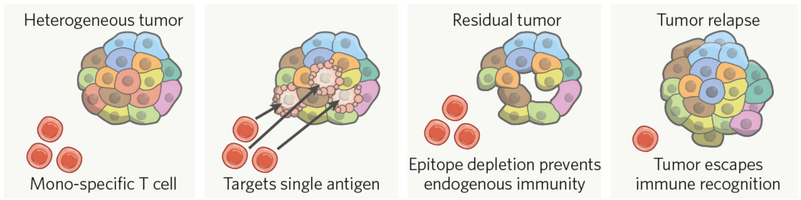
Rapid and Simplified Process for Manufacturing Multi-Tumor-Associated Antigen Specific T Cells

Anastasiya Smith, Tara Shahim, Jeannette Crisostomo, Eric A. Smith, Anna Wilga-Savitski, Jennifer Pickering, Tsvetelina Hoang, Juan Vera.
Marker Therapeutics, Inc., Houston, Texas, 77027, USA.

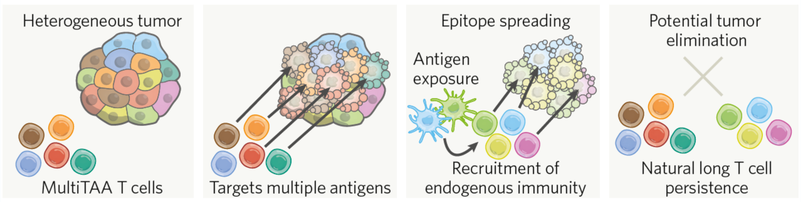
Introduction

AML is a malignant neoplasm of the myeloid lineage arising in the bone marrow and outgrowing normal hematopoietic elements. In 2022, there will be estimated 20,050 new cases of AML with ~11,500 deaths. Marker Therapeutics, Inc. has developed MT-401, a multi-tumor-associated antigen (multiTAA)-specific allogeneic T cell product capable of recognizing multiple targets expressed on the tumor simultaneously, minimizing tumor escape. Here we demonstrate how additional process improvements streamlined the manufacturing process and resulted in products with superior T cell phenotype and potency, both of which have the potential to enhance clinical responses.

MultiTAA-Specific T Cell Therapy



The lack of one AML antigen with sufficient tumor specificity leads to tumor immune escape.



In contrast to single-target T cells, Marker's multiTAA-specific T cells recognize up to 4 antigens for a more potent and durable anti-tumor response.

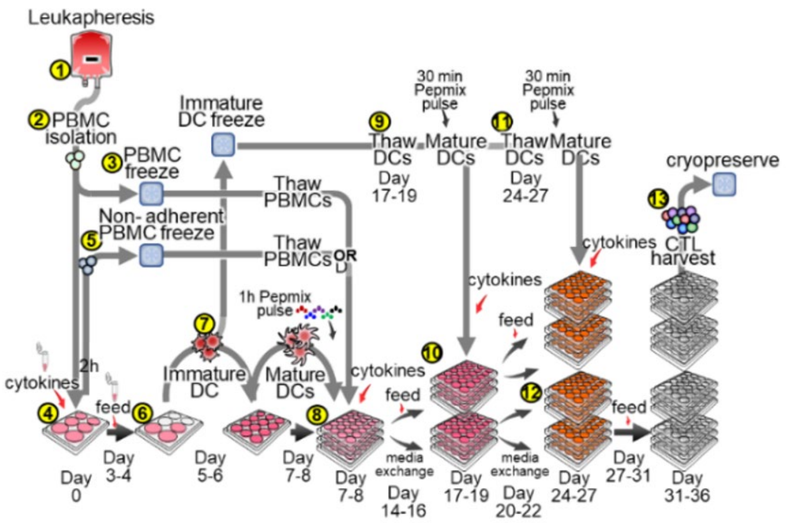
Objective

To streamline the manufacturing process and produce T cells with superior phenotype and potency, both of which have the potential to enhance clinical responses.

