

Background

Heat Biologics (Heat) has developed a next generation cellular vaccine platform that incorporates a tumor antigen chaperone (gp96-Ig) in a tumor cell line and a host of over-expressed cancer associated neoantigens. Viagenpumatucl-L (HS110), a human lung adenocarcinoma cell line, stably transfected to express gp96-Ig, is being tested in a phase 1/2 clinical trial (NCT02439450) for NSCLC. Heat has recently developed HS130, an allogeneic cell-based vaccine, designed to secrete tumor-associated antigens along with a costimulatory molecule, OX40L. Preclinical results of mouse HS130 (mHS130) in combination with mouse HS110 (mHS110) has shown a potent anti-tumor effector and memory CD8+ T cell response, followed by tumor regression. In our current study, we further characterized the role of mHS110 and mHS130 in combination with an agonist TNFRSF25 monoclonal antibody (mAb), PTX35. PTX35 is a potent stimulator of effector and memory CD8+ T cell responses, which taken together with HS110 and HS130 has the potential of treating human cancers. To study expansion, contraction, and maintenance of tumor-specific CD8+ T cell responses, mHS110 and/or mHS130, in combination with different doses of mouse-IgG1-PTX35 (mPTX35) was administered to C57BL/6 mice that were adoptively transferred with syngeneic OVA-specific T cells (OT-I). Mice were then challenged with murine melanoma tumors (B16F10-OVA) to characterize the tumor-specific immune cells in the periphery, spleen, and tumor-microenvironment that were involved in tumor regression. Combination of mPTX-35 with mHS-110 and mHS130 increased the expansion of tumor-specific CD8+ T-cells, in a mPTX-35 dose-dependent manner. This cellular expansion was significantly higher in the 1 mg/kg dose of mPTX-35 and far exceeded the additive value of mPTX-35, mHS130, and mHS110 treatment alone. Systemic administration of mPTX35, in combination with mHS110 and mHS130, led to a significant increase in the expansion of activated CD8+ T cells in the blood and stimulated activation of effector memory CD8 T cells residing in the spleen. Importantly, this combination resulted in higher frequencies of tumor infiltrating lymphocytes (TILs), which enhanced regression of established B16F10-OVA tumors and increased overall survival. These results strongly suggest that mPTX35 synergizes with mHS110 and mHS130 to amplify activated tumor-specific CD8+ T cells, program a strong memory response, and allow for tumor regression. We also assessed if PD1 antagonist therapies further amplified our responses and found that checkpoint inhibition (CPI) synergizes effectively with PTX35 and mHS110. Therefore, the combinations of these treatments along with CPI may translate into an efficacious approach to treating human cancers.

PTX35 Synergizes with Gp96-Ig/OX40L-Ig to Enhance Activated Tumor-Specific CD8+ T Cells

