Generation of a Novel, Allogeneic Cell-based, Gp96-Ig/OX40L Cancer Vaccine, Improves Anti-Tumor Immunity and Long-Term Memory T-cell Generation

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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. Single dose vaccination with mHS110 identified that 300 ng to 3000 ng of secreted gp96-Ig provided sufficient anti-tumor CD8+ T-cell expansion, with the greatest expansion observed on day 7, post-immunization. To identify the best ratio of mHS-110 to mHS-130; a dose ratio study was performed. Fixed numbers of mHS-110 (300 ng of secreted gp96-Ig) were matched with 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-0.5 ratio (300 ng of secreted gp96-Ig to 150 ng of secreted OX40L-Ig). These animals were subsequently boosted 14-days post-immunization, and we similarly found that the 1-to-0.5 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.

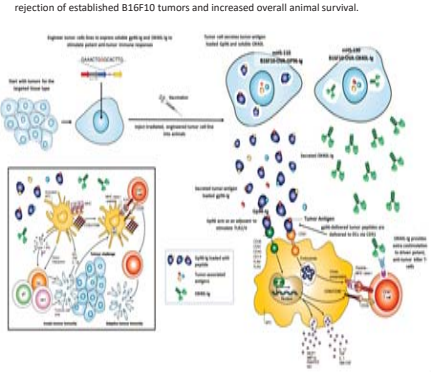


Figure 1: Mechanism of Action
Tissue-matched, allogeneic tumor lines are engineered to express soluble versions of heat-shock protein, gp96, and a potent costimulatory molecule, OX40L (HS-110 and HS-130, respectively). The KDEL endoplasmic reticulum retention sequence of gp96 was replaced by an IgG1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

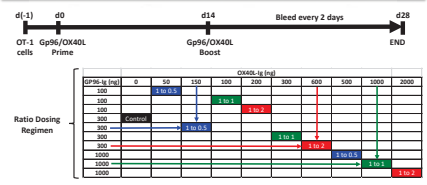


Figure 2: Variable Ratio; Dose Escalation Study to Determine Optimum Anti-Tumor CD8+ T-cell Expansion
T-cell receptor transgenic mouse CD8+ (OT-I) cells were isolated from in-house bred OT-I GFP mice using Easy Sep Mouse CD8+ T Cell isolation kit and injected into each C57BL/6 mouse intravenously (i.v)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

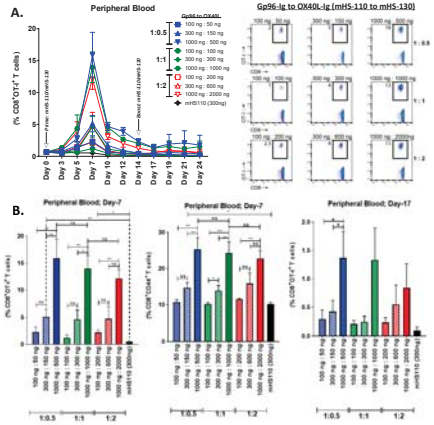


Figure 3: CD8+ T-cell Expansion and CD44 Expression Correlates with gp96/OX40L-Ig Exposure
Mice were tail bled consecutively on days post-immunization and boosts, and then stained for flow cytometry. **(A)** Graph shows mean ± SEM for each dose and ratio group tested per the legend and color map. Representative 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 ± 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

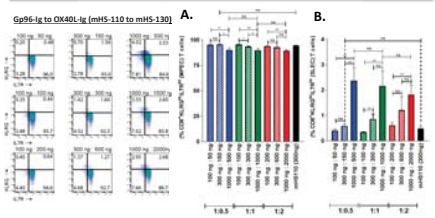


Figure 4: Dose-dependent generation of MPECs and SLECs upon vaccination with mHS-110 and mHS-130
Mice were tail bled consecutively on days post-immunization and boosts, and then stained for flow cytometry. Graph shows mean ± SEM for each dose and ratio group tested per the legend and color map. Representative 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

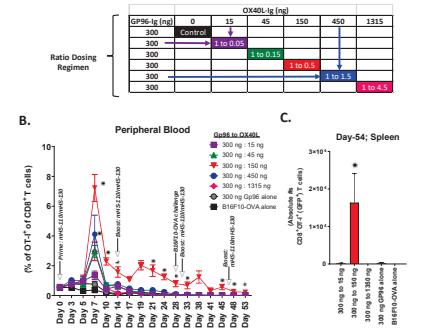
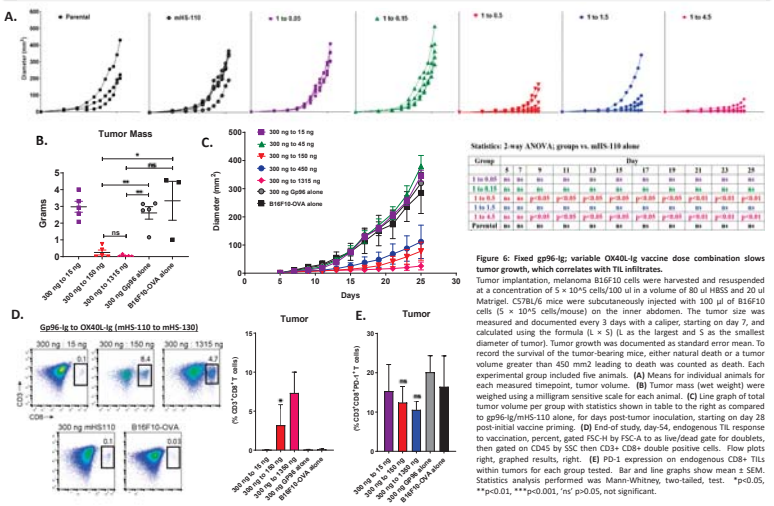


Figure 5: Fixed gp96-Ig: variable OX40L-Ig vaccine dose combination yields
(A) Experimental system setup and vaccination cell sources are the same as described in figure 2. **(B)** Graph shows mean ± SEM for each dose and ratio group tested per the legend and color map. **(C)** Absolute cell numbers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

Group	Day 5	7	9	11	13	15	17	19	21	23	25
1 to 0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1 to 0.15	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1 to 0.5	ns	ns	p<0.01	p<0.01	p<0.05	p<0.05	p<0.05	p<0.01	p<0.01	p<0.01	p<0.01
1 to 1.5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
1 to 4.5	ns	ns	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01	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 ul in a volume of 80 ul HBSS and 20 ul 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 × 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 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 ± 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-0.5 and 1-to-1; with 1-to-0.5 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 active biological dose for this combination is 100 ng of gp96-Ig to 50 ng of OX40L

Acknowledgments & Contact Info

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