

PRECLINICAL DEVELOPMENT OF A NOVEL, ALLOGENEIC, OX40L-SECRETING, THERAPEUTIC CANCER VACCINE FOR USE IN COMBINATION WITH A GP96-SECRETING VACCINE FOR SOLID TUMORS

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Background

Heat Biologics is focused on developing a next generation cellular vaccine platform that incorporates a tumor antigen chaperone (gp96-Ig) with costimulation into a single tumor cell line overexpressing a host of cancer associated neoantigens. Viagenpumatulcel-L (HS-110), a human lung adenocarcinoma cell line stably transfected to express gp96-Ig, is being tested in a phase 1/2 clinical trial (NCT#02439450) for NSCLC. A similar line was generated that complements HS-110, providing costimulation in the form of secreted OX40-Ig (HS-130). As a model, mouse surrogates of each human product (mHS-110 and mHS-130) were generated to activate and expand adoptively transferred OVA-specific T cells (OT-1) responding to tumor challenge with a B16F10 cell line over-expressing OVA.¹ To identify the best ratio of mHS-110 to mHS-130, multiple dose ratio and dose escalation studies were performed to measure T cell expansion (peripheral and intratumoral) in the context of tumor challenge. Combining mHS-110/gp96-Ig with mHS-130/OX40L-Ig at a ratio of 1 to 1.3, i.e, 339 ng/10⁶ cells/24 hrs of gp96-Ig to 441 ng/10⁶ cells/24 hrs of OX40L-Ig provided the best dose combination for long-term survival and expansion of tumor specific CD8⁺ T-cells, tumor infiltrating lymphocytes (TILs) and greatest tumor growth inhibition (TGI). The no observed effect level (NOEL) was determined to be 38 ng of gp96-Ig to 50 ng of OX40L-Ig with the minimum active biological effect level (MABEL) being 113 ng gp96-Ig to 147 ng OX40L-Ig. Dosing mice with mHS-130, HS-130 and an OX40 agonist as a positive control, the no observed adverse event level (NOAEL) was determined to be 20 mg/kg for the OX40 agonist antibody, 219.5 ng/animal or 0.01097 mg/kg of human OX40L-Ig/24 hrs for HS-130, and 387.2 ng/animal or 0.01936 mg/kg of mouse OX40L-Ig/24 hrs. The serum cytokines detected in response to cellular injection were those frequently associated with acute innate immune responses (CXCL10, MCP-3, CCL4, TNFalpha, CXCL1). Best dose group combination mHS-110/gp96-Ig to mHS-130/OX40L-Ig was at a ratio of 1 to 1.3, at a dose rate of 339 ng/10⁶ cells/24 hrs of gp96-Ig to 441 ng/10⁶ cells/24 hrs of OX40L-Ig, with a NOEL of 38 ng gp96-Ig to 50 ng OX40L-Ig, a MABEL of 113 ng gp96-Ig to 147 ng OX40L-Ig. These results support the clinical translation of this approach of combining a T cell activation platform with costimulation.

