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Evaluating Buoyancy Activated Cell Sorting (BACS) for T cell Isolation in CAR-T Manufacturing

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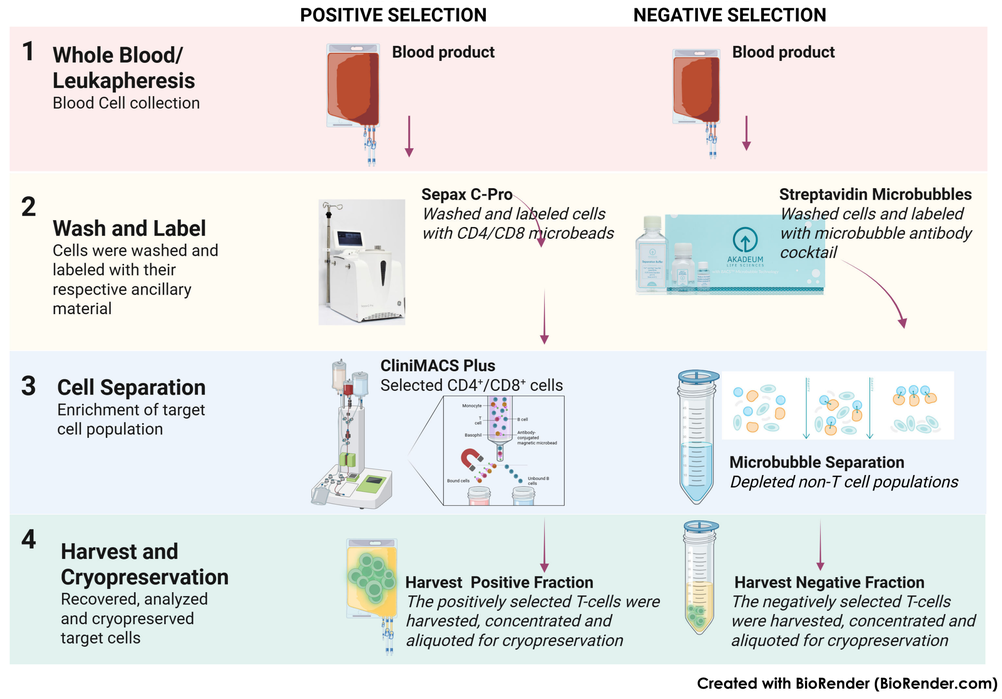
INTRODUCTION

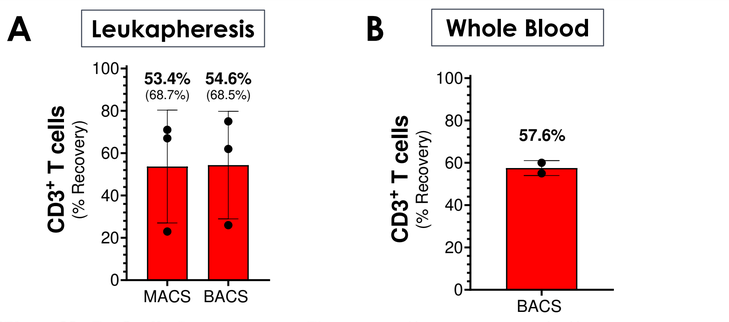
- As the CAR T and TCR landscape evolves, robust manufacturing solutions that enable increased access to these transformative therapies through reduced cost and complexity must be developed. Rigorous process development and characterization is critical when identifying these solutions to deliver a safe and potent drug product to patients.
- Traditional autologous CAR T and TCR cell therapy manufacturing begins with T cell isolation from patient leukapheresis material. The most common isolation technique used is Magnetic Activated Cell Sorting (MACS), a process by which magnetic nanoparticles, coated with antibodies, select specific cells that are then separated by application of a magnetic force. MACS cell separation requires costly equipment, magnetic columns and reagents, and skilled manufacturing operators to perform multiple complex steps.
- An alternative cell separation strategy that may improve manufacturability of these therapies is the isolation of T cells by negative selection using BACS. Buoyancy Activated Cell Sorting (BACS) utilizes antibody coated microbubbles to bind and remove contaminating cell types in the starting material (B cells, monocytes, granulocytes and red blood cells). The microbubbles are devoid of animal material and produced under cGMP conditions making them suitable for use in cell therapy. They are composed of a thin glass polystyrene microsphere which encapsulates a gaseous sphere rendering them buoyant in the surrounding buffer. The microbubble bound cells float to the top layer of the cell suspension leaving the "untouched" T cells at the bottom and allowing both the microbubbles and bound cells to be easily removed by evacuating the top layer of buffer.
- In addition to effective T cell isolation, unlike direct immunomagnetic labelling which leaves the beads attached to target cells and may interfere with characterization assays such as flow cytometry, negatively selected T cells are free of bound antibody and more suitable for a wide variety of downstream assays immediately post selection. This potential benefit may also render negatively selected cells to be more appropriate for shorter T cell processes.
- The purpose of this work was to evaluate the feasibility of this novel BACS technology and characterize the performance of T cells isolated using BACS to those isolated using MACS in a GMP representative CAR T manufacturing process. Additionally, we evaluate the performance of these GMP Microbubbles to isolate untouched T cells directly from whole blood. Whole blood is a potential alternative starting material source that is less invasive and costly than traditional leukapheresis but poses a processing challenge due to the large volume of red blood cells.

