Combination Immunotherapy: T-cell costimulation (OX40L, ICOSL, and 4-1BBL) secreted locally by Gp96-Ig vaccines, elicits robust antigen-specific, memory T cell responses and tumor elimination

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Abstract

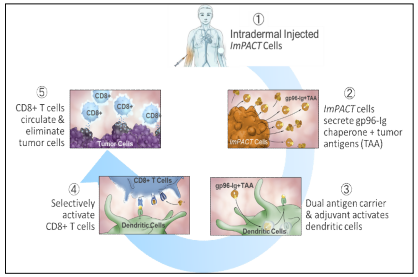
The recent clinical success with individual cancer immunotherapeutics has generated interest in combining treatments to produce more durable and even permanent responses.

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 new vaccines that co-express gp96-Ig along with either *Fc-OX40L*, *Fc-ICOSL* or *Fc-4-1BBL*, all within the same allogeneic cell line (***Com*PACT**).

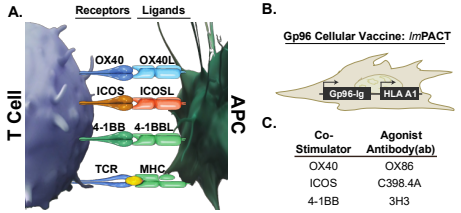
All ***Com*PACT** versions provide unique and significant antigen-specific and memory T cell activation. *ComPACT/OX40L* generated the most robust CD4+ and CD8+ T cell responses, particularly when compared to systemic delivery of an OX40 agonist antibody, which led to proliferation of non-specific CD4+ T cells, Tregs, and systemic inflammatory cytokine production. Importantly, ComPACT/OX40L led to high frequencies of IFNγ+, TNFα+, granzyme-b+, and IL-2+ antigen-specific CD8+ T cells at both priming and boosting, which enhanced rejection of established murine melanoma (B16.F10) and colon cancer (CT26) tumors and significantly increased 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



C.

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 Agonist mAbs

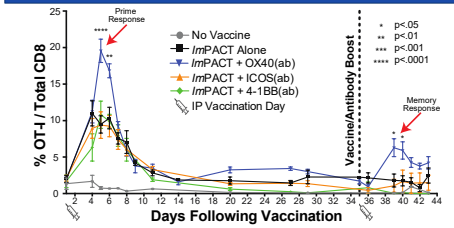


Figure 2. OX40 antibody synergizes with gp96-Ig vaccine resulting in T cell expansion. Mice adoptively transferred with OT-I (EGFP) cells via tail vein injection on day -1, were vaccinated with *Im*PACT +/- agonistic antibodies for OX40, ICOS, or 4-1BB, and analyzed by flow cytometry. Mice were boosted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

*Com*PACT : New Vaccine Combining Gp96-Ig with Fc-OX40L, Fc-ICOSL, or 4-1BBL

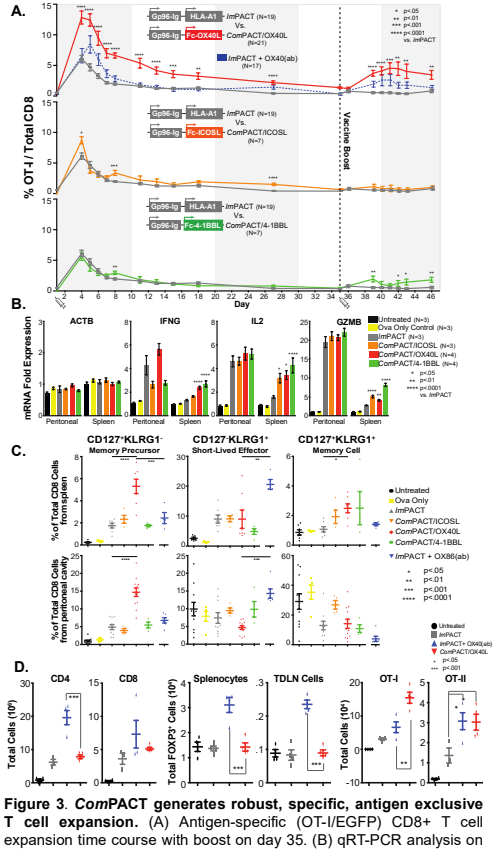


Figure 3. *Com*PACT generates robust, specific, antigen exclusive T cell expansion. (A) Antigen-specific (OT-I/EGFP) CD8+ T cell expansion time course with boost on day 35. (B) qRT-PCR analysis on splenocytes (right) and peritoneal cells (left) harvested on day 8 following treatment. (C) Flow cytometry analysis on memory precursor effector cell (MPEC) populations in splenocytes (top) and peritoneal (bottom) cells on day 8. (D) Comparison of immune cell activation following either *Com*PACT/OX40L or OX40 agonist antibody treatment.

*Com*PACT/OX40L Enhances Anti-tumor Immunity

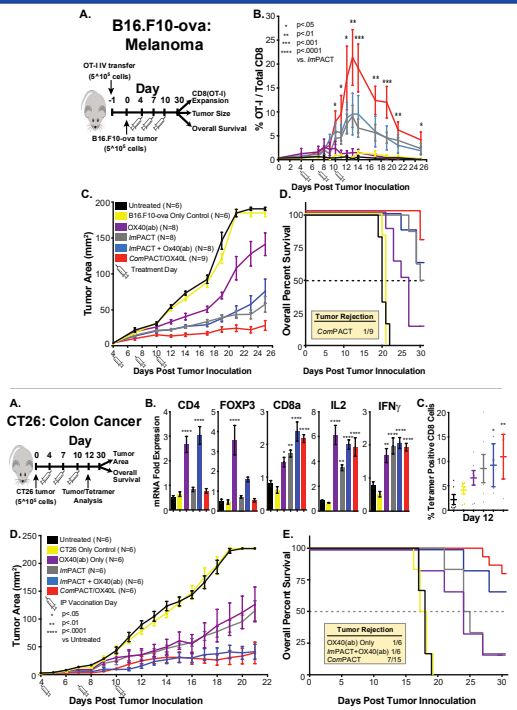


Figure 5. *Com*PACT treatment results in antigen-specific CD8+ tumor infiltration, block in tumor growth, increase in survival, and significant tumor rejection.

Top: (A) B16.F10 – Murine melanoma tumor model. $5x10^5$ 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 – Murine colon cancer model. $5x10^5$ 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) Percentage 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 novel, next-generation cancer immunotherapy vaccines to Gp96-Ig, which we call ***Com*PACT**, incorporating T cell co-stimulators Fc-OX40L, Fc-ICOSL, or Fc-4-1BBL.

-*Com*PACT/OX40L stimulates higher frequency proliferation of antigen-specific CD4+ and CD8+ T cells at both prime and boost, and more MPEC, than the non-specific activation observed with OX40 agonist antibodies.

-*Com*PACT/OX40L results in superior tumor rejection and improved overall survival in both immunogenic and non-immunogenic tumor model systems.

-*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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