Mechanism of Action

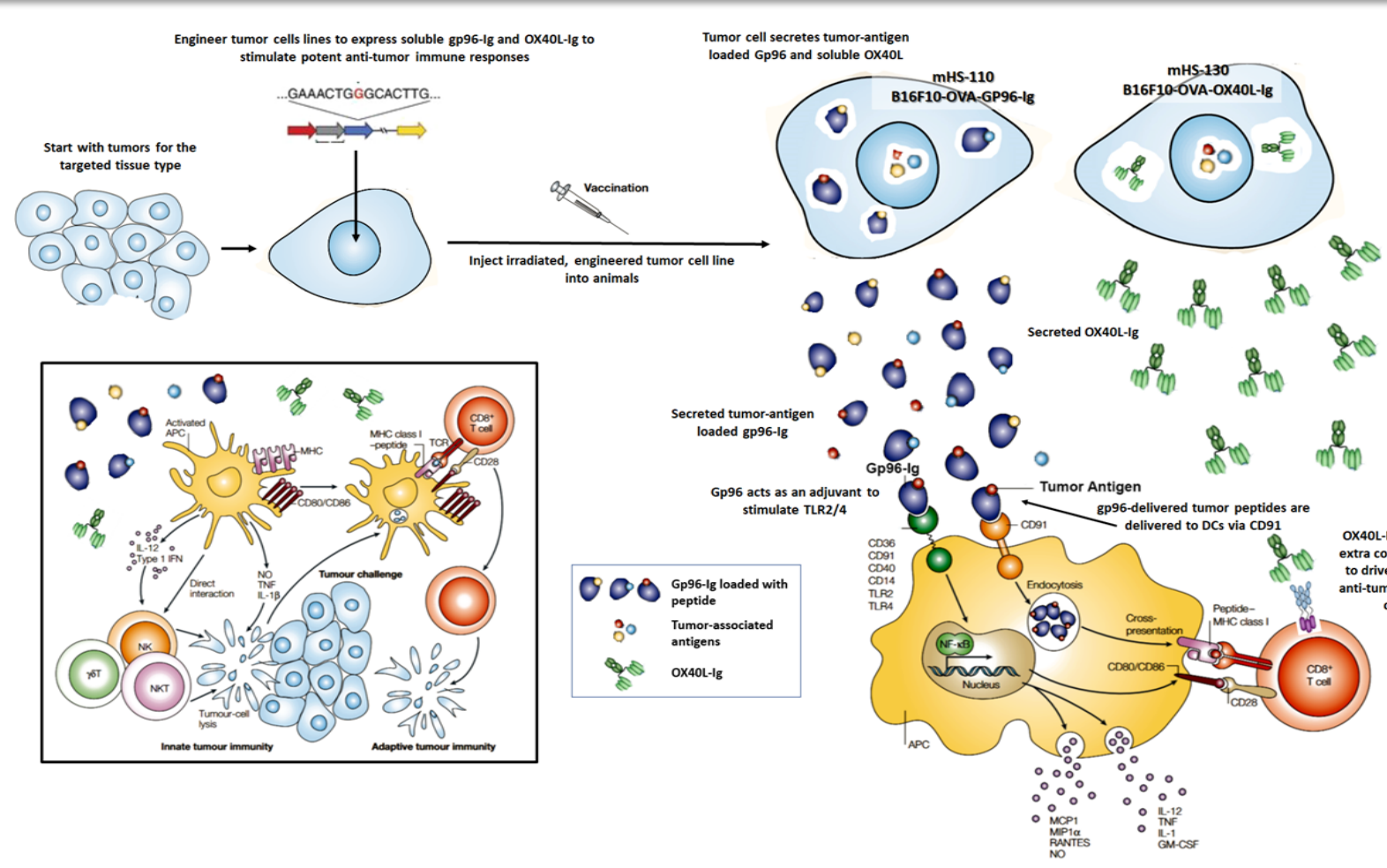


Figure 1: Mechanism of Action
Tissue-matched, allogeneic tumor lines are engineered to express soluble versions of heat-shock protein, gp96, and a potent costimulatory molecule, OX40L (HS-110 and HS-130, respectively). The KDEL endoplasmic reticulum retention sequence of gp96 was replaced by an IgG1 Fc chain. Since gp96 is responsible for folding endogenous proteins, many of which are destined for MHC-I loading; sequestered tumor peptides are delivered to antigen presenting cells (APCs) for CD8⁺ T-cell cross-presentation. Gp96 is a danger associated molecular pattern (DAMP) that stimulates inflammatory cascades via TLR2/4 stimulation and via uptake by scavenger receptor CD91. With the addition of a costimulatory molecule, OX40L fused with an IgG1 Fc chain, anti-tumor CD8⁺ T-cell responses are further enhanced. Activation of DCs by tumor-antigen loaded gp96 and costimulation by OX40L can enhance the innate and adaptive arms of immunity, via pro-inflammatory cytokines. *Figure adapted from Nicchitta CV, Nature reviews Immunology, Vol-3, May 2003*

