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Scalable Hematopoietic Stem Cell Expansion in Stirred-Tank Bioreactors for Advancing Cell Immunotherapies

ImmuneBridge

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Introduction

- **HSCs as a Source:** Hematopoietic stem cells (HSCs) differentiate into NK, T, and myeloid cells and are foundational for scalable, off-the-shelf allogeneic immune cell therapies. Among available sources, cord blood (CB) contains a higher proportion of primitive CD34⁺ cells with superior proliferative capacity and greater stemness compared to bone marrow (BM) or peripheral blood (PB), making it an ideal source for clinical manufacturing.
- **Challenges:** Conventional 2D culture systems are inefficient, costly, and labor-intensive, lacking scalability for large-scale clinical and commercial production.
- **Approach:** To overcome these challenges, our study developed an efficient and scalable platform using the following methodology:
  - **Process development and optimization:** CD34⁺ cells were expanded using the Ambr®15 cell culture system to identify optimal conditions and establish a scalable, cost-effective workflow for cord blood-derived HSC manufacturing.
  - **HSC differentiation into NK cells:** To assess the effect of upstream CD34⁺ culture conditions on NK cell development, expanded HSCs were differentiated within the Ambr®15 system and evaluated for key phenotypic markers and functional characteristics.

Experimental Overview: HSCs Expansion Workflow

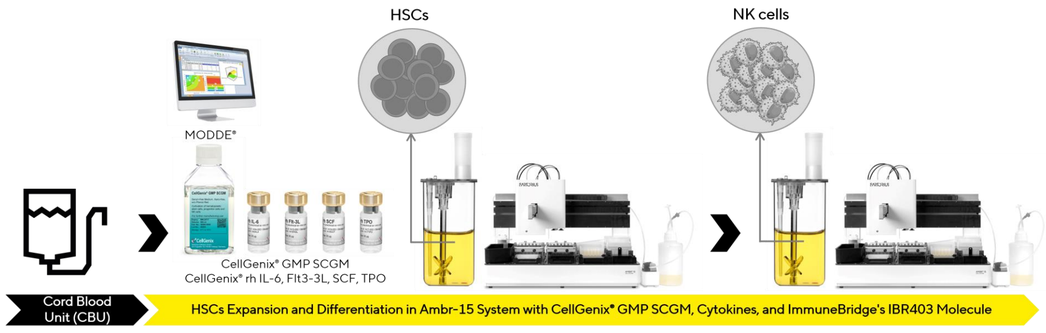


Figure 1: Workflow of cord blood-derived HSC expansion and NK cell differentiation.

Method and Results:

A. Process Optimization- Experimental Design Considerations

- **Media:** GMP-grade CellGenix® SCGM was evaluated against RUO control media, used in 2D cultures, to assess consistency and support CD34⁺ expansion. Cultures were supplemented with IBR403, SCF, Flt3-L, TPO, and IL-6- cytokines known to promote HSC survival, proliferation, and maintenance of stemness.
- **Seeding Strategy:** Cord blood units (CBUs) were thawed and cultured under two distinct conditions to evaluate timing and dilution effects on CD34⁺ cell expansion:
  - Early seeding (Figure 2): Undiluted cells were inoculated into Ambr®15 on Day 3 post-thaw.
  - Late seeding (Figure 3): Cells remained in static culture until Day 7, then inoculated into Ambr®15 after 1:5 dilution.
- **Feeding Strategy:** Feeding rates of 0.5, 1.0, and 2.0 VVD (vessel volumes per day)were mimicked in Ambr®15 using discrete medium exchange to replicate cytokine removal similar to continuous perfusion.

