THE UNIVERSITY OF TEXAS MD Anderson Cancer Center Children's Cancer Hospital

Rapid Personalized Manufacture (RPM) of *Sleeping Beauty* System-generated NY-ESO-1-specific TCR-T Cells Co-expressing Membrane-bound IL-15 Yields Antitumor Responses

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BACKGROUND

Redirecting T-cell Specificity Toward Solid Tumor Antigens

- Chimeric antigen receptor (CAR) redirected T-cells have demonstrated clinical effectiveness and notable successes with certain hematological malignancies
  - Ziopharm with MD Anderson has cleared IND to infuse CD19-specific CAR-T the day after gene transfer using a process termed "rapid personalized manufacture" (RPM, NCT03579888)
- CAR T-cell targets are limited to a few homogeneous cell-surface antigens which exposes a critical need for specificity to intracellular tumor-associated antigens (TAA)
  - Enables access to a more expansive TAA repertoire, as intracellular proteins comprise the majority of the proteome, to target a greater diversity of solid cancers (and myelomas) with improved therapeutic potential
- Targeting intracellular proteins is a capability exclusive to T-cell receptors (TCRs), either endogenous or introduced, that recognize processed peptides from intracellular proteins presented in the context of an individual's major histocompatibility complex (MHC) molecule
- TCR-based cancer immunotherapy is now an emerging approach to targeting solid cancers but still has several hurdles to overcome to be commercialized

Scale-up and Costs of TCR T-cell Therapy

- T cells genetically modified with virus require (i) recombinant retrovirus/lentivirus and (ii) *ex vivo* replication/propagation
- Current manufacture protocols add complexity of cost and time to produce patient-derived products
- The *Sleeping Beauty* (SB) non-viral gene transfer system circumvents these cell product manufacture challenges
  - Successfully tested in humans to express a CD19-specific CAR (Kebriaei *et al.* JCI. 2016, PMID: 27482888)
  - Quiescent T cells can be stably genetically modified using SB system DNA plasmids
  - Eliminates the need to propagate cells in tissue culture

Improving TCR T-cells with Cytokine Co-signaling

- Interleukin 15 (IL-15)
  - Homeostatic cytokine that supports long-lived memory T cells
  - A component within the inflammatory milieu of the tumor microenvironment that promotes antitumor responses
  - Expression is correlated with remission in lymphoma, T cell infiltration in tumors, and enhances *in vivo* antitumor activity
- Co-expression of a membrane-bound IL-15/IL-15Rα fusion molecule (mbIL15) significantly enhances *in vivo* persistence and antitumor activity of CAR⁺ T cells (Hurton *et al.* PNAS. 2016, PMID: 27849617)

Two-day Generation of Genetically-modified T Cells with Rapid Personalized Manufacture (RPM)

- Current manufacture is based on gene transfer in T cells that undergo requisite *ex vivo* activation and propagation to integrate transgenes from virus to yield clinical numbers of T cells
- Alternatively, T cells can be electroporated with DNA plasmids from SB system to co-express transgenes of interest whereby TCR-T cells are rapidly manufactured by RPM and ready for adoptive transfer without *ex vivo* activation and propagation using (**Figure 1**).

