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Weldable Workflow: Streamlining Cytokine Use For Closed-System CAR-T Manufacturing

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Abstract

Background: CAR-T cell manufacturing currently requires reconstitution and open handling of lyophilized cytokines to supplement media. Open processes necessitate extra cleanroom time, higher ISO requirements and manual interventions that increase the risk of user error and contamination. Transitioning to fully closed workflows is limited by the need for cytokines in a sterile weldable format. This study evaluated ProPaks, ready-to-use GMP liquid cytokines in weldable, single-use bags for integration into closed-system processes. We directly compared IL-7 and IL-15 as liquid-bagged GMP cytokines to lyophilized GMP cytokines across open research-scale and closed 1 liter manufacturing-scale formats to support T cell expansion, viability, and generation of desirable memory subsets including stem cell memory (T_{SCM}) and central memory (T_{CM}) cells.

Methods: GMP IL-7 and IL-15 cytokines were assessed in lyophilized and liquid-bagged formats by recovering cytokines into bottled or 1 L bagged GMP Human T cell media. Across multiple donors, purified $CD4^+$ and $CD8^+$ T cells were evaluated for expansion, viability, CD19-CAR lentiviral transduction efficiency, generation of T_{SCM} and T_{CM} subsets, and in vitro potency assays including bioluminescent tumor-killing and IFN-γ secretion. Scalability was examined by comparing an open-format 6-well plate to a closed 1 L G-Rex® bioreactor.

Results: Liquid-bagged cytokines were added to media 5× faster than lyophilized cytokines requiring reconstitution and aliquoting. Liquid bagged cytokines were fully recovered into bagged media and demonstrated equivalent bioactivity to lyophilized cytokines, supporting robust T cell expansion and viability. Equivalent T cell expansion was observed between the open and closed manufacturing processes. Both cytokine formats across research and manufacturing scales sustained efficient CD19-CAR transduction and generated comparable frequencies of T_{SCM} and T_{CM} subsets. CAR-T cells produced in both workflows displayed high potency, confirmed by target cell killing and IFN-γ secretion.

Conclusions: This study highlights the translatability of GMP cytokines from lyophilized to liquid-bagged formats, as well as the scalability of T cell manufacturing from open research-scale to closed 1 liter clinical-scale production. GMP-grade IL-7 and IL-15 supplied in weldable, single-use liquid bags streamline cytokine handling in closed-system CAR-T manufacturing. Closed system cytokines reduce contamination risk, manual interventions, and cleanroom burden while maintaining equivalent CAR-T expansion, phenotype, and functional potency compared to lyophilized cytokines. This approach supports the continued shift toward fully closed, efficient manufacturing workflows.

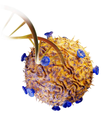
Materials and Methods

Media and Culture

T cells were cultured in GMP Human T cell media supplemented with 5% Human AB Serum and 10 ng/mL of IL-7 and IL-15 cytokines and cultured in G-Rex bioreactors (ScaleReady™).
T cells were activated, transduced with CD19-CAR and eGFP encoding lentivirus, and expanded for a total of 8 days.

Cytokine Quantification

Media containing IL-7 and IL-15 cytokines from lyophilized or bagged-liquid formats were run on human cytokine-specific Simple Plex™ cartridges on the Ella™ Instrument. Manual standard curves were generated with lot matched cytokines.



T Cell Analysis

T cell counts and viability were collected on a NucleoCounter® NC-200™ cell counter. T cells were stained with LIVE/DEAD™, antibodies against CD3, CD4, CD8, CD45RA, CD45RO, CD62L, CCR7 and a CD19-fluorokine protein, and assessed by flow cytometry. Transposition efficiency was determined as LIVE/DEAD$^-$ $CD3^+$ $eGFP^+$ and/or CD19-CAR^+ cells. T cell subsets are identified as: Stem Cell Memory (T_{SCM}, $CD45RA^+/CCR7^+$), Central Memory (T_{CM}, $CD45RA^-/CCR7^+$), Effector Memory (T_{EM}, $CD45RA^-/CCR7^-$) and Effector (T_{EFF}, $CD45RA^+/CCR7^-$) cells.

