Intratumoral injections of G100 (synthetic TLR4 agonist) increase trafficking of lentiviral vector-induced antigen-specific CD8 T cells to the tumor microenvironment

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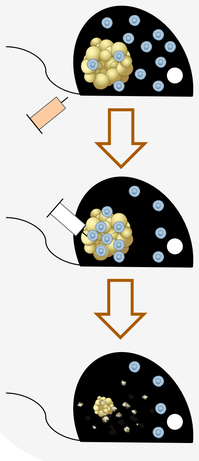
Introduction

The clinical efficacy of tumor-specific effector T cells can be limited by their proper trafficking to the site of the tumor and the immunosuppressed local environment. Strategies to improve homing of effector cells to tumors and to enhance activity of these effector cells could further unlock the potential of active cancer immunotherapy. G100 is the synthetic TLR4 agonist glucopyranosyl lipid adjuvant (GLA) formulated with 2% oil-in-water stable emulsion and has been shown to induce T cell homing chemokines CXCL9 and CXCL10. We assessed here whether intratumoral injections of G100 could improve trafficking of tumor antigen-specific CD8 T cells to the tumor microenvironment (TME), thereby achieving better anti-tumor control.

Mice immunized with ZVex/OVA, a lentiviral vector expressing ovalbumin, generated 8-9% tumor antigen-specific effector and memory CD8 T cells, which remained detectable at low levels even up to 35 days post-immunization. Tumor-infiltrating lymphocytes (TILs) isolated from B16/OVA-tumor-bearing mice treated with ZVex/OVA alone contained an average of 16.6% antigen-specific CD8 T cells, whereas those from mice treated with ZVex/OVA and G100 had 25.9%. While ZVex/OVA-induced antigen-specific CD8 T cells infiltrated the tumor without G100, most of these CD8 T cells did not remain in the TME over time. Intratumoral injections of G100 not only increased the total number of effector CD8 T cells within the TME but also kept the CD8 T cells within the TME over time. Furthermore, tumor-bearing mice treated with ZVex/OVA and G100 had significantly improved survival with slower growing tumors.

Intratumoral injections of G100 improved ZVex-induced therapeutic efficacy by increasing trafficking of effector T cells to the TME. Because G100 also stimulates antigen presentation and maturation of dendritic cells, intratumoral G100 following vector-induced generation of antigen-specific CD8 T cells or adoptive transfer of CAR or TCR T cells may be an effective way to increase the therapeutic efficacy of cancer immunotherapy.

Proposed Mechanism of Action



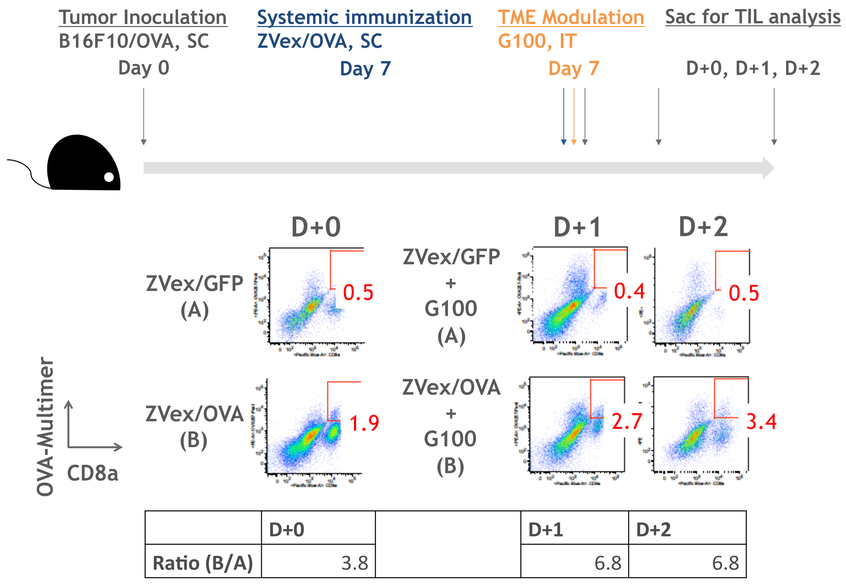
ZVex™ generates systemic Ag-specific CD8 T cells.

Local administration of **G100** recruits CTLs and maintains a pool of CTLs within the tumor.

