Phenotypic and Functional Impacts of Successive Antigen Stimulation on T Cells: Implications for Clinical Manufacturing of TAA-T cell Products

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INTRODUCTION

Adoptive cell therapy using ex vivo-expanded tumor-associated antigen-specific T-cells (TAA-T) is a promising approach for the treatment of various cancers. Generation of TAA-T cells involves iterative rounds of lymphocyte stimulation with target antigens (WT1, PRAME, and Survivin) via co-culture with peptide-pulsed dendritic cells (DCs). Current practices for manufacturing of TAA-T cells, utilized in multiple phase 1 clinical trials, rely upon cellular expansion for determining the number of stimulations – generally 2 rounds of stimulation, with an optional 3rd stimulation if further expansion is required to meet clinical dose. However, the impact of these varying rounds of TAA stimulation on lymphocytes has not been fully characterized. Here we evaluate the phenotypic and functional characteristics of TAA-T cells generated from healthy donors (n=18) at each of the 3 stimulations.

METHODS

To manufacture TAA-T cells, monocyte and lymphocyte populations were isolated from fresh, healthy donor apheresis samples using the Elutra® cell separation system. Monocytes were plated in T25 culture flasks in the presence of IL-4 and GM-CSF. On Day 5, DCs were matured by adding IL-1β, IL-4, IL-6, GM-CSF, LPS, and TNF-α. On Day 6, mature DCs were harvested, pulsed with TAA peptides, and co-cultured with lymphocytes at a 1:10 (DC:Lymph) ratio in G-Rep® culture flasks. After 7-day co-culture, CTLs were harvested, and stimulated a 2nd time with mature, peptide-pulsed DCs at a 1:20 ratio. A final 3rd stimulation was completed as before at a ratio of 1:20-1:40. In-process stimulation was taken fresh [flow] or cryopreserved (EUPSHOT) for future analysis.

RESULTS

~5-fold mean cellular expansion observed at each of the 3 rounds of stimulation

![Figure 2. Total cellular expansion at each of 3 stimulations, measured by Trypan Blue exclusion dye. Lymphocytes were stimulated with peptide-pulsed DCs for 7 days. Fold expansion calculated as the # cells harvested/# cells initiated.](image)

Significant enrichment of CD2+ T cells, with a strong selection for CD8+ T cells (p<0.0001)

![Figure 3. Cellular phenotyping by flow cytometry. Median frequencies were compared between sampling timepoints using Wilcoxon match-pairs sign rank test.](image)

CONCLUSION

While further research is needed to determine the most appropriate assays for evaluating anti-tumor functionality and potency of TAA-T-cells, these data suggest that 3 rounds of antigen stimulation produce a more potent drug product while maintaining other important phenotypic characteristics.

![Figure 4. Cellular memory phenotyping by flow cytometry. Summary phenotypes of individual TAA-T cell lines (n=18) across sampling timepoints. Th - Naive; TCM - Central Memory; TCF - Effector Memory; TCM - Terminally Effector Memory](image)

![Figure 5. Specificity of TAA-T cell products determined by RIPA EUPSHOT assay. (A) In-process specificity to total TAA Pepmix. (B) Specificity to individual TAA peptides (WT1, PRAME, and Survivin) at each of 3 stimulations. Statistical differences determined using Wilcoxon match-pairs sign rank test.](image)