Abbreviated T-Cell Activation on the Automated Clinimacs Prodigy Device Enhances Bispecific CD19/22 Chimeric Antigen Receptor T-Cell Viability and Fold Expansion, Reducing Total Culture Duration

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BACKGROUND

As clinical applications for Chimeric Antigen Receptor (CAR) T-cell therapy expand, cell manufacturing incorporating closed-system, automated instruments are supplanting traditional open-system, labor-intensive culture methods. The CliniMACS Prodigy (Miltenyi Biotec), a closed-system automated device, has demonstrated success in the production of CAR T-cells from T-cell enrichment, activation, viral transduction, and expansion to downstream harvest for cryopreservation/fresh infusion. The duration of T-cell activation/viral transduction, and total T-cell culture duration vary across centers (2-5 days and 7-12 days, respectively) and merit evaluation prior to routine use.

STUDY AIMS

To **compare** bispecific CD19/22 **CAR-T** cell **product purity and potency** across the 2 manufacturing methods.

Original method (OM): TransAct CD3/CD28 reagent mediated **T-cell activation/stimulation** and lentiviral transduction (MSCV-CAR1922-WPRE; Lentigen Inc.) was **terminated with a wash step at Day 5.**

Modified method (MM): TransAct CD3/CD28 reagent mediated T-cell activation/stimulation and lentiviral transduction (MSCV-CAR1922-WPRE; Lentigen Inc.) was terminated with a **wash step earlier, at Day 3.**

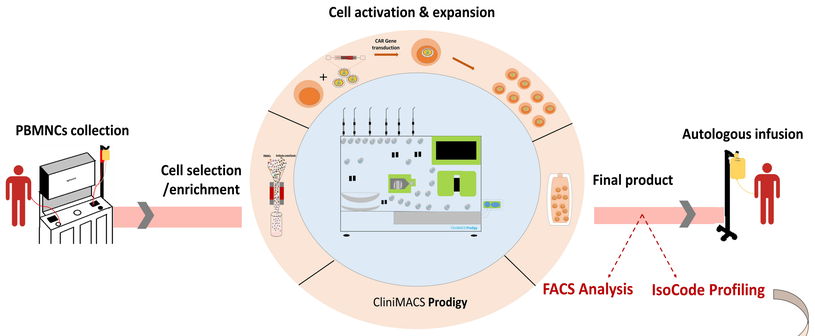
Hypothesis: In the OM runs (with more prolonged T-cell stimulation and activation), the relatively more sensitive patient cells experience suboptimal viability and cell expansion

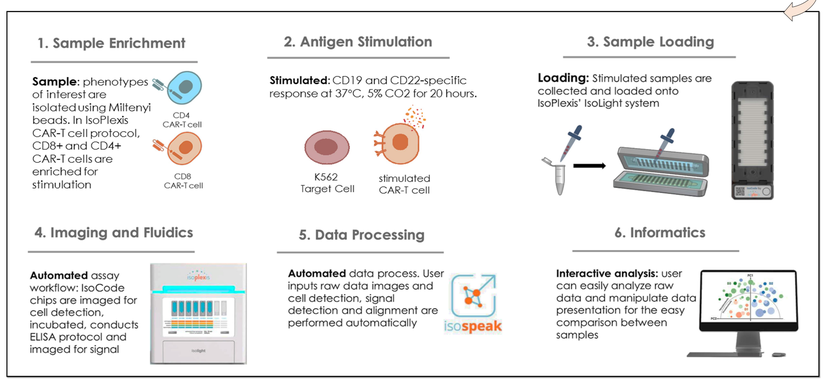
METHODS

A total of **4 apheresis products** were evaluated using the **MM** and compared with the **4 prior runs using the OM**.

Products were obtained from live or deceased patients with disease profiles similar to patients on the clinical trial. Other process parameters (enrichment for CD4/CD8 subsets, in-process media changes with GMP-TexMACS Medium supplemented with human IL-2 (200IU/mL) and 3% human AB serum) were kept unchanged across the 2 methods. Cells were harvested between days 7-12, when dose criteria were met.

