Intratumoral expression of IL-12 from a dendritic cell-targeting chimeric lentiviral vector from the ZVex™ platform cures established tumors in multiple models and induces systemic anti-tumor responses

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#4884

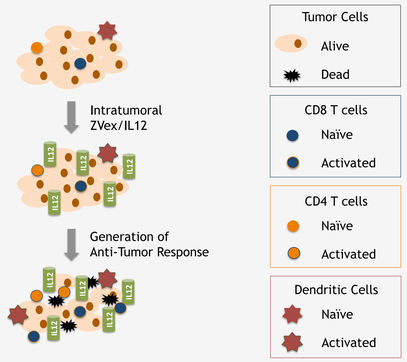
Introduction

Interleukin 12 (IL-12), produced by antigen-presenting cells, plays a pivotal role in the interplay between the innate and adaptive arms of the immune system. IL-12 treatment has been shown to augment cytotoxic T lymphocyte (CTL) and T-helper 1 responses and anti-tumor effects. However, its use as a systemic therapeutic agent is limited due to toxicity. Intratumoral administration of IL-12 is thus being explored as a local alternative route of administration. Here, we evaluated whether targeting expression of IL-12 to intratumoral dendritic cells (DC), thus mimicking the cytokine's physiological biosynthesis and localization, would result in local and systemic immune responses and tumor control in preclinical models.

Tumor cells (B16 melanoma, CT26 colon carcinoma, 4T1 breast cancer, A20 lymphoma, and P815 mastocytoma) were implanted unilaterally, and for some models also bilaterally, to study local and systemic (abscopal) effects of therapy. A chimeric third-generation lentiviral vector from the ZVex™ platform, pseudotyped with the DC-tropic envelope glycoprotein of Sindbis virus, was engineered to express murine IL-12 (ZVex/mIL12). ZVex/mIL12 was administered as a single intratumoral injection into palpable tumors, alone or in combination with G100, an investigative agent containing the synthetic TLR4-agonist glucopyranosyl lipid A (GLA). In some models, systemic anti-CTLA4 treatment was added to enhance therapeutic efficacy. Animals were monitored 2-3 times per week for tumor size and survival.

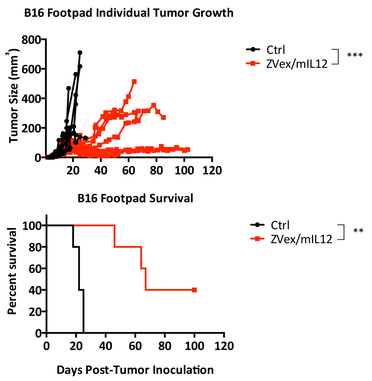
A single intratumoral injection of ZVex/mIL12 resulted in growth delay or complete tumor regression in all murine tumor models investigated and was accompanied by survival benefit. In the very aggressive 4T1 breast cancer model, the addition of G100 further enhanced therapeutic efficacy, even inducing complete tumor regression in one animal. Using the B16 and the 4T1 models, abscopal effects were observed after treatment with a single dose of ZVex/mIL12 or with ZVex/mIL12 combined with G100, respectively. The addition of anti-CTLA4 resulted in enhanced ZVex/mIL12-mediated anti-tumor efficacy in the primary treated tumors of the B16 model. These results suggest that intratumoral administration of IL-12 can modulate the tumor microenvironment to induce local and systemic immunity and that it can be combined with other therapies.

