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Gp96-Ig/costimulator (OX40L, ICOSL, or 4-1BBL) Combination Vaccine Improves T cell Priming and Enhances Immunity, Memory, and Tumor Elimination

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Abstract

The excitement in the field of immuno-oncology over the last several years, driven largely by the clinical success of checkpoint inhibitors, is tempered by the fact that only 10-40% of patients respond to these drugs given as monotherapy. It is widely believed that to improve efficacy and patient outcome, new approaches that combine treatments with more than one functionality are needed.

We have developed a next generation cellular vaccine platform – referred to as *Com*PACT (COMbination Pan-Antigen Cytotoxic Therapy), that incorporates a tumor antigen chaperone (gp96-Ig) with T cell costimulation (Fc-OX40L), into a single tumor cell line that secretes them both (recently published in *Cancer Immunology Research*, 2016).

*Com*PACT primes both antigen-specific CD4+ and CD8+ T cells, and stimulates 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 murine melanoma (B16.F10) and colon cancer (CT26) tumors and increased overall survival.

Here, we have assessed *Com*PACT in a 3rd tumor model (MC38 – colorectal carcinoma) and show that it synergizes effectively with PD1 and PD-L1 antagonist antibody therapies, amplifying antigen-specific T cells, programming a memory response, and eliminating tumors. *Com*PACT/αPD1 or αPD-L1 combinations may therefore translate into an efficacious approach to treat human cancers.

Gp96-Ig / T Cell Co-stimulator Synergy

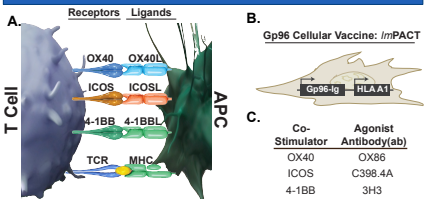


Figure 1. Testing synergy between *Im*PACT and T cell co-stimulators. (A) Diagram of co-stimulator receptors and ligands on T cells and antigen presenting cells (APC). (B) Schematic of gp96-Ig *Im*PACT vaccine. (C) Co-stimulator antibodies analyzed.

*Im*PACT Synergy with OX40 Agonist

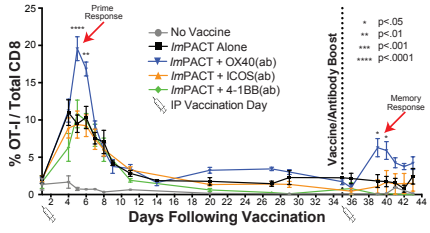


Figure 2. OX40 antibody synergizes with gp96-Ig vaccine resulting in T cell expansion. Mice adoptively transferred with OT-I (EGFP) cells via tail vein injection (day -1), were vaccinated with *Im*PACT +/- agonistic antibodies for OX40, ICOS or 4-1BB, and analyzed by flow cytometry. Mice were boosted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

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

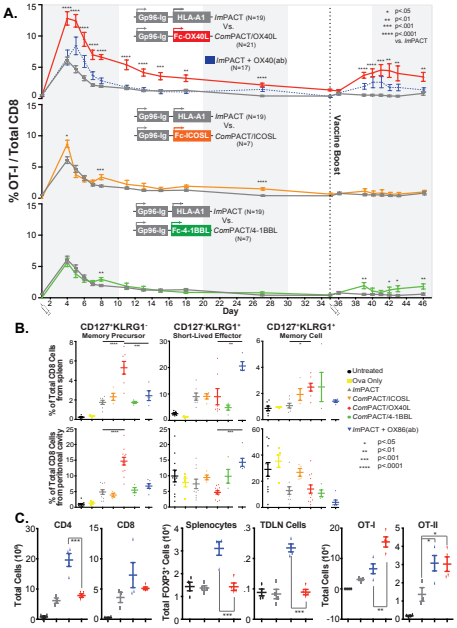
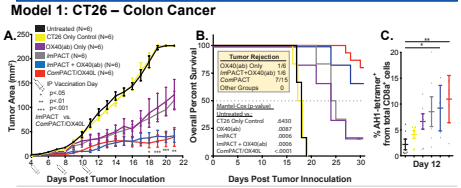


Figure 3. *Com*PACT amplifies antigen exclusive T cells. (A) OT-I (CD8+) T cell expansion time-course with boost on day 35. (B) Memory phenotype analysis by flow cytometry in splenocytes (top) and peritoneal (bottom) cells on day 8. (C) Immune cell activation after either *Com*PACT/OX40L or OX40 agonist antibody treatment.

