Automating Closed System Purification of White Blood Cells for T Cell Therapy Manufacturing

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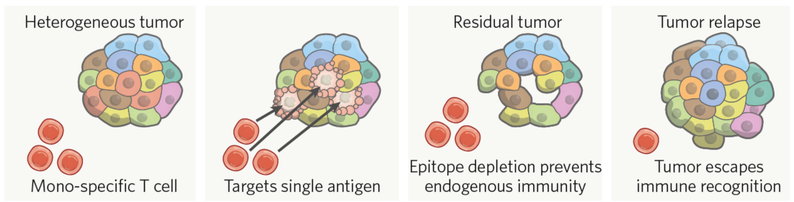
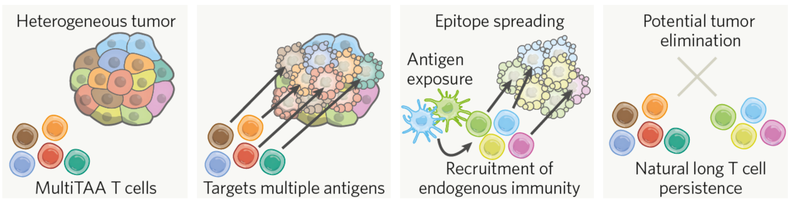
Introduction

Marker Therapeutics, Inc. has developed MT-401, a multi-tumor-associated antigen (multiTAA)-specific allogeneic T cell product capable of recognizing multiple tumor targets expressed simultaneously, minimizing tumor escape. One of the biggest challenges for cellular immunotherapies is the standardization of cell processing methods, which is crucial for product manufacturing. We compared two methods, manual and automated for isolation of white blood cells (WBCs) that serve as the starting material for manufacturing of multiTAA-specific T cells.

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.

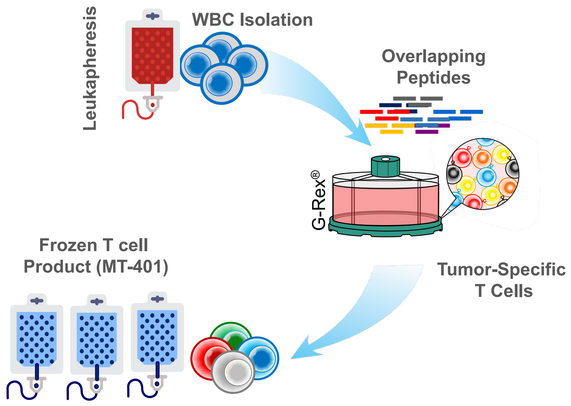
Objective

To compare WBC cell yield and characteristics of multiTAA-specific T cells generated from WBCs isolated using manual density gradient or Lovo, a closed system cell processing instrument.

Methods

MultiTAA-specific T cell manufacturing begins with the purification of WBCs from leukapheresis material. T cells are cultured with peptides spanning the entire primary sequences of 4 tumor antigens in a G-Rex® device to stimulate and expand antigen-specific T cells.

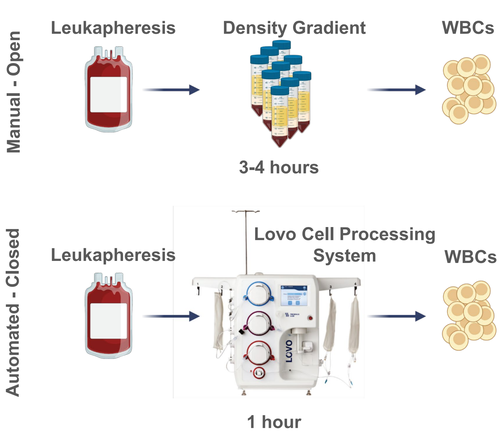
MT-401 Manufacturing Process



MT-401 is an allogeneic product composed of antigen-specific T cells recognizing four different antigens: WT1, Survivin, PRAME, and NY-ESO-1.

The classical method for separation of WBCs from blood-derived products, utilizes centrifugation in density gradient medium such as Ficoll™, which is an operator dependent, time consuming, and open process. The Lovo cell processing system allows for automated, functionally closed cell processing through spinning membrane filtration without the need for a density gradient medium.