Proposed Mechanism of Action

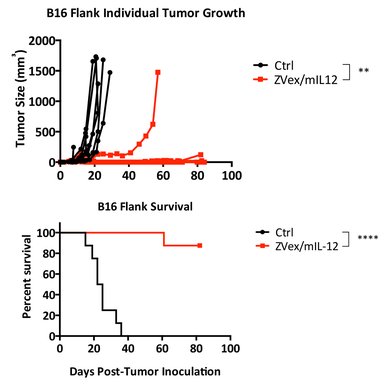


ZVex/mIL12 delayed tumor growth in multiple tumor models

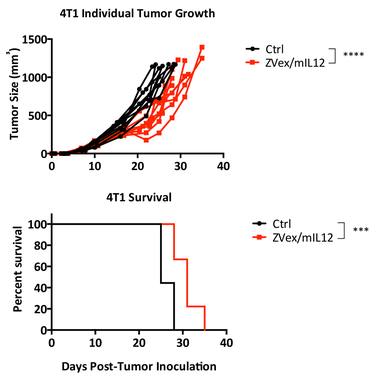
A) B16 Melanoma Model, Footpad



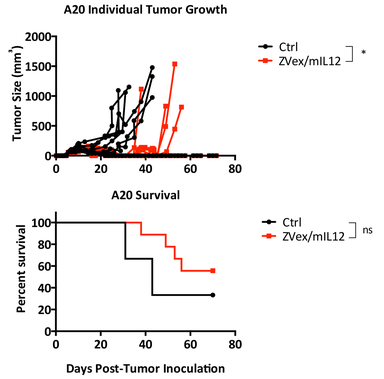
B) B16 Melanoma Model, Flank



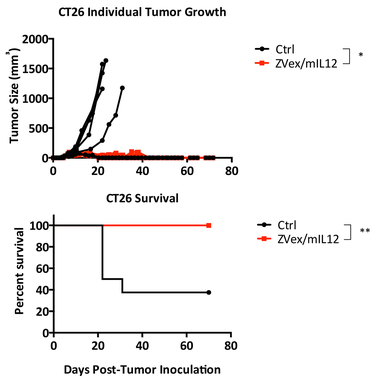
C) 4T1 Breast Cancer Model



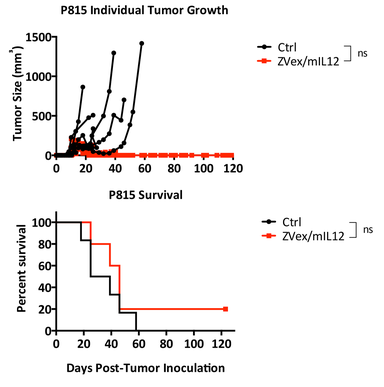
D) A20 Lymphoma Model



E) CT26 Colon Carcinoma Model



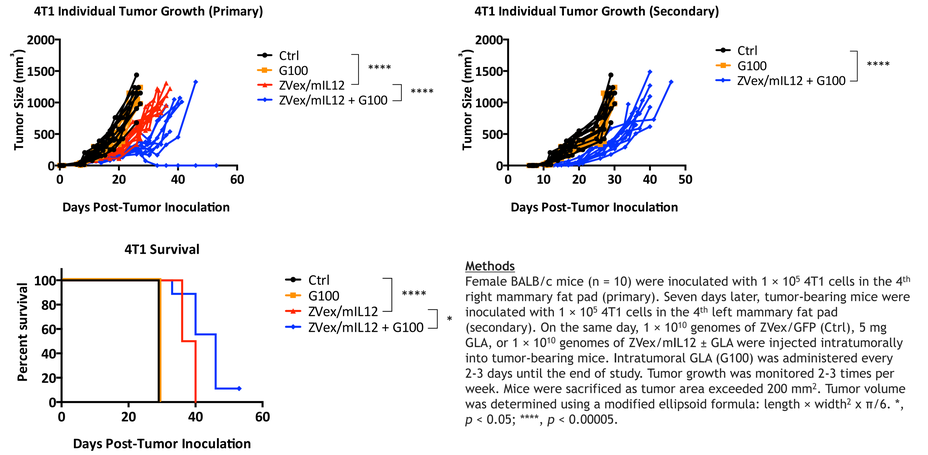
F) P815 Mastocytoma Model



Methods
Female mice (A, B: C57BL/6; C, D, E: BALB/c; F: DBA/2; n = 10) were inoculated with tumor cells (A: 1 × 10^6 B16F10, footpad SC; B: 1 × 10^5 B16F10, flank SC; C: 1 × 10^5 4T1, mammary fat pad; D: 5 × 10^6 A20, flank SC; E: 1 × 10^5 CT26, flank SC; F: 1 × 10^4 P815, flank SC). Seven days later, 1 × 10^{10} genomes of ZVex/GFP (Ctrl) or ZVex/mIL12 were injected intratumorally into tumor-bearing mice. Tumor growth was monitored 2-3 times per week. Mice were sacrificed as tumor area exceeded 100 mm² (footpad) or 200 mm² (flank). Tumor volume was determined using a modified ellipsoid formula: length × width² x π/6. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$; ****, $p < 0.00005$; ns, not significant.

