Locally secreted Fc-OX40L is superior to systemic, antibody mediated, OX40 co-stimulation for combination immunotherapy

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Abstract

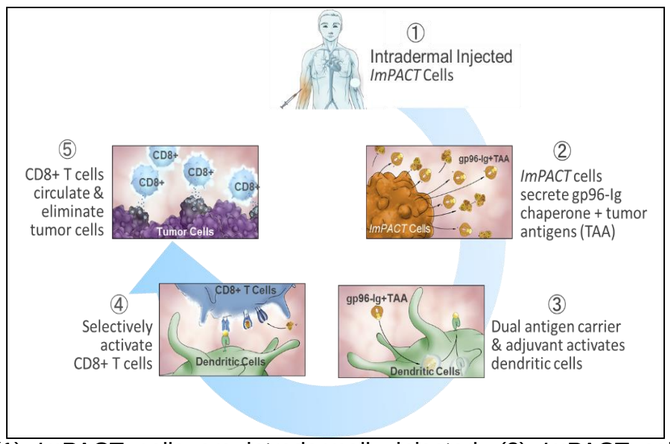
The recent clinical success with individual cancer immunotherapeutics has generated interest in combining treatments to produce more durable and even permanent responses.

Here we test the synergy between our allogeneic, **gp96-Ig** secreting, cell-based vaccine (***Im*PACT**) and various T cell co-stimulator agonist antibodies, and identified OX40 as a potent inducer of antigen-specific CD8 T cell proliferation when combined with *Im*PACT.

Next, we developed a new vaccine that co-expresses gp96-Ig along with Fc-OX40L, all within the same allogeneic cell line (***Com*PACT**).

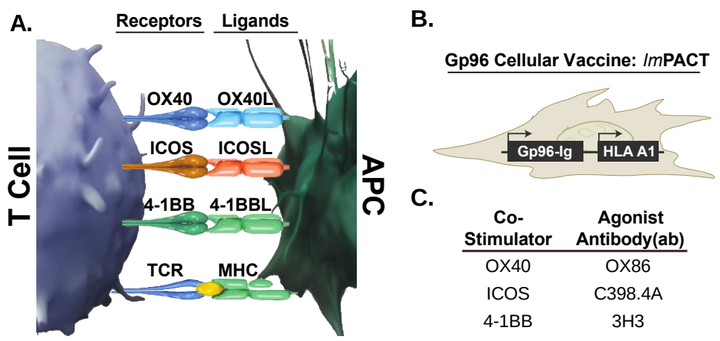
*Com*PACT provides significant priming of both antigen-specific CD4+ and CD8+ T cells, which is related to more potent activation of $CD127^{+}KLRG\text{-}1^{-}$ memory precursor cells. Systemic administration of OX40 antibodies led to proliferation of non-specific CD4+ T cells, Tregs and systemic inflammatory cytokine production. Importantly, *Com*PACT led to high frequencies of $IFN\gamma^{+}$, $TNF\alpha^{+}$, granzyme-b^{+} and IL-2^{+} antigen-specific CD8+ T cells at both priming and boosting, which enhanced rejection of established B16F10 (melanoma) and CT26 (colon cancer) tumors, significantly increasing overall survival.

Vaccine Mechanism of Action



(1) *Im*PACT cells are intradermally injected. (2) *Im*PACT cells secrete TAA-gp96-Ig complexes, which act as a dual antigen carrier and adjuvant, resulting in (3) dendritic cell activation. (4) CD8+ T cells are then selectively activated, (4) which circulate and eliminate tumor cells with overlapping TAA profiles.

Gp96-Ig / T Cell Co-stimulator Synergy



Co-Stimulator	Agonist Antibody(ab)
OX40	OX86
ICOS	C398.4A
4-1BB	3H3

Figure 1. Testing synergy between *Im*PACT and T cell co-stimulators. (A) Diagram of co-stimulator receptors and ligands on T cells and antigen presenting cells (APC). (B) Schematic of gp96-Ig *Im*PACT vaccine. (C) Co-stimulator antibodies analyzed.

