

Abstract & Background

Heat Biologics' technology is focused on developing a next generation cellular vaccine that incorporates a tumor antigen chaperone (gp96-Ig) with T-cell costimulation (OX40L-Ig) into a single tumor cell line. Viagenpumatucel-L (HS-110; ImPACT), a human lung adenocarcinoma cell line, stably transfected to express gp96-Ig is being tested in a Phase 2 clinical trial (NCT#02439450) with checkpoint inhibition for NSCLC. A similar line is being generated that will complement HS-110, providing costimulation in the form of secreted OX40-Ig (HS-130). To model how the addition of human HS-130 to HS-110 may impact anti-tumor immune responses, we generated mouse surrogates of these human lines and established an analogous system that treats tumor-bearing animals with tissue-matched irradiated mouse cancer cell lines (melanoma; B16F10) expressing gp96-Ig (mHS-110) and OX40L-Ig (mHS-130); both expressing ovalbumin (OVA), as our model tumor-associated antigen. To identify the best ratio of mHS-110 to mHS-130; a dose ratio study was performed. Fixed levels of gp96-Ig (mHS-110) measured as the total nanograms of gp96-Ig secreted over a 24 hour period, 113 ng in this study, were matched against different ratios of mHS-130 (OX40L-Ig). Similar to our single dose vaccination study, our results demonstrated that the peak CD8+ T-cell expansion occurred on day 7 post-immunization, and that the addition of mHS-130 further boosted anti-tumor CD8+ T-cell expansion by 3-fold when the ratios of mHS-110 to mHS-130 were at a 1-to-1.3 ratio (113 ng of secreted gp96-Ig to 147 ng of secreted OX40L-Ig). These animals were subsequently boosted 14-days post-immunization, and we similarly found that the 1-to-1.3 ratio of mHS-110 to mHS-130 gave the maximum expansion of CD8+ T cell responses, peaking on days 19-21 and contracting thereafter. Importantly, these ratios led to higher frequencies of antigen-specific CD8+ T cells at both priming and boosting, which enhanced rejection of established B16F10 tumors and increased overall animal survival. The minimal anticipated biological effect level (MABEL) for this dose combination is 113 ng of gp96-Ig (mHS-110) to 147 ng of OX40L-Ig (mHS-130), with the no observed effect level (NOEL) being 38 ng of gp96-Ig to 50 ng of OX40L-Ig, in mice.

