

PTX-35, a Potential First-in-class Agonist, Reduced the Suppressive Activity of Regulatory T cells and Enhanced CD4+ T cell Effector Responses in the Presence of Tumor Antigens in a Murine Melanoma Model



Khalid W. Kalim¹, Vikas Tahiliani¹, Jayalakshmi Miriyala¹, Patrick J. Dillon¹, Anh M. Trinh¹, Abra N. Chaney¹, Matthew M. Seavey¹, Eric P. Dixon¹, Rahul R. Jasuja², Jeff Hutchins¹

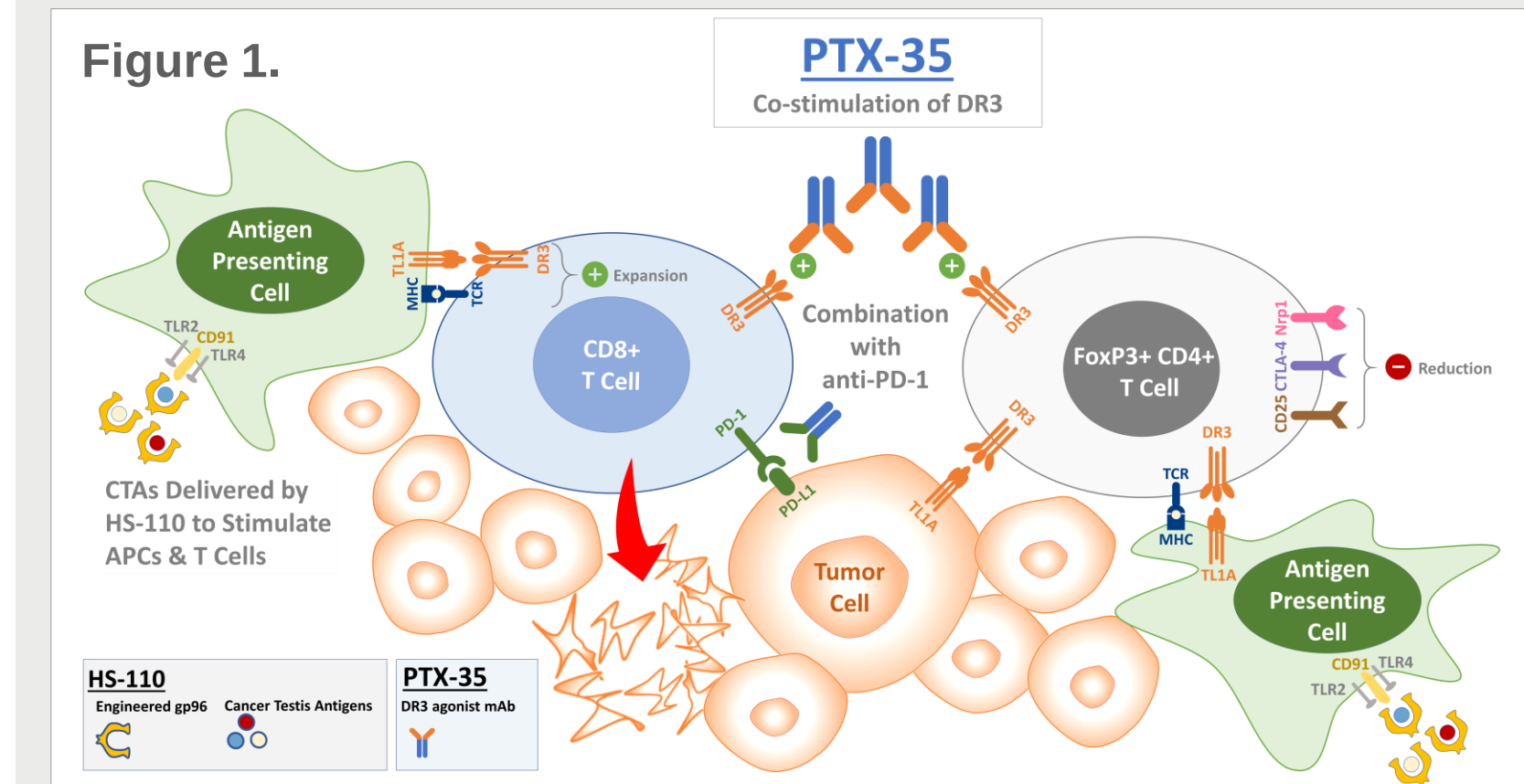
¹Heat Biologics, Inc., Suite 400, 627 Davis Drive, Morrisville, NC 27560; ²Pelican Therapeutics, 8122 Datapoint Drive, San Antonio, TX 78229



INTRODUCTION

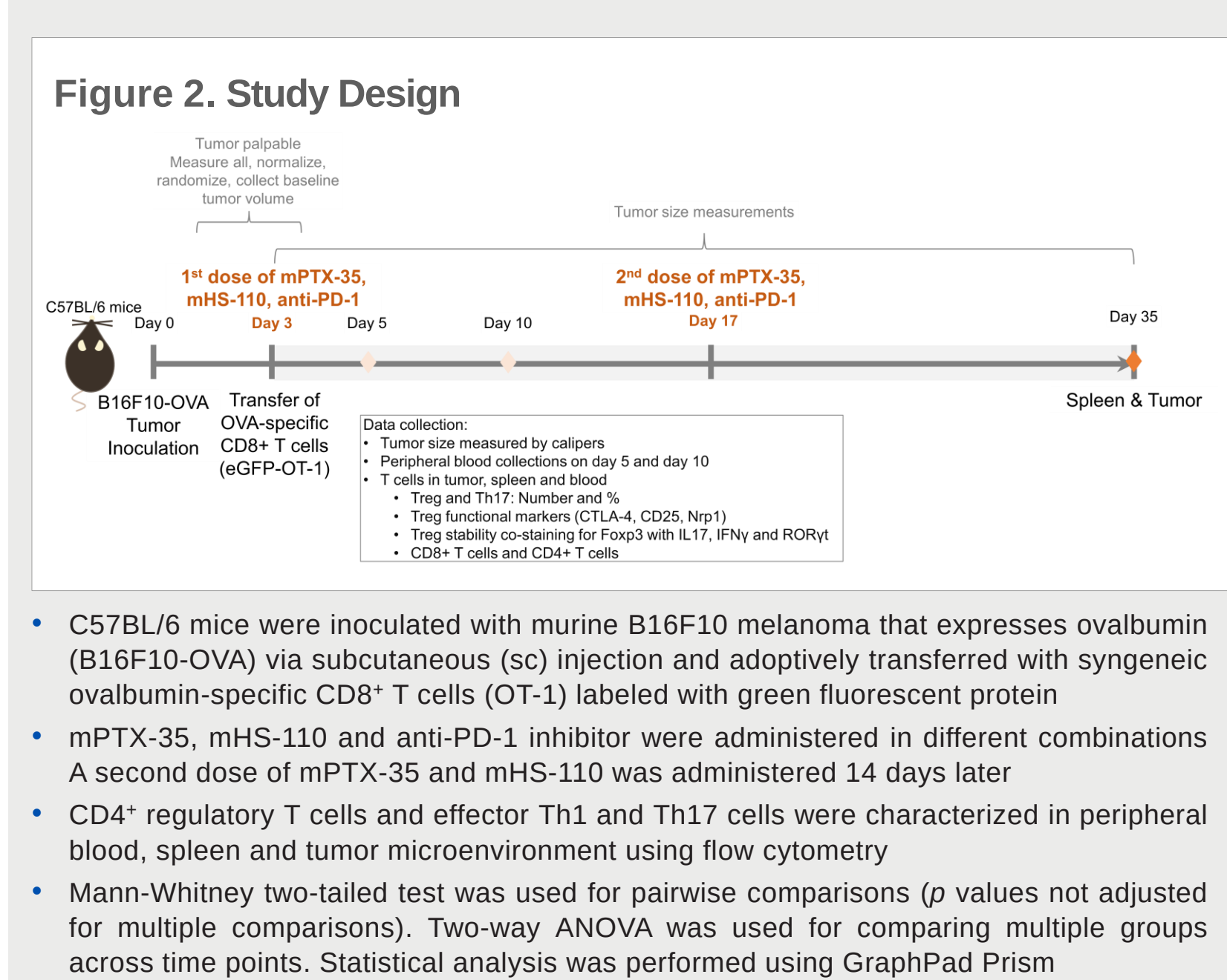
- PTX-35, a potential first-in-class Tumor Necrosis Factor Receptor Super Family 25 (TNFRSF25) agonist antibody, is currently in a Phase 1 clinical trial in patients with solid tumors
- TNFRSF25, also known as Death Receptor 3 (DR3), is a member of the TNF receptor superfamily. Stimulation of TNFRSF25 results in expansion of antigen-experienced CD8⁺ T-cells. TNFRSF25 is preferentially expressed by activated and antigen-experienced T lymphocytes
- Tumor cells evolve multiple mechanisms to escape immune surveillance, one of which is to establish a tolerogenic microenvironment by recruiting regulatory T cells (Tregs)
- This study aimed to characterize the impact of mouse PTX-35 (mPTX-35) on tumor growth, Treg functionality and stability, including the propensity to be converted to effector T cells as well as CD4⁺ Th1, Th17 responses in the tumor microenvironment and in the periphery