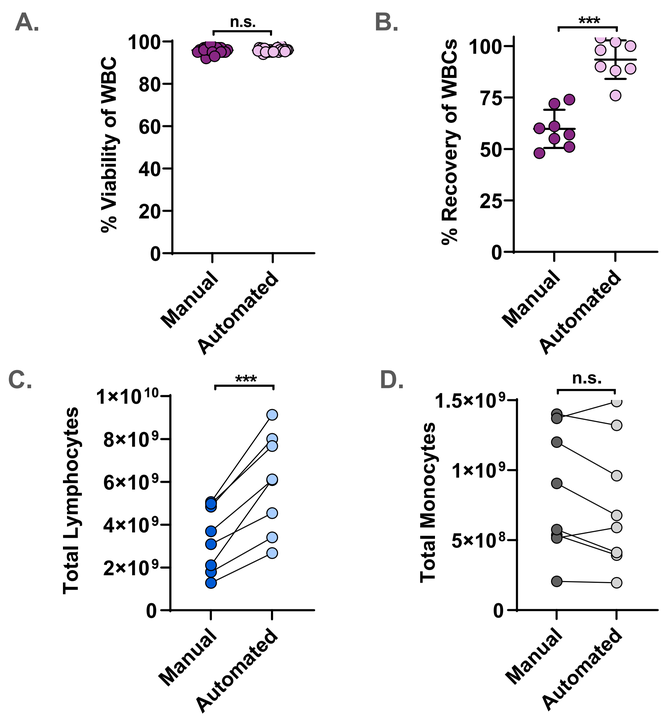
Isolation of WBCs From Leukapheresis



The multiTAA-specific T cell product manufacturing process begins with the isolation of WBCs from the donor leukapheresis material. We performed a comparability study between isolation of WBCs using manual density gradient (3-4 hours to complete procedure) and the automated Lovo cell processing system (1 hour to complete procedure).

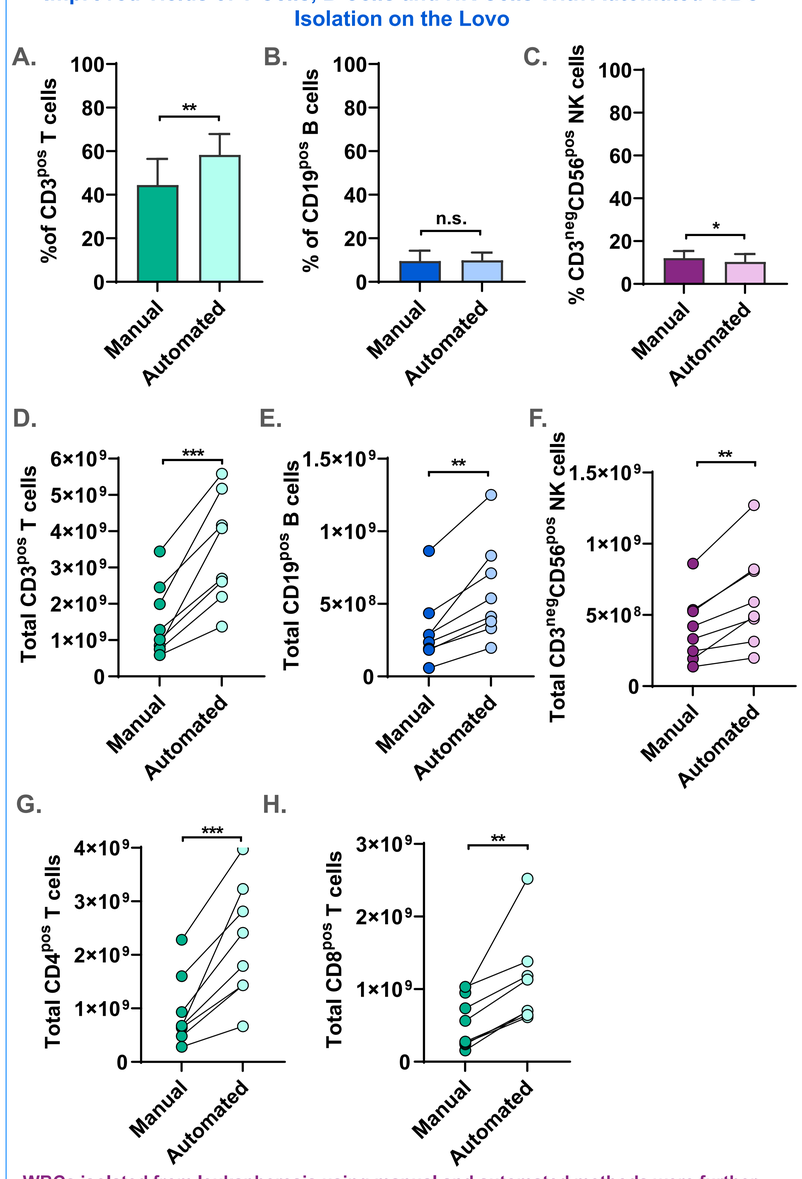
Results

WBC Isolation Using Lovo Results in Equivalent Viability But Significantly Higher Yield of WBCs Compared to Manual Density Gradient



Leukapheresis material from eight commercially sourced healthy donors was split into equal volumes and processed manually using Ficoll™ and automatically using the Lovo. The Cellometer and flow cytometry were used to determine % viability of WBCs (A), % recovery of WBCs (B), yield of lymphocytes (C), and yield of monocytes (D) after processing leukapheresis material using both methods.