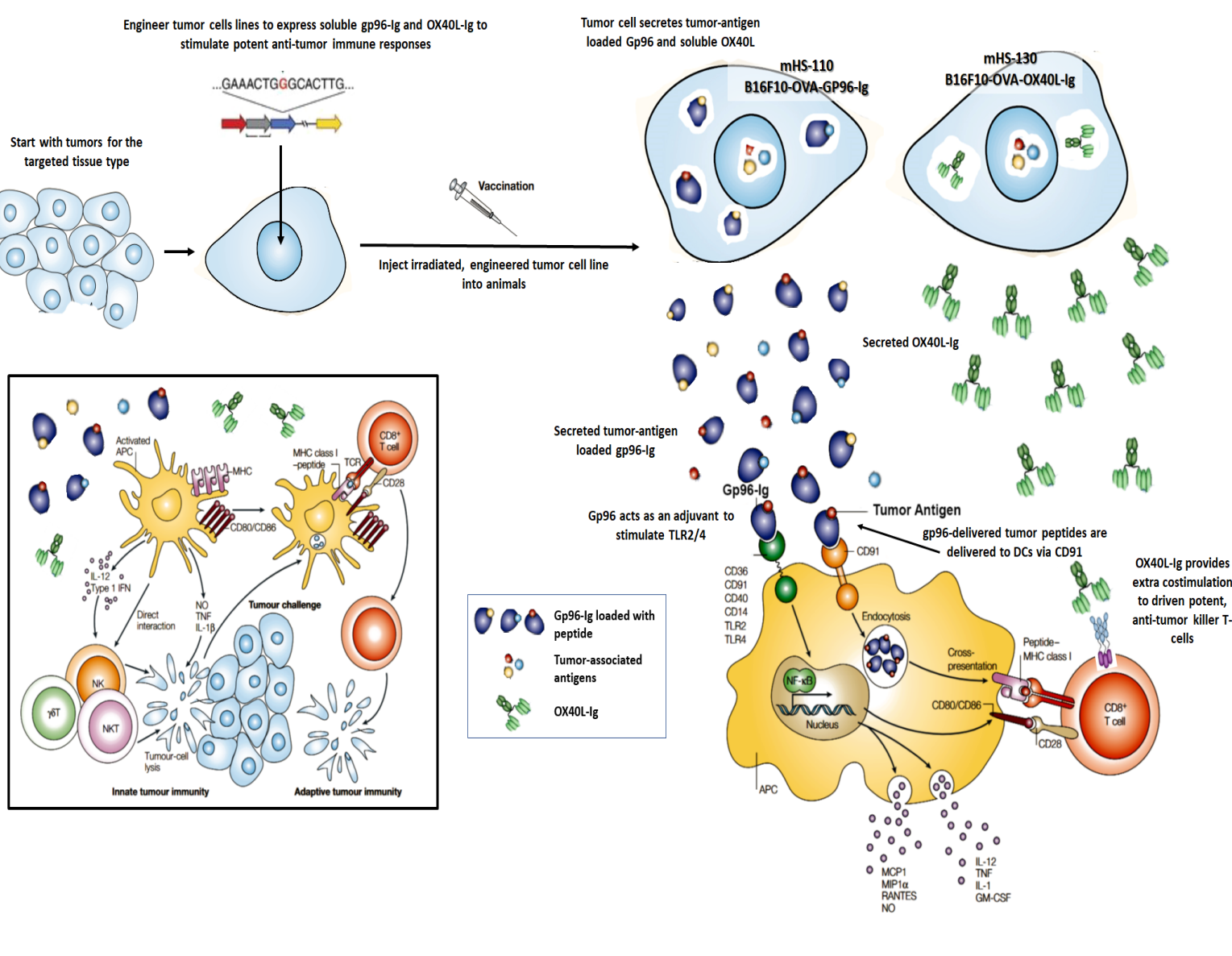


Figure 1: Mechanism of Action
Tissue-matched, allogeneic tumor lines are engineered to express soluble versions of heat-shock protein, gp96, and a potent costimulatory molecule, OX40L (HS-110 and HS-130, respectively). The KDEL endoplasmic reticulum retention sequence of gp96 was replaced by an IgG1 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 to Predict Optimal Anti-Tumor Responses