METHODS

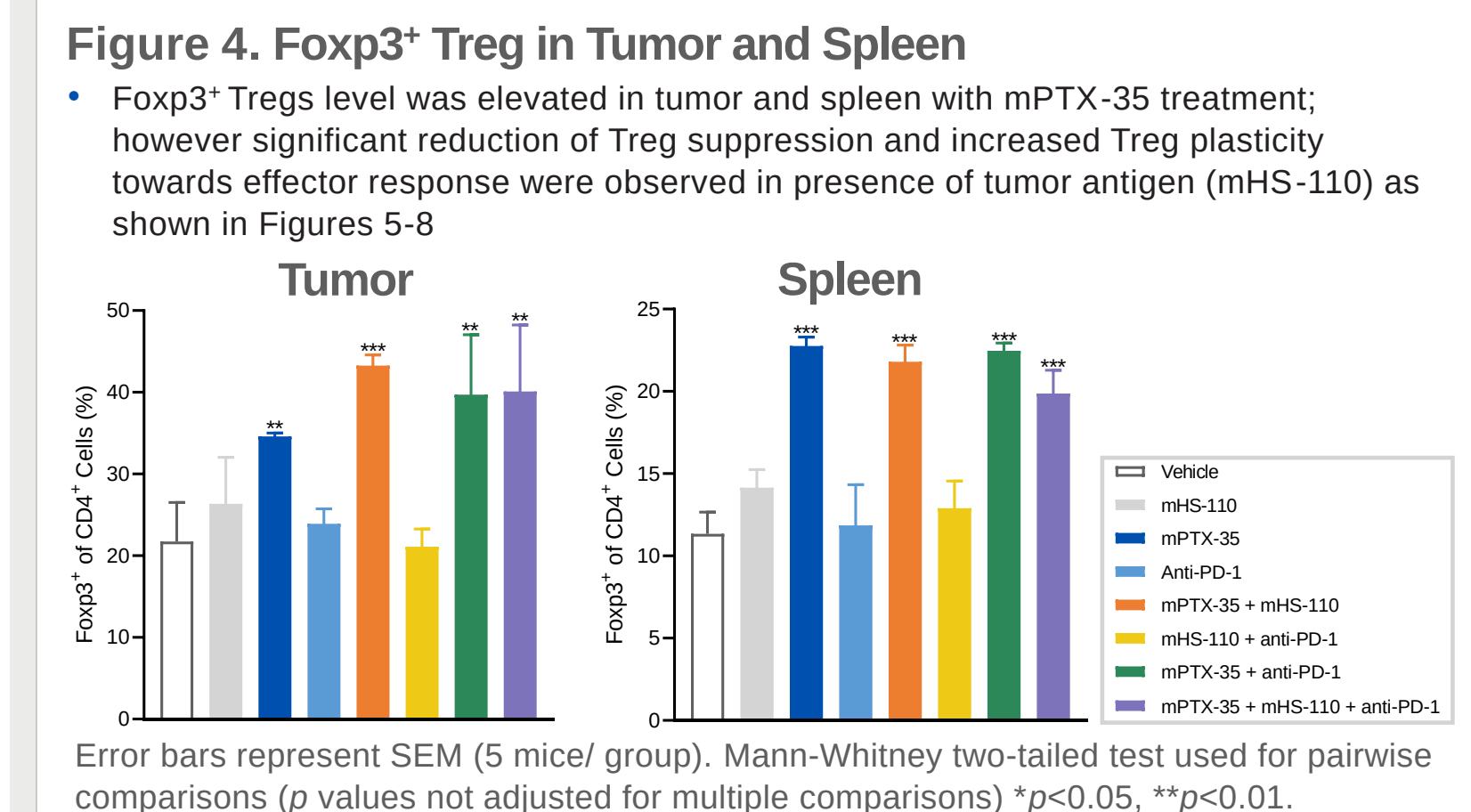
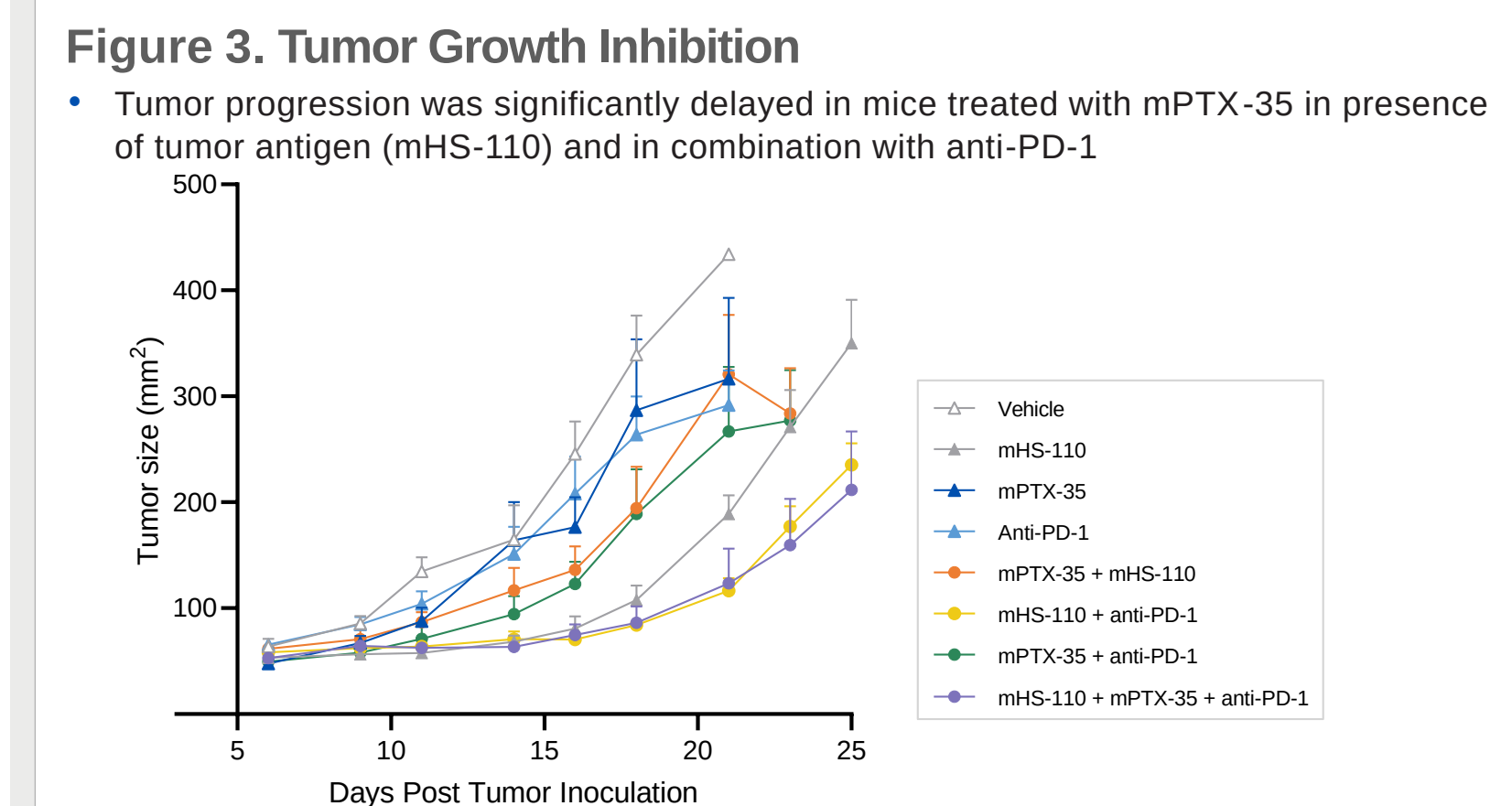