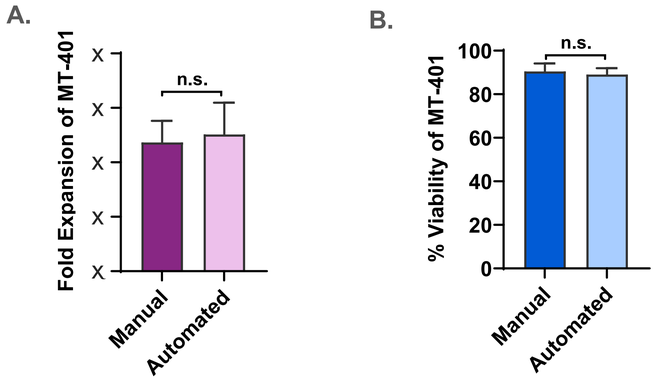
Improved Yields of T Cells, B Cells and NK Cells With Automated WBC Isolation on the Lovo



WBCs isolated from leukapheresis using manual and automated methods were further assessed by flow cytometry to determine the impact of processing methods on frequencies of T cells (A), B cells (B) and NK cells (C), and total recovered T cells (D), B cells (E), NK cells (F) CD4pos T cells (G) and CD8pos T cells (H).

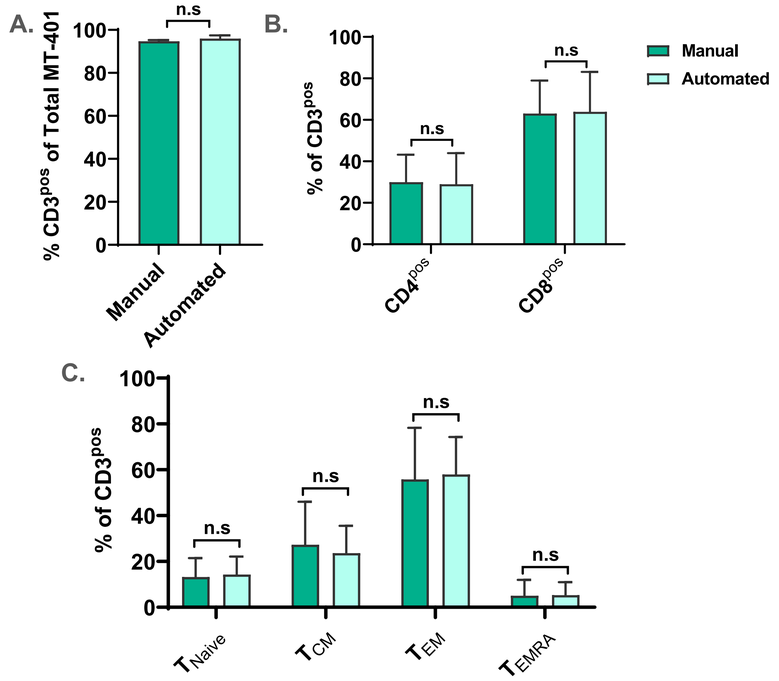
Results

Comparable Fold Expansion and Viability of MultiTAA-Specific T Cells Generated From WBCs Isolated by Manual or Automated Methods



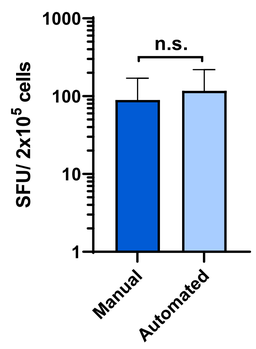
MultiTAA-specific T cells from three healthy donors were manufactured from WBCs isolated using manual Ficoll™ or automated Lovo methods. Graphs demonstrate the fold expansion (A) and % viability (B), as determined by Cellometer.

Comparable Phenotype of MultiTAA-Specific T Cells Generated From WBCs Isolated by Manual or Automated Methods



MultiTAA-specific T cells manufactured from WBCs isolated using manual Ficoll™ or automated Lovo methods were further analyzed by flow cytometry for %CD3pos T cells (A), %CD4pos and CD8pos T cells out of CD3pos T cells (B) and memory populations within CD3pos T cells (C) in MT-401.

Comparable Antigen Specificity of MultiTAA-Specific T Cells Generated From WBCs Isolated by Manual or Automated Methods



MultiTAA-specific T cells manufactured from WBCs isolated using manual Ficoll™ or automated Lovo methods were assessed for specificity by IFN-γ ELISpot analysis.

Conclusions

- Isolation of WBCs using the Lovo significantly improves cell yield without negatively impacting cell viability, resulting in larger quantities of valuable starting material compared to the manual density gradient method.
- Lymphocyte yield is significantly higher when WBCs are isolated using the Lovo compared to the manual method.
- WBCs isolated using the Lovo produce multiTAA-specific T cells with equivalent phenotype and specificity to those isolated with the manual method.
- Implementation of the Lovo allows closing of the manufacturing process, reduction of operator error and variability, as well as increased recovery of valuable starting material, without affecting the quality of the final product.