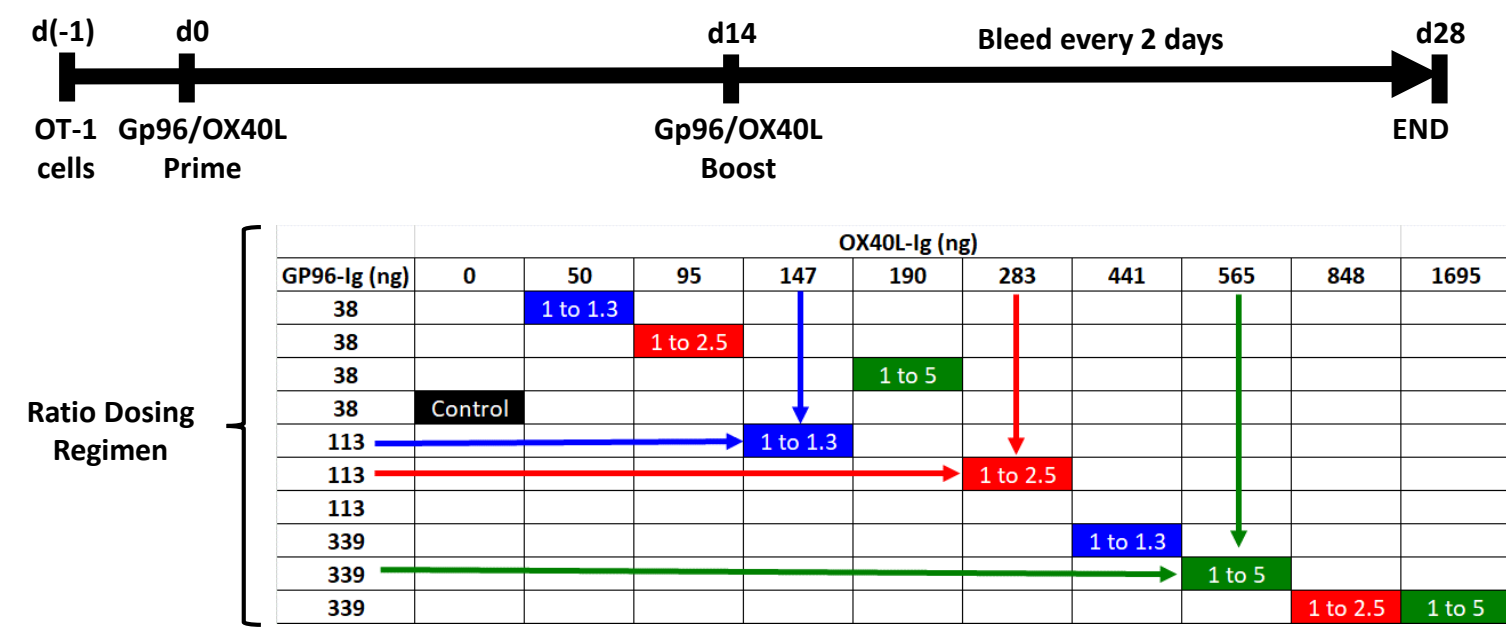


Figure 2: Variable Ratio; Dose Escalation Study to Determine Optimum Anti-Tumor CD8+ T-cell Expansion
T-cell receptor transgenic mouse CD8+ (OT-I) cells were isolated from in-house bred OT-I-GFP mice using Easy Sep Mouse CD8+ T Cell isolation kit and injected into each C57BL/6 mouse intravenously (i.v.) 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 µg/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.