Finding the Best Dosing Ratio – gp96 to OX40L

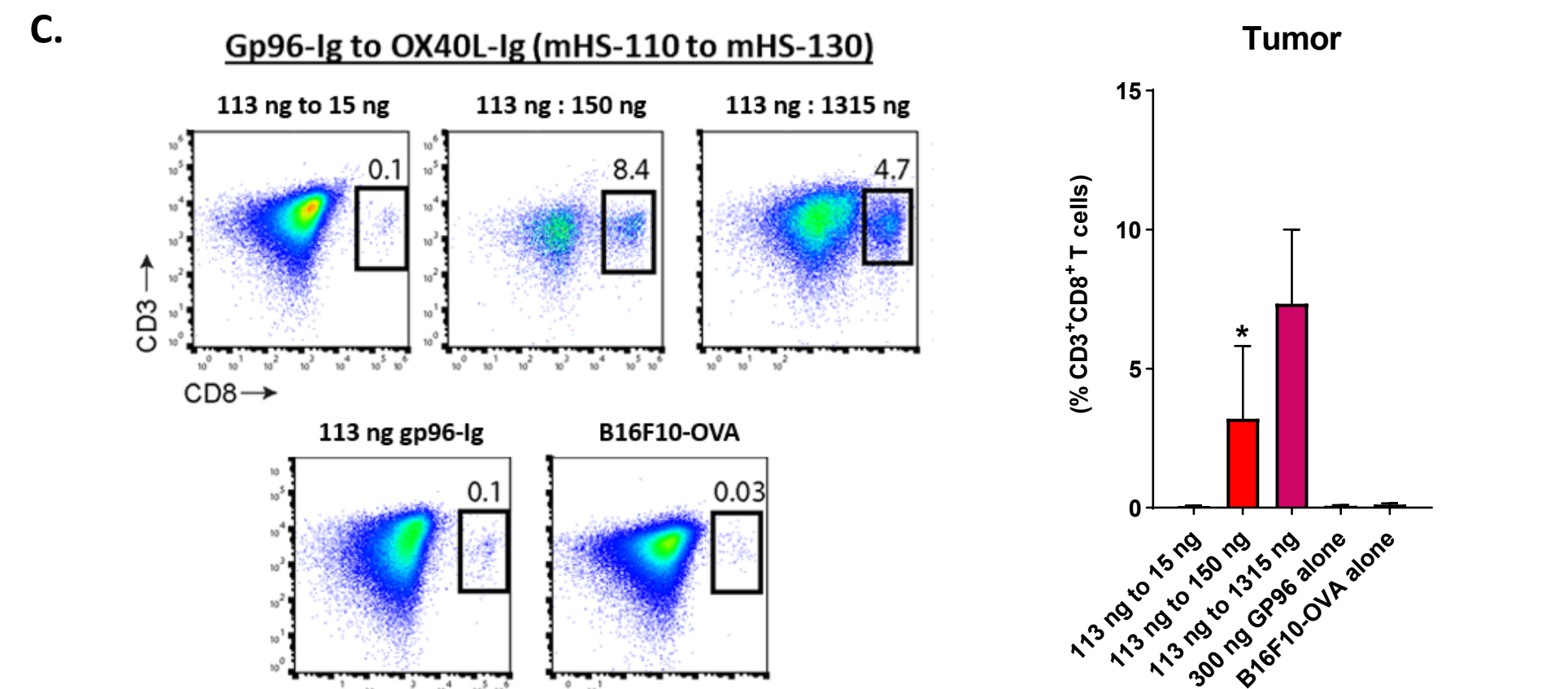
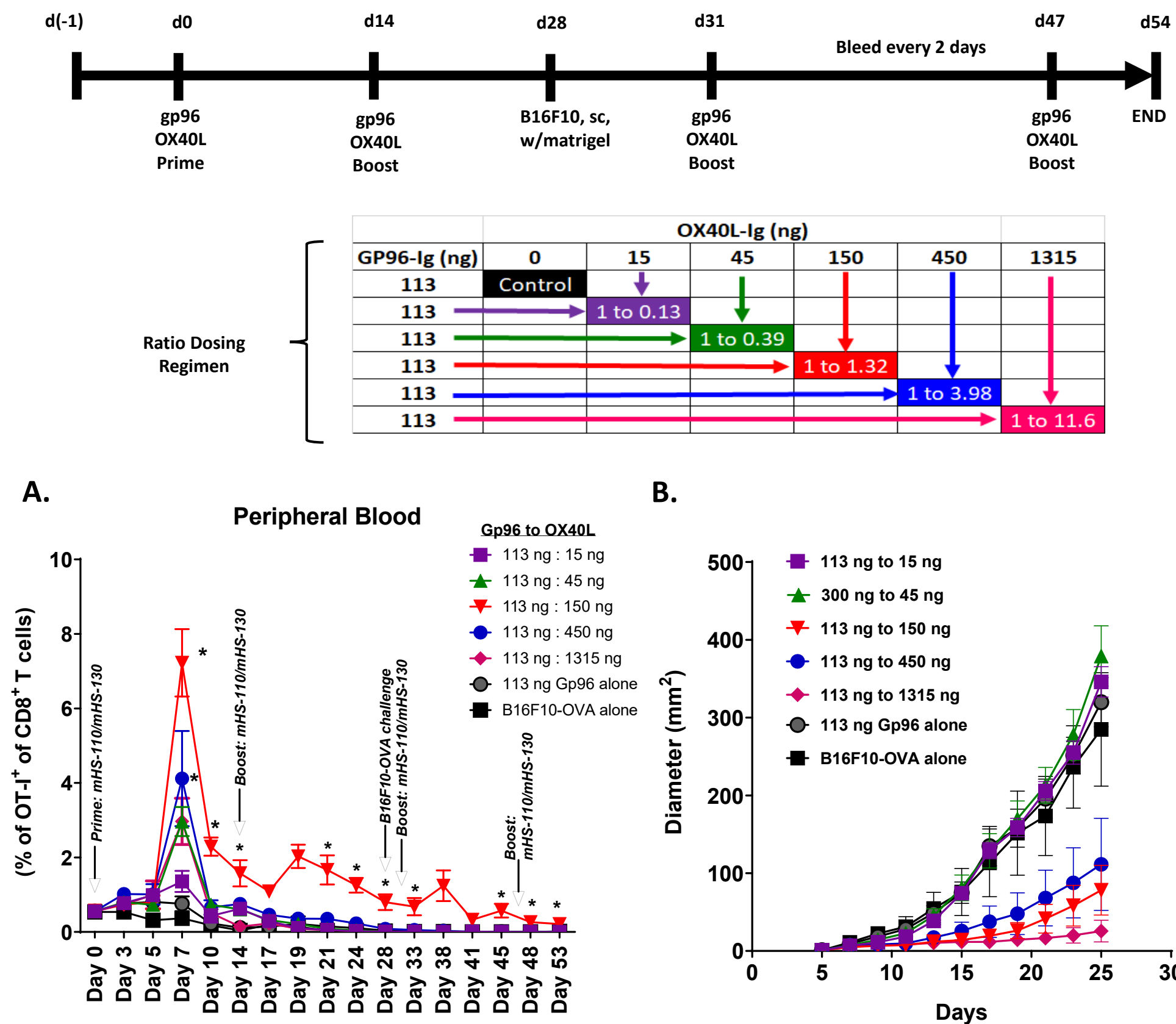


