Integration of MHC MACSimers in workflows to analyze antigen-specific T cells in research, analytical development and QC of cellular products

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Introduction

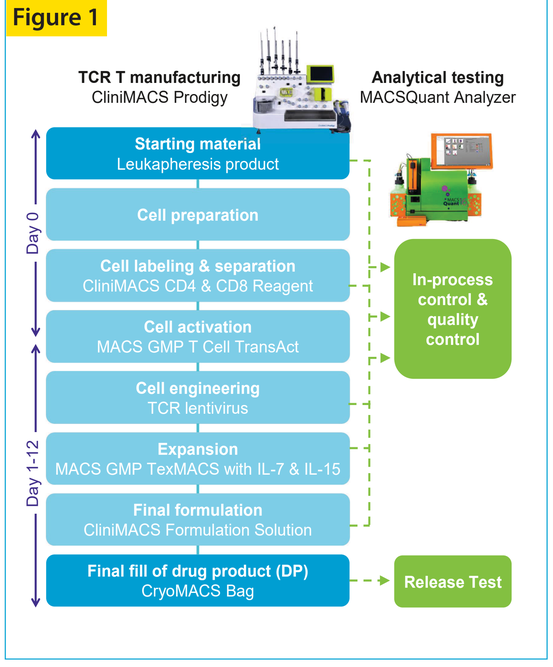
Antigen-specific T cells play a pivotal role in the immune system. Their function is to identify and react to specific antigens: distinct molecules present on pathogens, infected cells, cancer cells or other foreign entities. Upon encountering their corresponding antigen through their T cell receptor (TCR), these cells become activated and trigger an immune response.
MHC multimers are powerful tools used to identify antigen-specific T cells. These reagents are made by combining major histocompatibility complex (MHC) molecules with specific antigen-derived peptides and fluorophores. Each T cell has a unique T cell receptor (TCR) that recognizes a specific antigen presented by an MHC molecule. MHC multimers are designed to bind to these TCRs with high specificity. By using MHC multimers, scientists can quantify the frequency of antigen-specific T cells in a sample and study their characteristics, such as their activation state or cytokine production. This method is particularly useful for studying immune responses in infections, cancer, and autoimmune diseases. MHC multimers are invaluable tools for identifying antigen-specific T cells.
Our innovative MHC MACSimers enhance cell therapy development across all stages, from early discovery (like TCR and epitope screening) to in-process control (IPC), quality control (QC) of cell products, and immunomonitoring. They are designed for flexibility, reliability, and seamless integration into development workflows.

Overview of the MHC MACSimer and MACSpep portfolio

MHC MACSimer FleX Kits	MACSpep Single Peptides	Peptide-loaded MACSimers
"Load-it-yourself" MACSimers	MHC class I and class II binding peptides	Pre-loaded "off-the-shelf" MACSimers
Flexible TCR and epitope screening	For MACSimer FleX Kit loading	For highly standardized T cell analytics
Two colors for flow cytometry	Expansion of Ag-specific T cells	Two colors for flow cytometry
(PE/Vio® 667, more will follow)	Cancer, infection, autoimmune, and many more epitopes	(PE/Vio® 667, more will follow)