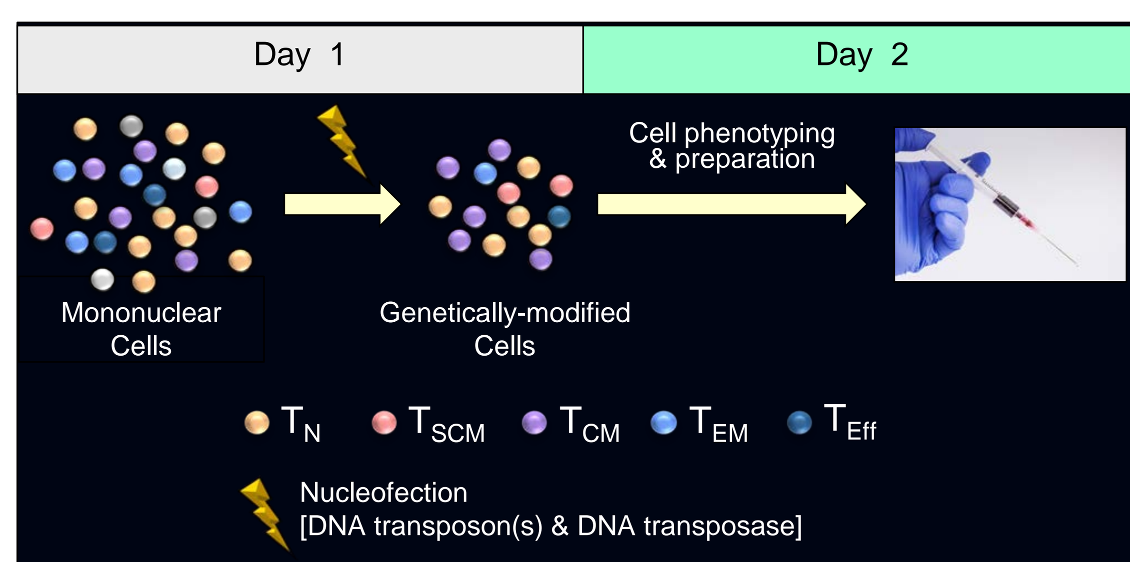

Fig. 1 Very rapid manufacture of T cells under RPM. The RPM approach can produce genetically-modified T cells in less than 2 days from gene transfer. This manufacturing process does not rely on activating and propagating T cells prior to electroporation. Thus, the T cells can be "simply" infused. T_N: naïve, T_{SCM}: stem cell memory, T_{CM}: central memory, T_{EM}: effector memory, and T_{Eff}: effector T cells.

Two-day Generation of Genetically-modified T Cells with RPM (*cont.*)

- Electroporated T cells manufactured under RPM are infused less than two days after gene transfer
  - SB-derived transposition results in stable integration
  - mbIL15 promotes T-cell survival *in vivo*
- Less manipulation of the product may preserve less differentiated T-cell subsets, thus potentially improving potency
- **We hypothesized that with the co-expression of mbIL15: (i) therapeutically effective TCR⁺ T cells could be generated and very rapidly infused without being activated and propagated *ex vivo* and (ii) there would be enhanced persistence *in vivo* of modified-T cells with a desirable memory phenotype**