- Activation of TNFRSF25 by PTX-35 is context dependent. Anti-tumor activity has been demonstrated in presence of tumor antigens provided by HS-110. This is independent of whether an anti-PD-1 inhibitor is present
- mHS-110 is a genetically engineered murine B16F10 melanoma cell line that expresses a tumor antigen ovalbumin (OVA) chaperoned by heat shock protein gp96
- Not all Tregs are suppressive
 - Only functional Tregs that express CTLA-4 and CD25 are responsible for the suppressive activity of the Tregs
 - Neuropilin-1 (NRP1) is a co-receptor protein highly expressed on Treg cells that has been involved in cancer immune evasion
- Compromised Treg stability can result in increased Treg plasticity and enhanced effector responses

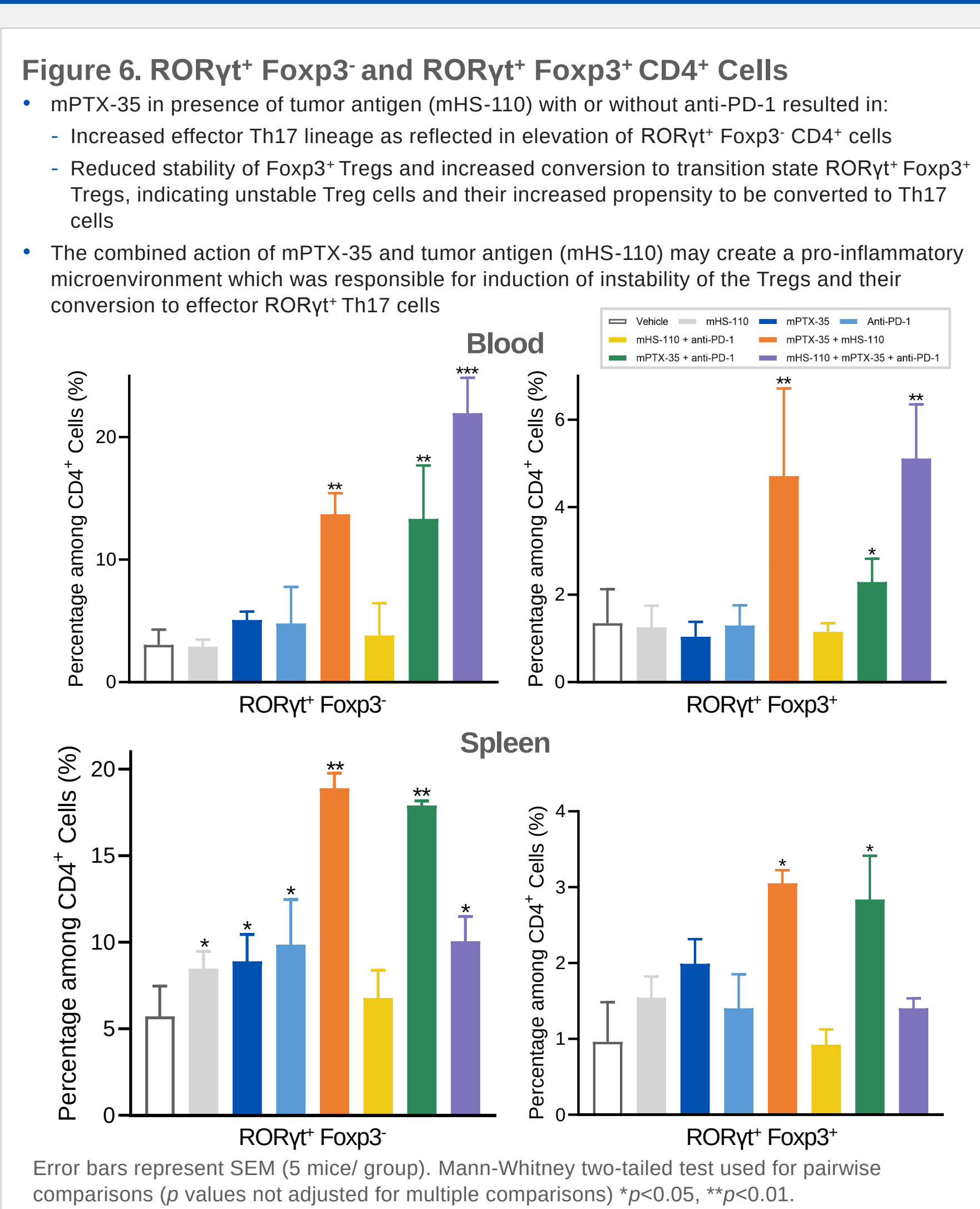
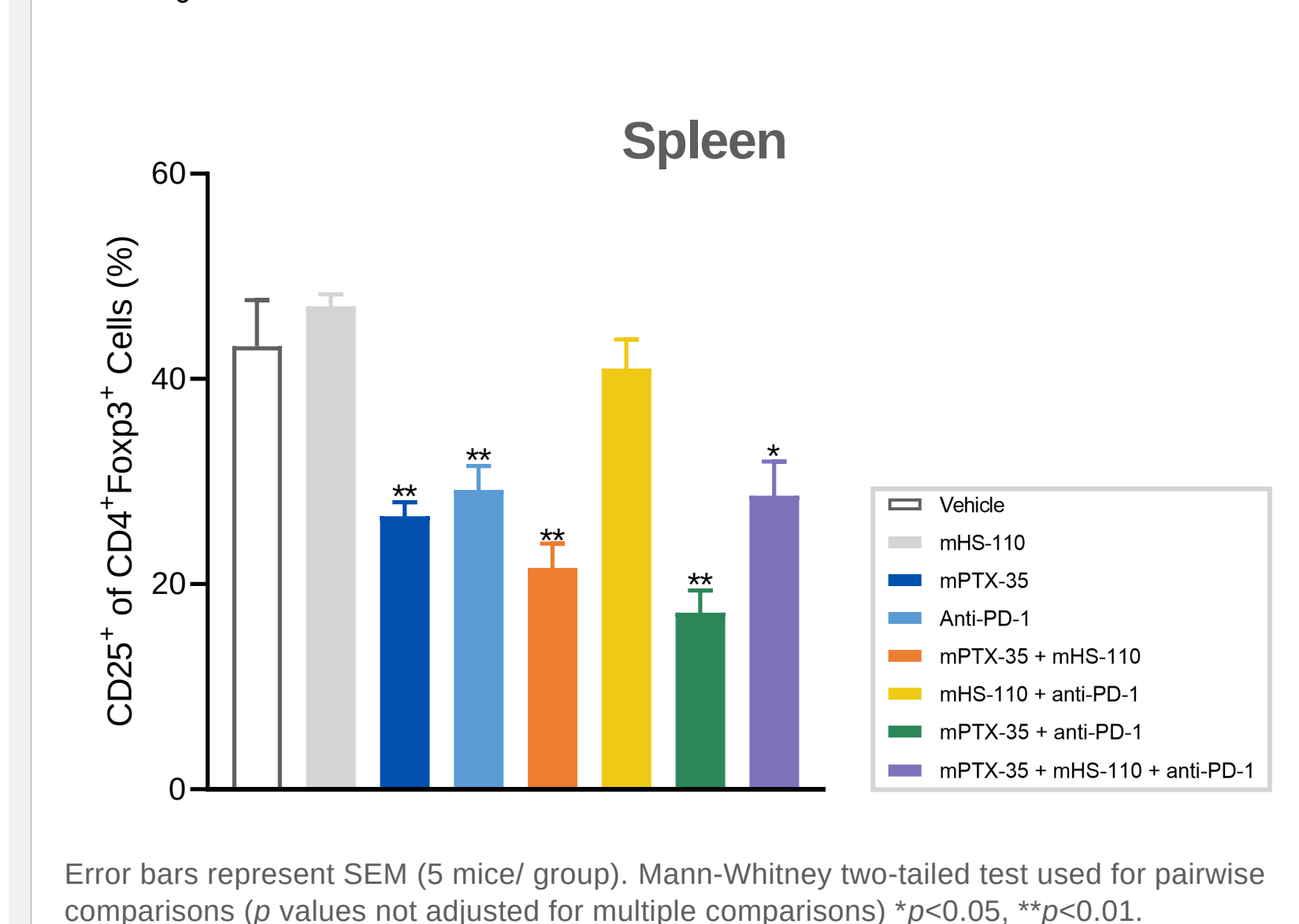
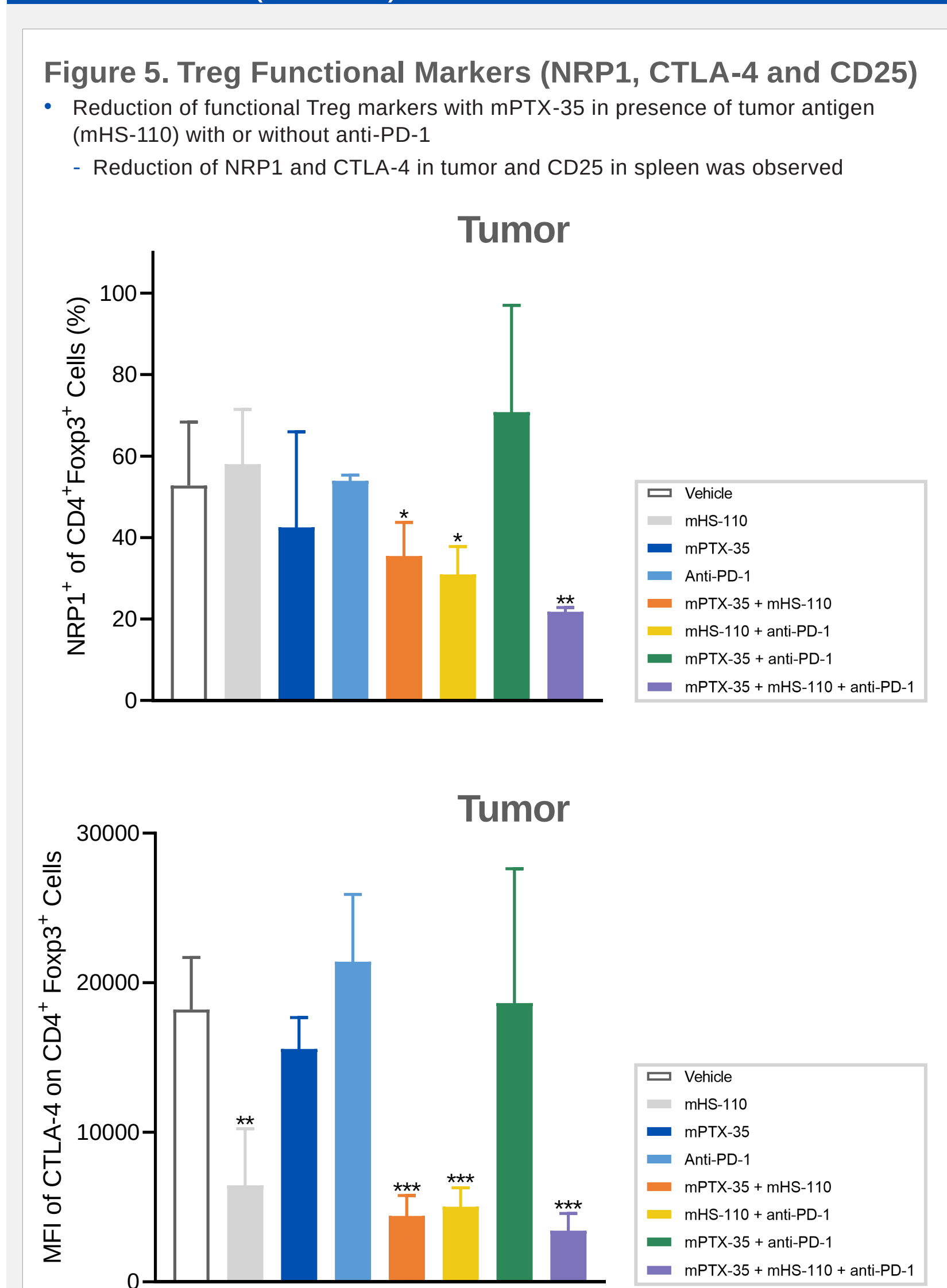
METHODS (cont'd)



