Novel MHC multimer technology for sensitive discovery, analysis, and enrichment of antigen-specific T cells to advance TCR-based cancer immunotherapies

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Background

Antigen-specific T cells play a central role in the immune response against cancer. Precise identification and characterization of antigen-specific T cell receptors (TCRs) and their cognate peptide epitopes are critical for selecting candidates for TCR-based therapies and for monitoring T cell-mediated immune responses in patients. Given the highly individualized nature of cancer-specific T cell responses, shaped by the genetic diversity of the major histocompatibility complex, flexible and reliable TCR detection reagents are of utmost importance for successful therapy developments.

Methods

TCR-based therapy development demands analytical reagents that deliver consistent, reliable performance across diverse workflow stages. To meet this challenge, we developed MHC MACSimers, a novel MHC multimer technology engineered for optimized stability, enhanced binding avidity, and peptide-loading capabilities. Designed to support the entire TCR-based cancer therapy pipeline, from early discovery and translational research, to epitope screening, quality control, and patient monitoring, this poster highlights the seamless integration of MHC MACSimers across all phases of cancer drug development.

Results

1 Optimized T cell detection sensitivity and flexibility for cancer research and TCR discovery

In the early stages of TCR-based therapy developments, such as translational research, TCR discovery, and drug target screening, flexible and highly sensitive MHC reagents are essential. MHC MACSimer FleX Kits offer researchers and clinicians the versatility to screen a broad range of peptides, including cancer-associated antigens, through advanced peptide-loading technology. Additionally, MHC MACSimers incorporate REAlease® technology, allowing for peptide-MHC staining removal after flow cytometry sorting, which maximizes flexibility for downstream assays during research and early discovery (Figure 1A). Thanks to their unique reagent design, MHC MACSimers outperform commonly used MHC Tetramers, delivering superior staining performance and enhanced T cell detection sensitivity (Figure 1B).

Figure 1

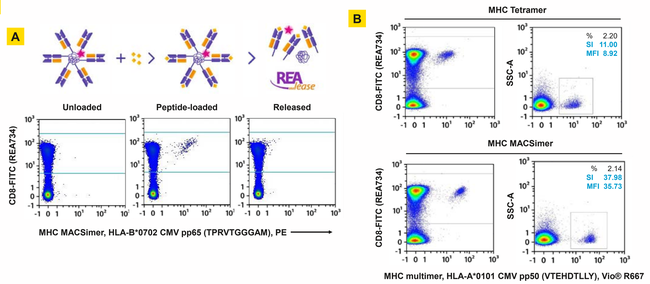


Figure 1: Optimized T cell detection sensitivity and flexibility. A) MHC MACSimer B*0702 FleX Kit, human, PE was loaded with MACSpep CMV pp65 according to standard loading protocol. Human HLA-B*0702-positive CMV-reactive CD8+ T cells were expanded and spiked into human PBMC. Cells were stained with unloaded MHC MACSimers as a control (left panel), or with peptide-loaded MHC MACSimers to detect virus-specific T cells (middle panel). In a third approach, MHC MACSimers were released from the T cells using REAlease® technology (right panel). B) MHC MACSimers in comparison to conventional MHC Tetramers. Human HLA-A*0101-positive CMV-reactive CD8+ T cells were expanded and spiked into human PBMC. Flow cytometry staining was performed using conventional MHC tetramers (based on biotinylated MHC monomers multimerized on streptavidin, upper panels) versus MHC MACSimers (optimized MHC MACSimer technology based on covalent conjugation, lower panels), both coupled to Vio® R667 for direct comparison of staining performance (stain index [SI] and mean fluorescent index [MFI]). Dot plots depict frequency of antigen-specific T cells among all viable CD8+ cells. CD8-FITC staining was performed with CD8 Antibody, anti-human, REAfinity™ (REA734). Measurements were performed using the MACSQuant® Analyzer.

2 Reliable sorting and enrichment of antigen-specific T cells across all drug development stages

Antigen-specific T cells in humans are rare and highly diverse, posing significant challenges for analysis. Yet, effectively isolating and enriching cancer-specific T cells is crucial for immunological and genetic studies that deepen our understanding of tumor-specific immune responses throughout all stages of drug development. MHC MACSimers empower reliable TCR-based cell sorting using flow cytometry (Figure 2A) and magnetic-activated cell sorting (by MACS® technology) (Figure 2B).

Figure 2

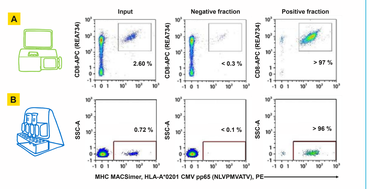


Figure 2: TCR-based sorting of antigen-specific CD8 T cells with MHC MACSimer. A) Flow cytometry-based sorting with the MACSQuant® Tyto Cell Sorter. Antigen-specific T cells from human PBMCs were stained with MHC MACSimer A*0201 CMV pp65, human, PE and subsequently co-stained with antibodies (CD14, CD20, CD4, CD8, and DAPI). Cells were sorted and analyzed by flow cytometry. B) Magnetic cell sorting using MACS® Technology. Antigen-specific T cells from human PBMCs were stained with MHC MACSimer A*0201 CMV pp65 (NLVPMVATV), human, PE and subsequently incubated with anti-PE MicroBeads. Magnetic separation of MHC MACSimer-positive cells was done using magnetic LS Columns. The original cell fraction (Input) is shown in comparison to negative and positive sorting fractions for both technologies.