METHODS

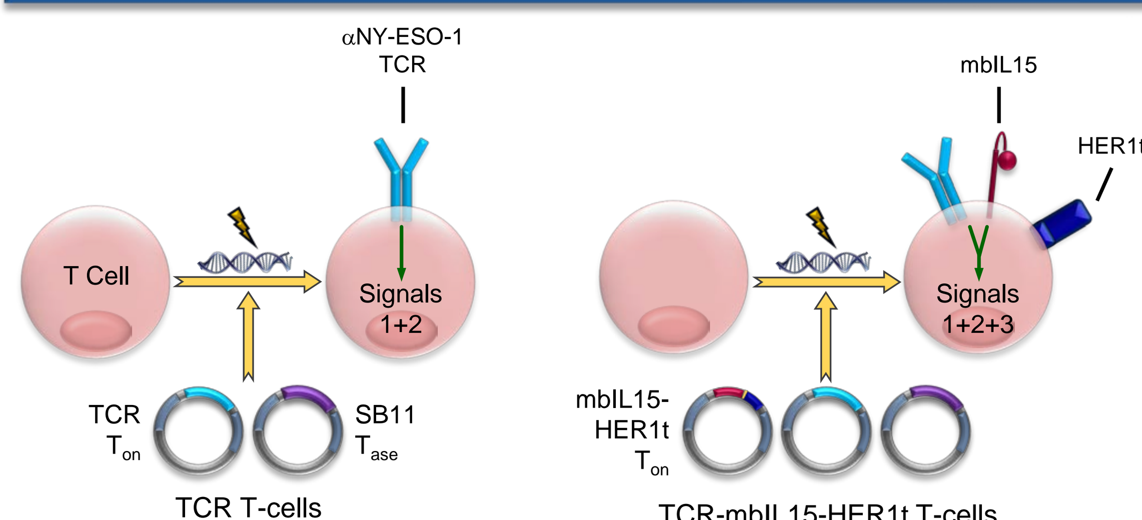

Fig. 2 Generation of T cells under RPM and by *ex vivo* propagation that express TCR (*left*) and TCR with mbIL15 and HER1t (*right*). Schematic of SB modification of T cells to generate TCR and TCR-mbIL15-HER1t T-cells. Mononuclear cells underwent nucleofection (electroporation), with SB11 transposase coded from DNA plasmid and: (i) NY-ESO-1-specific TCR or (ii) NY-ESO-1-specific TCR and mbIL15-HER1t (together in a bicistronic vector) coded from two separate SB DNA transposon plasmids. TCR engagement provides T-cell activation and co-stimulation (signals 1 & 2) while mbIL15 provides a 3rd stimulatory signal to enhance survival and initiate full activation of naïve T cells. Truncated HER1t safety switch, devoid of signaling capacity, enables selective depletion of mbIL15⁺ T cells. Electroporated T cells were placed overnight in culture (with no exogenous cytokines) prior to injection into mice for the RPM process. For the *ex vivo* propagated cells, the electroporated cells were recursively stimulated twice using K562-derived activating and propagating cells (AaPC) with cytokine supplementation.

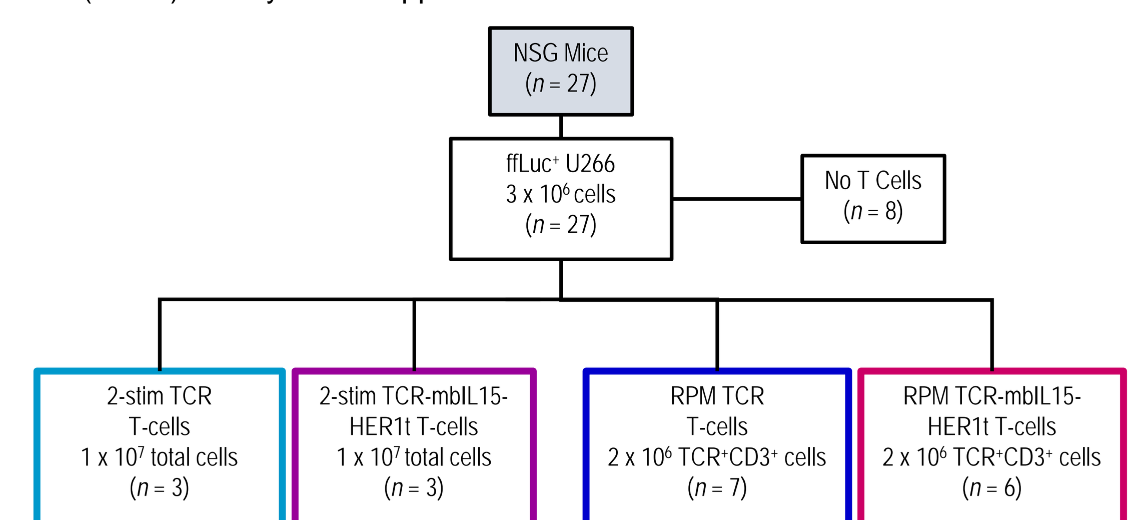

Fig. 3 Mouse model of established and disseminated multiple myeloma. On day 0, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice were intravenously (i.v.) injected with 3x10⁵ HLA A2⁺ NY-ESO-1⁺ U266 myeloma cells co-expressing firefly luciferase (ffLuc). Five days later, tumor bioburden was assessed via bioluminescence imaging (BLI) and mice were stratified into treatment groups. On day 6, a single i.v. injection of T-cells was administered for T-cell treatment groups: (i) 10⁷ total viable 2-stim αNY-ESO-1 TCR T-cells, (ii) 10⁷ total viable 2-stim αNY-ESO-1 TCR-mbIL15-HER1t T-cells, (iii) 2x10⁶ viable TCR⁺ RPM αNY-ESO-1 TCR T-cells, and (iv) 2x10⁶ viable TCR⁺ RPM αNY-ESO-1 TCR-mbIL15-HER1t T-cells. To calculate T-cell dosing, TCR cell surface expression was measured the day after electroporation, which is a sum of integrated and episomal expression. Tumor burden was serially monitored by BLI and mice were euthanized when moribund or at study end (day 66).

