Novel Dry Antibody Cocktail for Standardized Analytics of Engineered (CD19 CAR) T Cells, Enhancing Comparability and Efficiency in Patient Monitoring

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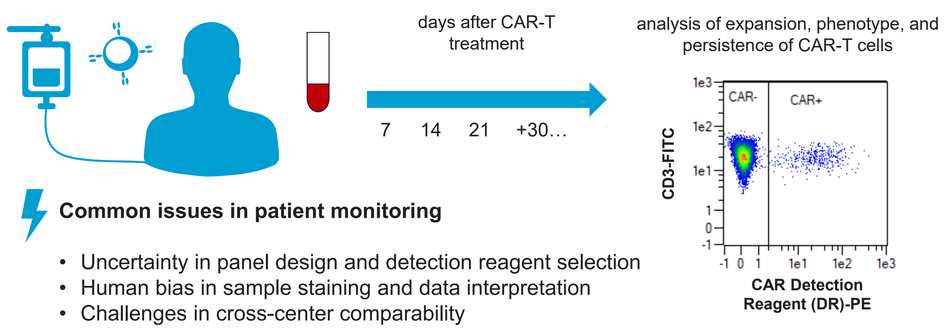
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Introduction and Aim

CD19 CAR T-cell therapy is an innovative treatment that involves modifying a patient's T cells to specifically target and destroy cancer cells expressing the CD19 protein, commonly found in certain types of blood cancers. Monitoring patients who receive this therapy is crucial for assessing therapy effectiveness, detecting potential side effects, and making timely adjustments to the treatment plan. Generating comparable results across different treatment centers worldwide is essential to ensure consistent patient care, but still represents a major challenge (Figure 1). Uniform monitoring enables clinicians to accurately track patient responses, compare outcomes across studies, and refine treatment protocols, ultimately improving the global standard of care for CAR-T and other engineered cell therapies, e.g., TCR-T cell therapy. Our aim is to enhance global comparability and efficiency in patient monitoring by developing innovative and robust flow cytometry reagents.

Figure 1: Common challenges of patient monitoring in engineered T cell therapy, e.g., CAR-T therapy

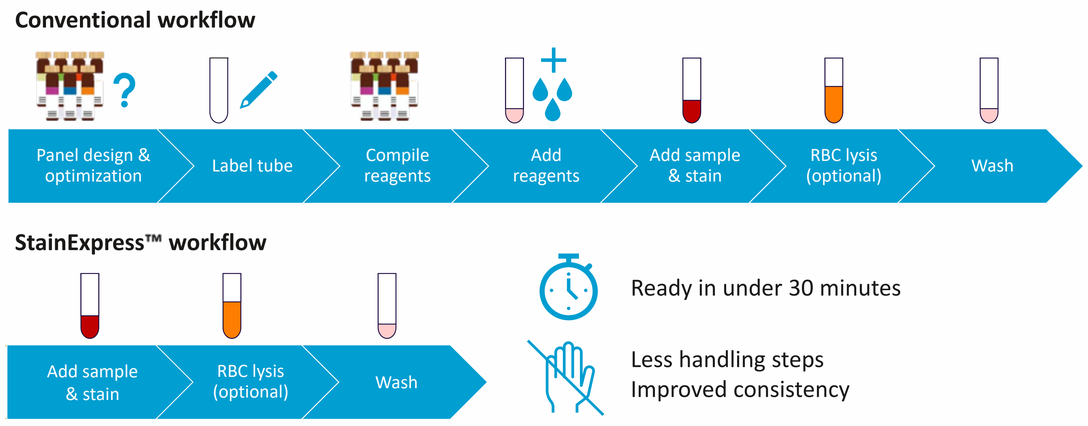


Experiments and Results

1 Fast and easy detection of CD19 CAR-T cells using the StainExpress™ CD19 CAR-T Monitoring Cocktail

The dried StainExpress™ Cocktails offer exceptional ease and speed. Whole blood samples are simply added to the StainExpress™ tube, lysed, washed, and ready for analysis in under 30 minutes. By eliminating tedious manual handling steps such as antibody pipetting, this approach mitigates the risks of human error and variability (Figure 2).