3 Robust TCR analytics during TCR-T cell optimization, manufacturing, and quality control

In the later stages of TCR-based therapy development, spanning TCR selection and optimization, cell manufacturing, and quality control, the importance of reagent standardization becomes paramount. Peptide-loaded MHC MACSimers are routine-ready reagents, rigorously quality-controlled before release, offering a reliable and robust tool for precise T cell detection. This includes critical applications like measuring T cell transduction efficiency in engineered TCR-T cell products. Figure 3 illustrates MHC MACSimer-based detection of engineered TCR-T cells produced via the CliniMACS® Prodigy TCT-LS process, expressing NY-ESO-1- and MAGE-A4-specific TCRs.

Figure 3A

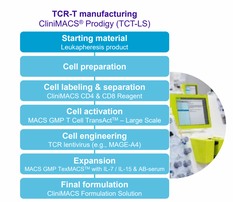


Figure 3B

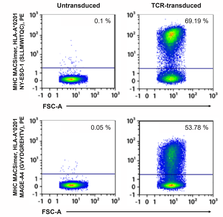


Figure 3: Analysis of TCR-transduction efficiency in human engineered TCR-T cells using MHC MACSimers. A) Engineered TCR-T cells were generated using the TCT manufacturing process on the CliniMACS® Prodigy platform. B) HLA-A*0201-positive NY-ESO1- or MAGE-A4-specific TCR-T cells were generated with a TCT small-scale process. Pan T cells were isolated from PBMCs, activated via T Cell TransAct™, and transduced with the respective lentivirus vectors, followed by an expansion period for 10 days. Afterwards, TCR-transduced T cells were stained with allele- and target-matched MHC MACSimers in PE (right panels). Untransduced (UTD) T cells served as a control (left panels). Antibody co-staining was performed with anti-CD8 VioGreen™ antibody (REA734) and DAPI (dilution 1:20). Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Dot plots depict the frequency of TCR-transduced versus UTD T cells among all viable CD8-positive cells.

4 Extended MHC MACSimer performance data for streamlined analytical reagent validation

As clinical cancer studies progress, the need for validated and standardized reagent workflows becomes critical. MHC MACSimers underwent extensive performance testing across key quality metrics, aligned with International Council for Harmonization (ICH) guidelines, covering reagent titration, linearity, specificity, and operator consistency. The results confirm that MHC MACSimers deliver exceptional robustness and sensitivity, making them the ideal solution for streamlined quality control of cell products and reliable patient monitoring throughout clinical trials (see Figure 4).

Figure 4A

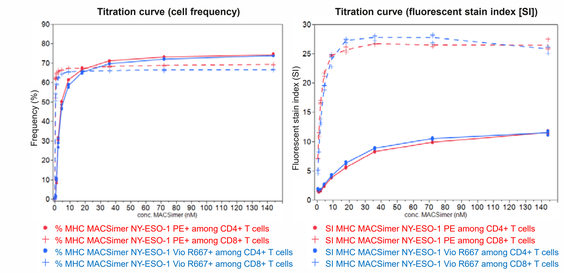


Figure 4B

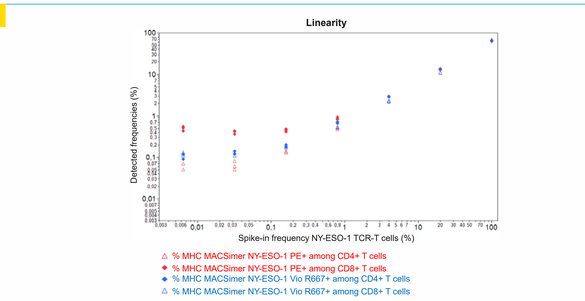


Figure 4C and D

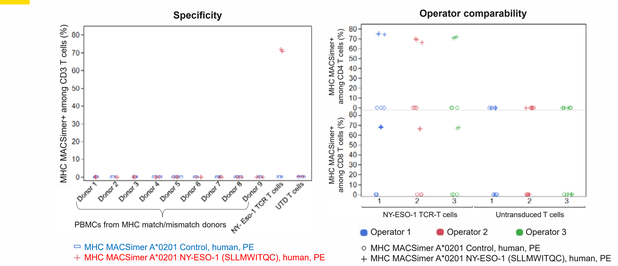


Figure 4: Performance data. All stainings were performed in triplicates using NY-ESO-1-TCR-engineered T cells or untransduced (UTD) T cells. MHC MACSimer A*0201 NY-ESO-1 (SLLMWITQC), human (in PE or Vio® R667) was used to detect TCRs. As negative control, MHC MACSimer A*0201 control reagent was used. Cells were co-stained with 7-AAD and REAfinity™ antibodies (CD4-VioGreen, CD3-FITC, CD8-VioBlue) and measured on the MQ® Analyzer. A) Titration: TCR-T cells were stained in a range from 0-144 nM. The left graph depicts frequencies of MACSimer+ cells under CD8+ or CD4+ and the right graph shows the stain index (SI). B) Linearity: TCR-T cells were spiked into UTD T cells. The graph shows frequencies of MACSimer+ cells among CD4+ and CD8+ cells plotted against the spike-in frequency. C) Specificity: TCR-T cells, UTD T cells, and PBMCs from nine MHC match/mismatch donors were stained with NY-ESO-1-specific MACSimers or MACSimer control reagents. Frequencies of MACSimer+ cells among CD3+ cells are shown. D) Operator comparability: Three operators performed stainings of TCR-T and UTD T cells with NY-ESO-1-specific MACSimers or MACSimer control reagents. Frequencies of MACSimer+ cells among CD4+ and CD8+ cells are shown.

Conclusion

MHC MACSimers are advanced TCR detection reagents designed to deliver highest performance, flexibility, and isolation efficiency across research workflows and various clinical stages of T cell-based cancer immunotherapy.

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