RESULTS

Ex Vivo Propagated TCR-T Cell Product Phenotype

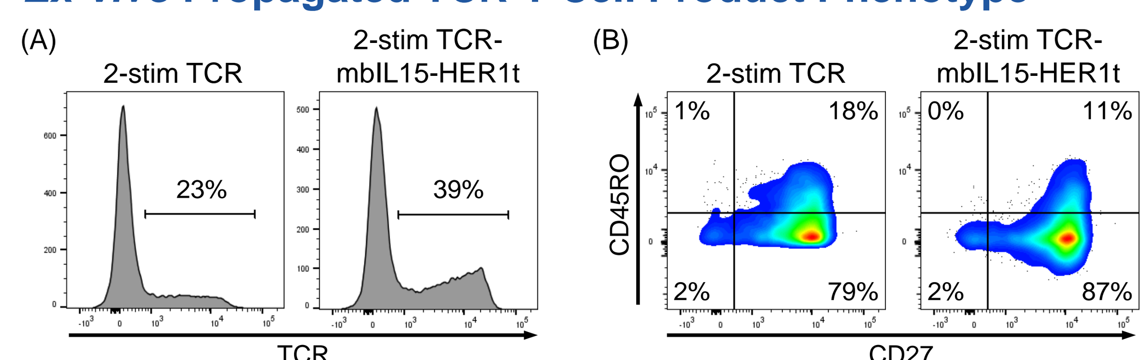

Fig. 4 Propagated TCR-T cells show stable transgene expression and maintain a less-differentiated phenotype. Recursively stimulated TCR-T cells on treatment day were assessed for (**A**) TCR expression of gated CD3⁺ cells (integrated expression), as well as (**B**) CD45RO and CD27 memory-associated markers, with gating on CD3⁺TCR⁺ cells.

Propagated TCR-mbIL15-HER1t T Cells Yield Potent Antitumor Activity

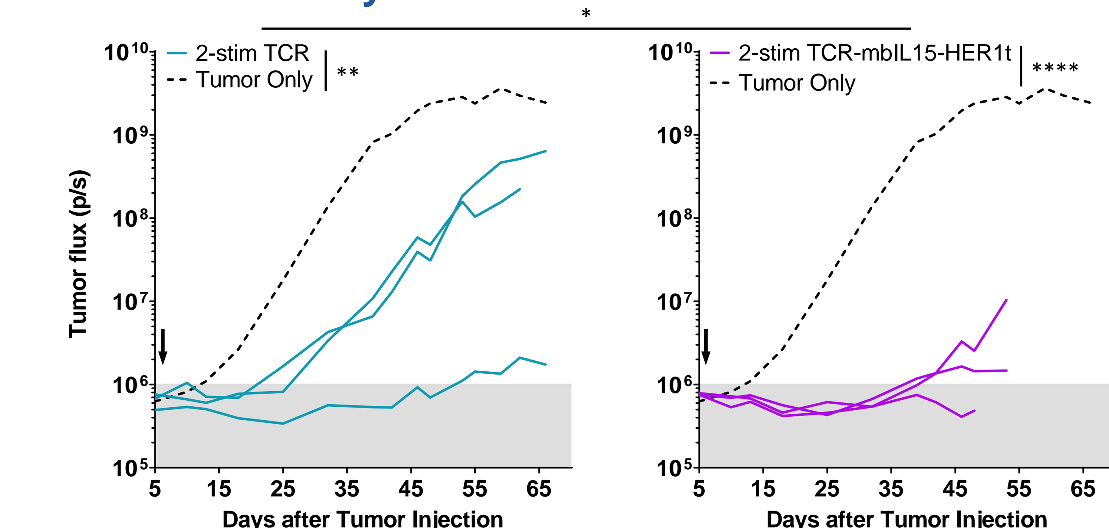

Fig. 5 Propagated TCR-mbIL15-HER1t T cells exhibit efficient tumor control and delay tumor outgrowth. Quantified tumor burden (ffLuc activity) was measured by BLI. Each colored line represents an individual animal. Dotted black line represents mean tumor flux for treatment control group. Arrows indicate day of modified T-cell injection. Shaded grey area represents twice the limit of detection (LOD), where LOD is based on BLI of mice without tumor but administered luciferin, and mice euthanized in this range were considered disease-free. Significance determined by one-way ANOVA (Tukey's post test) on log-transformed end-point tumor flux values. *$P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