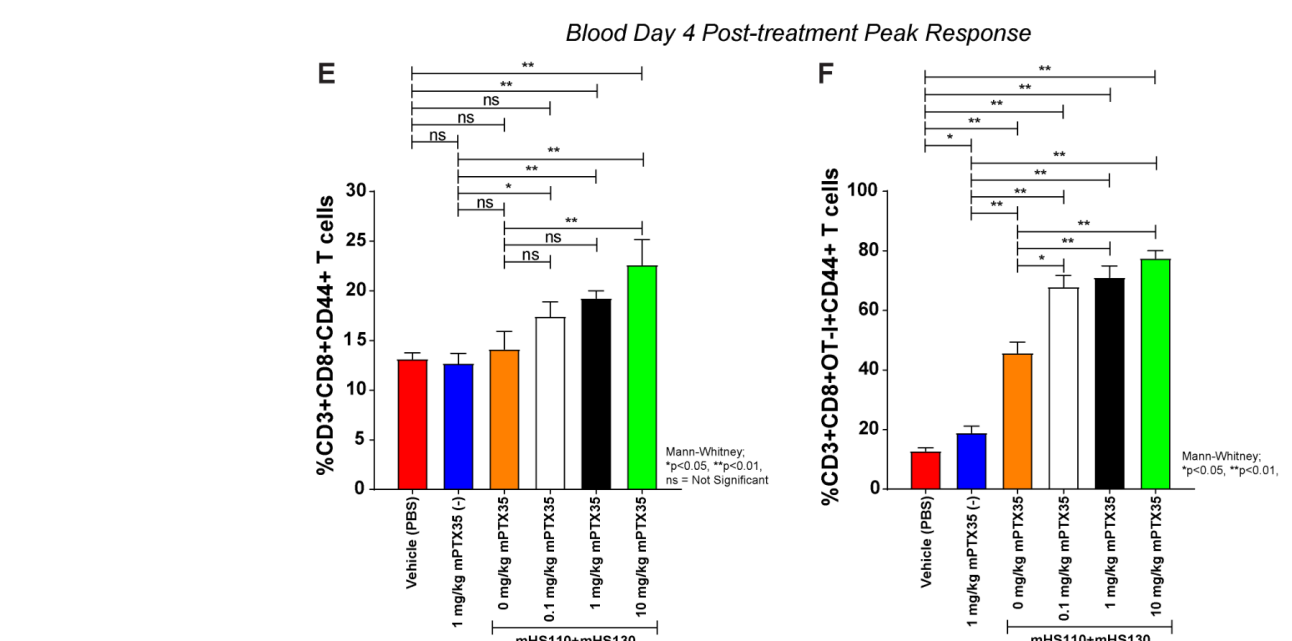