Figure 2: Fixed gp96-Ig; variable OX40L-Ig vaccine dose combination yields greatest tumor regression for the 1 to 1.3 ratio
Tumor implantation, melanoma B16F10 cells were harvested and resuspended at a concentration of 5×10^5 cells/100 μ l in a volume of 80 μ l HBSS and 20 μ l Matrigel. C57BL/6 mice were subcutaneously injected with 100 μ l of B16F10 cells (5×10^5 cells/mouse) on the inner abdomen. The tumor size was measured and documented every 2 days with a caliper, starting on day 7, and calculated using the formula ($L \times S$) (L as the largest and S as the smallest diameter of tumor). Tumor growth was documented as standard error mean. To record the survival of the tumor-bearing mice, either natural death or a tumor volume greater than 450 mm³ leading to death was counted as death. Each experimental group included five animals. (A) Graph shows mean $\pm$ SEM for each dose and ratio group tested per the legend and color map. (B) Line graph of total tumor volume per group with statistics shown in table to the right as compared to gp96-Ig/mHS-110 alone, for days post-tumor inoculation, starting on day 28 post-initial vaccine priming. (C) End-of study, day-54, endogenous TIL response to vaccination, percent, gated FSC-H by FSC-A to as live/dead gate for doublets, then gated on CD3⁺ CD8⁺ double positive cells. Flow plots right, graphed results, right. Bar and line graphs show mean $\pm$ SEM. Statistics analysis performed was Mann-Whitney, two-tailed, test. *p<0.05, **p<0.01, ***p<0.001, 'ns' p>0.05, not significant.

