Vector Engineered Immunotherapy Incorporating gp96-Ig and T Cell Costimulatory Fusion Proteins Elicits a Superior Antigen-Specific CD8+ T Cell Response

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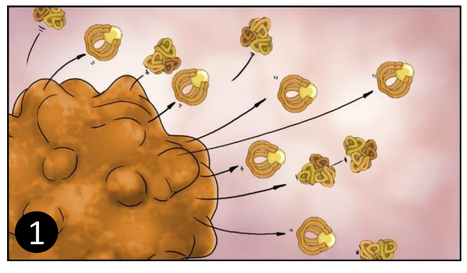
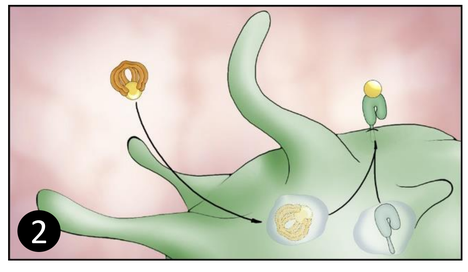
Abstract

Secretable heat-shock protein gp96-Ig based allogeneic cellular vaccines achieve high-frequency polyclonal CD8+ T cell responses to femto-molar concentrations of tumor antigens through antigen cross-priming *in vivo*. Multiple immunosuppressive mechanisms evolved by established tumors can dampen the activity of this vaccine approach, creating a demand for the development of combination immunotherapy approaches for patients with advanced disease. A systematic comparison of PD-1, PD-L1, CTLA-4 and LAG-3 blocking antibodies in mouse models of long-established B16-F10 melanoma demonstrated superior combination between gp96-Ig vaccination and PD-1 blockade as compared to other checkpoints. Triple combinations between gp96-Ig vaccination, PD-1 blockade and T cell costimulation using OX40, ICOS or 4-1BB agonists, provide synergistic anti-tumor benefit. Enthusiasm for the development of such triple combinations is tempered by the anticipated cost of these therapies. To circumvent this issue, we have taken a novel approach by re-engineering gp96-Ig expressing vectors that simultaneously co-express ICOSL-Ig, 41BBL-Ig or OX40L-Ig, providing costimulatory benefit without the need for additional antibody therapy. We find that the co-secretion of gp96-Ig and these costimulatory fusion proteins in allogeneic cell lines, results in enhanced activation of antigen-specific CD8+ T cells. Thus, combination immunotherapy can be achieved by vector re-engineering, obviating the need for vaccine/antibody/fusion protein regimens, and importantly may reduce both total cost of therapy and the risk of systemic toxicity.

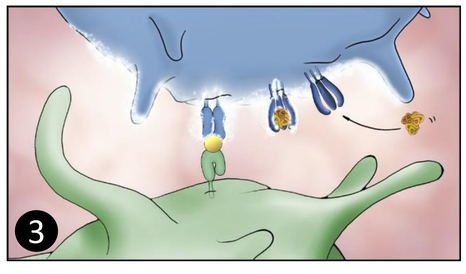
Mechanism of Action



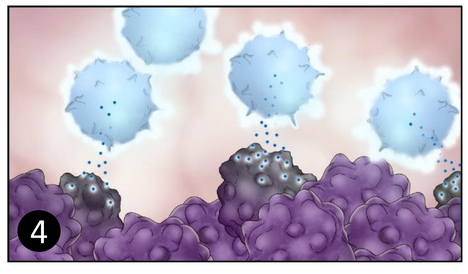
Vaccine cells secrete gp96-Ig together with cell-derived shared tumor antigens