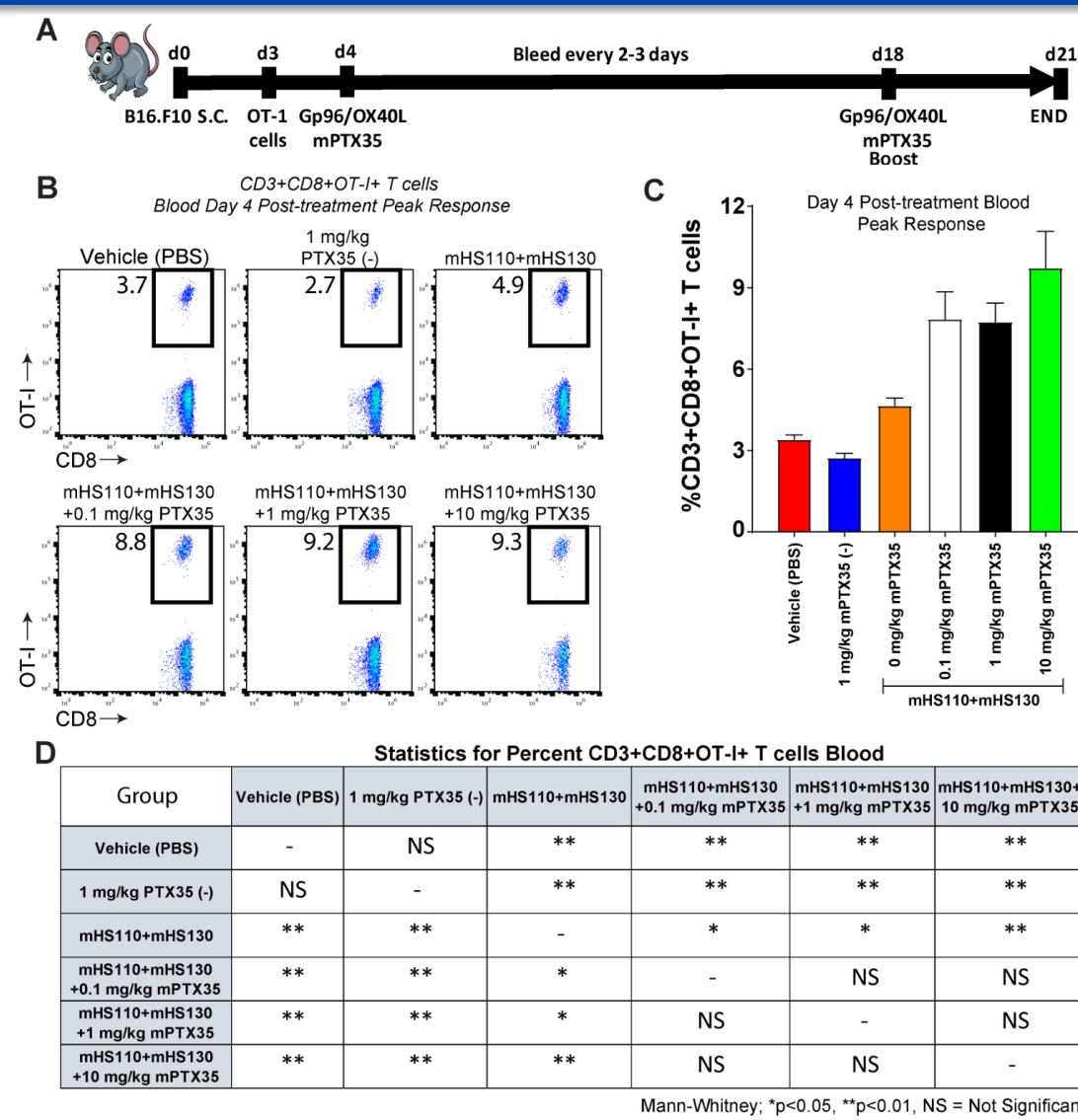