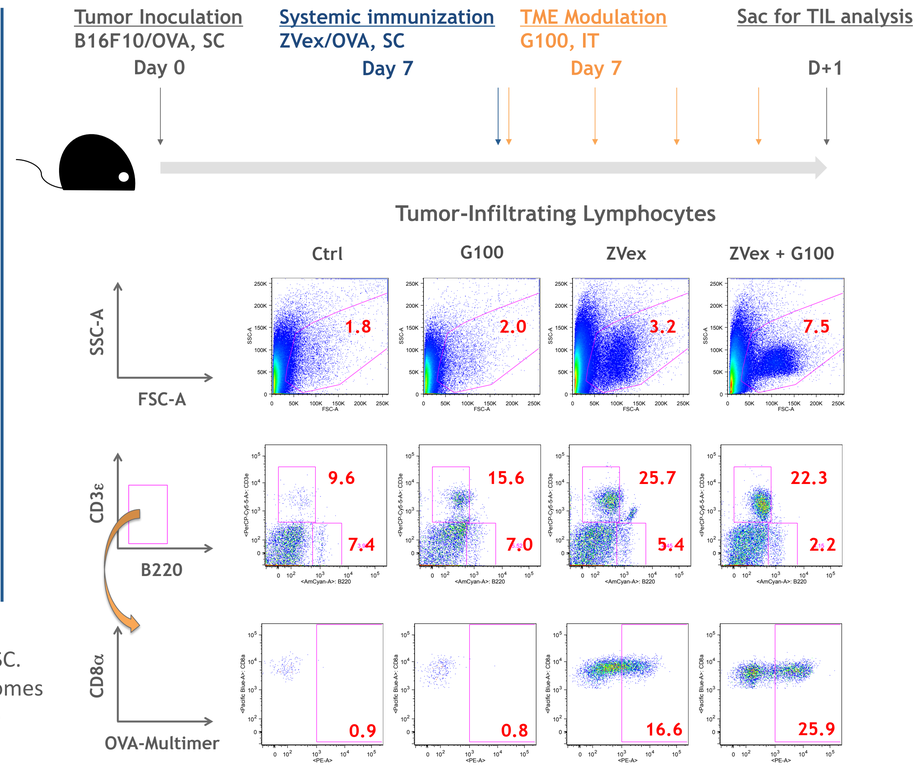
Recruited CTLs proliferate and lyse tumor cells, decreasing tumor burden.

Intratumoral injections of G100 increase proliferation of tumor-infiltrating, ZVex-induced antigen-specific CD8 T cells

Intratumoral injections of G100 recruit ZVex-primed CD8 T cells to the tumor

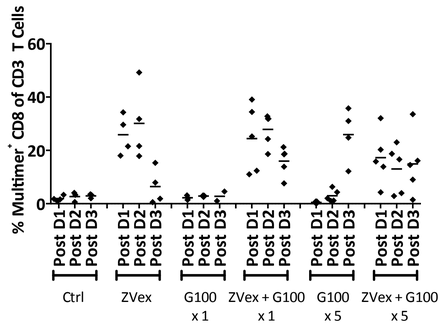


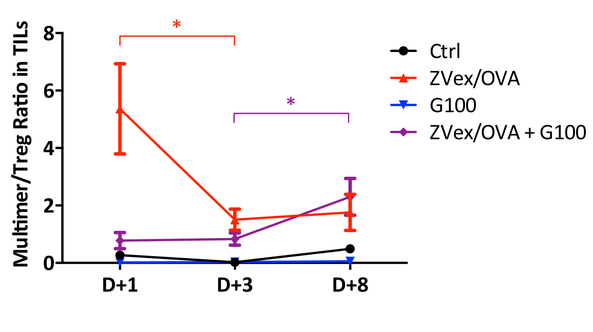
	D+0		D+1	D+2
Ratio (B/A)	3.8		6.8	6.8



Methods
C57BL/6J mice (5-10 females/group) were inoculated with 1 x 10^5 B16/OVA cells, flank SC. When tumors became palpable (Day 7), mice were immunized with 1 x 10^{10} vector genomes of ZVex/OVA, SC, and/or 5 μg G100, IT. Day 0, 1, or 2 post-last G100 injection, TILs were isolated and stained for flow cytometry analysis.