Methods

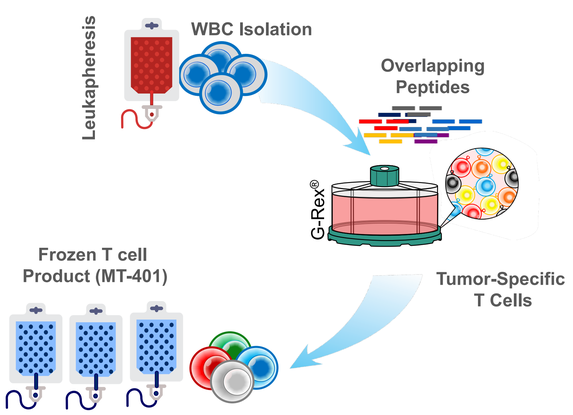
To commercialize the multiTAA-specific T cell therapy, the complexity and duration of the manufacturing process needed to be reduced.

Original multiTAA-Specific T Cell Therapy Manufacturing Process



The original process for generating multiTAA-specific T cells was lengthy, required many interventions and was not compatible with a closed system manufacturing.

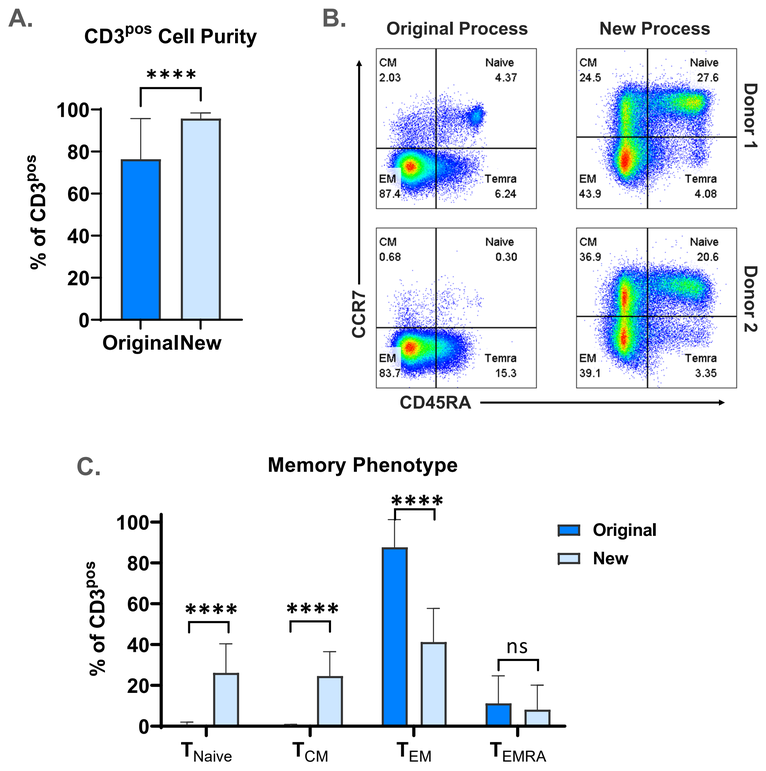
New MT-401 Manufacturing Process



The new 9-day manufacturing process requires only one stimulation and eliminates the need to generate dendritic cells.