CD8+ T-cell Expansion Over Time with gp96/OX40L-Ig Prime & Boost

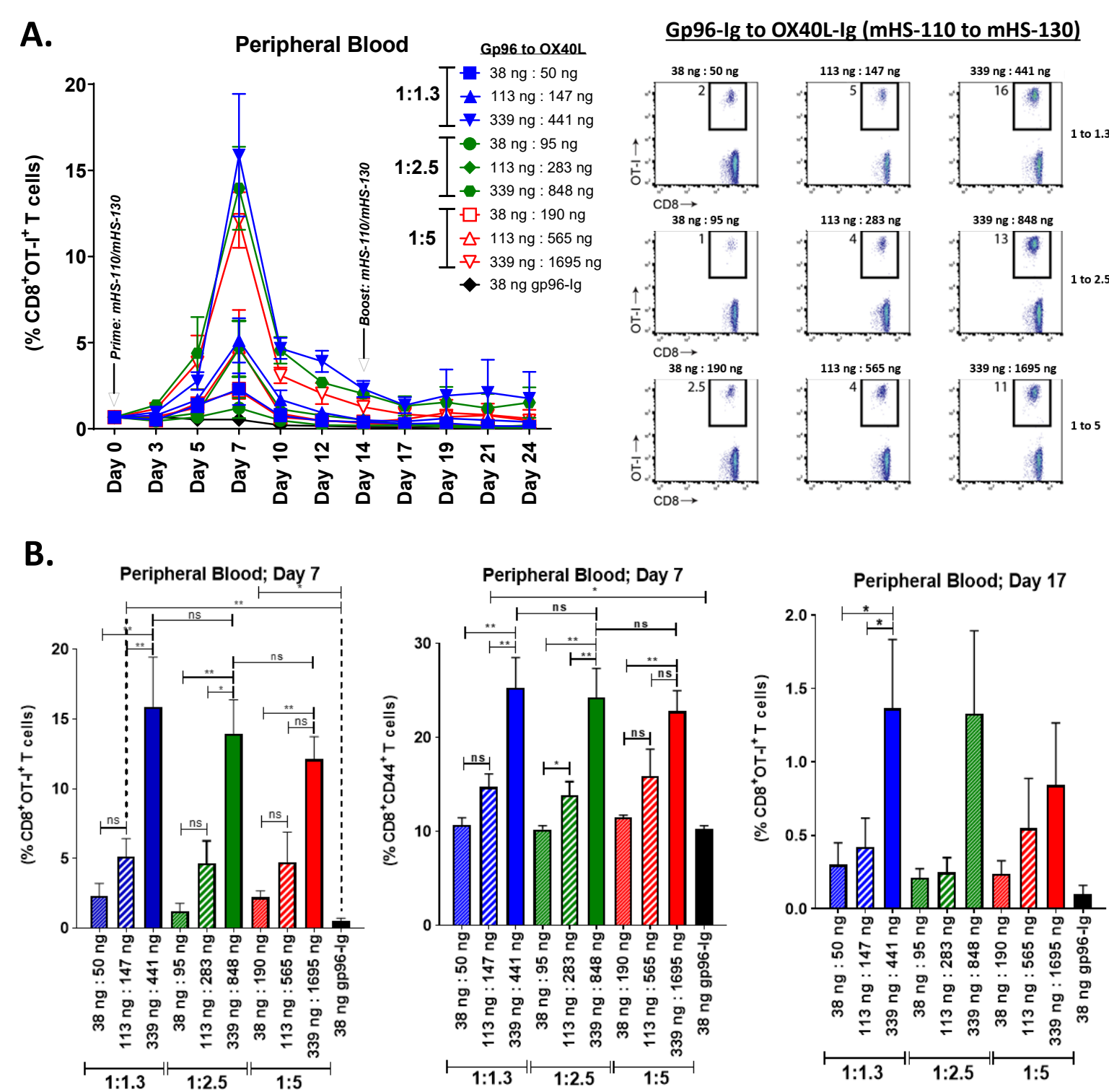


Figure 3: CD8+ T-cell Expansion and CD44 Expression Correlates with gp96/OX40L-Ig Exposure
Mice were tail bled consecutively on days post-immunization and boosts, and then stained for flow cytometry. (A) Graph shows mean $\pm$ SEM for each dose and ratio group tested per the legend and color map. Representative flow plots are shown in the right panel. (B) Percent OT-1 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). 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.