Functional MABEL & NOEL Dose Ratio Determination: Tumor Growth Inhibition

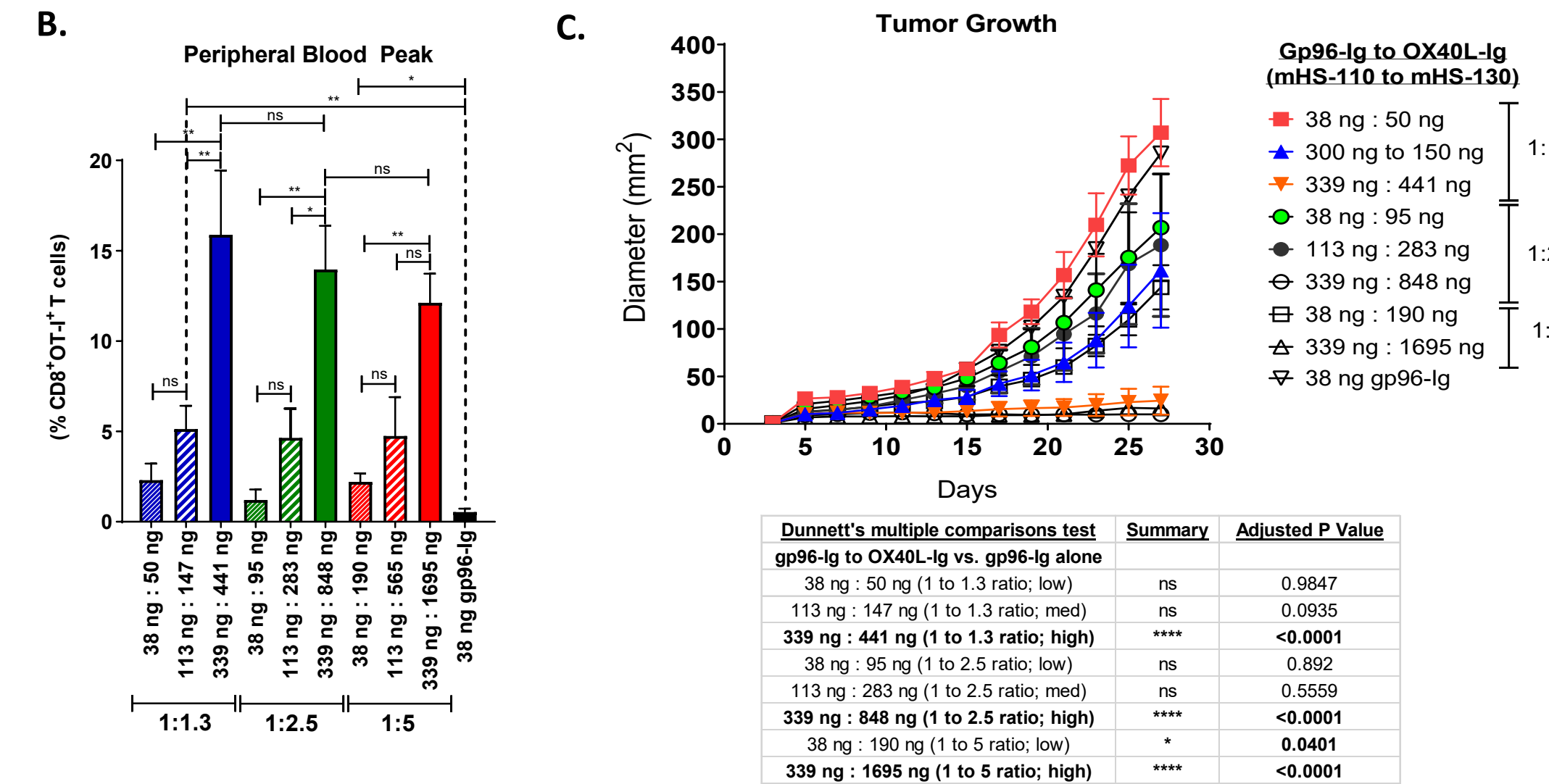
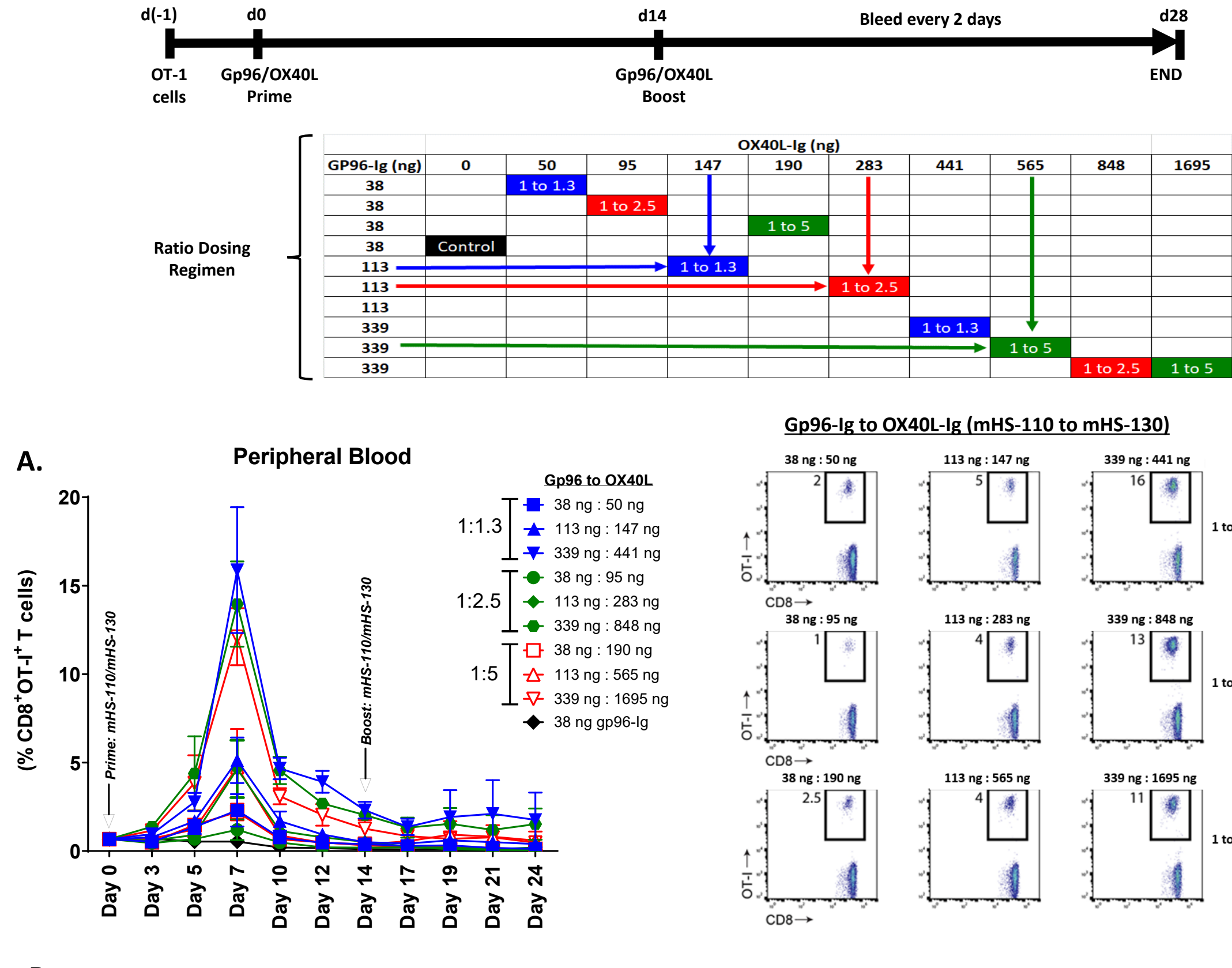