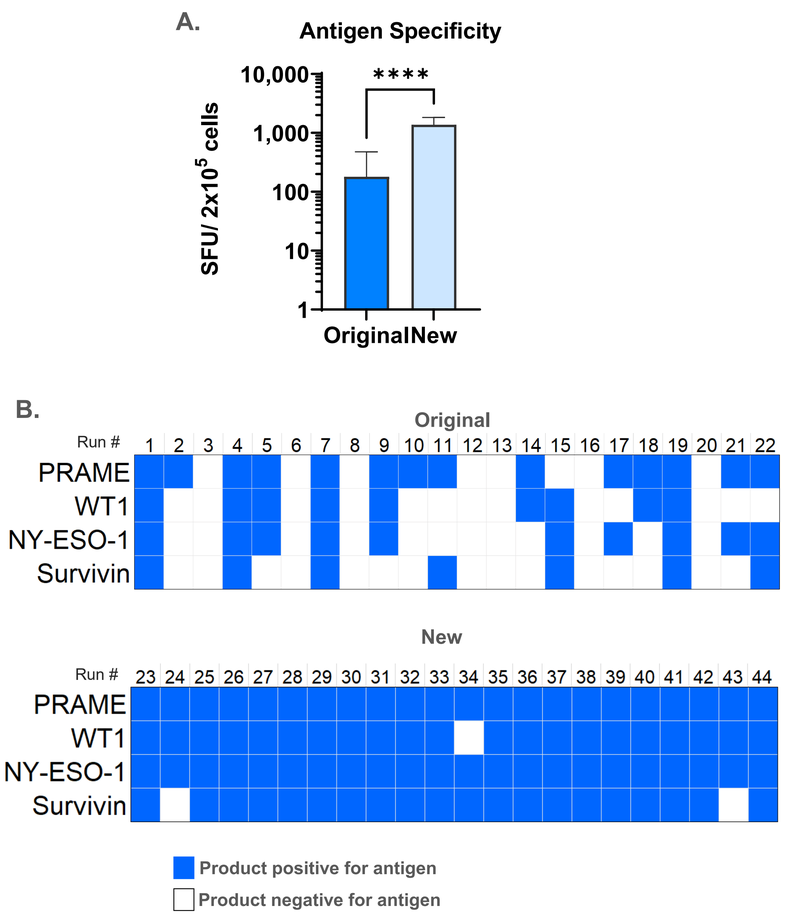
Results

New Manufacturing Process Yields Products With Higher Purity, Improved Memory Phenotype and Greater Antigen Specificity Compared to Old Process



To evaluate how the new manufacturing process affects T cell purity and phenotype multiTAA-specific T cells from healthy donors were manufactured using the original process with dendritic cells and three stimulations (Original) or with one stimulation and no dendritic cells (New). Graphs depict: Comparison of the CD3pos T cell purity of the final products (A) representative flow cytometry plots highlighting T cell memory populations in two donors manufactured using the original and new process (B), four T cell memory populations from products manufacturing using the original and new process as measured by flow cytometry (C).

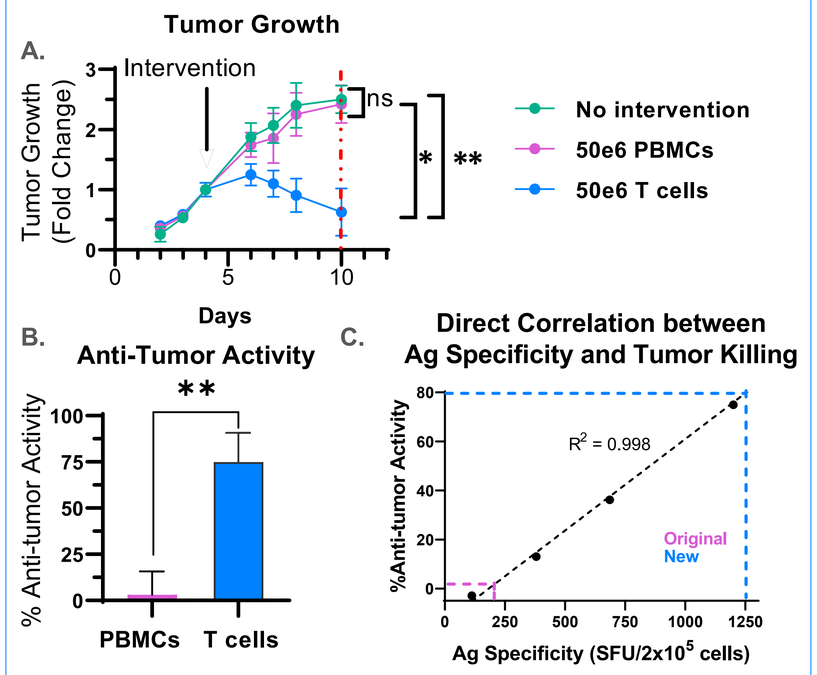
Process Evolution Yields Increased Specificity and Diversity Against All 4 Tumor Targets



Process improvements have resulted in increased antigen specificity and numbers of products with specificity for multiple tumor antigens, as determined by IFN-γ ELISpot analysis.