Generation of Memory and Effector T-cell Populations

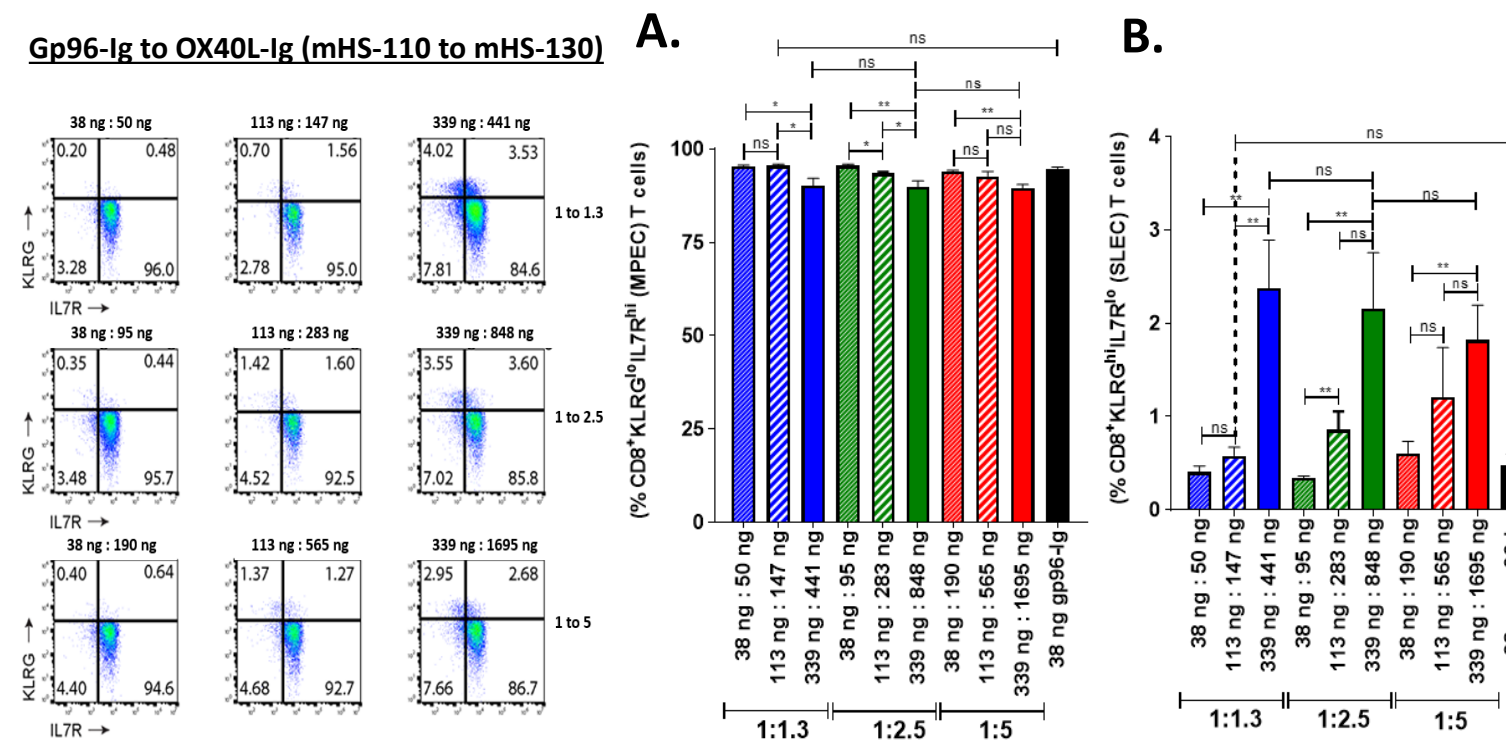


Figure 4: Dose-dependent generation of MPECs and SLECs upon vaccination with mHS-110 and mHS-130
Mice were tail bled consecutively on days post-immunization and boosts, and then stained for flow cytometry. Graph shows mean $\pm$ SEM for each dose and ratio group tested per the legend and color map. Representative flow plots are shown in the left panel. (A) Memory Precursor Effector Cells (MPEC); (B) Short Lived Effector Cells (SLEC) for endogenous CD8+ T-cell populations. Statistics analysis performed was Mann-Whitney, two-tailed, test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, 'ns' $p > 0.05$, not significant.