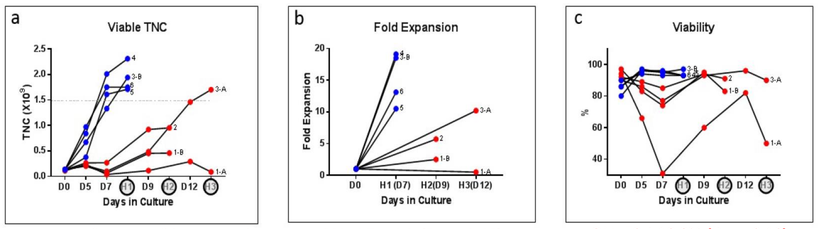
Transduction by Protein L expression, viability and cell phenotype (CD3, CD4/CD8) were measured by **flow cytometry**. For potency analysis, antigen-specific polyfunctional cytokine upregulation in CAR-T cell products was measured on 5 samples by the **single-cell 32-plex IsoCode chip proteomics.**





RESULTS

Significantly higher Viable TNC, Fold Expansion, Viability, Final CD3% and Transduction Efficiency in products manufactured using the MM compared to OM; Comparable CD4/CD8 ratios across the 2 methods.



OM: 0.8x10⁹ ± 0.7x10⁹ (Days 9-12)
MM:1.93x10⁹ ± 0.27x10⁹ (Day 7)

OM: 4.7 ± 4.2 (Days 9-12)
MM: 15.3 ± 4.2 (Day 7)

OM: 79 ± 19.3% (Days 9-12)
MM: 94 ± 2% (Day 7)

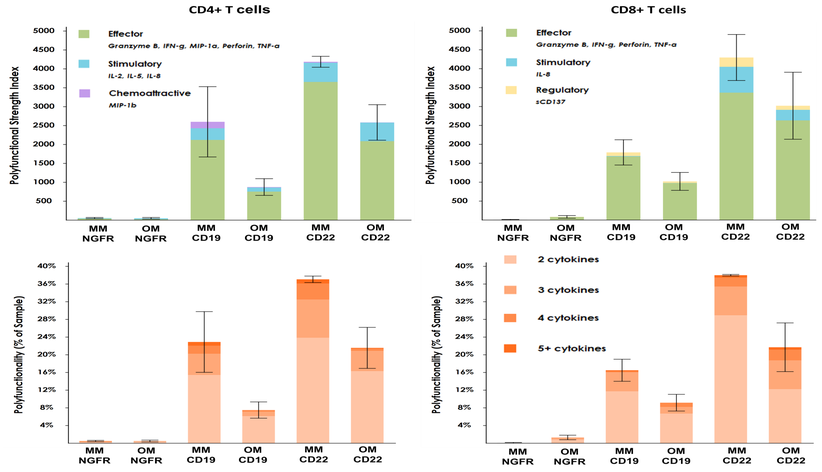
OM: 96.7 ± 3.3% (Days 9-12)
MM: 99.7 ± 0.3% (Day 7)

OM: 2.6 ± 1.3 (Days 9-12)
MM: 5.6 ± 3.6 (Day 7)

OM: 71.9 ± 5.3% (Days 9-12)
MM: 79.7 ± 3.9% (Day 7)

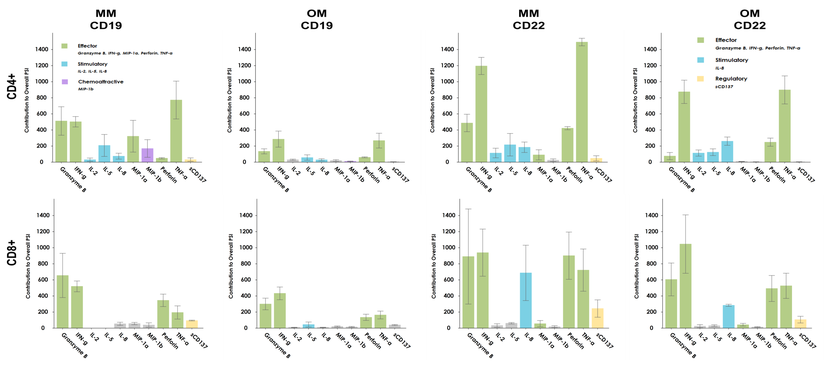
Old method (D5 wash) **Modified method (D3 wash)** **H1, H2, H3: harvest days depending on cell dose**

Consistently more robust polyfunctional response of CD4+ or CD8+ CAR-T cell products to CD19 or CD22 with MM compared to OM.