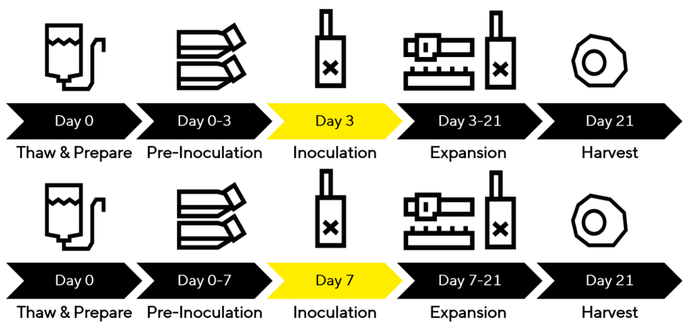
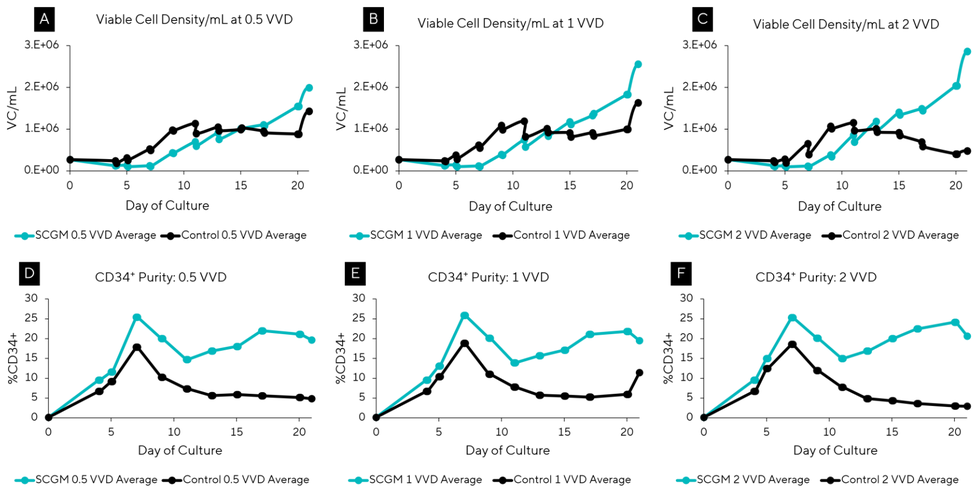


Figure 2: Early seeding workflow – Day 3 inoculation into Ambr®15

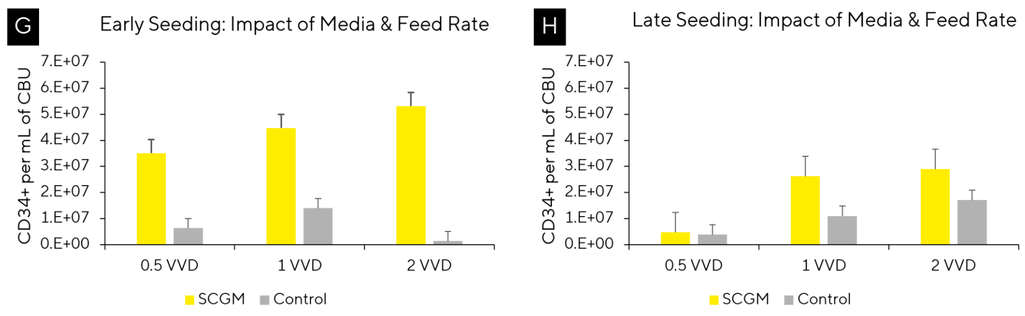
Figure 3: Late seeding workflow – Day 7 inoculation into Ambr®15

Process Optimization- Results

- **Higher Cell Growth (Figures A – C):**
  HSCs cultured in GMP-grade SCGM demonstrated consistently higher viable cell density (VCD) across all VVD conditions, with over 2× greater VCD at harvest compared to RUO control media.
- **Improved CD34⁺ Purity (Figures D – F):**
  Cultures in SCGM showed a marked increase in CD34⁺ purity over time, with the most significant gains observed at 1.0 and 2.0 VVD feeding rates.



- **Early Seeding Advantage (Figures G – H):**
  Early seeding resulted in a 3× increase in CD34⁺ purity (13.2% vs 4.5%) and nearly 3× higher viable cell density (1.8E6 vs 6.6E5 vc/mL) compared to late seeding workflow. The highest overall CD34⁺ yield (>6E7 cells/mL of input CBU) was achieved under early seeding with GMP-grade SCGM at 2 VVD.



B. MODDE®- Driven Cytokine DOE : Optimizing CD34⁺ Expansion & Validation Runs

- **DOE identified reduced cytokine combinations for efficient CD34⁺ expansion in SCGM:**
  Cytokine inputs (0–100 ng/mL each for SCF, TPO, Flt3-L, IL-6) were evaluated across 3 donors and 2 feeding rates (0.5 & 2 VVD). The design focused on low cytokine input to achieve cost efficiency while maintaining CD34⁺ expansion outcomes. While higher cytokine levels produced the greatest CD34⁺ yield, the SCGM – DOE Optimized (R10) condition achieved 70–80% of the yield of SCGM – 100%, at approximately 45% of the cytokine cost.