Figure 2: (A) C57BL/6 mice (n=5 mice/group) were injected subcutaneously (S.C.) with 5E⁶ B16.F10 melanoma tumors and 3 days later, adoptively transferred with 1E⁶ syngeneic OT-I transgenic CD8+ T cells. Mice were subsequently treated on days 4 and 18 post tumor inoculation. (B, C) Percentage of CD3+CD8+OT-I+ T cells were enumerated in the peripheral blood on day 4 post-treatment. CD3+CD8+OT-I+ T cells are presented as the mean ± SEM of 5 mice/group or as a representative FACS plot. (D) The Mann-Whitney two-tailed test was used to determine statistical significance. *p<0.05, **p<0.01, NS = Not Significant. Percentage of (E) CD3+CD8+CD44+ T cells or (F) CD3+CD8+OT-I+CD44+ T cells were enumerated on day 4 post-treatment and are presented as the mean ± SEM of 5 mice/group.

PTX35 plus Gp96-Ig/OX40L-Ig Augments Effector Memory CD8+ T cell Responses

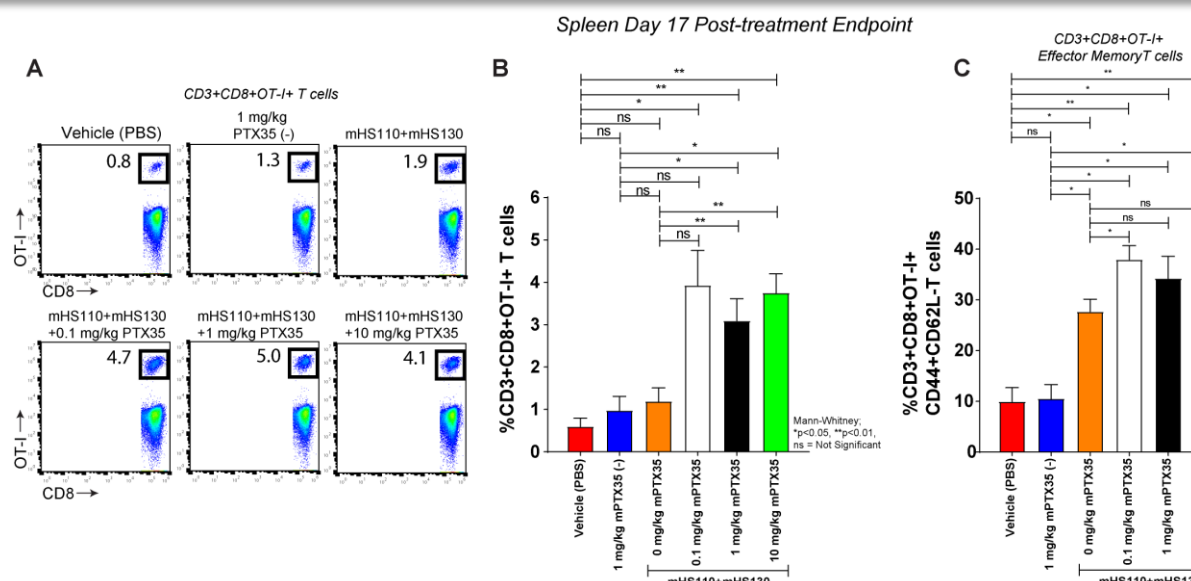


Figure 3: C57BL/6 mice (n=5 mice/group) bearing B16.F10 melanoma tumors and adoptively transferred with 1E⁶ syngeneic OT-I transgenic CD8+ T cells were sacrificed on day 21 post-tumor inoculation when mice reached tumor burden. (A, B) The percentage of CD3+CD8+OT-I+ T cells were enumerated on day 17 post-treatment in the spleen. CD3+CD8+OT-I+ T cells are presented as the mean ± SEM of an experiment containing 5 mice/group or as a representative FACS plot from the spleen. (C) CD3+CD8+OT-I+CD62L+ T cells were enumerated on day 17 post-treatment and are presented as the mean ± SEM of an experiment containing 5 mice/group. Mann-Whitney two-tailed test was used to determine statistical significance. *p<0.05, **p<0.01, NS = Not Significant.

Tumor-Infiltrating Lymphocytes (TILs) are Markedly Increased After PTX35 Addition