MATERIALS AND METHODS



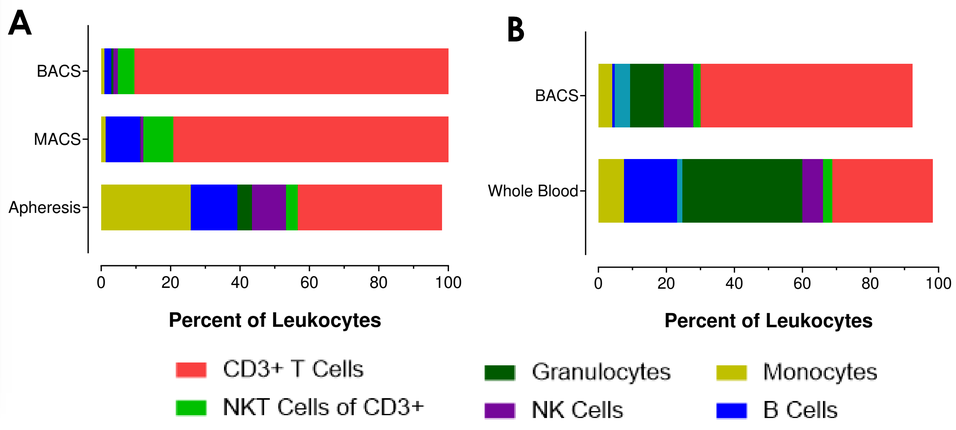
[Fig. 1] Selection Process. The above diagram describes the unit operation for **Magnetic Activated Cell Sorting (MACS)** for positive selection and **Buoyancy Activated Cell Sorting** (BACS) for negative selection. Both methods were used to isolate T-cells, either by positive or negative cell separation, respectively.

T CELL RECOVERY



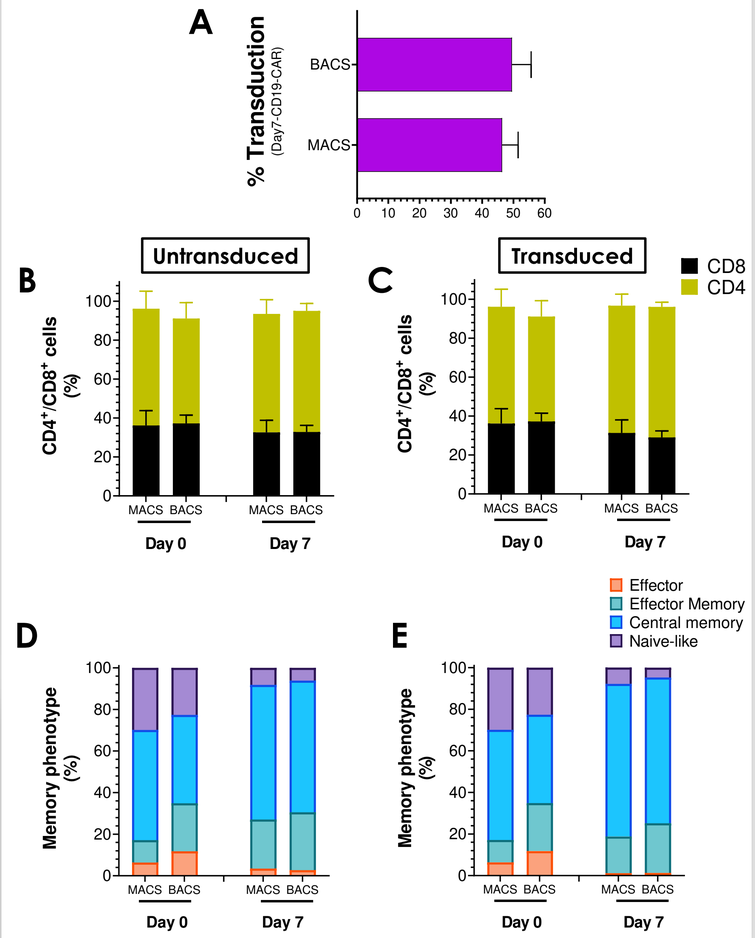
[Fig. 2] T Cell Recovery. The results represent the average percent recovery of $CD3^+$ T cells from Leukapheresis (A), and Whole Blood (B), respectively.