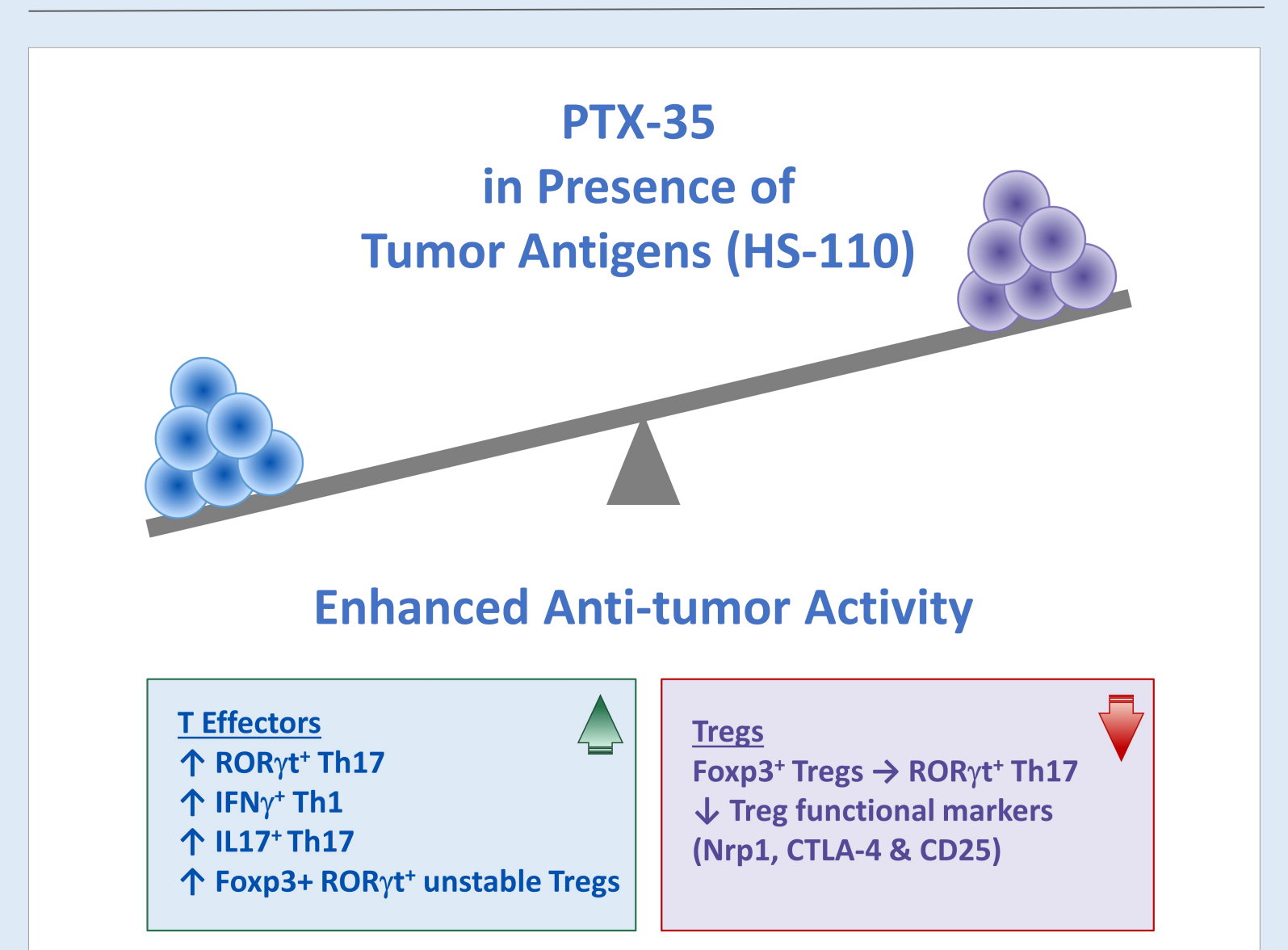
RESULTS



RESULTS (cont'd)