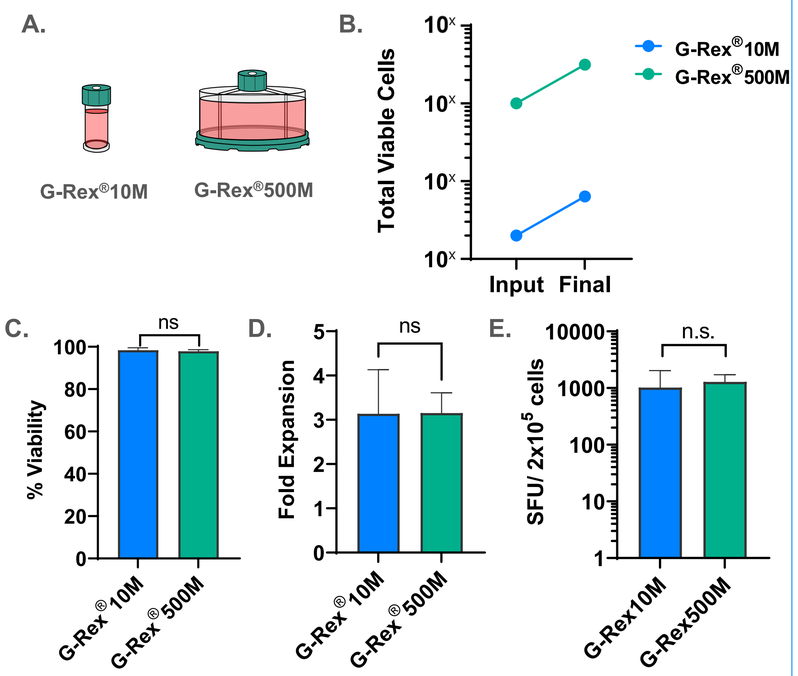
Results

MT-401 In Vitro Efficacy Against a Leukemic Tumor Model



To evaluate the ability of MT-401 (manufactured using the new process) to kill AML target cells, MT-401 was co-cultured with luciferase-expressing THP-1 AML cells. After 4 days, THP-1 cells were split into 3 groups: 1) MT-401 treatment; 2) control PBMC treatment (same donor as product); and 3) untreated (A). These results show significant anti-tumor effects of MT-401 (B). % Anti-tumor activity = 100 – [100 * (fold change in growth of treatment condition / fold change in growth of no treatment condition)] (B). Anti-tumor activity correlates with antigen specificity as measured by luciferase-based killing assay and IFN-γ ELISpot (C).

Manufacturing Scale-up for Large Scale Production Using G-Rex®500M



To generate large quantities of T cells without increasing the number of vessels, the larger G-Rex®500M device was tested and T cell expansion kinetics and antigen specificity were evaluated. MultiTAA-specific T cells from healthy donors were seeded at the same cell density in a G-Rex®10M (surface area 10 cm²) or G-Rex®500M (surface area 500 cm²) (A). Assessment of total viable cells after expansion confirmed that G-Rex® devices of different sizes are linearly scalable when the initial seeding cell density and media volume are normalized by surface area (B). Percent viability and (C) Fold expansion as determined using a Cellometer (D) and antigen specificity as determined by IFN-γ ELISpot analysis.(D) revealed no statistically significant differences between products generated in either a G-Rex®10M or a G-Rex®500M.

Conclusions

- We have shortened the manufacturing of multiTAA-specific T cells to 9 days and eliminated the need to generate DCs prior to T cell stimulation.
- The improved manufacturing process produces superior T cells with significantly higher CD3pos cell purity, increased naïve and central memory phenotype, greater antigen specificity and diversity for all four tumor antigens and anti-tumor activity compared to T cells generated using the original process.
- We demonstrated that the new process is scalable to a G-Rex®500M device without affecting the fold expansion and viability of the final product.
- These process improvements significantly reduced the number of interventions needed during manufacturing, thereby decreasing both the possibility of manufacturing failures and product manufacturing time, which translates to faster patient treatment.