Optimizing Perfusion Parameters Using Quality by Design for the Intensified Production of CAR-T Cells in a Single-Use Automated Stirred-Tank Bioreactor

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Introduction

- The *ex vivo* expansion of patient chimeric antigen receptor (CAR) T cells to therapeutic doses is often challenging and represents the longest phase of manufacturing.
- Scalable expansion processes that maximize CAR-T cell yields and reduce vein-to-vein time are therefore required to meet rising demand at reduced costs.
- This study systematically investigates the optimization of perfusion parameters to maximize CAR-T cell growth and quality in a single-use, automated stirred-tank bioreactor, with a view to reducing process time for autologous processes and increasing yields for allogeneic modalities.

Methods

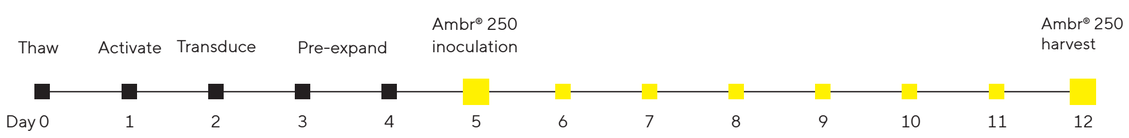
- A design of experiments (DOE) approach was applied to assess the impact of critical perfusion parameters A and B, and donor variability on the expansion of CAR-T cells in the Ambr® 250 High Throughput Perfusion single-use stirred-tank bioreactor. A total of seventeen week-long perfusion cultures were performed in serum-free 4Cell® Nutri-T Medium.
- Anti-CD19 CAR-T cells were generated via retronectin-assisted lentiviral transduction and pre-expanded in static flasks before inoculation into the bioreactor.
- Measured outcomes included daily cell counts and immunophenotypic characterization of T cells at inoculation and harvest in the Ambr® 250.

Table 1: *Factors and levels investigated in the perfusion DOE.*

Factor	Level		
	Low	Mid	High
Perfusion Parameter A	1	2	3
Perfusion Parameter B	0.25	0.5	1
Healthy Donor	1	2	3

Image 1: *Ambr® 250 High Throughput Perfusion unbaffled ATF vessel*

Figure 1: *Experimental overview.*



Perfusion Supports up to 4.5× Improvement in Final Cell Yields

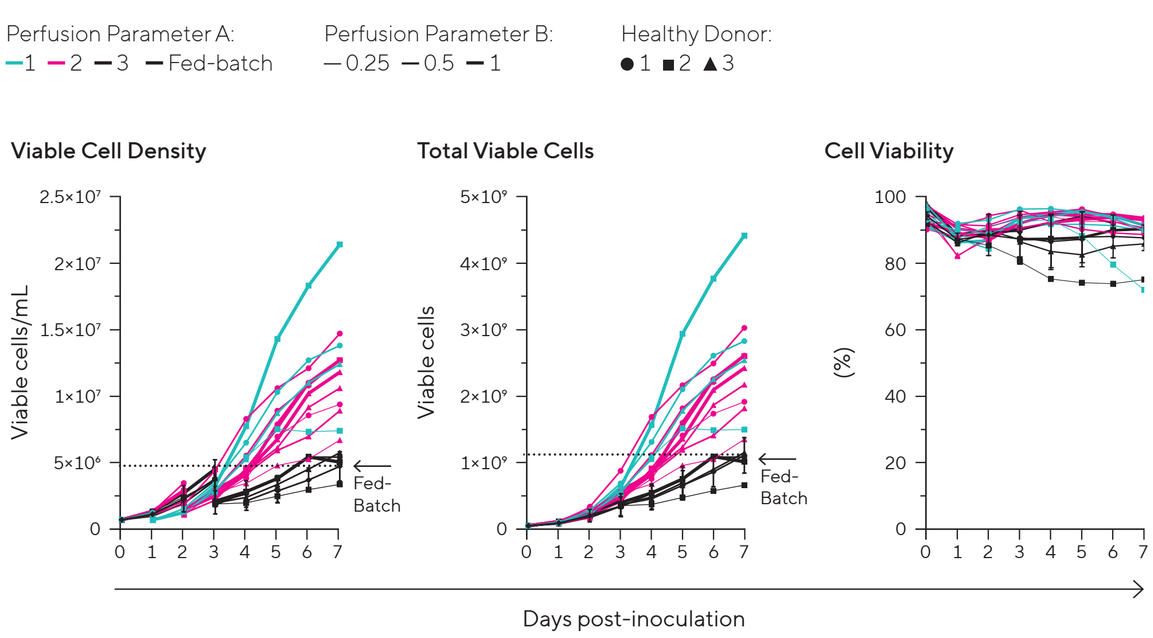


Figure 2: *Implementing perfusion significantly improved cell growth compared to fed-batch, while maintaining > 90% cell viability.*
Daily viable cell density, cell yield, and viability counts for all 17 DOE bioreactor runs.