CONCLUSIONS



- In a B16F10 melanoma mouse model, mPTX-35 in presence of tumor antigen (mHS-110) resulted in:
 - ✓ Reduced expression of Treg functional markers (NRP1, CTLA-4 and CD25) which could be responsible for reduced suppressive activity of the Treg cells
 - ✓ Compromised Treg stability including increased Treg plasticity and enhanced CD4⁺ T cell effector Th1 and Th17 responses
 - ✓ These changes in Treg cell phenotype appear to be associated with delayed tumor progression
- Stimulating TNFRSF25 by PTX-35 in presence of tumor antigens, resulted in reduction of Treg suppression and enhancement of T effector response
- PTX-35 is being evaluated in first-in-human Phase 1 study in patients with solid tumors ([NCT04430348](https://clinicaltrials.gov/ct2/show/study/NCT04430348))

REFERENCES

Schreiber et al. Immunol Res. 2013 Dec;57(1-3):3-11
Elkord et al. Expert Opin. Biol. Ther. 2010; 10: 1573-1586
Schreiber et al. J Clin Invest. 2010; 120(10): 3629-3640
Nishikii et al. Blood. 2016 Dec 15;128(24):2846-2858
Lee et al. Current Opinion in Immunology. 2009. Volume 21, 274-280
Chen et al. Front. Immunol. 2019
Hung et al. J Exp. Med. 1998 Dec 21; 188(12): 2357-2368

ACKNOWLEDGMENTS

We appreciate CPRIT for grant (DP160012) that partially funded this work.
*Corresponding Author: Eric Dixon <edixon@heatbio.com>



An electronic version of the poster and any supporting materials can be viewed by scanning the QR code or via [this link](https://doi.org/10.1158/1078-0432.CCR-21-0000). The QR code/link is intended to provide scientific information for individual reference. The PDF should not be altered or reproduced in any way.



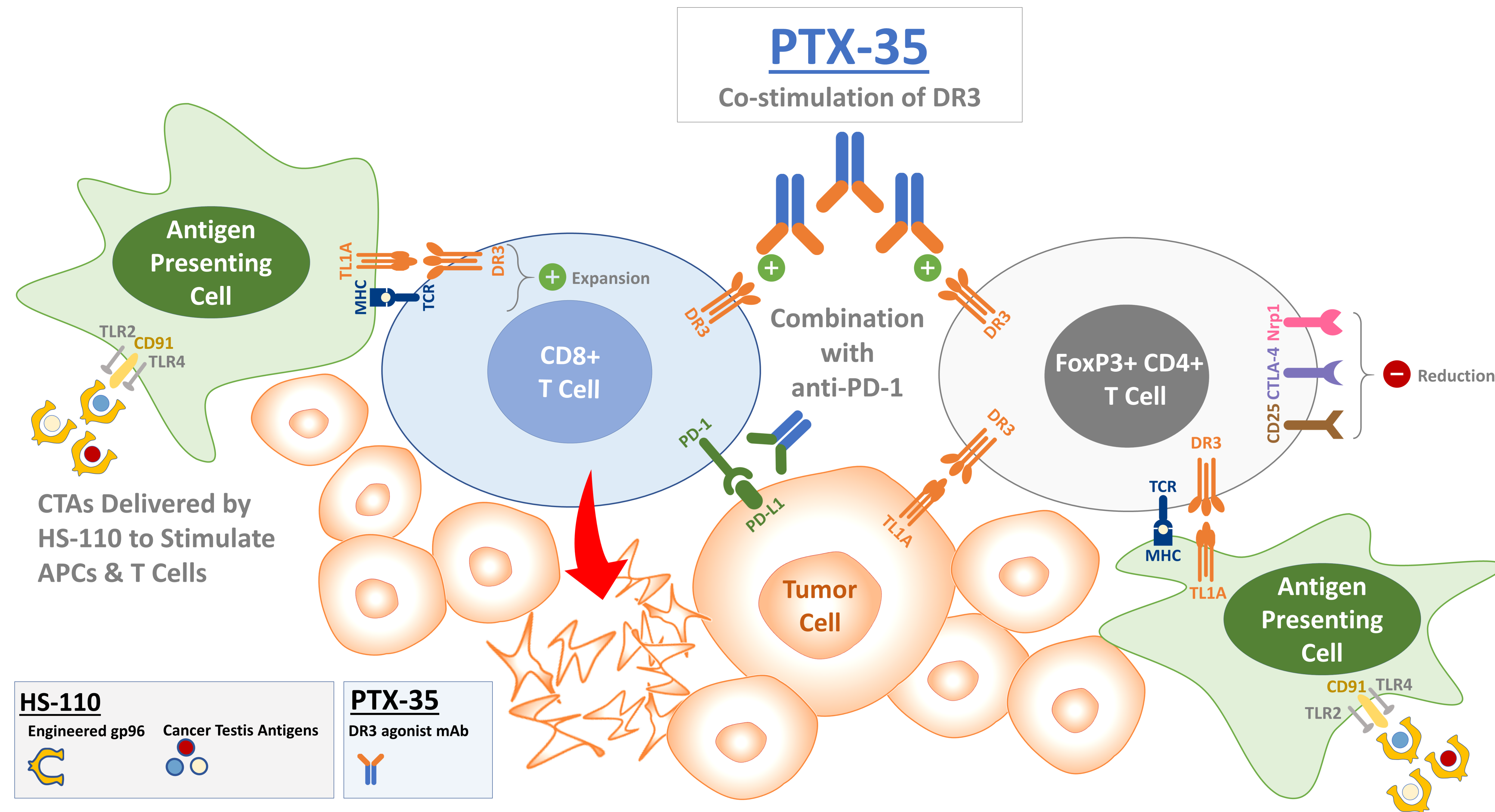
CANCER PREVENTION & RESEARCH
INSTITUTE OF TEXAS

INTRODUCTION

- PTX-35, a potential first-in-class Tumor Necrosis Factor Receptor Super Family 25 (TNFRSF25) agonist antibody, is currently in a Phase 1 clinical trial in patients with solid tumors
- TNFRSF25, also known as Death Receptor 3 (DR3), is a member of the TNF receptor superfamily. Stimulation of TNFRSF25 results in expansion of antigen-experienced CD8⁺ T-cells. TNFRSF25 is preferentially expressed by activated and antigen-experienced T lymphocytes
- Tumor cells evolve multiple mechanisms to escape immune surveillance, one of which is to establish a tolerogenic microenvironment by recruiting regulatory T cells (Tregs)
- This study aimed to characterize the impact of mouse PTX-35 (mPTX-35) on tumor growth, Treg functionality and stability, including the propensity to be converted to effector T cells as well as CD4⁺ Th1, Th17 responses in the tumor microenvironment and in the periphery

METHODS

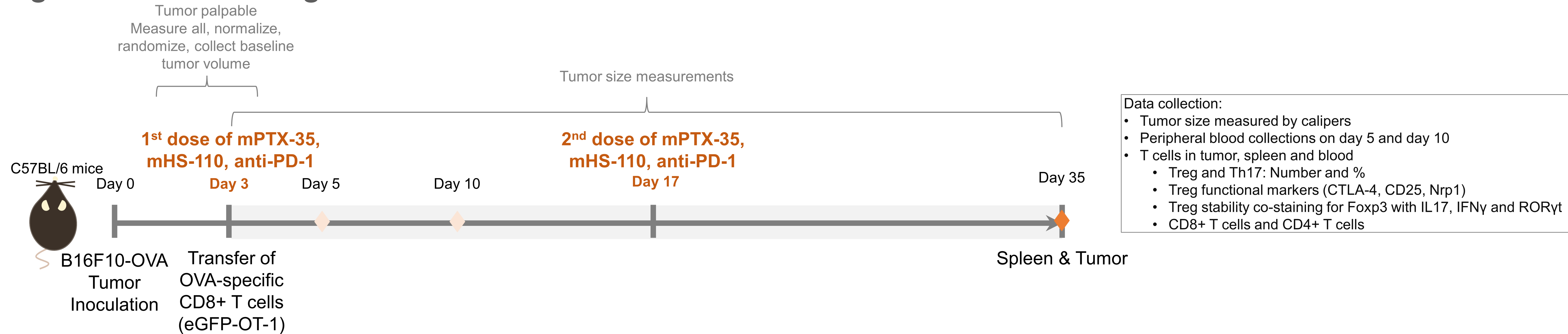
Figure 1.