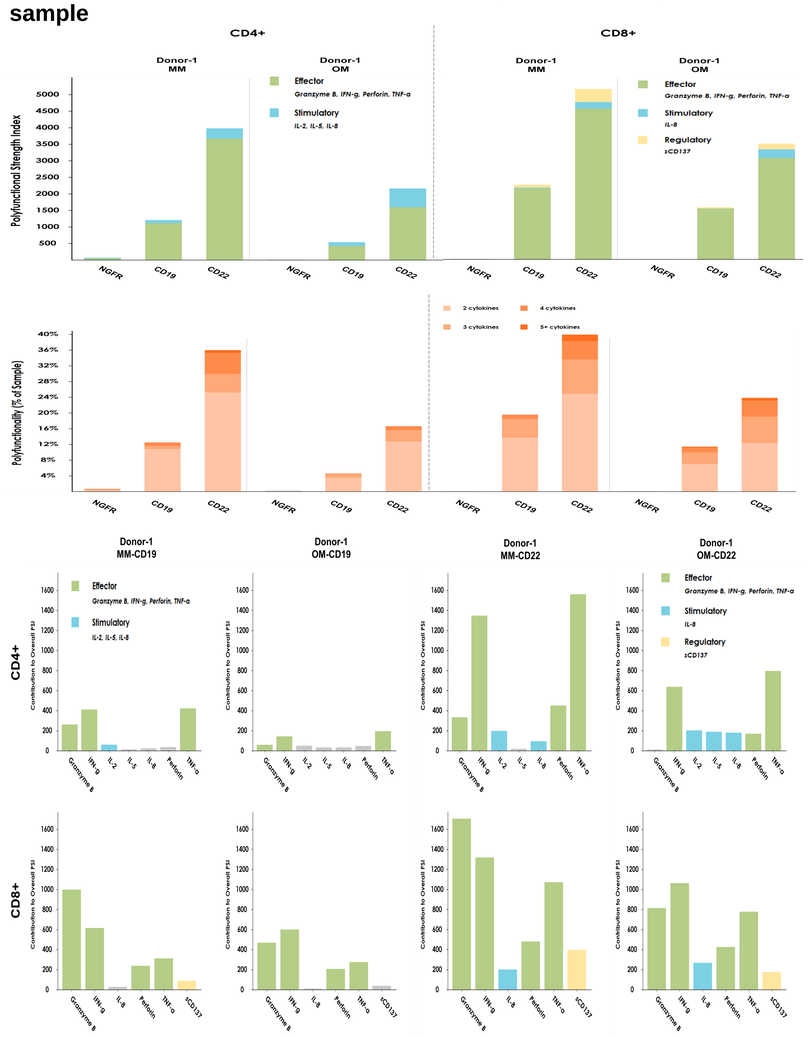


Polyfunctionality : co-secreting at least 2 or more proteins per single cell

IFN-γ, TNF-α: Primary drivers for enhanced CD4 PSI induced by CD19/ CD22 antigen; Granzyme B, IFN-γ, Perforin, TNF-α: primary drivers for enhanced CD8 PSI by CD19 or CD22 antigen.



Higher increase of Polyfuctional Strength Index (PSI) with the MM than the OM in both CD4+ and CD8+ CAR-T cell products in a paired donor sample



CONCLUSIONS

- Our data demonstrate that the modified CD19/CD22 Bispecific CAR T-cell manufacturing method (MM) which terminated T-cell activation/transduction by culture Day 3, resulted in reproducible and robust CAR T cell production, even in the relatively more sensitive patient cells.
- Viability, Viable TNC recovery, CD3% and Protein L expression were consistently higher with the modified method (MM) compared to the old method (OM). All final products using the MM met product release criteria.
- In addition, final product dose requirements were consistently met by culture Day 7 when using the MM, augmenting process efficiency. Consequently, we have adopted the MM for the manufacture of clinical CD19/CD22 Bispecific CAR T cells.
- Compared to the OM, the MM promotes more robust polyfunctional response of CD4+ or CD8+ CAR-T cell products to CD19 or CD22. These results were consistent in a paired patient sample set analyzed separately.
- Additional potency studies have been initiated to compare differences in T-cell subsets, activation/exhaustion/senescence and differentiation markers, and the metabolic activity of cells manufactured by the 2 methods.

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