Propagated TCR-T Cell Persistence & Memory Composition

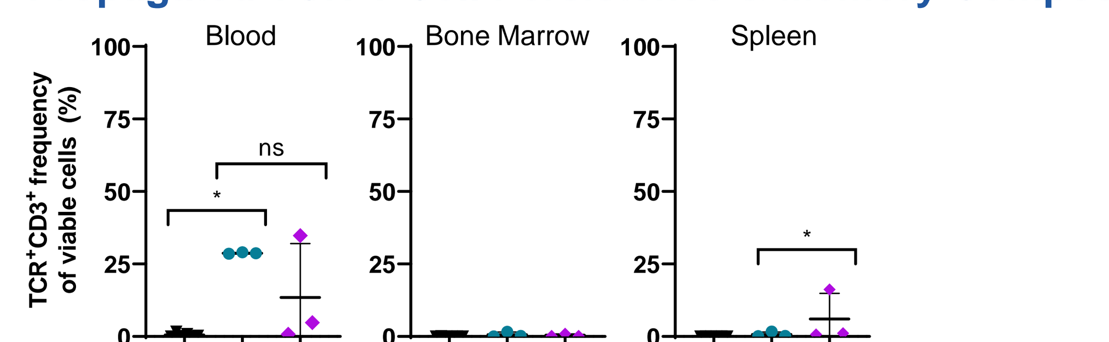

Fig. 6 Modest engraftment of 2-stim TCR ± mbIL15 T cells persisting with desirable memory phenotype at treatment endpoint. Peripheral blood and tissues of moribund mice were analyzed to assess persistence of genetically modified T cells (*top*), and frequencies of memory subsets delineated by CD45RO and CD27 expression (*bottom*). Memory subset data is gated on TCR⁺CD3⁺ cells. *$P < 0.05$, ns = not significant, Kruskal-Wallis and T tests, respectively.

RPM T-cell Infusion Product Phenotype

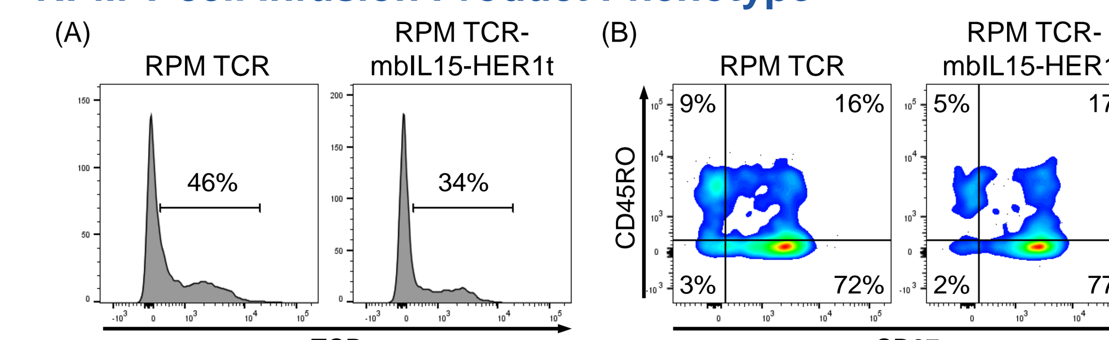

Fig. 7 RPM TCR-T cells show transgene expression and maintain a less differentiated phenotype at 18 hours after genetic modification. RPM TCR-T cells on treatment day were assessed for (**A**) TCR expression of gated CD3⁺ cells (a sum of integrated and episomal expression), as well as (**B**) CD45RO and CD27 memory-associated markers, with gating on CD3⁺TCR⁺ cells.

A Single Low-dose of Unpropagated RPM TCR-mbIL15-HER1t T-cells Demonstrate Potent Tumor Rejection