Variable Ratio; Fixed Dose Vaccine Combination with Tumor Challenge

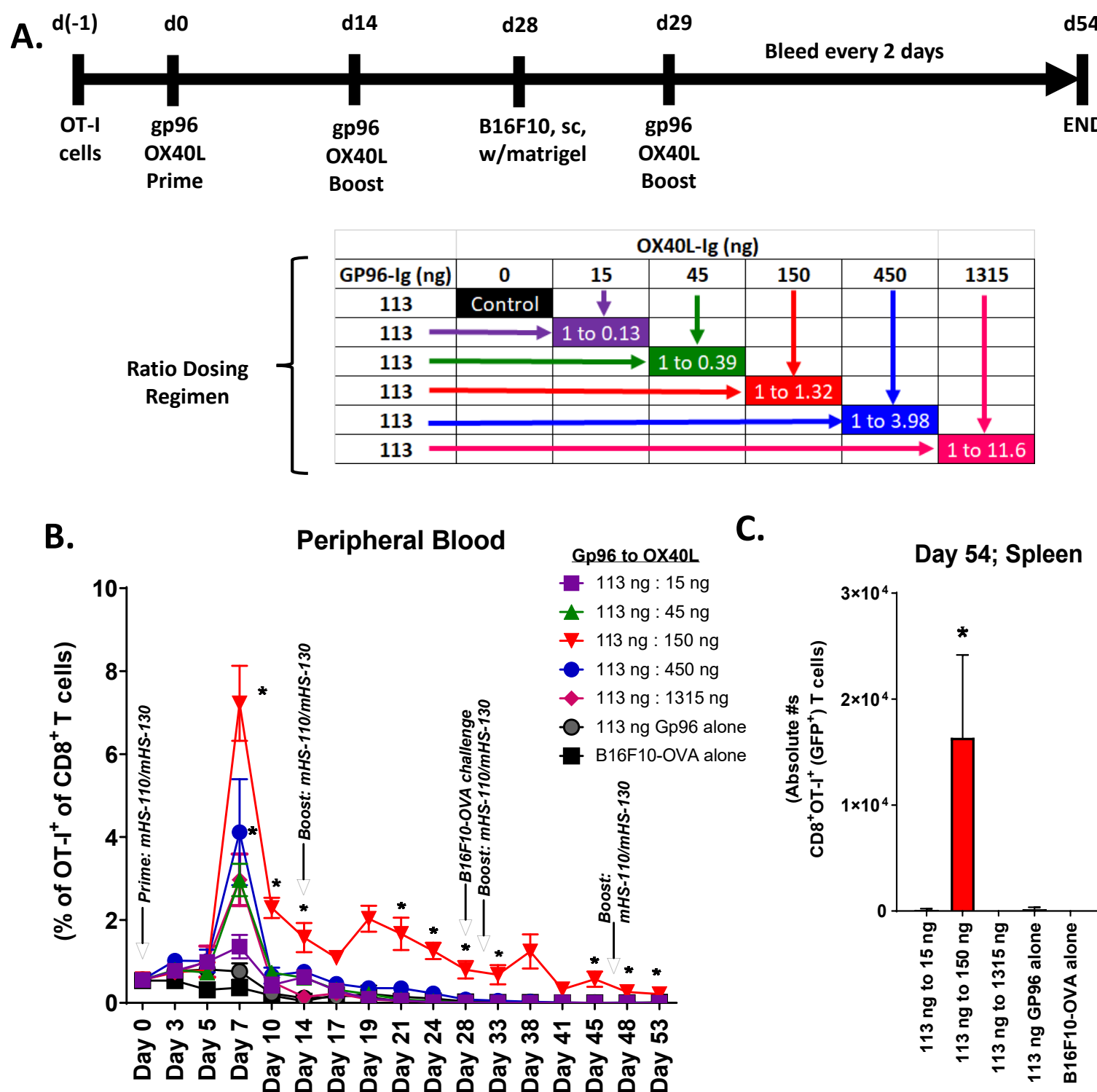
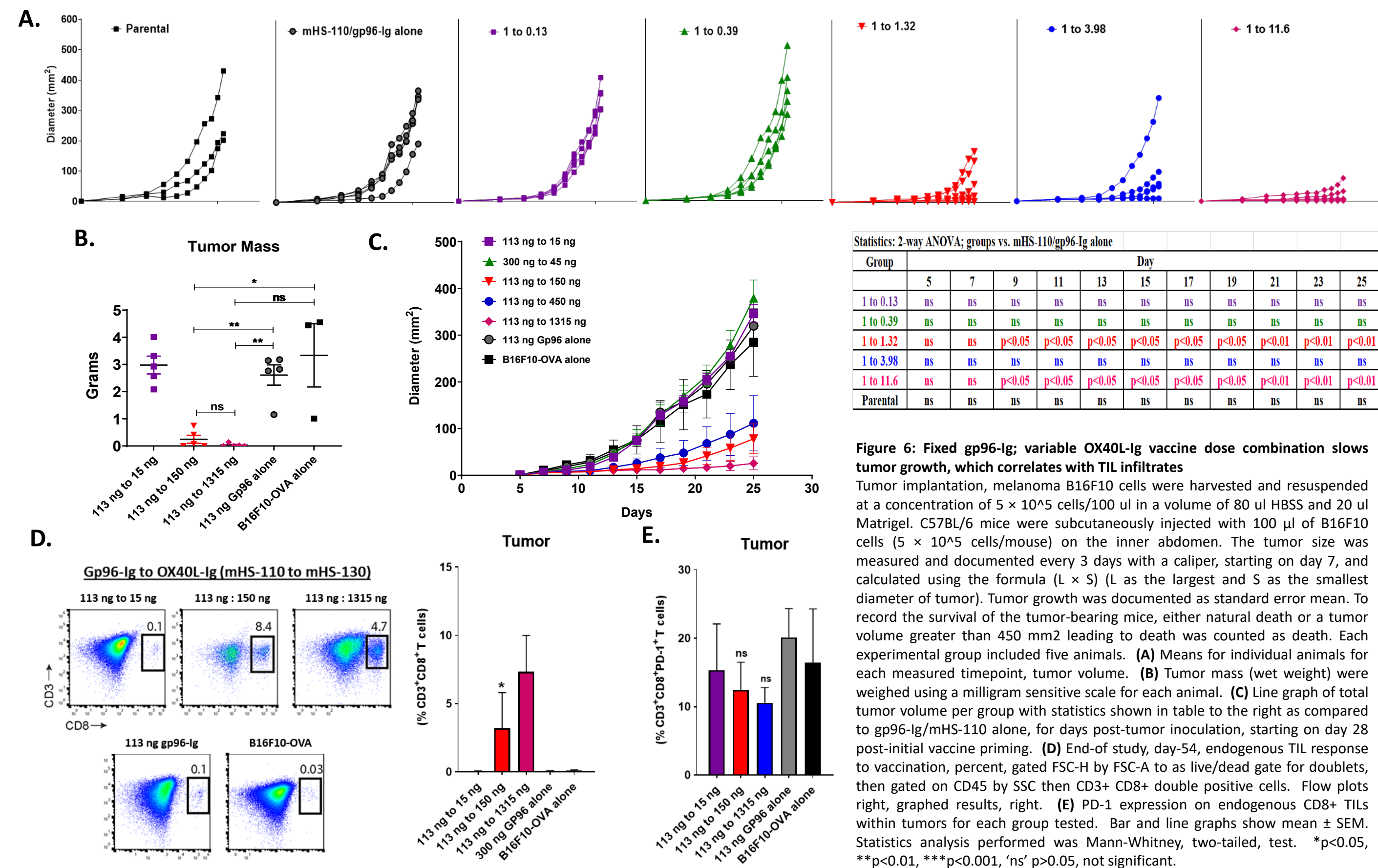