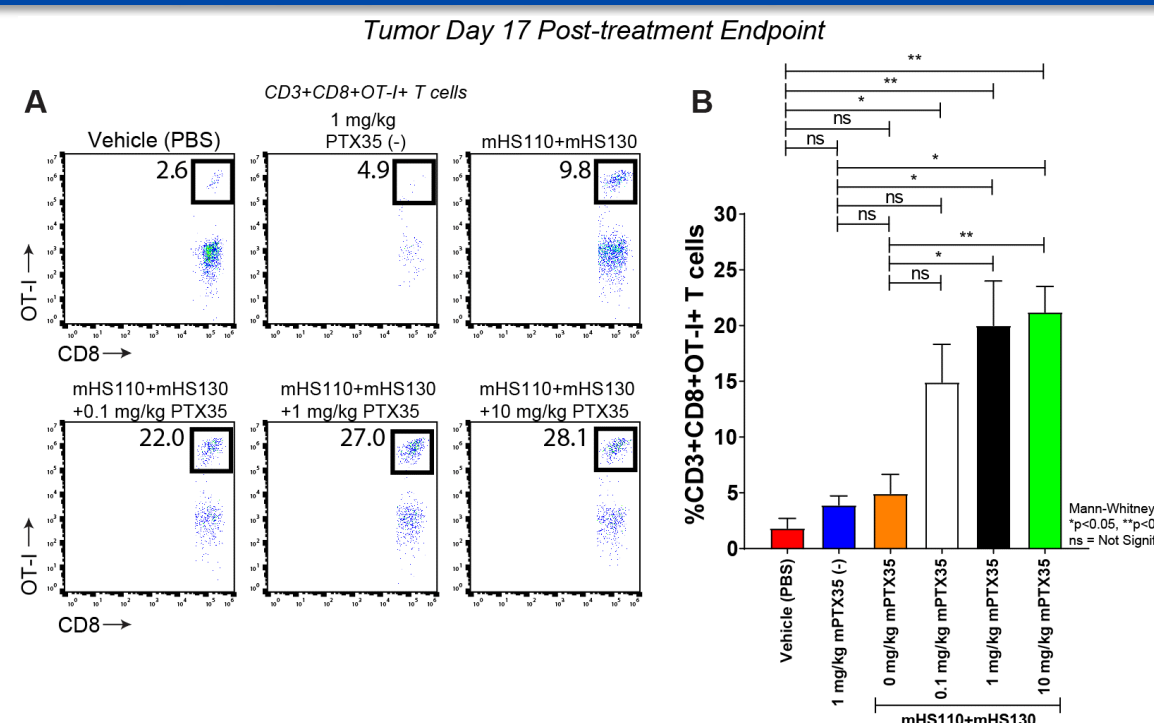


Figure 4: C57BL/6 mice (n=5 mice/group) bearing B16.F10 melanoma tumors were adoptively transferred with 1E⁶ syngeneic OT-I transgenic CD8+ T cells and sacrificed on day 21 post-tumor inoculation. (A, B) The percentage of CD3+CD8+OT-I+ T cells were enumerated on day 17 post-treatment. CD3+CD8+OT-I+ T cells are presented as the mean ± SEM of 5 mice/group or as a representative FACS plot. Mann-Whitney was used to determine statistical significance. *p<0.05, **p<0.01, NS = Not Significant.

Significantly Increased Levels of TILs Results in Substantial Tumor Growth Inhibition

