#605

Potency of Gp96-Ig/OX40L-Fc Cell-Based Combination Vaccine in Cancer Immunotherapy

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Abstract

The breakthrough discoveries of checkpoint inhibitors in the field of tumor immunology have driven the clinical success of immunotherapies for cancer, despite their beneficial efficacy in only a small portion of patients. This is due in part to immuno-evasive mechanisms and the inability of the immune system to recognize tumor antigens as foreign.

As a therapeutic approach to effectively present these tumor antigens in order to elicit an anti-tumor immune response, we previously designed and characterized an allogenic, gp96-Ig secreting, cell-based vaccine (*Im*PACT); currently being assessed in a phase II study in non-muscle invasive bladder cancer and a phase Ib study in non-small cell lung cancer – the latter, in combination with the PD-1 antagonist Nivolumab.

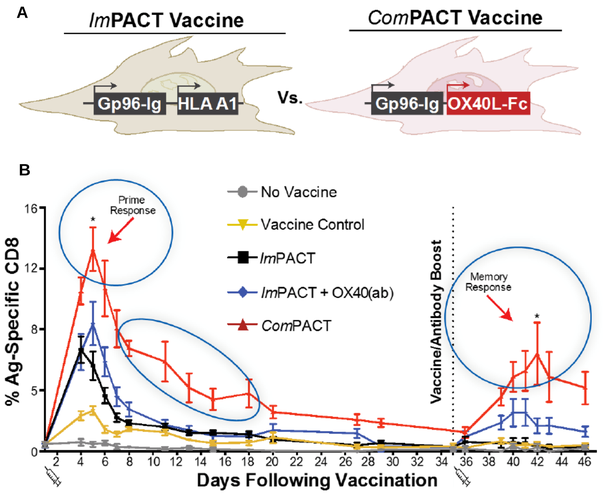
We recently characterized a 'next-generation' vaccine (*Com*PACT) that combines the tumor antigen chaperone Gp96-Ig along with the T cell costimulator OX40L-Fc, which are both secreted from the same cell (*Fromm et. al. Cancer Immunology Research. 2016*). In preclinical assays, *Com*PACT is effective at stimulating CD4+ and CD8+ antigen-specific T cell expansion, the programing of a durable memory T cell phenotype, and the elimination of melanoma and colon tumors. This anti-tumor efficacy is enhanced when *Com*PACT is combined with checkpoint inhibition (anti-PD1 or anti-PDL1).

To support manufacturing and clinical efforts of both *Im*PACT and *Com*PACT, in anticipation of phase III expansion and/or new trial initiation, we have developed novel potency assays to quantify the biologically active form of Gp96-Ig and the *in vitro* activity of OX40L-Fc on T cell costimulation.

It has been shown that gp96 can interact with toll-like receptors (TLR) and that this interaction results in the activation of the NF-κB pathway. Since THP1 cells express abundant TLR2/4, we engineered a THP1 cell line to express luciferase that is regulated by NF-κB response elements. Furthermore, we utilized the human T cell line, Jurkat, as host cells in which to also express NF-κB-luciferase, to quantify OX40L-Fc costimulation. Jurkat/ NF-κB-luciferase cells primed with CD3 and CD28, and subsequently cultured with *Com*PACT-secreted OX40L-Fc, results in a dose dependent increase in NF-κB (luciferase) expression.