Optimal Perfusion Parameters Are Donor-Dependent

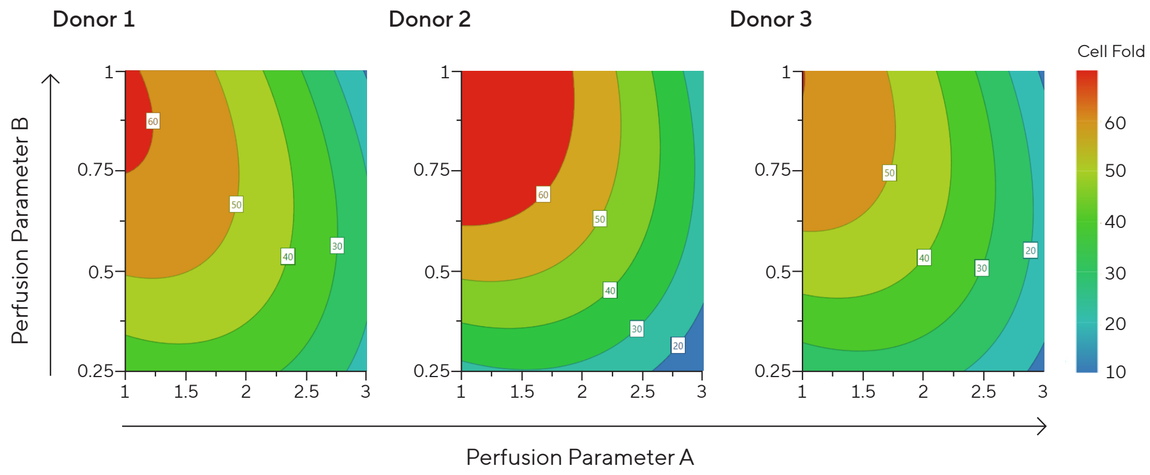


Figure 3: *The optimal combination of perfusion parameters for the expansion of CAR-T cells varies by healthy donor.*
Contour plots generated in MODDE® software model effects of perfusion parameters A and B on CAR-T cell fold-expansion in 4Cell® Nutri-T Medium in Ambr® 250.

Harvested CD8+ T Cells Are Rich in Naïve|Central Memory Subsets

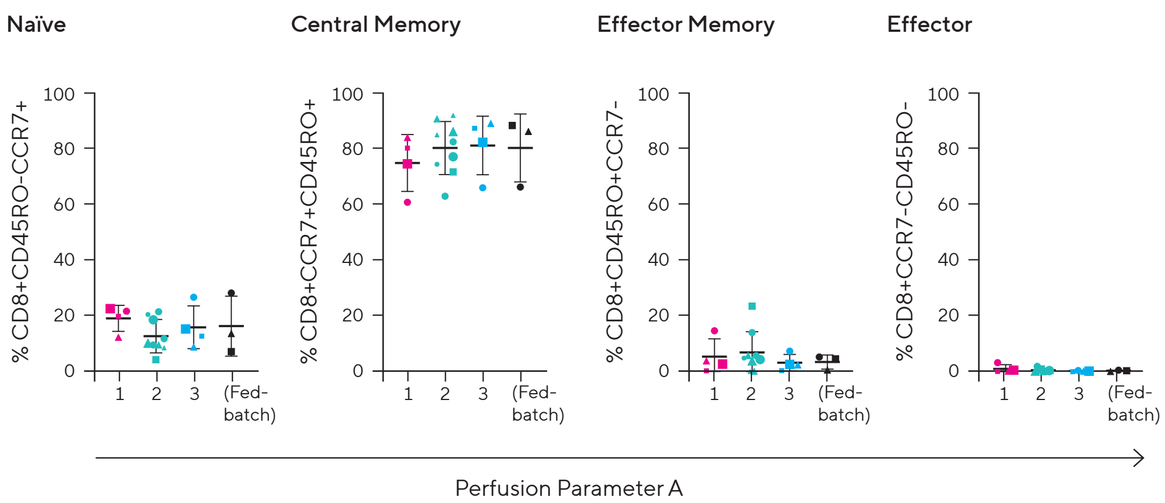


Figure 4: *CD8+ T cells harvested from the Ambr® 250 were predominantly of naïve and central memory subsets, irrespective of the perfusion parameters.*
Differentiation marker expression for CD8+ T cells harvested from 17 DOE bioreactor runs.

Perfusion Could Reduce Time-To-Patient by up to 50%

a)

Process	Total doses produced[1]	Expansion time for 1st dose	Avg. medium for 1 dose	Vessel required
Fed-batch	1	~7 days	~250 mL	1 × Ambr® 250
Optimized perfusion	4	~3 days	~400 mL	1 × Ambr® 250

Assumes 1e9 viable cells per dose. Data shown is specific to the expansion phase only. [1]Total doses produced over a 1-week expansion in Ambr® 250.

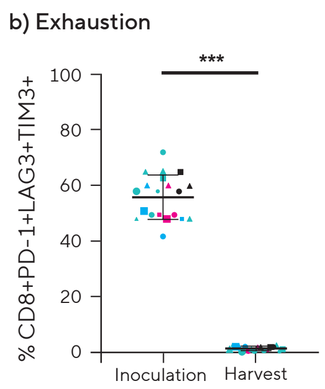


Figure 5: *Implementing perfusion significantly improved process efficiencies and throughput, without negatively impacting cell exhaustion at harvest.*
a) Process efficiencies for fed-batch vs optimized perfusion process in the Ambr® 250;
b) Exhaustion marker expression of CD8+ T cells harvested from 17 DOE bioreactor runs.

Conclusion

- Optimizing perfusion parameters in the Ambr® 250 stirred-tank bioreactor led to 4.5× higher CAR-T cell yields than the fed-batch process, and reduced the expansion time required to reach a therapeutic CAR-T dose of 1e9 viable cells by up to 50%.
- Irrespective of the perfusion conditions, the majority of harvested CD8+ T cells were in naïve|central memory subsets and displayed very low exhaustion marker expression.
- This work highlights the benefits of perfusion versus fed-batch when intensifying the production of high-quality CAR-T cells in stirred-tank bioreactors. In addition, it emphasizes the importance of optimizing the perfusion parameters to maximize cell yields, reduce costs and shorten process timelines.