PROCESS PHENOTYPE



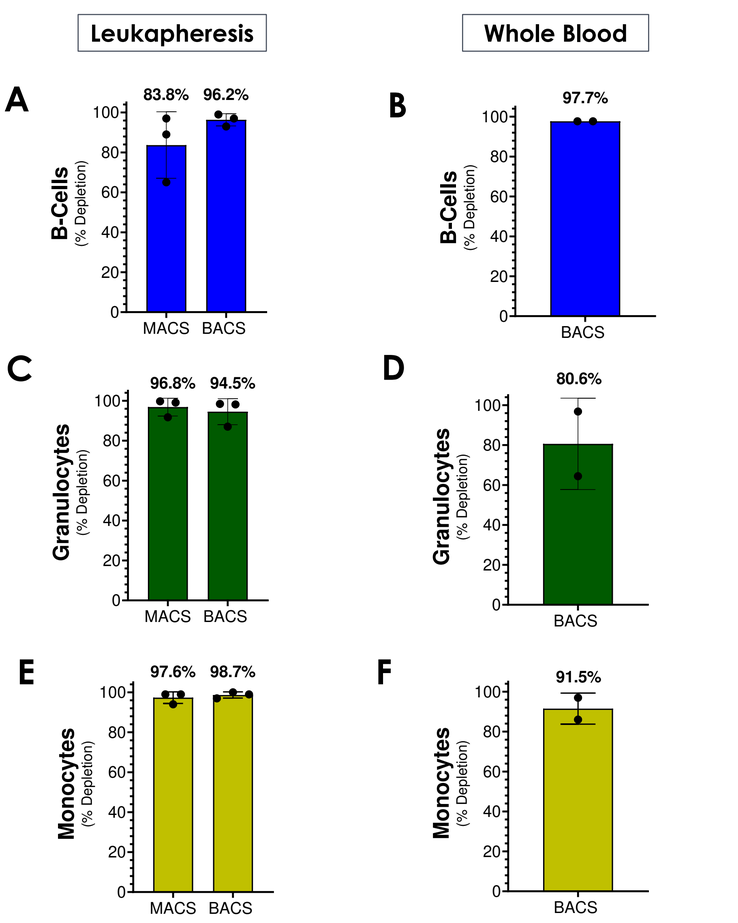
[Fig. 3] Leukocyte Purity. Figure 3A, depicts the Leukapheresis phenotype for pre-and-post cell separation. Figure 3B, depicts the Whole Blood phenotype for pre-and-post cell separation. The groups compared are Apheresis, Magnetic Activated Cell Sorting (MACS) positive fraction, and Apheresis/Whole Blood Buoyancy Activated Cell Sorting (BACS) negative fraction. The results represent the mean process phenotype and T cell purity across 2 donors for Whole Blood and 3 donors for Leukapheresis, respectively.

CAR EXPRESSION AND MEMORY PHENOTYPE



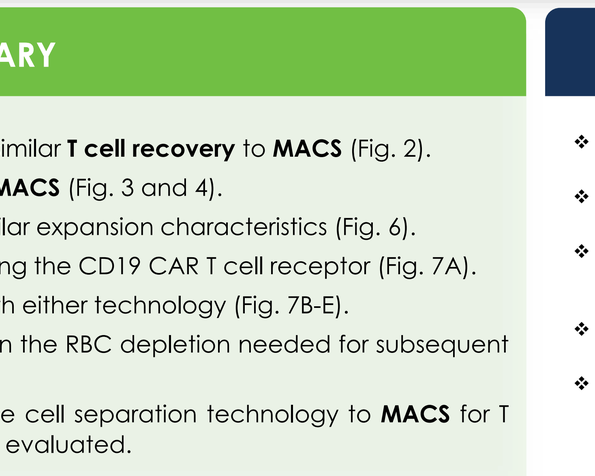
[Fig. 7] Transduction Efficiency and Drug product phenotype. Transduction efficiency was similar independent of T cell isolation process (A). Transduced CD19 CAR T-cells generated from MACS and BACS starting material had similar CD4/CD8 (B & C) ratios and Memory phenotype (D & E). Drug product from Whole Blood enriched T cells were not evaluated.