Figure 2: Significant time savings and reduction of variability with StainExpress™ Cocktails



2 Optimized gating strategy for CD19 CAR-T cell monitoring using the StainExpress™ Cocktails

The gating strategy ensures accurate analysis by systematically excluding debris, doublets, residual erythrocytes, and dead cells. It focuses on identifying CD3⁺ cells and differentiating between CAR⁺ and CAR⁻ populations, further categorizing them into CD4⁺ and CD8⁺ cells for detailed analysis (Figure 3). Our CAR detection reagents (CAR DR) enable background-free analysis of whole blood samples by utilizing REAfinity® Recombinant Antibody Technology, which eliminates non-specific Fc receptor binding by immune cells. The CD19 CAR FMC63 Idiotype Antibody, PE, REAfinity™ (clone: REA1297), used for CAR detection, exhibits high specificity for FMC63-based CD19 CARs, including commercial therapies like Yescarta® and Kymriah®. Its recombinant nature ensures consistent performance across different lots and compatibility with various panels. In summary, the benefits of REAfinity® Recombinant Technology include 1) enhanced specificity for accurate targeting, 2) superior reproducibility for reliable performance across all batches, and 3) outstanding sensitivity for low levels of target cells.

Figure 3: Detection of CD19 CAR-T cells using the StainExpress™ CD19 CAR-T Monitoring Cocktail

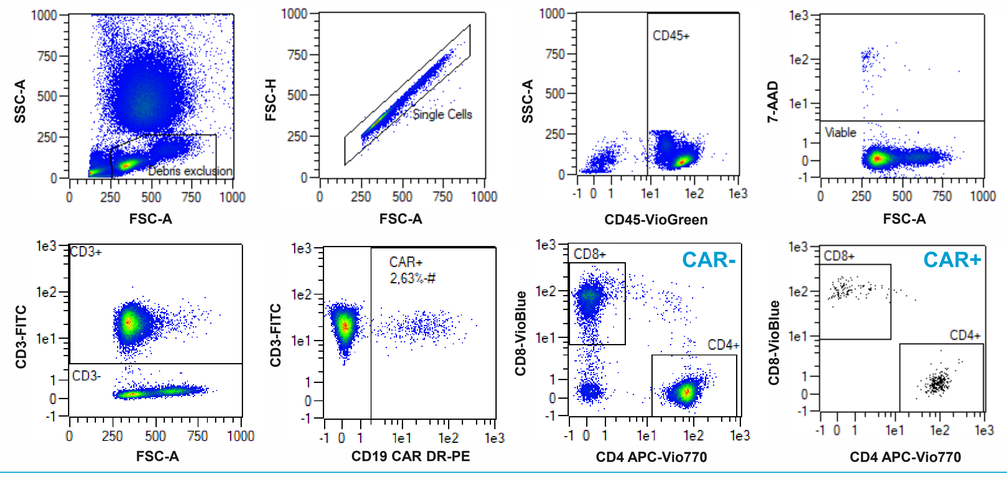


Fig 3: CD19 CAR-T cells, generated within 12 days using the CliniMACS Prodigy® T Cell Transduction process, were spiked into healthy donor lysed blood samples and stained with the StainExpress™ CD19 CAR-T Monitoring Cocktail. Staining was conducted at 19–25 °C for 10 min, washed once, followed by analysis at the MACSQuant® Analyzer 16. Debris and doublets were excluded. CD45+ cells were selected to exclude erythrocytes. Dead cells were excluded by 7-AAD staining. CD3+ cells were identified and distinguished into CAR+ and CAR– populations, followed by categorization into CD4+ and CD8+ T cells.