Gp96-Ig/antigen complexes are taken up by APC and antigens transferred to MHC I

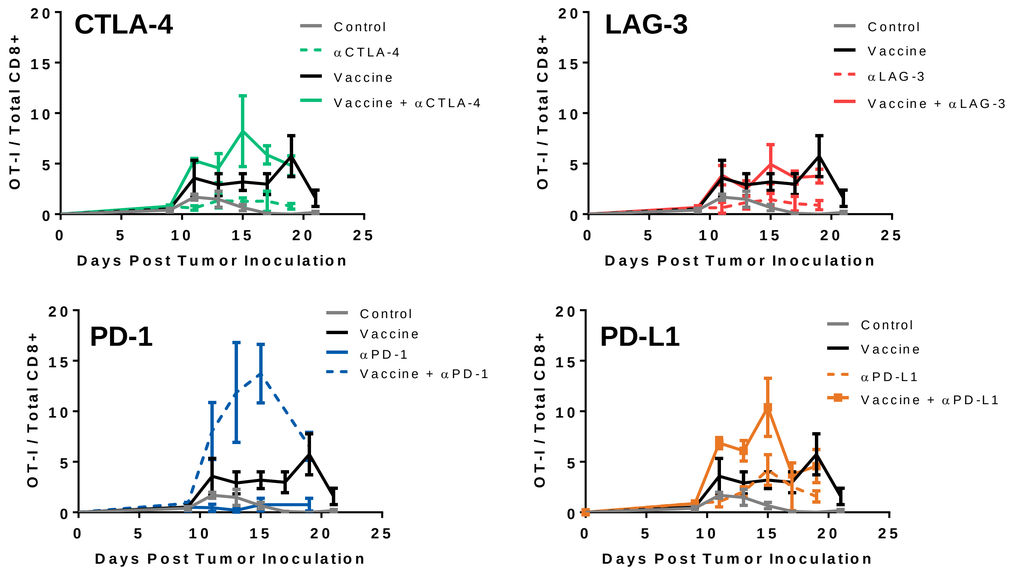


Antigen cross-presentation leads to exclusive activation of CD8+ T cells



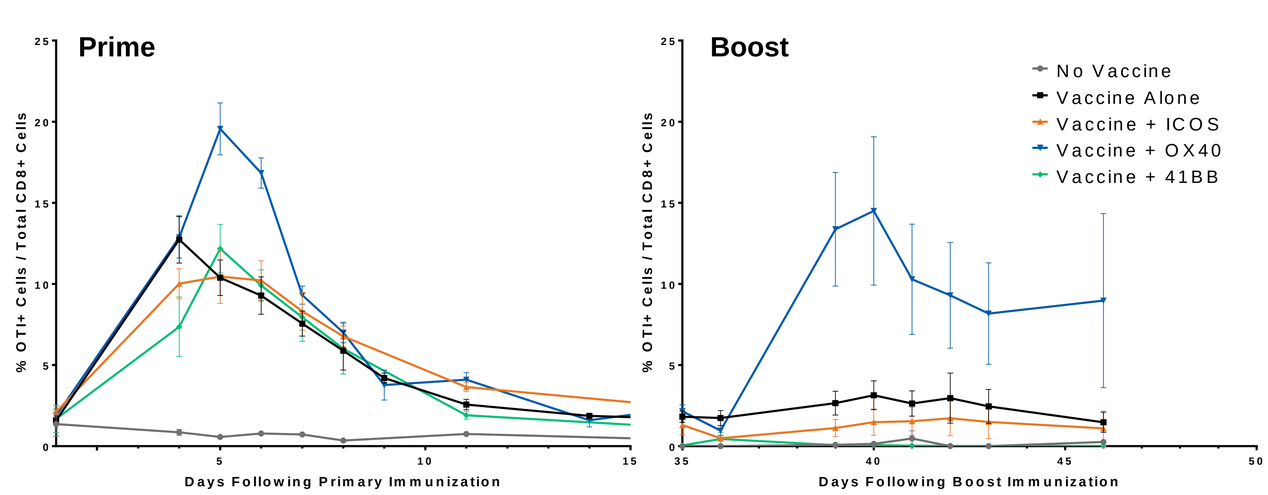
Activated CD8+ T cells can recognize shared tumor antigens on distant tumors

Evaluation of CTLA-4, LAG-3, PD-1 & PD-L1



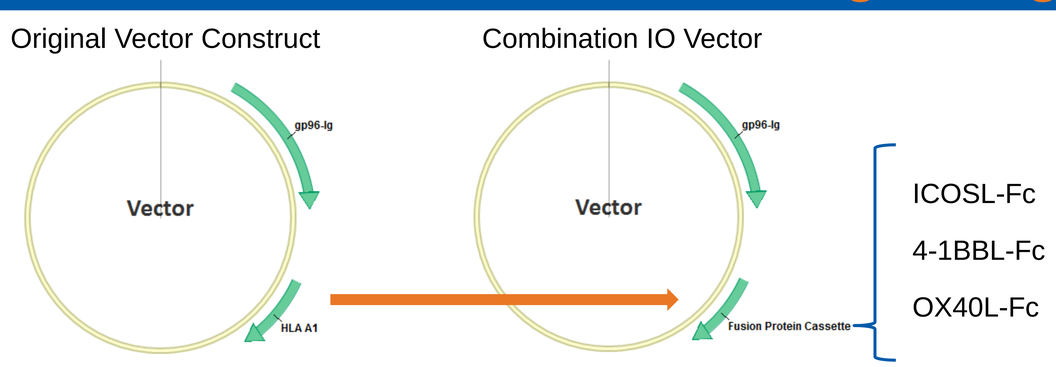
Mechanistic synergy between gp96-Ig vaccination and checkpoint inhibition. C57BL/6 mice were adoptively transferred with a mixed population of ova-specific CD8+ and CD4+ T cells (OT-I and OT-II, respectively) on day -2, and then inoculated with B16-F10 tumors on day 0 ($2x10^5$ cells). Mice were immunized with 3T3-ova (control, 10^6 cells) or 3T3-ova-gp96-Ig (vaccine, 10^6 cells) on day 9, alone or in combination with the indicated antibody (100 µg). The frequency of OT-I (above) and OT-II (data not shown) in the peripheral blood was monitored on the indicated days.

Evaluation of ICOS, 4-1BB & OX40



Mechanistic proof of concept for selection of optimized combination IO vector. C57BL/6 mice were adoptively transferred with ova-specific CD8+ T cells (OT-I) on day -2. Mice were then immunized with ova expressing gp96-Ig vaccines together with T cell costimulatory agonists to ICOS, OX40 or 4-1BB at the time of priming (day 0) as well as boosting (day 35). The frequency of OT-I was monitored in the peripheral blood on the indicated days.

Vaccine + Costimulator Vector Re-Engineering



Vector re-engineering strategy to incorporate vaccine and T cell costimulatory fusion protein. Prior studies demonstrated potential synergy between various T cell costimulatory ligands and gp96-Ig based vaccines. The original gp96-Ig vector was re-engineered to generate a cell-based combination IO product secreting both the gp96-Ig fusion protein and various T cell costimulatory fusion proteins.