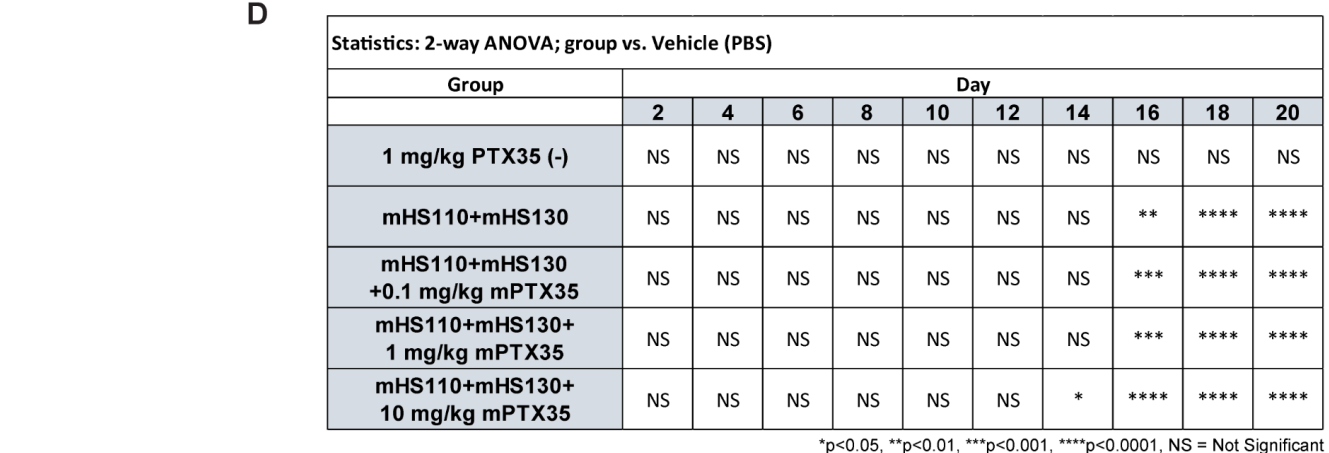
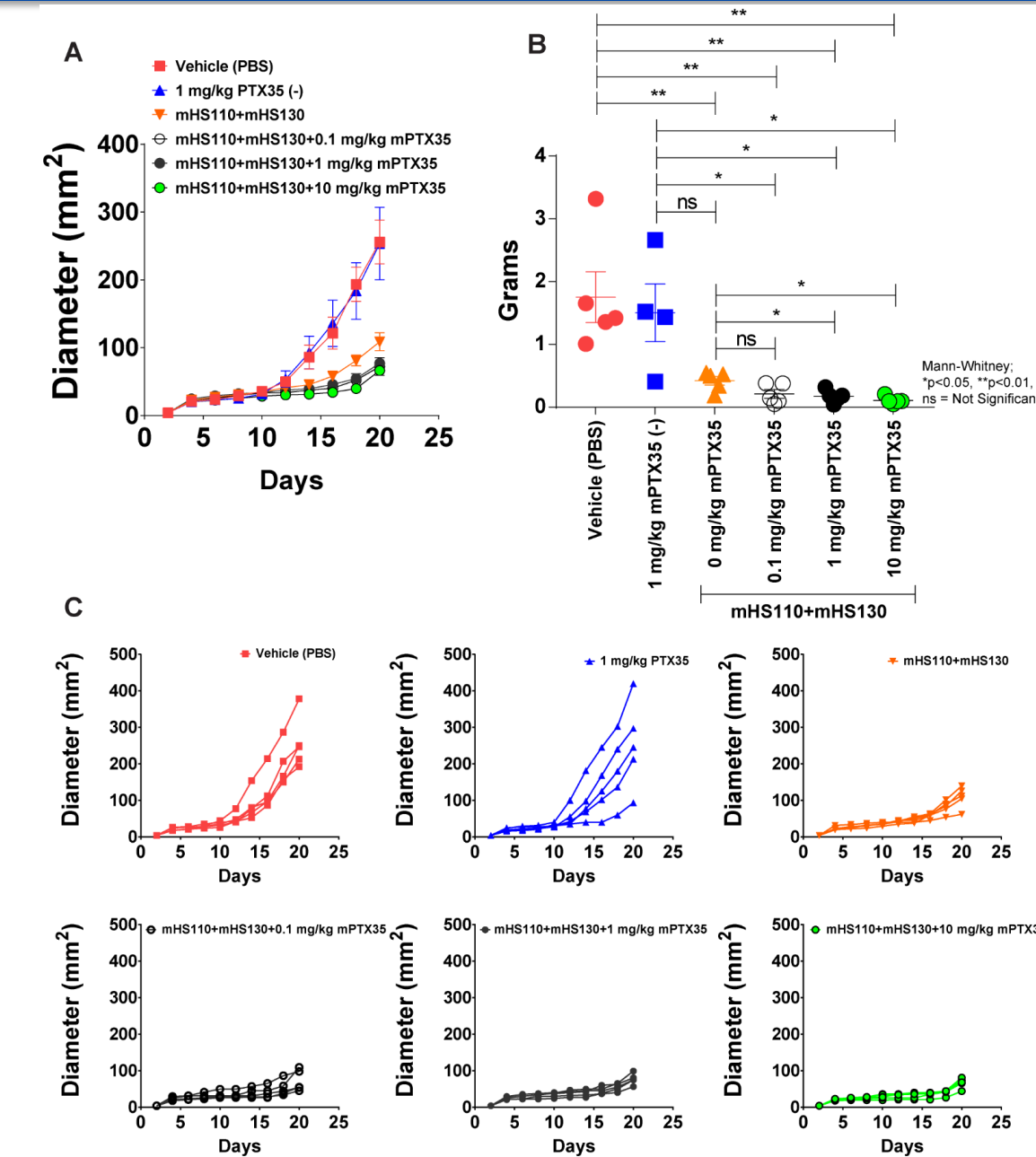