RESIDUAL IMPURITTIES



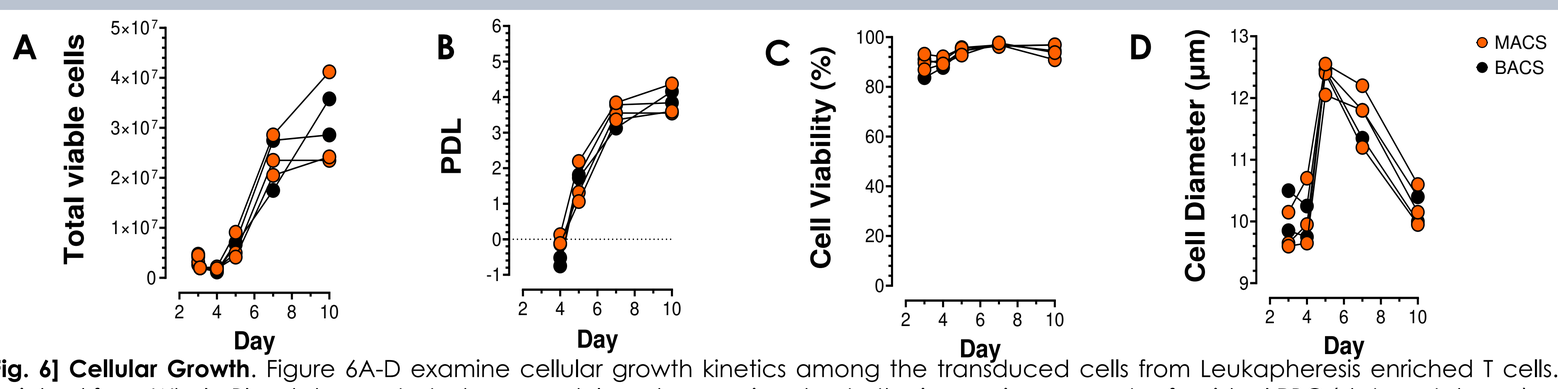
[Fig. 4] Leukocyte Depletion. The results represent the average percent depletion of B-cells, Granulocytes, and Monocytes from Leukapheresis (A, C, E; 3 donors), and Whole Blood (B, D, F; 2 donors), respectively.

RED BLOOD CELL REDUCTION



[Fig. 5] Red Blood Cell Reduction. The results represent the mean volume reduction of Red Blood Cell (A, B) per 100mL starting material processed by different selection methods. Aph, Leukapheresis; WB, Whole Blood.

GROWTH KINETICS



[Fig. 6] Cellular Growth. Figure 6A-D examine cellular growth kinetics among the transduced cells from Leukapheresis enriched T cells. T cells enriched from Whole Blood demonstrated poor or delayed expansion due to the increasing amounts of residual RBC (data not shown).

RESULTS AND SUMMARY

- Starting with either **Leukapheresis** or **Whole Blood**, **BACS** yielded similar **T cell recovery** to **MACS** (Fig. 2).
- **BACS** produced $CD3^+$ T-cells with **higher purity** in comparison to **MACS** (Fig. 3 and 4).
- The growth kinetics between **BACS** and **MACS** demonstrated similar expansion characteristics (Fig. 6).
- **BACS and MACS** isolated T cells were **equally efficient** at expressing the CD19 CAR T cell receptor (Fig. 7A).
- There was no **phenotypic difference** between groups isolated with either technology (Fig. 7B-E).
- Additional optimization of **BACS** Microbubbles is needed to attain the RBC depletion needed for subsequent drug product manufacturing from **Whole Blood** (Fig. 5).

In summary, **BACS** has great potential to be used as an alternative cell separation technology to **MACS** for T cell isolation. Additional T cell functional characterization should be evaluated.

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