Methods

Figure 1



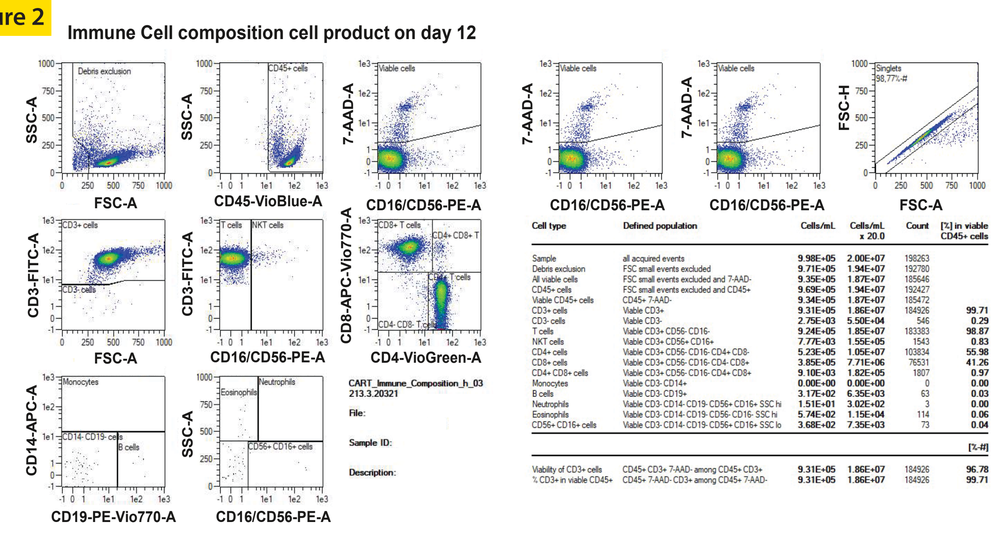
TCR T cell therapy is a promising immunotherapy that includes genetically modifying a patient's T cells to recognize tumor-associated antigens presented by MHC on cancer cells, offering advantages in targeting a broader range of intracellular antigens and managing tumor heterogeneity compared to CAR-T cell therapy and small-molecule inhibitors.

Generation of engineered TCR T cells
Frozen Leukapheresis from healthy donors were used to generate TCR T cells using the GMP-compliant CliniMACS Prodigy® platform, which enables an automated and closed engineering of T cells in a highly reproducible manner. During the manufacturing process, CD4+ and CD8+ T cells were isolated magnetically with MACS® Cell Separation system and activated with MACS® GMP T Cell TransAct™ following by the transduction with the respective TCR lentiviral vectors. For expansion T cells were cultured in TexMACS™ GMP Medium supplemented with inactivated human AB serum, IL-7 and IL-15.
Well established flow cytometric assays comprising antibody panels or pre-formulated cocktails (StainExpress™ products) and additional tools for automated data acquisition and analysis (Express Mode) are already used in clinical trials for CAR T cell therapies. These assays were transferred and adapted to the TCR T workflow. All used antibody fluorochrome conjugates are based on REAfinity Recombinant Antibodies. The combination of all these tools offers high reproducibilty and reliability.