Figure 5: C57BL/6 mice (n=5 mice/group) were injected subcutaneously (S.C.) with 5E⁶ B16.F10 melanoma tumors and 3 days later, adoptively transferred with 1E⁶ syngeneic OT-I transgenic CD8+ T cells. Mice were subsequently treated on days 4 and 18 post tumor inoculation and monitored for (A, C) tumor growth inhibition (TGI). The tumor size was measured and documented every 2 days with a caliper, starting on day 2, and calculated using the formula (L × S) (L as the largest and S as the smallest diameter of tumor). (B) Tumors were extracted on day 21 post-tumor inoculation when mice reached tumor burden and tumor mass (grams) was recorded. (D) 2-way ANOVA was performed to determine statistical significance. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS = Not Significant.

Checkpoint Inhibition (αPD1) along with Gp96-Ig and PTX35 Generates Significant Anti-Tumor immunity

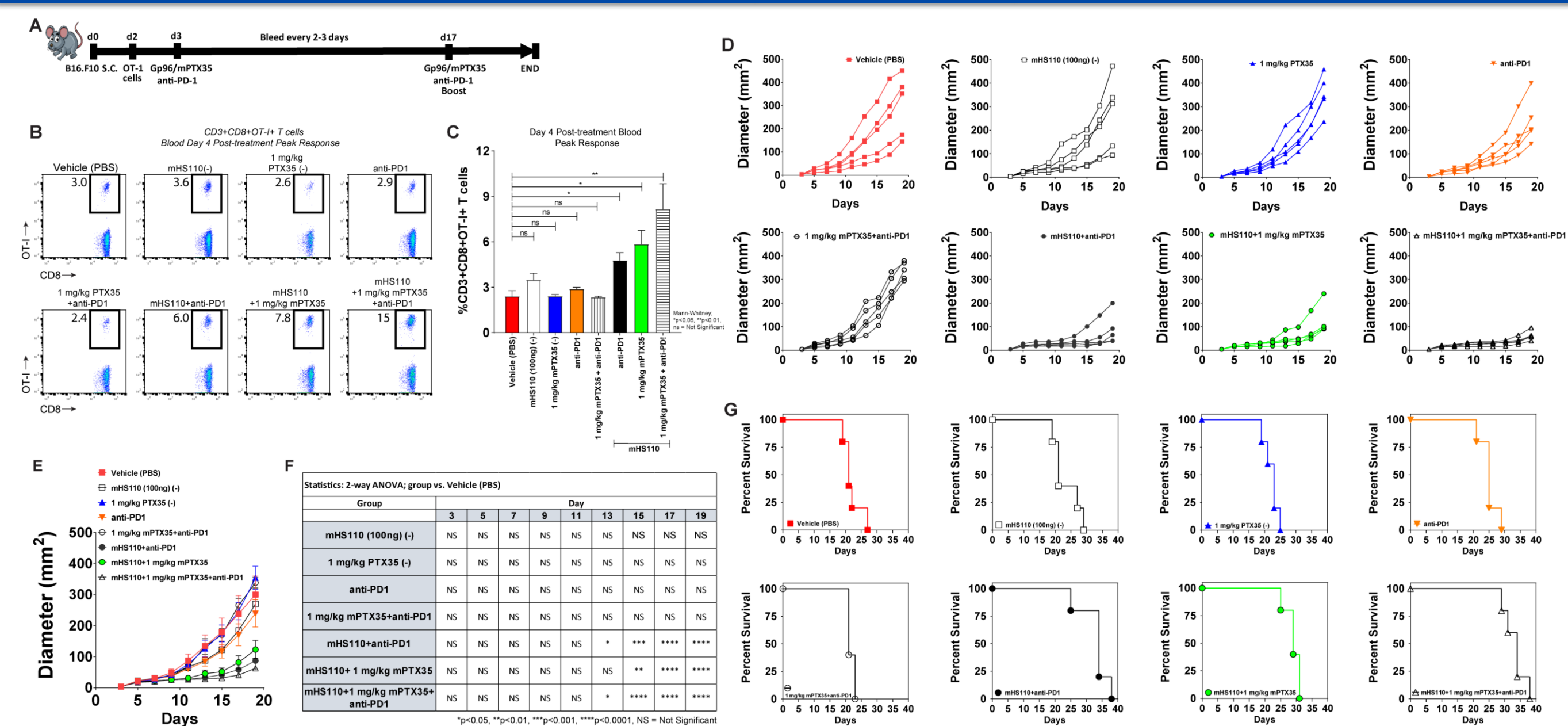


Figure 6: (A) C57BL/6 mice (n=5 mice/group) were injected subcutaneously (S.C.) with 5E⁶ B16.F10 melanoma tumors and 2 days later, adoptively transferred with 1E⁶ syngeneic OT-I transgenic CD8+ T cells. Mice were subsequently treated on days 3 and 17 post-tumor inoculation and monitored for tumor growth inhibition (TGI). (B, C) The percentage of CD3+CD8+OT-I+ T cells were enumerated in the peripheral blood during the peak of CD8+ T cell responses on day 4 post-treatment. CD3+CD8+OT-I+ T cells are presented as the mean ± SEM of an experiment containing 5 mice/group or as a representative FACS plot from the peripheral blood. (D, E) The tumor size was measured and documented every 2 days with a caliper, starting on day 3, and calculated using the formula (L × S) (L as the largest and S as the smallest diameter of tumor). (F) 2-way ANOVA was performed to determine statistical significance. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, NS = Not Significant. (G) Overall survival was monitored over 38 days.