Figure 3: Variable Ratio; Dose Escalation Study to Determine Optimum Anti-Tumor CD8⁺ T-cell Expansion and Tumor Growth Inhibition
T-cell receptor transgenic mouse CD8⁺ (OT-I) cells were isolated from in-house bred OT-I-GFP mice using Easy Sep Mouse CD8⁺ T Cell isolation kit and injected into each C57BL/6 mouse intravenously (i.v) through lateral tail vein with 1 million OT-I cells suspended in HBSS. One day after injecting OT-I, all the mice were tail bled for baseline and 4 hours later, mHS-110 (B16F10-OVA-gp96-Ig) and mHS-130 (B16F10-OVA-OX40L-Ig) were treated with 10 μ g/mL of Mitomycin-C for 3 hours and given intraperitoneally (i.p.) to each group accordingly. Animals were dosed based on nanogram expression level (determined by measuring the supernatants of culturing per 1 million cells in a 24-hour period) of gp96-Ig or OX40L-Ig. Mice were tail bled consecutively on days post-immunization and boosts, and then stained for flow cytometry. (A) Graph shows mean $\pm$ SEM for each dose and ratio group tested per the legend and color map. Representative flow plots are shown in the right panel. (B) Percent OT-I CD8⁺ T-cells in the peripheral blood for days 7 and 17 (before and after boost), plus expression of CD44 on endogenous CD8⁺ T-cells (middle panel). (C) Tumor growth inhibition of B16F10-OVA implanted tumors. Graphs show mean $\pm$ SEM. Statistics analysis performed was Mann-Whitney, two-tailed, test. *p<0.05, **p<0.01, ***p<0.001, 'ns' p>0.05, not significant.

Nonclinical Data Summary

Type	Design/System	Result/Value
Primary pharmacodynamics POC	mHS-110 + OX40, ICOS, 4-1BB mAb	Best synergy with OX40 mAb No Treg induction with OX40 mAb
	mHS-110 + OX40L-Ig, ICOSL-Ig, 4-1BBL-Ig	Superior CD8 ⁺ T-cell expansion and activity seen with OX40L-Ig synergy with mHS-110
	Tumor growth inhibition (TGI) B16F10 CT26 4T1	Best TGI seen with OX40L-Ig combination with mHS-110. Long-term memory of CD8 ⁺ T-cells confirmed
Dose Range Finding Pharmacology	Tumor growth inhibition Cell expansion	Using fixed doses of mHS-110, best dose ratio is 1.3 of mHS-130 to 1 of gp96-Ig (mHS-110) best, next being 2.5 to 1
In vitro immuno-toxicity	Human Jurkat cell lines transfected with OX40 receptor	Similar EC50 between mouse and human OX40L-Ig for OX40 (135 vs. 106 ng/mL)
In vivo immuno-toxicity	Normal C57BL/6 mice Dose escalation 1.0E+04 to 2.0E+07 cells 2 ng to 7,745 ng/animal	NOAEL of 219.5 ng/animal or 0.01097 mg/kg for human HS-130 NOAEL of 387.2 ng/animal or 0.01936 mg/kg for mouse HS-130

Conclusions

- Combination of an allogeneic tumor line expressing gp96-Ig and OX40L-Ig is a potent stimulator of anti-tumor CD8⁺ T-cell immune responses in animals
- Best ratio of gp96-Ig to OX40L-Ig falls within the range of 1-to-1.3 and 1-to-2.5; with 1-to-1.3 providing the best expansion and anti-tumor immunity
- Addition of OX40L-Ig secreting cells to gp96-Ig provides a synergistic impact on both transferred and endogenous tumor specific T-cells
- The minimal anticipated biological effect level (MABEL) for this dose combination is 113 ng of gp96-Ig to 147 ng of OX40L-Ig, with the no observed effect level (NOEL) being 38 ng of gp96-Ig to 50 ng of OX40L-Ig

Acknowledgments & Contact Information

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