- Activation of TNFRSF25 by PTX-35 is context dependent. Anti-tumor activity has been demonstrated in presence of tumor antigens provided by HS-110. This is independent of whether an anti-PD-1 inhibitor is present
- mHS-110 is a genetically engineered murine B16F10 melanoma cell line that expresses a tumor antigen ovalbumin (OVA) chaperoned by heat shock protein gp96
- Not all Tregs are suppressive
 - Only functional Tregs that express CTLA-4 and CD25 are responsible for the suppressive activity of the Tregs
 - Neuropilin-1 (NRP1) is a co-receptor protein highly expressed on Treg cells that has been involved in cancer immune evasion
- Compromised Treg stability can result in increased Treg plasticity and enhanced effector responses

METHODS (cont'd)

Figure 2. Study Design

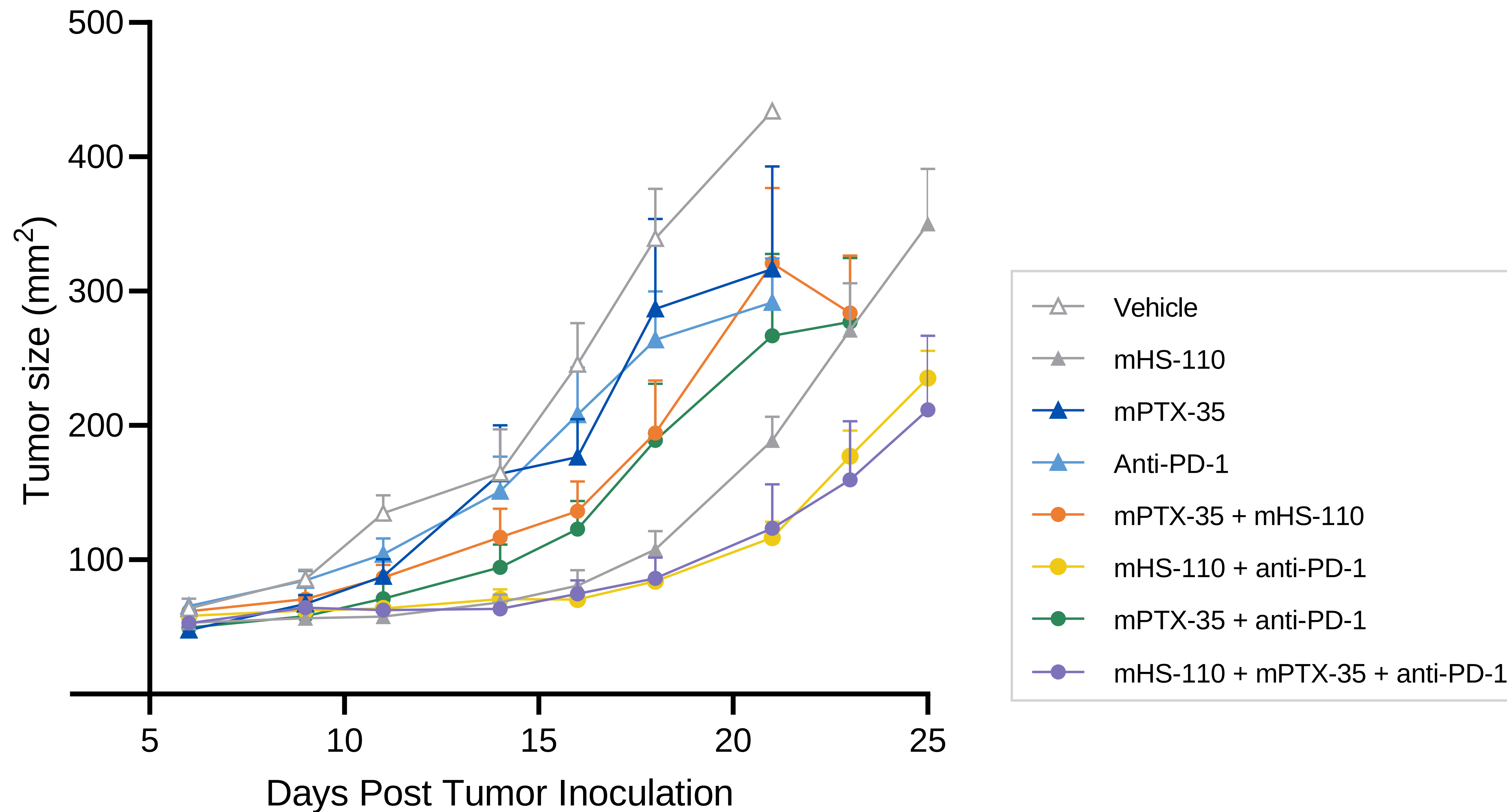


- C57BL/6 mice were inoculated with murine B16F10 melanoma that expresses ovalbumin (B16F10-OVA) via subcutaneous (sc) injection and adoptively transferred with syngeneic ovalbumin-specific CD8+ T cells (OT-1) labeled with green fluorescent protein
- mPTX-35, mHS-110 and anti-PD-1 inhibitor were administered in different combinations A second dose of mPTX-35 and mHS-110 was administered 14 days later
- CD4+ regulatory T cells and effector Th1 and Th17 cells were characterized in peripheral blood, spleen and tumor microenvironment using flow cytometry
- Mann-Whitney two-tailed test was used for pairwise comparisons (p values not adjusted for multiple comparisons). Two-way ANOVA was used for comparing multiple groups across time points. Statistical analysis was performed using GraphPad Prism

RESULTS

Figure 3. Tumor Growth Inhibition

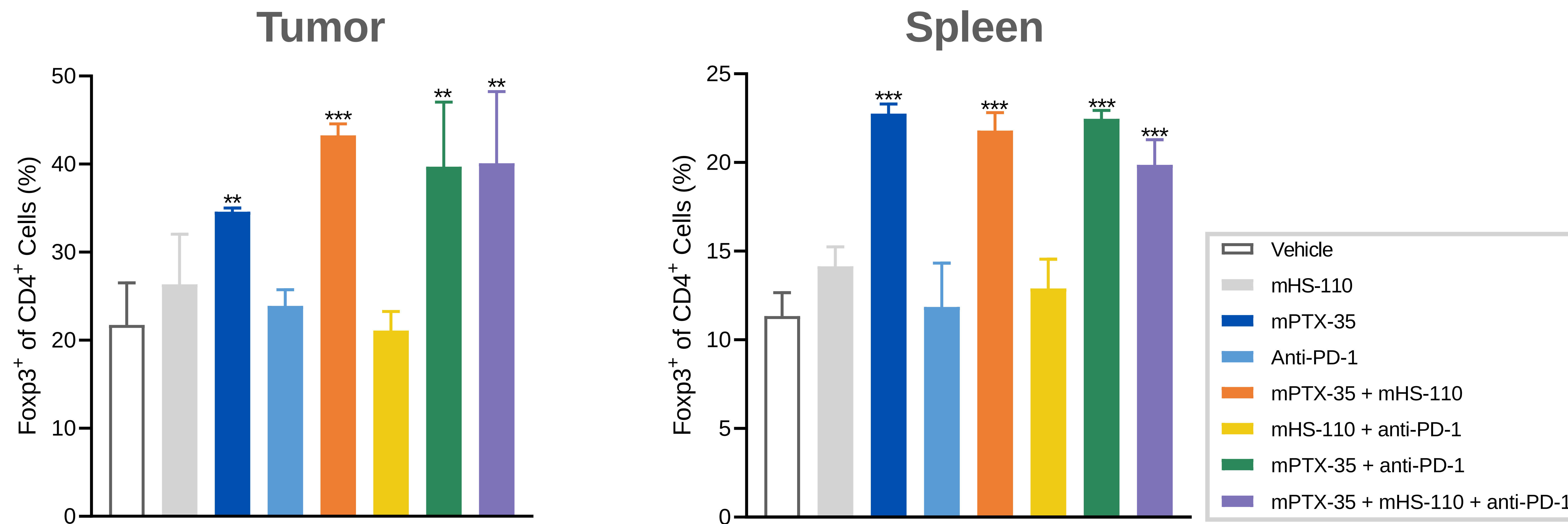
- Tumor progression was significantly delayed in mice treated with mPTX-35 in presence of tumor antigen (mHS-110) and in combination with anti-PD-1