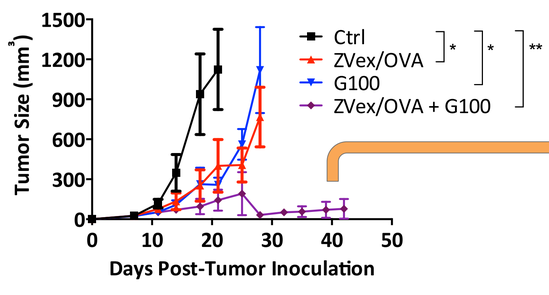
Intratumoral injections of G100 maintain a pool of ZVex-primed CD8 T cells within the tumor



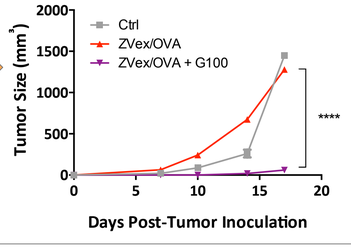


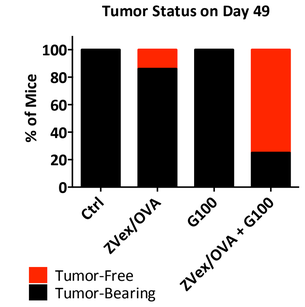
Methods
C57BL/6J mice (5-10 females/group) were inoculated with 1 x 10^5 B16/OVA cells, flank SC. When tumors became palpable (Day 7), mice were immunized with 1 x 10^{10} vector genomes of ZVex/OVA, SC, and/or 5 μg G100, IT. Day 1, 2, 3, or 8 post-last G100 injection, TILs were isolated and stained for flow cytometry analysis. Error bars represent Mean ± SEM. * $p < 0.05$.

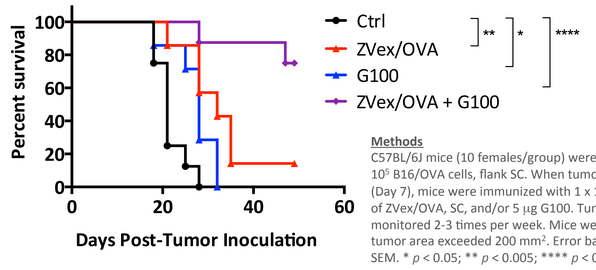
Intratumoral injections of G100 enhance ZVex-induced anti-tumor efficacy



Mice with regressed B16/OVA tumors rejected B16 tumor re-challenge

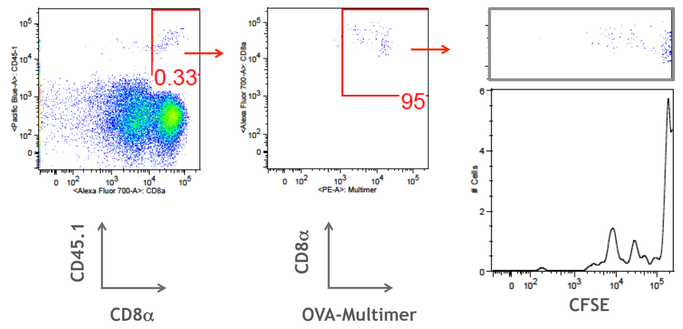






Methods
C57BL/6J mice (10 females/group) were inoculated with 1 x 10^5 B16/OVA cells, flank SC. When tumors became palpable (Day 7), mice were immunized with 1 x 10^{10} vector genomes of ZVex/OVA, SC, and/or 5 μg G100. Tumor growth was monitored 2-3 times per week. Mice were sacrificed as tumor area exceeded 200 mm^2. Error bars represent Mean ± SEM. * $p < 0.05$; ** $p < 0.005$; **** $p < 0.005$.

Intratumoral injections of G100 increase proliferation of ZVex-primed CD8 T cells



Methods
On Day 0, B6.SJL donor mice (5 females/group, CD45.1) were immunized with 1 x 10^{10} vector genomes of ZVex/OVA, SC. C57BL/6J recipient mice (CD45.2) were inoculated with 1 x 10^5 B16/OVA cells, flank SC. On Day 14, CD8 T cells isolated from donor mice were stained with CFSE and adoptively transferred to tumor-bearing recipient mice. On Day 15, tumor-bearing mice were administered intratumoral injections of 5 μg G100. TILs were isolated and stained for flow cytometry analysis 3 days post-G100 injection.

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