Results

1 Quantification of immune cell subsets

Figure 2

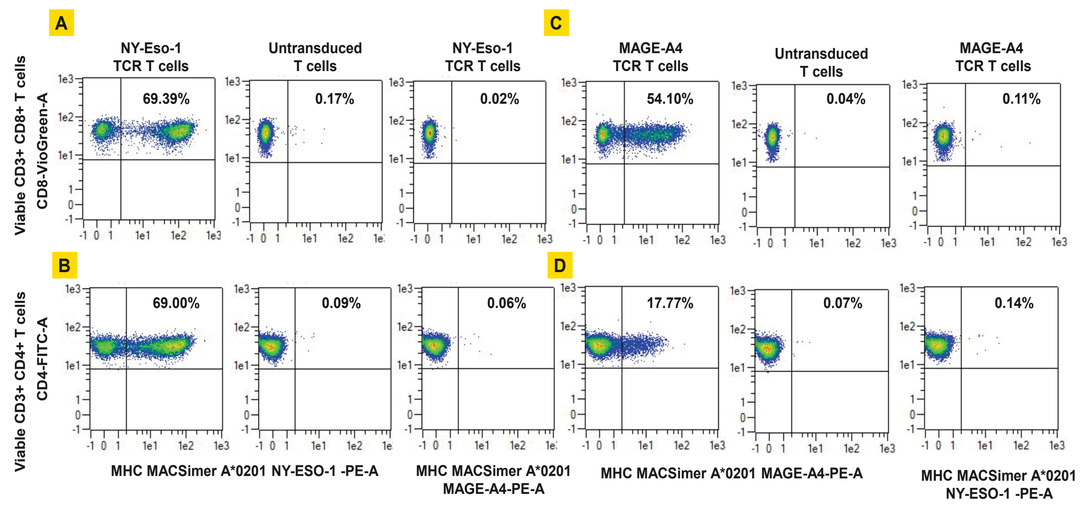


Cell type	Defined population	Cells/mL	Cells/mL x 20.0	Count	[%] in viable CD45+ cells
Sample	all acquired events	9.98E+05	2.00E+07	198263	
Debris exclusion	FSC small events excluded	9.71E+05	1.94E+07	192780	
All viable cells	FSC small events excluded and 7-AAD-	9.35E+05	1.87E+07	185646	
CD45+ cells	FSC small events excluded and CD45+	9.69E+05	1.94E+07	192427	
Viable CD45+ cells	CD45+ 7-AAD-	9.34E+05	1.87E+07	185472	
CD3+ cells	Viable CD3+	9.31E+05	1.86E+07	184926	99.71
CD3- cells	Viable CD3-	2.75E+03	5.50E+04	546	0.29
T cells	Viable CD3+ CD56- CD16-	9.24E+05	1.85E+07	183383	98.87
NKT cells	Viable CD3+ CD56+ CD16+	7.77E+03	1.55E+05	1543	0.83
CD4+ cells	Viable CD3+ CD56- CD16- CD4+ CD8-	5.23E+05	1.05E+07	103834	55.98
CD8+ cells	Viable CD3+ CD56- CD16- CD4- CD8+	3.85E+05	7.71E+06	76531	41.26
CD4+ CD8+ cells	Viable CD3+ CD56- CD16- CD4+ CD8+	9.10E+03	1.82E+05	1807	0.97
Monocytes	Viable CD3- CD14+	0.00E+00	0.00E+00	0	0.00
B cells	Viable CD3- CD19+	3.17E+02	6.35E+03	63	0.03
Neutrophils	Viable CD3- CD14- CD19- CD56+ CD16+ SSC hi	1.51E+01	3.02E+02	3	0.00
Eosinophils	Viable CD3- CD14- CD19- CD56- CD16- SSC hi	5.74E+02	1.15E+04	114	0.06
CD56+ CD16+ cells	Viable CD3- CD14- CD19- CD56+ CD16+ SSC lo	3.68E+02	7.35E+03	73	0.04
					[%-#]
Viability of CD3+ cells	CD45+ CD3+ 7-AAD- among CD45+ CD3+	9.31E+05	1.86E+07	184926	96.78
% CD3+ in viable CD45+	CD45+ 7-AAD- CD3+ among CD45+ 7-AAD-	9.31E+05	1.86E+07	184926	99.71

Staining of starting material, such as whole blood or leukapheresis products (fresh or frozen), with the "Immune cell composition" flow panel and automated sample acquisition and analysis using Express Mode "CART_Immune_Composition_h_03" enables the enumeration of various cellular subsets. The same panel was applied to the final cell product, as illustrated in figure 2. NY-Eso-1 TCR-engineered T cells from day 12 cultures were stained using the "Immune cell composition" flow panel, which include 7-AAD and the following antibody fluorochrome conjugates: CD45-VioBlue, CD56-PE, CD16-PE, CD3-FITC, CD4-VioGreen, CD8-APC-Vio770, CD14-APC, and CD19-PE-Vio770. Sample acquisition and flow cytometric analysis were performed automatically using the Express Mode "CART_Immune_Composition_h_03" on the MACSQuant® Analyzer, allowing for the assessment of cellular impurities and the quantification of T cells.

