Zvex/[hIL12] also did not produce detectable levels of hIL12p70 above baseline in the plasma of immunized mice.

## Multigenome ZVex induces quadrivalent response w/o antigenic competition




**Methods**: For immunogenicity assays, female mice (Figs. E-H and M-P, Balb/C) (Figs. I-L, C57BL/6) were immunized with single MAGE-A expressing ZVex, ZVex/[MultiMAGE] (MAGE-A1, A3, A4 and A10) or ZVex/[MultiMAGE, mIL12]. 14 days post-immunization, splenocytes were isolated, re-stimulated with relevant peptides, and stained for flow cytometry analysis. Bars and error bars represent mean + SEM.
**Conclusion**: (Figs. E-H) Mice immunized with ZVex encoding single *MAGE-As* developed a response similar in magnitude and specificity to those immunized with ZVex/[MultiMAGE]. (Figs. I-P) The addition of mIL12 into ZVex/[MultiMAGE] enhanced CD8 and CD4 specific T cell responses to MAGE-A antigens.

## IL12 enhances ZVex/[MultiMAGE]-induced T cell responses