RESULTS

Figure 4. Foxp3⁺ Treg in Tumor and Spleen

- Foxp3⁺ Tregs level was elevated in tumor and spleen with mPTX-35 treatment
- However **significant reduction of Treg suppression and increased Treg plasticity towards effector response were observed in presence of tumor antigen (mHS-110) as shown in Figures 5-8**

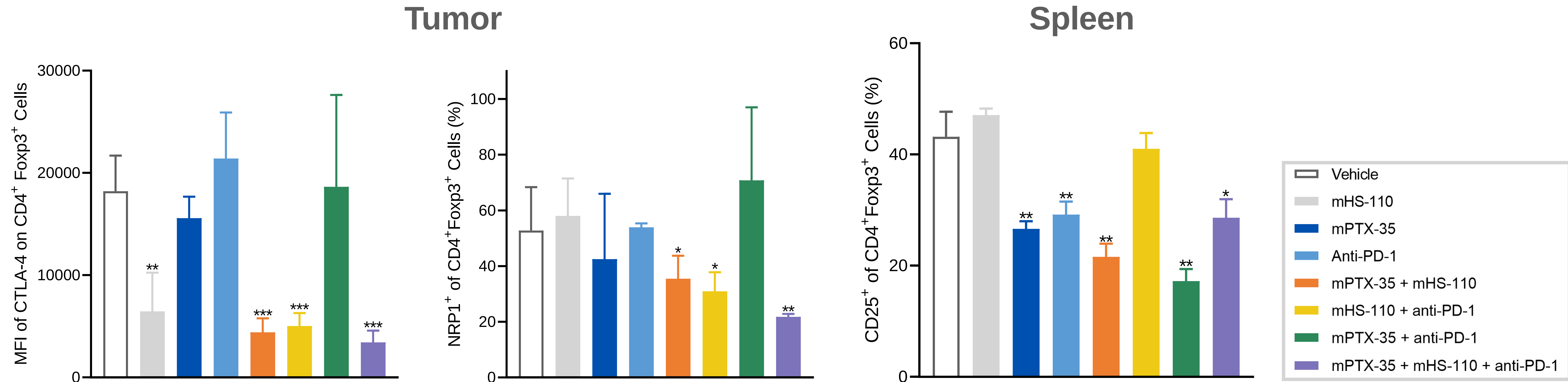


Error bars represent SEM (5 mice/ group). Mann-Whitney two-tailed test used for pairwise comparisons (*p* values not adjusted for multiple comparisons) **p*<0.05, ***p*<0.01.

RESULTS

Figure 5. Treg Functional Markers (NRP1, CTLA-4 and CD25)

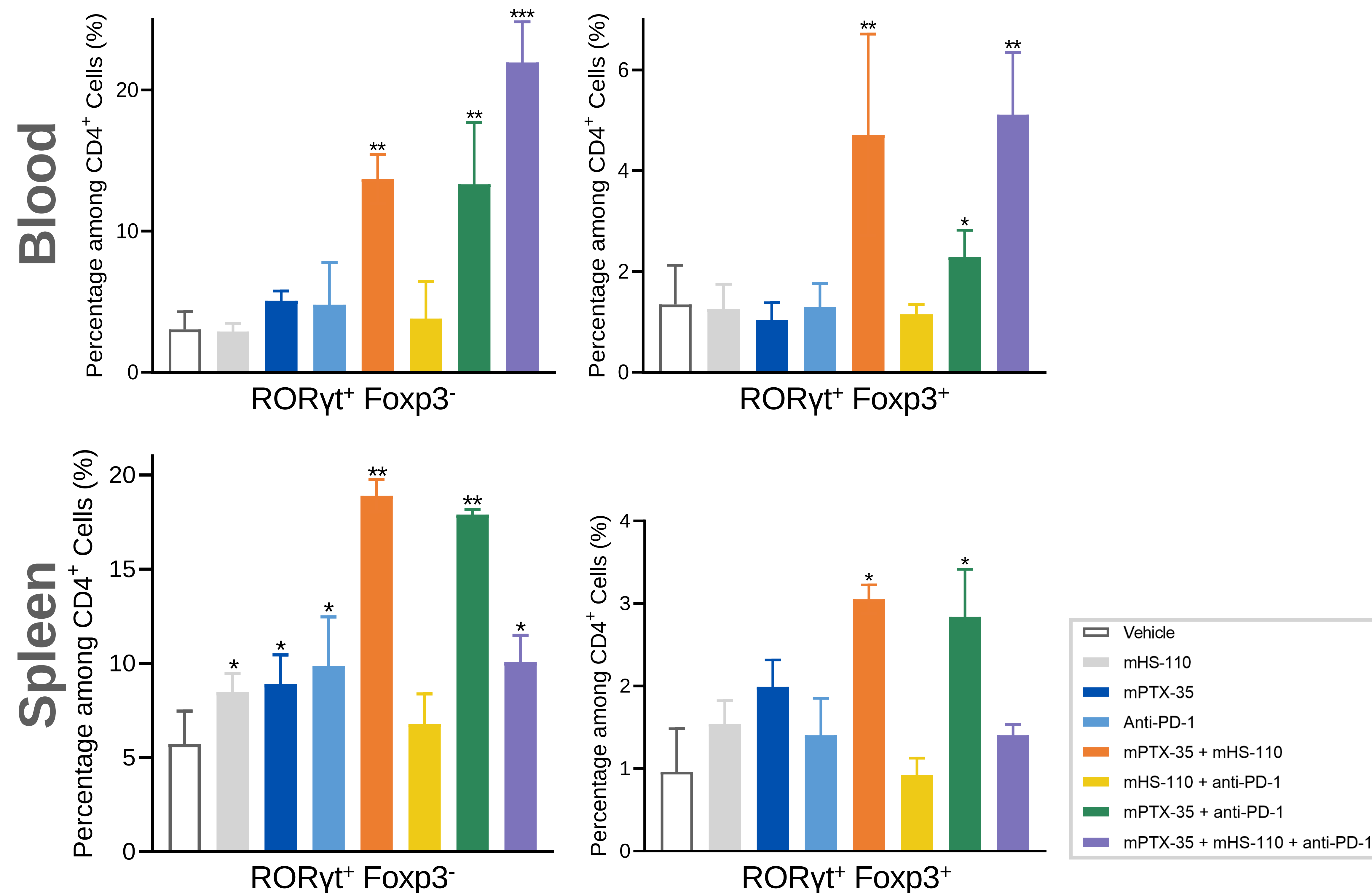
- Reduction of functional Treg markers with mPTX-35 in presence of tumor antigen (mHS-110) with or without anti-PD-1
 - Reduction of NRP1 and CTLA-4 in tumor
 - Reduction of CD25 in spleen



Error bars represent SEM (5 mice/ group). Mann-Whitney two-tailed test used for pairwise comparisons (p values not adjusted for multiple comparisons) * $p < 0.05$, ** $p < 0.01$.