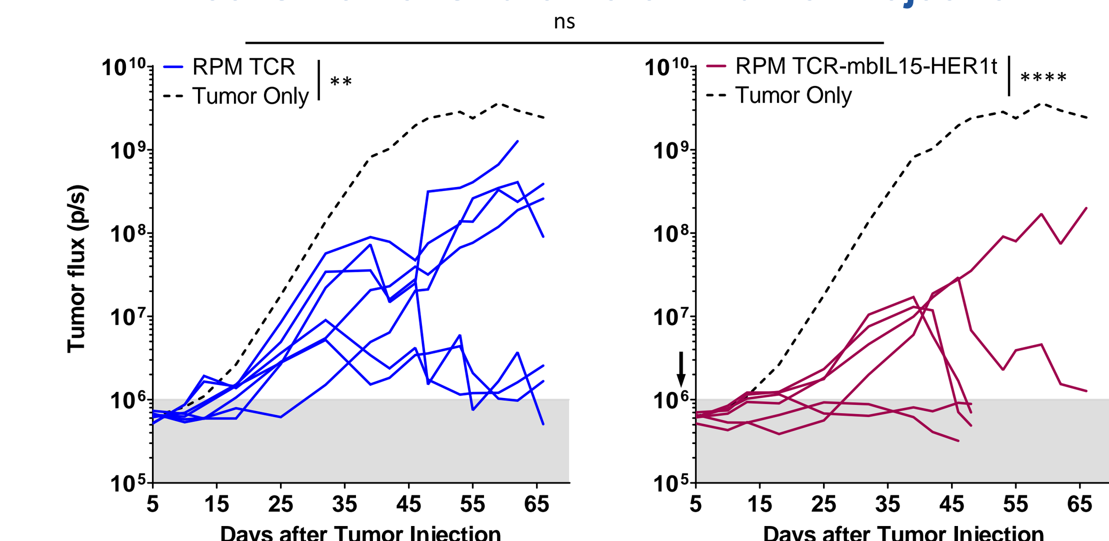

Fig. 8 RPM TCR-mbIL15-HER1t T cells exhibit delayed tumor control but reject tumor. Quantified tumor burden (ffLuc activity) was measured by BLI. Each colored line represents an individual animal. Dotted black line represents mean tumor flux for treatment control group. Arrows indicate the day genetically modified cells were injected. Shaded grey area represents twice the LOD, where LOD is based on BLI of mice without tumor but administered luciferin, and mice euthanized in this range were considered disease-free. Mice reaching <2X LOD flux became moribund due to onset of xenogenic graft-versus-host disease (xGvHD), as determined by additional assessment, and were euthanized. ** $P < 0.01$, **** $P < 0.0001$; one-way ANOVA (Tukey's post test).

RPM T-cells Increase Survival

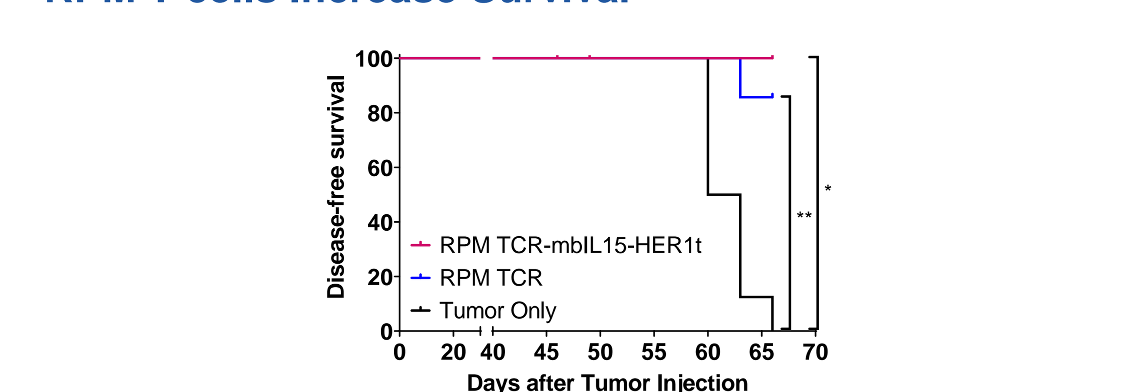

Fig. 9 RPM T cell-treatment significantly improves survival. Kaplan Meier survival curves show disease-free survival, whereby mice were considered disease-free when tumor flux was below twice the LOD (<10⁶ p/s). Thus, survival curves depict mice (with tumor) surviving to end of study and censored mice that reached 2X LOD that were euthanized at earlier timepoints. Significance determined by Gehan-Breslow-Wilcoxon test. *$P < 0.05$, ** $P < 0.01$.