3 Combination of the StainExpress™ T Cell Subsets Cocktail with a CAR detection reagent of choice and liquid antibodies

The StainExpress™ T Cell Subsets Cocktail offers exceptional versality and adaptability. It features a free PE channel allowing the user to incorporate any CAR detection reagent (CAR DR) of choice, e.g., BCMA CAR DR, for T cell analytics in BCMA CAR-T therapies such as Abecma® and Carvykti® (Figure 4A). The cocktail's flexibility further allows for easy expansion with liquid antibodies, for instance to detect CD19-positive malignant cells (Figure 4B), or to assess CAR-T cell differentiation (Figure 4C). Monitoring the presence of CD19-positive B cells is crucial in patients undergoing CD19 CAR-T therapy, as these cells should be eradicated by the treatment. The re-emergence of CD19-positive tumor cells suggests that the cancer is evading the therapy and may have developed resistance, which is a critical indication for potential treatment failure (Figure 4B). Additionally, assessing CD45RO and CCR7 expression is particularly valuable because these markers allow to distinguish between T cell subsets, which provides insight into the functional state of CAR-T cells. Specifically, these markers can be used to distinguish naïve T cells (CD45RO⁻ CCR7⁺), central memory T cells (CD45RO⁺ CCR7⁺), effector memory T cells (CD45RO⁺ CCR7⁻), and effector T cells/ terminally differentiated effector memory T cells (CD45RO⁻ CCR7⁻) (Figure 4C).

Figure 4: Combination of the StainExpress™ cocktail with CAR DRs and additional liquid antibodies

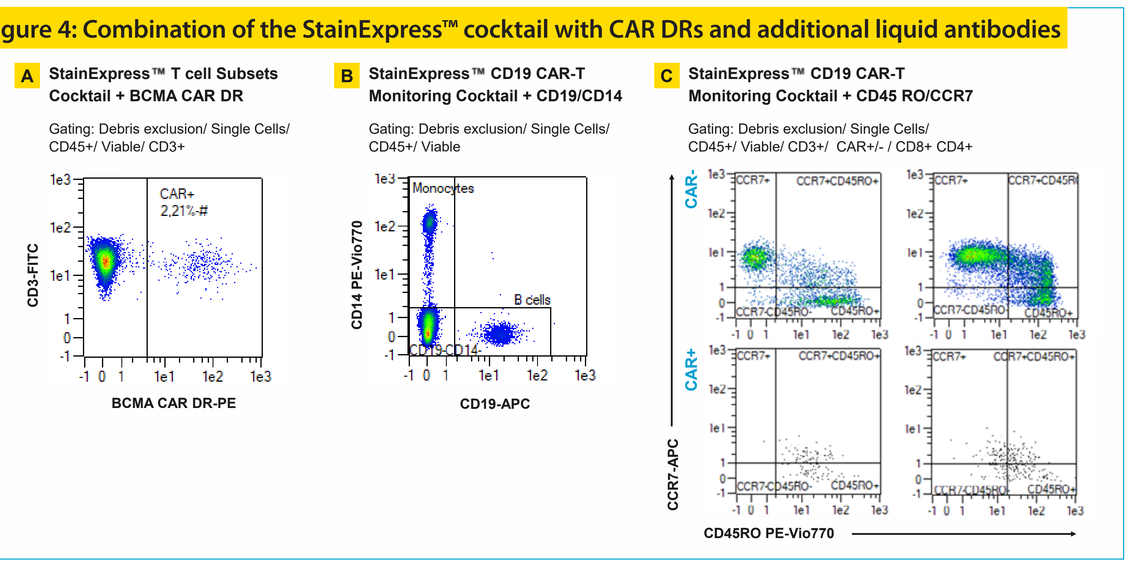


Fig 4: CAR-T cells generated within 12 days using the CliniMACS Prodigy® T Cell Transduction process were spiked into healthy donor lysed blood samples. Staining was conducted at 19–25 °C for 10 min, washed once, followed by analysis at the MACSQuant® Analyzer 16. (A) BCMA CAR-T cells were stained with Stain Express™ T Cell Subsets Cocktail plus BCMA CAR DR-PE. (B) CD19 CAR-T cells were stained with StainExpress™ CD19 CAR-T Monitoring Cocktail plus CD19-APC and CD14-PE-Vio770. (C) CD19 CAR-T cells were stained with StainExpress™ CD19 CAR-T Monitoring Cocktail plus CD45RO-PE-Vio770 and CCR7-APC.