RESULTS

Figure 6. RORyt⁺ Foxp3⁻ and RORyt⁺ Foxp3⁺ CD4⁺ Cells



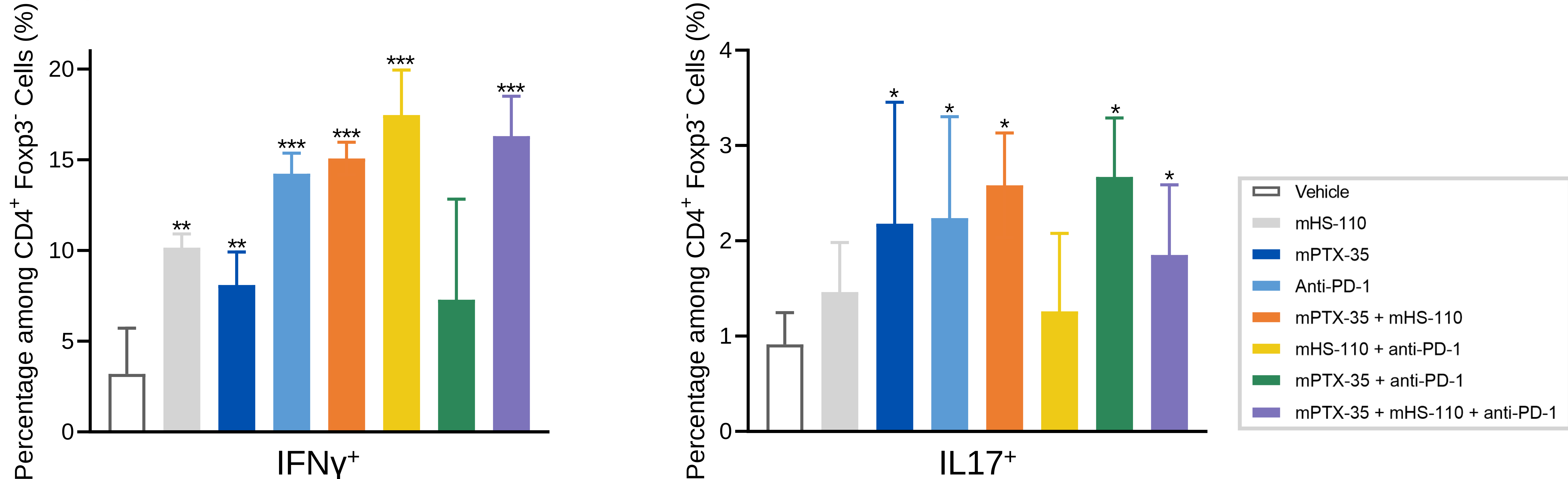
Error bars represent SEM (5 mice/ group). Mann-Whitney two-tailed test used for pairwise comparisons (*p* values not adjusted for multiple comparisons) **p*<0.05, ***p*<0.01.

- mPTX-35 in presence of tumor antigen (mHS-110) with or without anti-PD-1 resulted in:
 - Increased effector Th17 lineage as reflected in elevation of RORyt⁺ Foxp3⁻ CD4⁺ cells
 - Reduced stability of Foxp3⁺ Tregs and increased conversion to transition state RORyt⁺ Foxp3⁺ Tregs, indicating unstable Treg cells and their increased propensity to be converted to Th17 cells
- The combined action of mPTX-35 and tumor antigen (mHS-110) may create a pro-inflammatory microenvironment which was responsible for induction of instability of the Tregs and their conversion to effector RORyt⁺ Th17 cells

RESULTS

Figure 7. IFN γ ⁺ Th1 and IL17⁺ Th17 CD4⁺ Foxp3⁻ Cells in Tumor

- Effector CD4⁺ T cells comprised of IFN γ producing Th1 cells and IL17 producing Th17 cells play an important role in antitumor immunity
- Increased IFN γ ⁺ Th1 cells and IL17⁺ Th17 cells were observed with mPTX-35 treatment with or without tumor antigen (mHS-110)

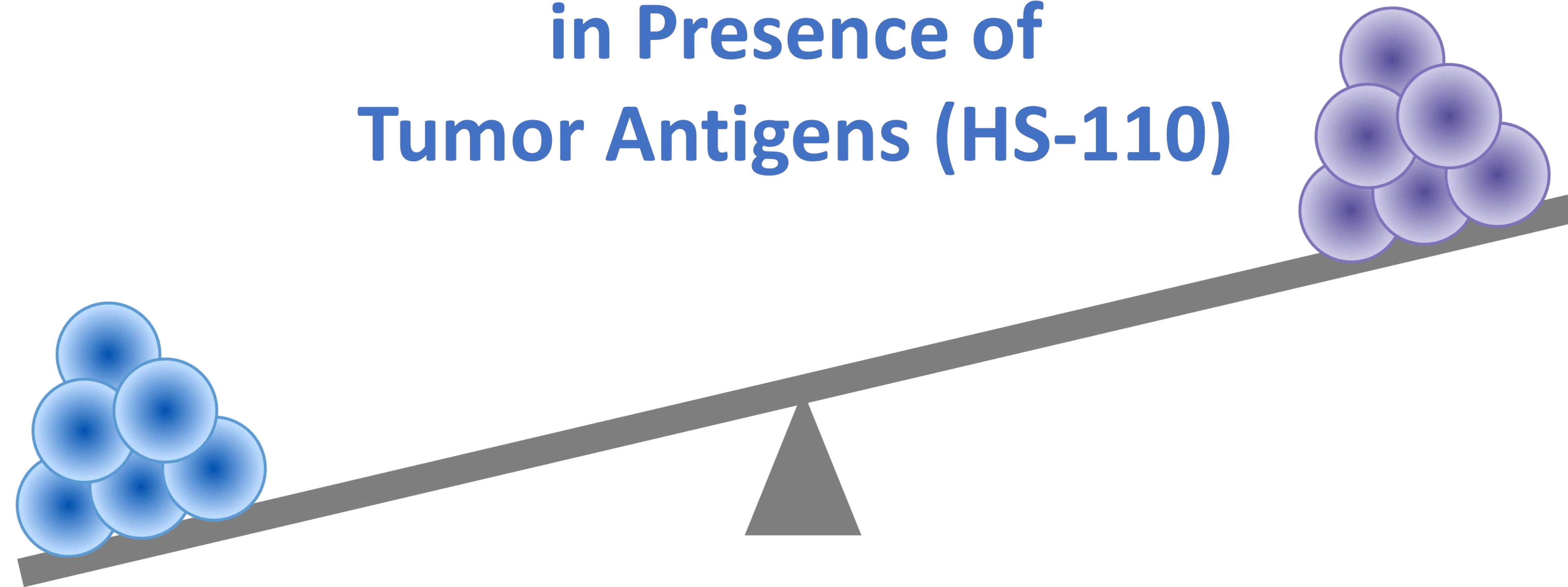


Error bars represent SEM (5 mice/ group). Mann-Whitney two-tailed test used for pairwise comparisons (*p* values not adjusted for multiple comparisons) **p*<0.05, ***p*<0.01.

Poster Presentation at the AACR Annual Meeting 2021 (April - May, 2021, Virtual)

CONCLUSION

PTX-35 in Presence of Tumor Antigens (HS-110)



Enhanced Anti-tumor Activity

T Effectors

↑ ROR γ t⁺ Th17
↑ IFN γ ⁺ Th1
↑ IL17⁺ Th17
↑ Foxp3⁺ ROR γ t⁺ unstable Tregs



Tregs

Foxp3⁺ Tregs → ROR γ t⁺ Th17
↓ Treg functional markers
(Nrp1, CTLA-4 & CD25)



- In a B16F10 melanoma mouse model, mPTX-35 in presence of tumor antigen (mHS-110) resulted in:
 - ✓ Reduced expression of Treg functional markers (NRP1, CTLA-4 and CD25) which could be responsible for reduced suppressive activity of the Treg cells
 - ✓ Compromised Treg stability including increased Treg plasticity and enhanced CD4⁺ T cell effector Th1 and Th17 responses
 - ✓ These changes in Treg cell phenotype appear to be associated with delayed tumor progression
- Stimulating TNFRSF25 by PTX-35 in presence of tumor antigens, resulted in reduction of Treg suppression and enhancement of T effector response
- PTX-35 is being evaluated in first-in-human Phase 1 study in patients with solid tumors ([NCT04430348](https://clinicaltrials.gov/ct2/show/study/NCT04430348))