*Com*PACT Exhibits Strong Anti-tumor Efficacy

Model 1: CT26 – Colon Cancer



Model 2: B16.F10 – Melanoma

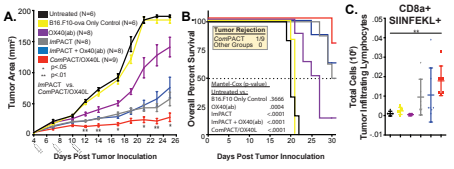


Figure 4. *Com*PACT increases TIL, blocks tumor growth, and increases survival. ***Top:*** CT26 – Murine colon cancer model. (A) Tumor growth, (B) overall survival, and (C) AH1-tetramer+ cells found in CD8+ splenocytes on day 12. ***Bottom:*** B16.F10 – Murine melanoma tumor model. (A) Tumor growth, (B) overall survival, and (C) SIINFEKL-tetramer+ intra-tumoral T-cells (TIL) on day 13.

*Com*PACT Synergizes with Checkpoint Inhibition and Generates Significant Anti-tumor Immunity

Model 3: MC38 – Colorectal Carcinoma

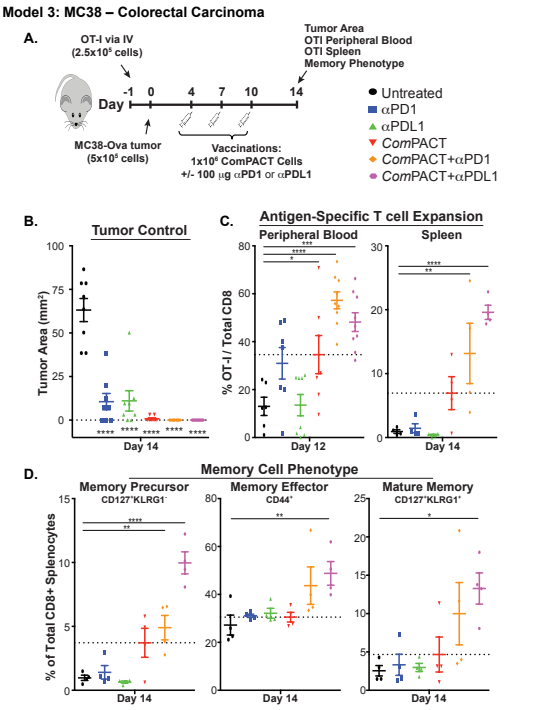


Figure 5. *Com*PACT synergizes with PD1/PDL1 checkpoint inhibition to enhance tumor rejection, antigen-specific T cell expansion, and memory response.
(A) Schematic of MC38 – Murine colorectal carcinoma model. *Com*PACT and checkpoint inhibition (both αPD1 and αPDL1) synergize to (B) control tumor growth, (C) amplify antigen-specific CD8+ T cells in the spleen and peripheral blood, and (D) generate a robust memory T cell response.

Tumor Models: *For all tumor models*: $5x10^5$ cells were injected into the rear flank on day 0 and mice were treated on days 4, 7 and 10 once tumors had established.

Statistical Analysis. One-way ANOVA was used for all sample group analyses. Significance is denoted by *: *p<.05, **p<.01, ***p<.001, and ****p<.0001. Sample sizes noted in experiments or from ≥3 distinct biological replicates with error as SEM.

Key Concepts

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

-***Com*PACT** demonstrates antigen specificity, without the off-target systemic inflammatory signature seen with OX40 agonist mAbs.

-***Com*PACT** synergizes with checkpoint inhibition **(αPD1 and αPDL1)** to maximize antigen-specific T cell proliferation, memory cell response, and tumor eradication.

-***Com*PACT** delivers a vaccine and co-stimulatory fusion protein in a single compound, and synergizes strongly with checkpoint inhibitors. Future combinations of the two may significantly improve patient outcomes.

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