Potency Assays

Cytotoxicity assays were performed by co-culturing CD19-CAR-T effector cells with $CD19^+$ Nalm-6 target cells at varying effector-to-target (E:T) ratios. Following overnight incubation, supernatants were collected, cells were lysed, and luminescence was measured after addition of assay buffer and substrate. Specific killing was calculated as: Killing (%) = [1 – (Sample Luminescence/Control Luminescence)] x 100.
Wells containing only target cells served as controls. Supernatants were analyzed using Simple Plex cartridges to detect Granzyme B, IL-2 IFN-γ, and TNF-α on the Ella.

Table 1: Key R&D Systems Reagents

Material	Catalog Number
GMP Human T Cell Media	CCM038-GMP-1L CCM038-GMP-1B
GMP Human AB Serum	HABS001-GMP-100ML HABS001-GMP-50B
Recombinant Human IL-7 GMP Protein, CF	BT-007-GMP
ProPak™ Recombinant Human IL-7 GMP Protein	PPK-007-GMP-010
Recombinant Human IL-15 GMP Protein, CF	BT-015-GMP
ProPak Recombinant Human IL-15 GMP Protein	PPK-015-GMP-010
G-Rex 6M Well Plate (ScaleReady)	80660M
G-Rex 100M-CS (ScaleReady)	81100-CS
Recombinant Human CD19 Protein, Atto 647N Conjugate	ATM9269-020
Simple Plex Human IL-7 Cartridge	SPCKB-PS-000506
Simple Plex Human IL-15 Cartridge	SPCKB-PS-000500
Simple Plex Cell Activation Panel 1	ST01C-CS-003222

Study Design

Comparison of open system, research scale to closed system clinical scale CAR-T cell workflows.

We designed experiments to directly compare open, research scale and closed, clinical scale CAR-T cell workflows.

Table 2: Summary of conditions and reagents for open, research scale and closed, clinical scale workflows in this study.

Workflow	G-Rex	Cell #	Media	huAB Serum	Cytokine
Open, R&D Scale		1e7			
Closed, Clinical Scale		1e8			

Results

GMP cytokines packaged in process-sized, single-use bags are successfully recovered into 1 liter media bags.

We assessed recovery of bagged-liquid GMP cytokines into bagged GMP Human T Cell Media. In parallel, GMP lyophilized cytokines were added to bottled media. Cytokine concentrations were measured using Simple Plex assays on the Ella platform using manual standard curves. We observed full recovery of cytokines in both formats.

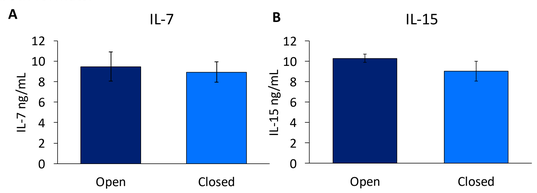


Figure 1: Simple Plex assays on the Ella Platform were used to quantify ProPak and lyophilized GMP cytokine concentrations in GMP Human T Cell Media for **A)** IL-7 and **B)** IL-15. Data is the average of 4 independent experiments with error bars ±SD.

Open and closed workflows support robust T cell expansion and viability with increased CAR-T yield at clinical scale.

We compared research scale open system workflow to closed system workflow to generate CAR-T cells. No transduction (No TD) samples were grown as controls in the open workflow. We observed robust T cell expansion and high viability in both workflows. Equivalent transduction efficiency was observed with 10x the yield of CAR-T cells in the clinical scale workflow.

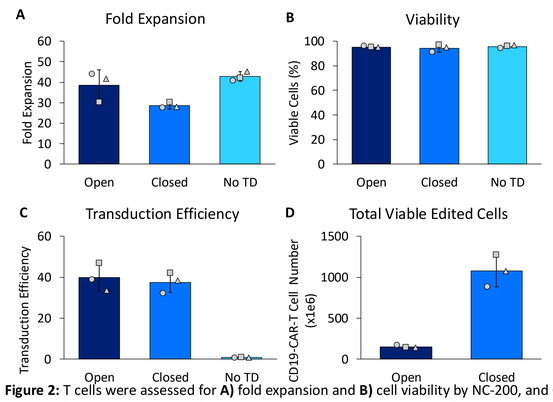


Figure 2: T cells were assessed for **A)** fold expansion and **B)** cell viability by NC-200, and **C)** transduction efficiency for CD19-CAR expression by flow cytometry. **D)** Total viable edited cells were calculated by multiplying total live cells by % of $CD19\text{-}CAR^+$. Data is the average of 3 donors, error bars ±SD. Donor 1 (circle), 2 (square) and 3 (triangle).