Figure 5: Fixed gp96-Ig; variable OX40L-Ig vaccine dose combination yields greatest expansion for the 1 to 1.3 ratio
(A) Experimental system setup and vaccination cell sources are the same as described in figure 2. (B) Graph shows mean $\pm$ SEM for each dose and ratio group tested per the legend and color map. (C) Absolute cell numbers for OT-1 CD8+ T-cells in the spleen for the final study day, 54. 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.

Combination Vaccine Regimen and Response to Tumor Challenge



Statistics: 2-way ANOVA; groups vs. mHS-110/gp96-Ig alone

Group	5	7	9	11	13	15	17	19	21	23	25
1 to 1.13	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1 to 0.39	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1 to 1.32	ns	ns	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.01	p<0.01	p<0.01	p<0.01
1 to 3.98	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1 to 11.6	ns	ns	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.01	p<0.01	p<0.01	p<0.01
Parental	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Figure 6: Fixed gp96-Ig; variable OX40L-Ig vaccine dose combination slows tumor growth, which correlates with TIL infiltrates
Tumor implantation, melanoma B16F10 cells were harvested and resuspended at a concentration of 5×10^5 cells/100 µl in a volume of 80 µl HBSS and 20 µl Matrigel. C57BL/6 mice were subcutaneously injected with 100 µl of B16F10 cells (5×10^5 cells/mouse) on the inner abdomen. The tumor size was measured and documented every 3 days with a caliper, starting on day 7, and calculated using the formula $(L \times S) / 2$ (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 mm² leading to death was counted as death. Each experimental group included five animals. (A) Means for individual animals for each measured timepoint, tumor volume. (B) Tumor mass (wet weight) were weighed using a milligram sensitive scale for each animal. (C) 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. (D) 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 CD45 by SSC then CD3+ CD8+ double positive cells. Flow plots right, graphed results, right. (E) PD-1 expression on endogenous CD8+ TILs within tumors for each group tested. 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.

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 Info

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*Corresponding Author: Matthew M. Seavey, Senior Director of Research, mseavey@heatbio.com

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