*Im*PACT Synergy with OX40, but not 4-1BB or ICOS Agonist mAbs

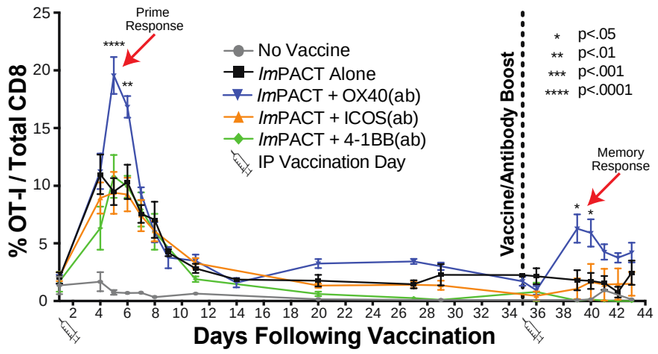


Figure 2. OX40 antibody synergizes with gp96-Ig vaccine resulting in T cell expansion. Mice transferred with OT-I (EGFP) cells via tail vein injection on day -1, were vaccinated with *Im*PACT +/- agonistic antibodies for OX40, ICOS or 4-1BB, and analyzed by flow cytometry. Mice were boosted on day 35.

*Com*PACT : New Vaccine Combining Gp96-Ig with OX40L-Fc

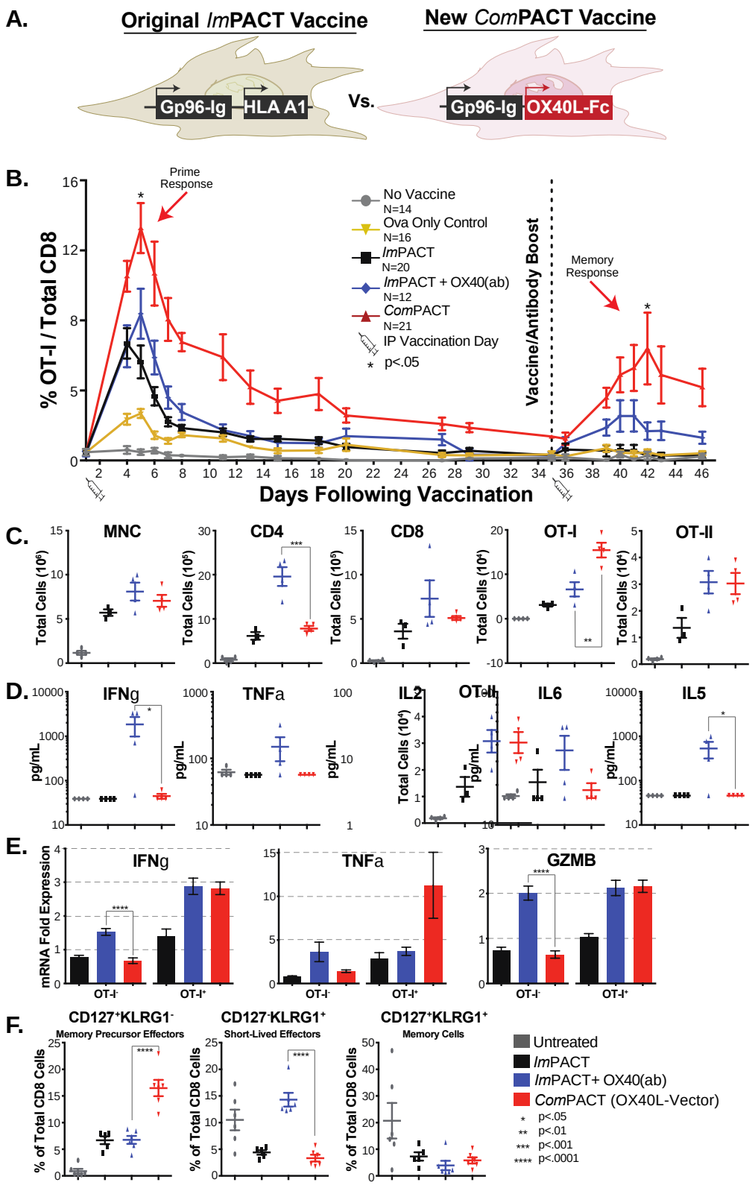
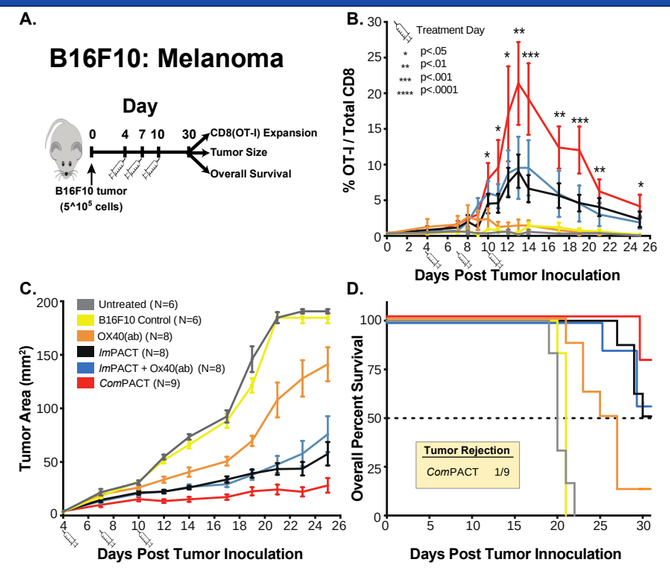