RPM T-cells Show Enhanced Persistence & Desirable Memory Phenotype

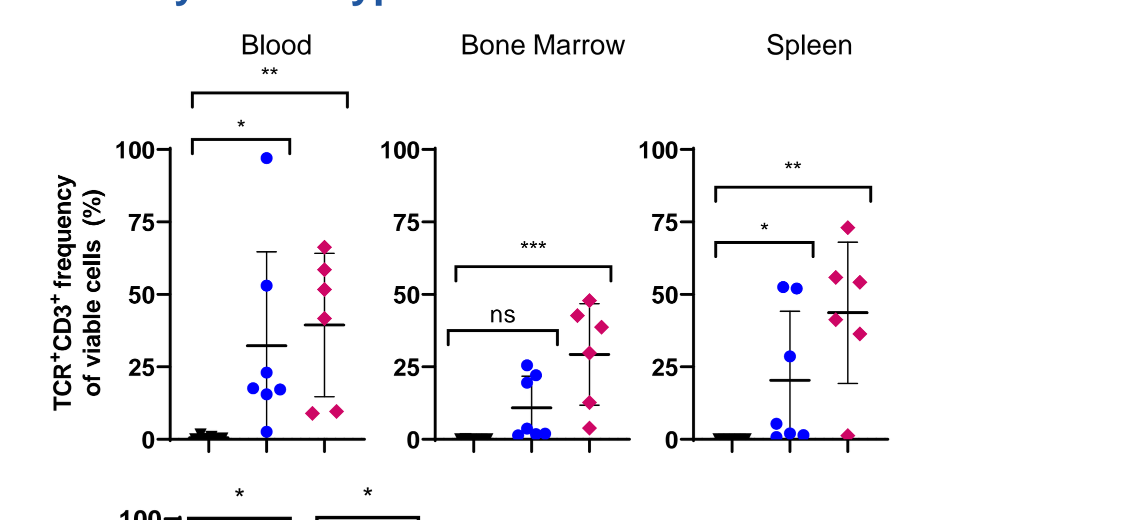

Fig. 10 RPM T-cells show a significant and tissue-distributed engraftment of TCR⁺ T cells, with RPM TCR-mbIL15-HER1t-treated mice possessing increased T cell frequencies of the less-differentiated CD45RO⁺CD27⁺ phenotype. Peripheral blood and tissues of moribund mice and mice at end of study (day 66) were analyzed to assess persistence of genetically modified T cells (*top*), and frequencies of memory subsets delineated by CD45RO and CD27 expression (*bottom*). Memory subset data is gated on TCR⁺CD3⁺ cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns = not significant; Kruskal-Wallis and T tests, respectively.

CONCLUSIONS

- 2-stim TCR and TCR-mbIL15-HER1t T cells can be generated with *ex vivo* manufacture using AaPC and show stable integrated expression of transgenes
- 2-stim TCR and TCR-mbIL15-HER1t T cells showed antitumor activity, but with 2-stim TCR-mbIL15-HER1t T cell-treated mice having significantly lower end-point tumor burden
- 2-stim T cells had modest persistence detected at endpoint but >60% of remaining TCR⁺ T cells were CD45RO⁺CD27⁺
- RPM TCR and TCR-mbIL15-HER1t T cells can be rapidly generated and adoptively transferred without the need for *ex vivo* activation and propagation (similar to CAR-T under RPM)
- With one low-dose, RPM T cells showed significant antitumor activity, with 67% of RPM TCR-mbIL15-HER1t T cell-treated mice attaining disease-free status (<2X LOD), and enhanced survival
- Recovered RPM T cells evidenced robust and distributed persistence of TCR⁺ T-cells that maintained a predominantly less differentiated phenotype
- These data support a clinical trial to rapidly manufacture genetically modified T cells under RPM
- Reducing the manufacture time of TCR⁺ T cells under RPM can:
  1. advance genetically modified cell-based therapies as a manufacturing platform with broad appeal
  2. shorten time to treatment
  3. decrease costs

Conflict of Interest Disclosures: **LVH:** *Ziopharm Oncology, Inc.:* Employment, patent & equity ownership; *M.D. Anderson Cancer Center:* Patent; **LZ:** *Ziopharm Oncology, Inc.:* Patent; **DCD:** *Ziopharm Oncology, Inc.:* Employment & equity ownership; **SO:** *Ziopharm Oncology, Inc.*: Patents; **LJNC:** *Targazyme, Inc.:* Equity Ownership; *Immatics:* Equity ownership; *City of Hope:* Patents & royalties; *Sangamo BioSciences:* Patents & royalties; *Ziopharm Oncology, Inc.:* Employment, equity ownership, patents; *MD Anderson Cancer Center:* Visiting scientist; **HS:** *Ziopharm Oncology, Inc.*: Patents.