2 Detection of engineered TCR T cells for research, analytical development and QC

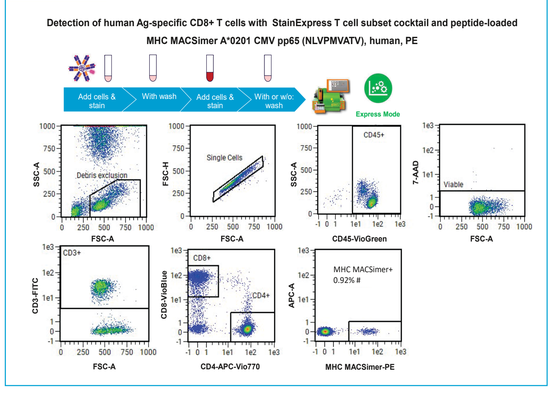
Figure 3



The transduction efficiency and the viability of small scale transduced T cells was determined during cell manufacturing and the final cell procduct. NY-Eso-1-, MAGE-A4 TCR-engineered T cells as well as untransduced T cells from day 10 cultures were stained with the MHC MACSimer A*0201 NY-ESO-1 (SLLMWITQC), human, PE and the MHC MACSimer A*0201 MAGE-A4 (GVYDGREHTV), human, PE, separately and washed afterwards. Next the cells were stained with a flow panel containing following antibody fluorochrome conjugates: CD8-VioGreen, CD4-FITC, and CD3-APC-Vio770. All used antibodies are anti-human and based on REAfinity Recombinant Antibodies. After a washing step the cells were analyzed on the MACSQuant® Analyzer after adding DAPI. The first plot (Figure 3 A, B, C, D) show the staining of the TCR engineered T cells with the respective peptide-loaded MHC MACSimer-PE, the second plot the staining of untransduced T cells and third the negative control staining (MHC MACSimer-PE loaded with a peptide of another tumor antigen) of TCR engineered T cells. Frequencies are given for MHC MACSimer-stained cells under CD8+ T cells (A and C) or CD4+ T cells (B and D).

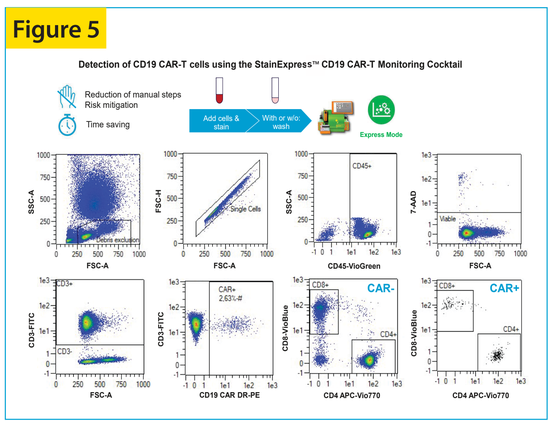
3 MHC MACSimer- and CAR- staining in combination with StainExpress™

Figure 4



Lysed whole blood from an A0201-specific, CMV pp65-reactive donor was stained with MHC MACSimer A0201 CMV pp65 (NLVPMVATV), human, PE. After staining, the cells were washed and transferred to the StainExpress cocktail tube "T cell subset", mixed, and incubated for another 10 minutes at room temperature. Following a final wash, the cells were analyzed using the MACSQuant® Analyzer 16.
The gating strategy involved excluding debris within lysed whole blood using an SSC low gate. Cells were plotted in FSC-H against FSC-A to exclude doublets. Erythrocytes and plasma cells were excluded by selecting CD45+ cells. Dead cells were excluded by selecting 7-AAD negative cells. Within the viable cells, CD3+ cells were identified and further divided into CD8+ and CD4+ T cells. Antigen-specific T cells within the CD8+ population were identified by staining with MHC MACSimer A*0201 CMV pp65 (NLVPMVATV), human, PE, showing a frequency of 0.92% of the parent population (Figure 4).

Figure 5



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 minutes, washed one time, followed by analysis using the MACSQuant® Analyzer 16. To ensure accurate analysis, debris was first excluded by gating on FSC versus SSC, encompassing lymphocytes and monocytes. Single cells were then identified, and doublets excluded using FSC-A and FSC-H gating. CD45+ leukocytes were gated to exclude residual erythrocytes and identify leukocytes. Dead cells were further excluded using 7-AAD staining. CD3+ cells were identified and distinguished into CAR+ and CAR– populations. CAR+ cells were subsequently categorized into CD4+ and CD8+ T cells, as were CAR– cells (figure 5).

Conclusion

We demonstrate that MHC MACSimers can reproducibly and reliably detect rare antigen-specific T cells within both CD4+ and CD8+ subpopulations. They can be combined and integrated into various processes to simplify assay workflows, such as dried antibody reagents and automated cell acquisition and flow analysis via Express Modes. Finally, peptide-loaded MHC MACSimers consistently and accurately detect TCR Ts in the context of IPC/QC of cell therapy products, e.g., directed against a common tumor antigen.

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