Figure 3. *Com*PACT generates greater antigen specific T cell expansion than OX40 antibody. (A) Schematic of new vaccine *Com*PACT. (B) Antigen specific (OT-I/EGFP) CD8 T cell expansion time course analyzed by flow following vaccination and boost by *Im*PACT +/- OX40(ab) or *Com*PACT (day 35). Mice were analyzed at day 8 by (C) peritoneal flow cytometry, (D) blood serum cytokines (E) T cell activation qRT-PCR on sorted CD8: $OT\text{-}I^{-}$/$OT\text{-}I^{+}$ cells and (F) flow on Memory Precursor Effector Cell (MPEC) populations.

*Com*PACT Generates Significant Anti-tumor Immunity



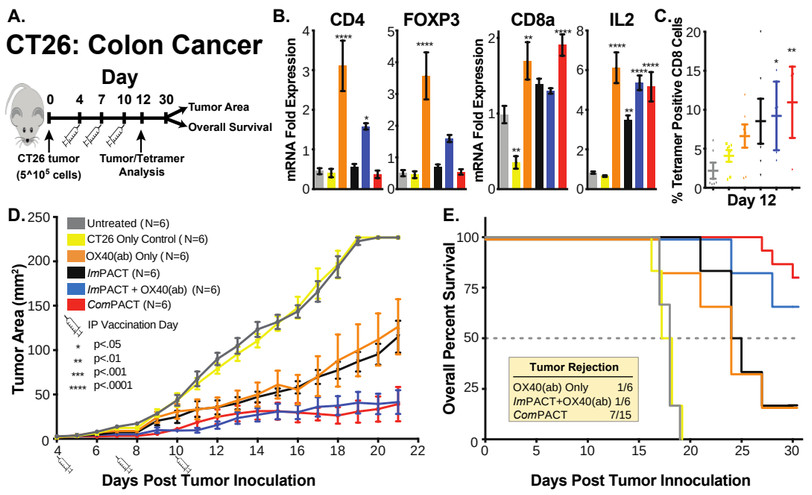


Figure 5. *Com*PACT treatment results in antigen-specific CD8+ tumor infiltration, block in tumor growth, increase in survival and significant tumor rejection.

Top: (A) B16F10 – Melanoma tumor model. 500k cells were injected into the rear flank on day 0 and mice were treated on days 4, 7 and 10. (B) CD8 (OT-I) expansion following vaccination. (C) Tumor growth and (D) overall survival.

Bottom: (A) CT26 – Colon cancer model. 500k cells were injected into the rear flank on day 0 and mice were treated on days 4, 7 and 10. (B) Day 12 genetic analysis of tumor isolated RNA. (C) Percent of AH1-tetramer+ cells found in CD8+ splenocytes on day 12. (D) Tumor growth and (E) overall survival.

Statistical Analysis. One-way ANOVA was used for all sample group analyses. Significance is denoted by *, signifying the following: *p<.05, **p<.01, ***p<.001, and ****p<.0001. Sample sizes are noted in experiments and represent a minimum of 3 distinct biological replicates with error as SEM.

Key Concepts

-We have developed a novel, next-generation cancer immunotherapy vaccine to Gp96-Ig, which we call ***Com*PACT**, incorporating T cell co-stimulator Fc-OX40L.

-*Com*PACT stimulates higher frequency proliferation of antigen-specific CD4+ and CD8+ T cells at both priming and boosting, and more MPEC, than OX40 agonist antibodies.

-*Com*PACT demonstrates greater antigen specificity, without off-target proliferation and systemic inflammatory cytokine stimulation seen with OX40 agonist mAbs.

-*Com*PACT delivers a vaccine and co-stimulatory fusion protein in a single compound, with superior specificity than traditional antibodies. This product may simplify the development of combination immunotherapeutics for oncology patients.

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