4 Combination of the StainExpress™ T Cell Subsets Cocktail with MHC MACSimers for TCR-based detection of T cells

For T cell receptor (TCR)-based detection of antigen-specific T cells, the StainExpress™ T Cell Subsets Cocktail can be perfectly combined with an MHC multimer (or "MHC MACSimer") of choice, e.g. in the context of TCR-T cell analytics for engineered autologous T cell therapies such as Tecelra® (Afami-cel), a melanoma-associated antigen A4 (MAGE-A4)-directed autologous T cell therapy recently approved for a solid tumor. Figure 5 shows the detection of antigen-specific T cells using MHC MACSimer A*0201 CMV pp65 (NLVPMVATV) in combination with the StainExpress™ T Cell Subsets Cocktail with optimized panel design and gating strategy.

Figure 5: TCR-based detection of antigen-specific T cells with StainExpress™ and MHC MACSimers

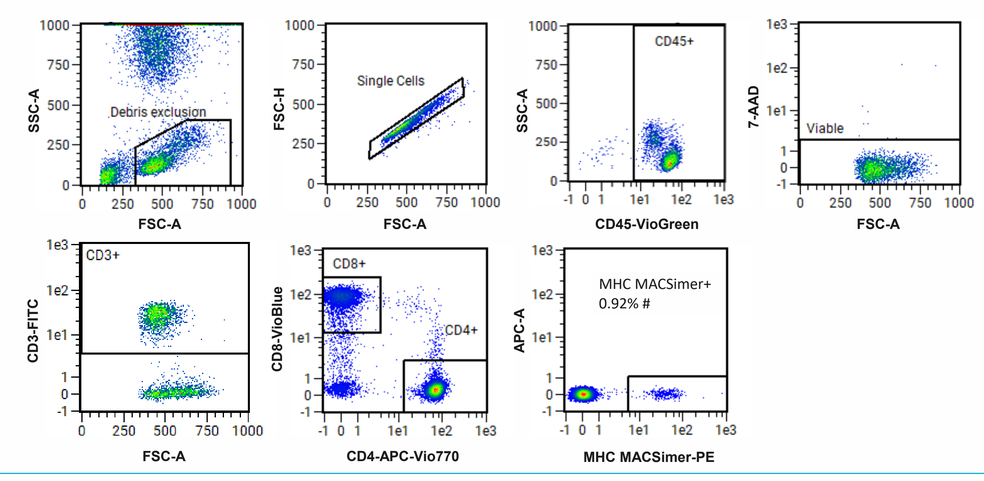


Fig 5: Lysed whole blood samples from a HLA-A*0201⁺ Cytomegalovirus (CMV)-reactive donor were stained with MHC MACSimer A*0201 CMV pp65 (NLVPMVATV), human, PE (at a final concentration of 60 nM) for 10 minutes at room temperature (RT). Afterwards cells were washed and transferred to the StainExpress™ tube, mixed, and incubated for 10 minutes at RT. After washing, cells were analyzed at the MACSQuant® Analyzer 16. Debris and doublets were excluded. Erythrocytes and plasma cells were excluded by selecting CD45⁺ cells. Dead cells were excluded by 7-AAD. CD3⁺ cells were identified and divided into CD8⁺ and CD4⁺. In the population of CD8⁺ T cells, antigen-specific T cells were identified as MHC MACSimer⁺.

Conclusion

This poster presents the StainExpress™ CD19 CAR-T Monitoring Cocktail, a dry antibody solution for standardized and efficient flow analysis. It reduces human bias and variability, providing robust results that are consistent across global treatment centers. Its flexible format, the StainExpress™ T Cell Subsets Cocktail, can be combined with any detection reagent of interest, ensuring applicability across various therapeutic contexts, including CAR-T therapies other than CD19, such as BCMA. In addition, the StainExpress™ T Cell Subsets Cocktail can be combined with MHC MACSimers for TCR-based detection of antigen-specific T cells, e.g., for TCR-T cell therapy monitoring. StainExpress™ key features are: 1) Simplified workflow and minimized manual handling, 2) optimized gating and reduced background, 3) adaptable for CAR-T and TCR-T cells, and compatible with liquid antibodies for advanced phenotyping that is consistent across global centers.

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