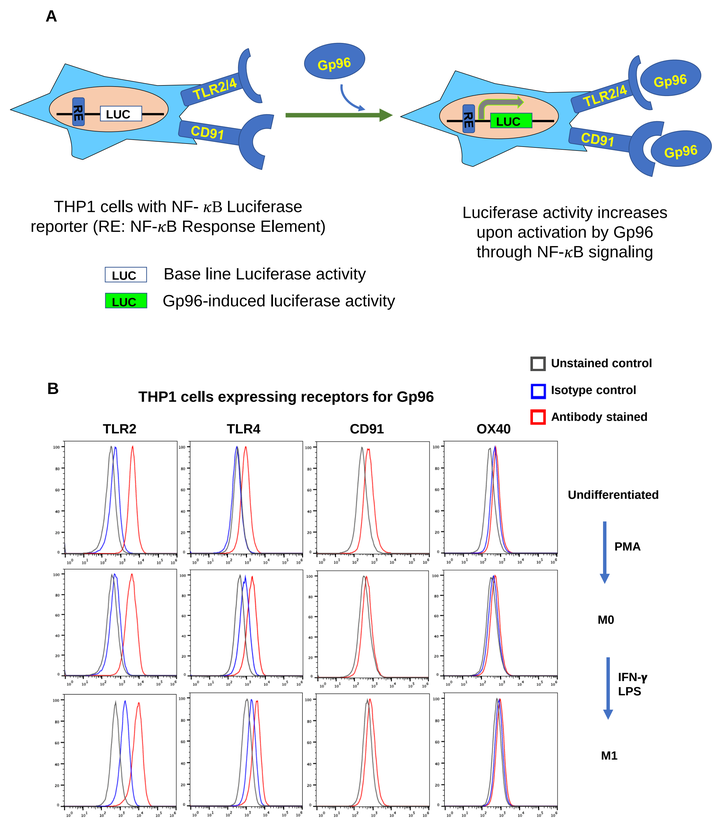
Our current data in both assays shows a correlation with the input of Gp96-Ig and OX40L-Fc, and may serve as an effective potency assay to facilitate the manufacturing of our vaccine product as it transitions into more advanced cancer immunotherapy clinical studies.

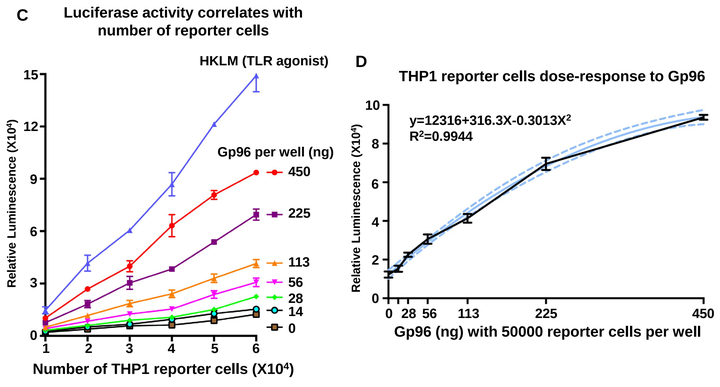
From *Im*PACT to *Com*PACT



A, Illustration of the design of *Im*PACT and *Com*PACT vaccines. **B**, *Com*PACT vaccine produces superior antigen-specific CD8+ T-cell expansion in pre-clinical model system. Antigen specific (OT-I/EGFP) CD8 T cell expansion analyzed by flow cytometry following vaccination and boost by *Im*PACT +/- OX40(ab) or *Com*PACT.
Fromm et. al. Cancer Immunology Research. 2016