Conclusions

- PTX35 synergizes with Gp96-Ig/OX40L-Ig to enhance activated tumor-specific CD8+ T cells and effector memory CD8+ T cells.
- Tumor-Infiltrating Lymphocytes (TILs) are significantly increased after PTX35 addition and results in a substantial reduction in tumor growth.
- Checkpoint inhibition (αPD1) with Gp96-Ig and PTX35 results in a significant increase in antigen-specific CD8+ T cell responses, reduction in tumor burden, and an increase in overall survival

Acknowledgments & Contact Info

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References

- Schreiber TH, Podack ER. Immunobiology of TNFSF15 and TNFRSF25. Immunologic Research, December 2013, Volume 57 (issue 1-3), pp. 3-11
- Schreiber T.H., Levy R.B., and Podack E.R.; (2010). Therapeutic Treg expansion in mice by TNFRSF25 prevents allergic lung inflammation. J Clin Invest.; 120(10):3629-3640
- Melero I, Hirschhorn-Cymerman D, Morales-Kastresana A, Sanmamed M.F., and Wolchok J.D.; (2013). Agonist Antibodies to TNFR Molecules That Costimulate T and NK Cells. Clin Cancer Res.; 19(5): 1044-53
- Slebiada T.J. et al.; (2011). Triggering of TNFRSF25 promotes CD8+ T-cell responses and anti-tumor immunity. Eur J of Immunol 41 (9), 2606-2611
- Schreiber T.H., et al.; (2014). Comparative combination cancer immunotherapy with vaccination and TNFRSF stimulation. Society of Immunotherapy of Cancer (SITC) Conference, Poster: <https://doi.org/10.1158/1078-0432.CCR-14-0005>
- Nishikii H. et al.; (2016). DR3 signaling modulates the function of Foxp3+ regulatory T cells and the severity of acute graft-versus-host disease. Blood 128 (24), 2846-2858
- Fromm G, de Silva S, Giffin L, Xu X, Rose J, Schreiber TH. Gp96-Ig/Costimulator (OX40L, ICOSL, or 4-1BBL) Combination Vaccine Improves T-cell Priming and Enhances Immunity, Memory, and Tumor Elimination. Cancer Immunol Res. 2016;4(9):766-778. doi:10.1158/2326-6066.CIR-15-0228