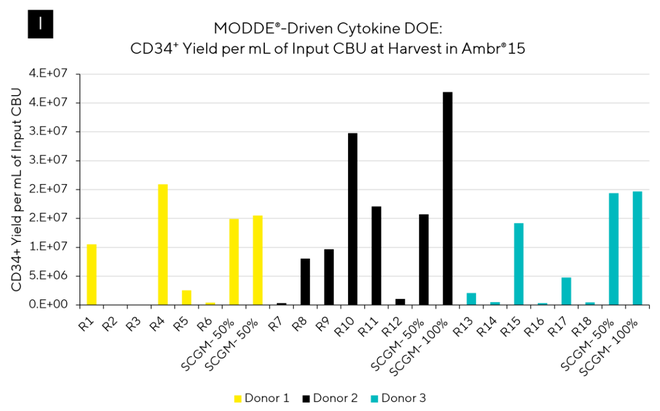
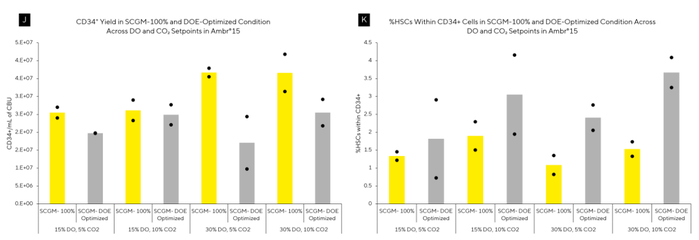


Figure I (Day 21):

- All experiment conditions were conducted in SCGM medium.
- Control conditions used SCGM with fixed cytokine levels: 100% (100 ng/mL each) and 50% (50 ng/mL each)
- DOE conditions (R1–R18), also in SCGM, explored variable cytokine combinations ranging from 0 to 100 ng/mL per cytokine, as designed using MODDE®

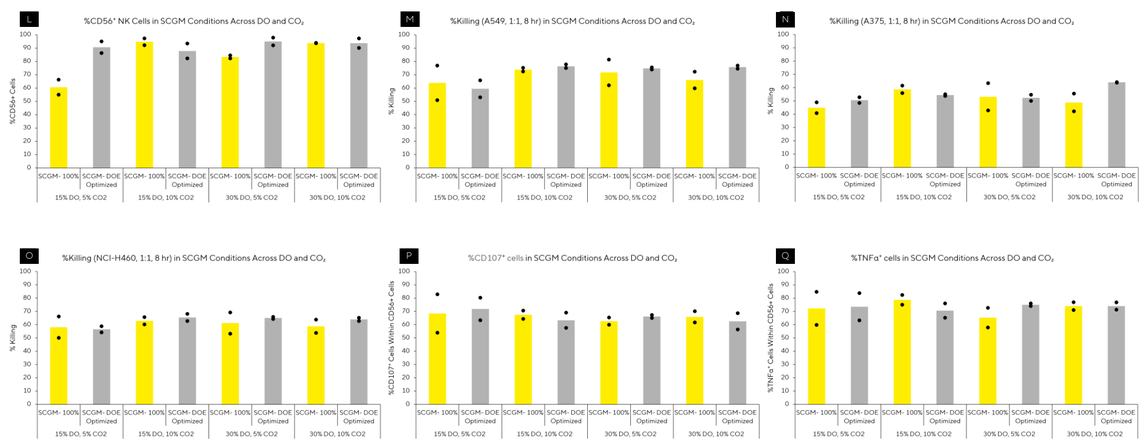


Figures J & K (Day 21):

- SCGM- DOE Optimized (R10) was confirmed as optimal. Gas setpoints (15% vs. 30% DO, 5% vs. 10% CO_2) had no significant impact on CD34⁺ yield. The optimized condition achieved >80% of the CD34⁺ yield with a consistently higher proportion of HSCs across 2 donors.

C. NK Cell Differentiation (CBU-HSCs): Purity, Cytotoxicity, & Cytokine Secretion

- As a proof of concept, cells were differentiated from CBU-HSCs to NK, achieving 86% purity after 28 days. Figures L- R, show NK differentiation metrics across purity, killing, and cytokine secretion for SCGM-100% and SCGM-DOE optimized (R10) conditions, under varying DO and CO2 bioreactor setpoints (N=2 donors).



Figures L – R:

- Target cell line: A549 (lung adenocarcinoma), NCI-H460 (large-cell lung carcinoma) and A375 (melanoma)
- In vitro-derived NK cells showed cytotoxicity with target killing rates of 50% for A375, 65% for A549, and 57% for NCI-H460. Stimulation induced IFN-γ (41.4%), CD107a (64%), and TNF-α (68%) expression.

Conclusion

- Successfully optimized expansion of CBU-derived HSCs and NK differentiation in Ambr®15 system with CellGenix® SCGM, CellGenix® cytokines (SCF, Flt3-L, TPO, IL-6), and IBR403.
- MODDE®-driven DOE and higher medium exchange rates enabled efficient CD34⁺ expansion with reduced cytokine input.
- Established a scalable, donor-independent platform for HSC-derived immune cell therapies targeting oncology and immune disorders.