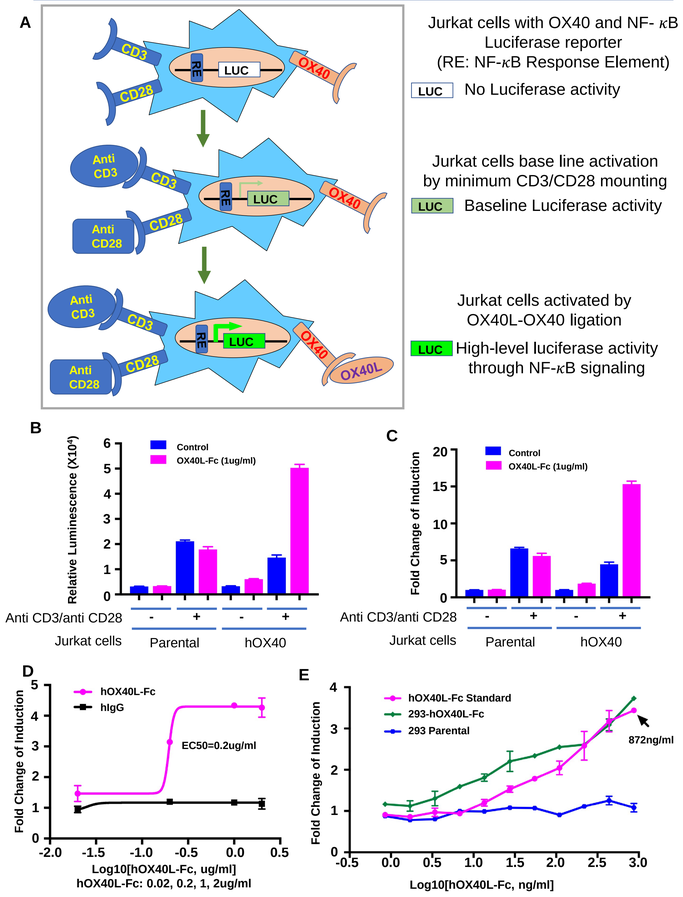
Potency of Gp96-Ig





A, Strategy of Gp96 potency assay. Gp96 induces NF-κB signaling through its known receptors including TLR2, 4, and CD91. Therefore NF-κB Luciferase reporter assay can be used to determine Gp96 activity. **B**, Gene expression was assessed by flow cytometry with cells unstained, isotype control, and specific antibodies stained. THP1 cells express a fair amount of TLR2 and 4 but expression of CD91 is marginally detectable in undifferentiated cells. Upon induction of differentiation, expression of TLR2, TLR4, and CD91 remain similar as undifferentiated cells. In addition, OX40 expression in THP1 cells is undetectable, which avoids the potential interference of OX40L-Fc from *Com*PACT vaccine in Gp96 potency assay. **C and D**, THP1 reporter cells (undifferentiated) response to purified Gp96 in a dose dependent manner determined by Luciferase activity assay.

Potency of OX40L-Fc



A, Strategy of OX40L potency assay based on T cell activation through TCR signaling. OX40L-OX40 ligation functions as a co-stimulator in TCR signaling, which enhances NF-κB signaling induced upon TCR activation. Therefore NF-κB Luciferase reporter assay can be used to determine OX40L activity. TCR signaling is activated by minimum CD3/CD28 mounting (**A, B, and C**). This activation is controlled by using the appropriate titration of anti CD3/anti CD28 so that T cell activation can be stimulated further upon OX40L-OX40 ligation (**B and C**). The further stimulation is OX40 dependent. **B**, Relative Luminescence induced by NF-κB signaling. **C,** Relative Luminescence units are normalized to reporter cells without any activation and the results are shown as fold change. **D**, Jurkat-hOX40 reporter cells response to purified hOX40L-Fc in a dose dependent manner by Luciferase activity assay. **E**, 293 cell line stably expressing hOX40-Fc was established to mimic the aspect of hOX40L-Fc in *Com*PACT vaccine. The condition medium yields 872ng/ml of hOX40L determined by standard ELISA (data not shown). Purified hOX40L-Fc standard was diluted with medium to 872ng/ml as the start concentration followed by a series of dilutions to be compared with condition medium from 293-hOX40L-Fc and Parental cells for OX40L activity. The reporter cells response to the standard and the condition medium from 293-hOX40L-Fc cells in a similar trend while condition medium from 293 parental cells does not stimulate NF-κB signaling in reporter cells.

Conclusion and Future Direction

1. *Im*PACT vaccine cells express approximate 800ng Gp96 in secretion form determined by standard ELISA (10^6 cells in 1ml medium for 24 hours). Similar yield of Gp96 is expected from *Com*PACT vaccine. This amount of Gp96 is above the linear range of the Luciferase reporter assay. Therefore, the potency assay is sufficiently sensitive to be adapted to determine Gp96 activity secreted from *Im*PACT and *Com*PACT. Evaluation of the Gp96 activity secreted from *Im*PACT vaccine is in process.

2. OX40L potency assay is specific for *Com*PACT vaccine. It has been reported that the reporter host cells for OX40L potency assay do not express TLR2/4 or CD91. Therefore, we don't expect that Gp96 production in *Com*PACT will interfere with the OX40L potency assay. Although the vaccine is not available yet, the established 293-hOX40L-Fc cells allow us to evaluate the potency assay, which can be adapted to evaluate the activity of OX40L-Fc produced by *Com*PACT.

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