ZVex/mIL12 in combination with G100

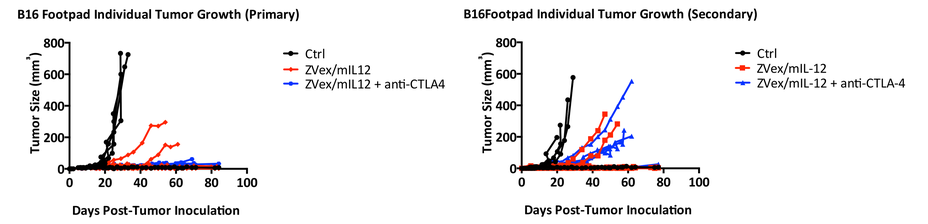
4T1 Breast Cancer Model



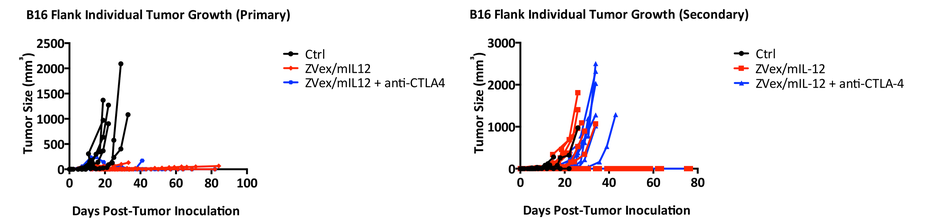
Methods
Female BALB/c mice (n = 10) were inoculated with 1 × 10^5 4T1 cells in the 4th right mammary fat pad (primary). Seven days later, tumor-bearing mice were inoculated with 1 × 10^5 4T1 cells in the 4th left mammary fat pad (secondary). On the same day, 1 × 10^{10} genomes of ZVex/GFP (Ctrl), 5 mg GLA, or 1 × 10^{10} genomes of ZVex/mIL12 ± GLA were injected intratumorally into tumor-bearing mice. Intratumoral GLA (G100) was administered every 2-3 days until the end of study. Tumor growth was monitored 2-3 times per week. Mice were sacrificed as tumor area exceeded 200 mm². Tumor volume was determined using a modified ellipsoid formula: length × width² x π/6. *, $p < 0.05$; ****, $p < 0.00005$.

ZVex/mIL12 in combination with anti-CTLA4 antibody

A) B16 Melanoma Model, Footpad



B) B16 Melanoma Model, Flank



Conclusions

✧ ***Intratumoral ZVex/mIL12 generates robust anti-tumor effect in all tumor models tested***
Tumor-bearing mice given a single intratumoral injection of ZVex/mIL12 resulted in delayed tumor growth or complete tumor regression in well-established tumor models, some known to be highly aggressive.

✧ ***Intratumoral ZVex/mIL12 mediates systemic (abscopal) tumor control***
In mice bearing B16 or 4T1 tumors in both lateral sites, ZVex/mIL12 administered into one tumor also delayed or completely controlled tumor growth in untreated secondary tumors.

✧ ***Intratumoral ZVex/mIL12 synergizes with other therapeutic platforms***
Combining ZVex/mIL12 with G100, both administered intratumorally, further improved tumor growth control and survival in the aggressive 4T1 model and also resulted in control of untreated secondary tumors. Similarly, when tested in the B16 model, combination with anti-CTLA4 antibody therapy further improved the ZVex/mIL12-mediated effects against primary and secondary B16 tumor growth.

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