High frequencies of T_{SCM} and T_{CM} subsets are achieved with low frequencies of PD-1 expression in both workflows.

Improved patient outcomes in CAR-T therapies are correlated with higher frequencies of T_{SCM} and T_{CM} cells and reduced exhaustion marker expression[1,2]. At the end of each workflow, we assessed T cell memory subset frequences in addition to PD-1 expression. $CD4^+$ and $CD8^+$ T cells demonstrated >60% combined frequencies of T_{SCM} and T_{CM} cells and low frequencies of $PD\text{-}1^+$ cells in both workflows.

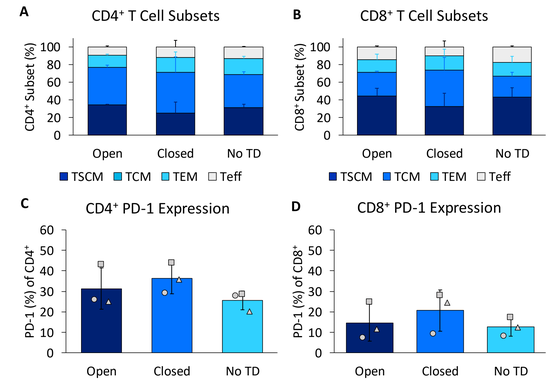


Figure 3: Flow cytometry analysis of T cell memory subset frequencies in **A)** $CD4^+$ and **B)** $CD8^+$ T cells. Flow cytometry analysis of PD-1 expression in **C)** $CD4^+$ and **D)** $CD8^+$ T cells. Data is the average of 3 donors, error bars ±SD. Donor 1 (circle), 2 (square) and 3 (triangle).

CD19-CAR-T cells generated in both workflows specifically kill $CD19^+$ target cells and exhibit robust cytokine secretion.

Potency assays were performed to assess the functionality of CAR-T cells generated in open and closed system workflows. CD19-CAR-T cells specifically killed $CD19^+$ Nalm-6 cells and were highly functional by secreting IFN-γ, Granzyme B, IL-2 and TNF-α.

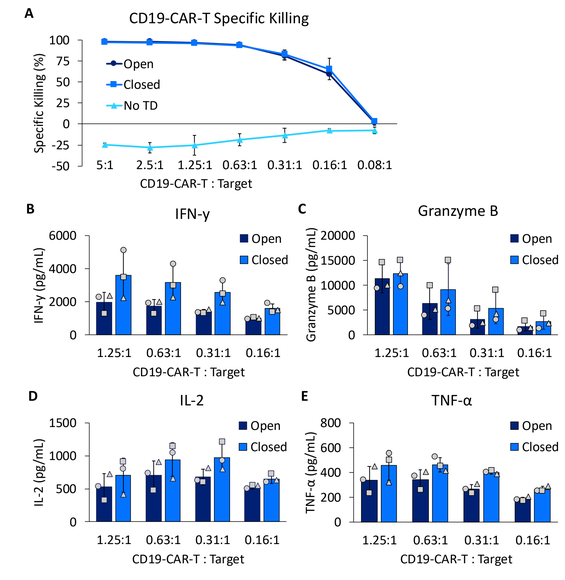


Figure 4: (A) Bioluminescence-based cell potency assay and **(B-E)** Simple Plex assays on the Ella platform quantify IFN-γ **(B)**, Granzyme B **(C)**, IL-2 **(D)** and TNF-α **(E)** secretion. Data is the average of 3 donors, error bars ±SD. (B-E) Donor 1 (circle), 2 (square) and 3 (triangle).

Conclusions

- Here we demonstrate an easy transition from open to closed CAR-T cell workflows with equivalent cytokine recovery and maintained high levels of T cell growth, viability and transduction efficiency.
- Research scale workflows are seamlessly transitioned to clinical-scale with 10x greater CD19-CAR-T cells in 100M-CS than 6M G-Rex plates.
- Both workflows support the generation of quality T cells with high frequencies of T_{SCM} and T_{CM} memory subsets and low PD-1 expression, which correlate with improved patient outcomes[1,2].
- Potent CAR-T cells were easily quantified with Simple Plex cartridges on the Ella.
- All in all, the closed system workflow enables scalable, streamlined, and contamination-controlled manufacturing of high-potency CAR-T cells.

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References

1. Deng, Q., *et al.* Nat. Med. 2020
2. Finney, C. *et al.* J. Clin. Invest. 2019