Increasing Lentiviral Transduction Efficiency: Towards Cost-Effective T Cell Therapy Manufacturing

Benjamin A. F. Blaha[1], Alexia Toufexi[1], Gregory Berger, Enas Hassan, and Nicholas R. Gaddum

Cell and Gene Therapy Catapult, 12th Floor Tower Wing, Guy's Hospital, Great Maze Pond, London, SE1 9RT, United Kingdom

1 Introduction

Challenge: High-cost manufacturing processes of Chimeric Antigen Receptor (CAR) T-cells therapies are prohibitively expensive (Fig. 1) [1,2]. Due to the cost of virus, transduction is a major cost driver in CAR T-cell manufacturing. Several bioprocessing parameters have been identified as potentially playing a role in transduction efficiency, such as the physical proximity of lentivirus particles to T cells. This proximity could be manipulated through (1) the number of cells and virus particles in the suspension; (2) the periods of agitation to encourage homogeneity; and (3) the surface-to-volume ratio in the transduction vessel [3]. However, limited research has been performed on identifying and optimising critical process parameters of transduction.

Aim: Investigate sensitivities of critical bioprocess parameters upon T-cell transduction.

Manufacturing Process of CAR T-cell Therapy

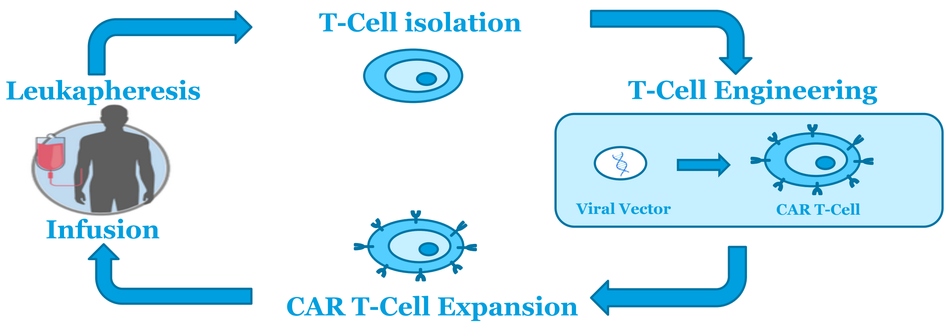


Figure 1. Schematic representation of the manufacturing process of CAR T-cell therapies.

2 Methods

A cryopreserved CD3+ T-cell bank generated from fresh leukapheresis was used. Over a period of four days, transduction of cells was achieved in 36 parallel culture conditions in wells using GFP lentiviral vectors (MOI=1). Investigated process parameters included (1) working volume (1-3 mL); (2) agitation frequency (0-8 cycles/day); (3) cell seeding concentration (0.5-1.0 viable cells/mL), and (4) activation agent (0.5-1.5 U/mL) (TransAct™, Miltenyi Biotec, Germany). Agitation was performed using orbital shakers at 500 rpm at a different number of frequency cycles of 30 min intervals per day. After cell counting and flow cytometry analysis, time course profiles and multivariate screening models were generated (Fig. 2).

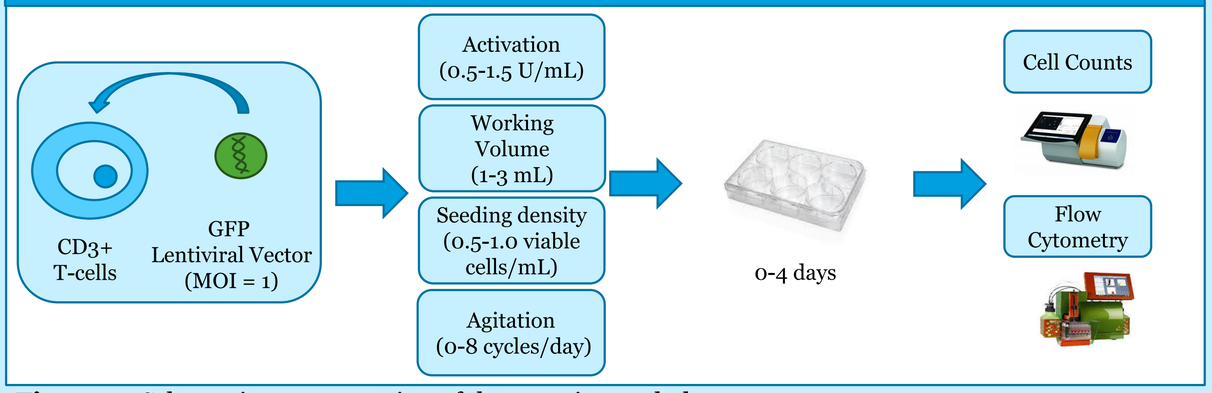


Figure 2. Schematic representation of the experimental plan.

Time course profiles

One-Factor-a-Time (OFAT)

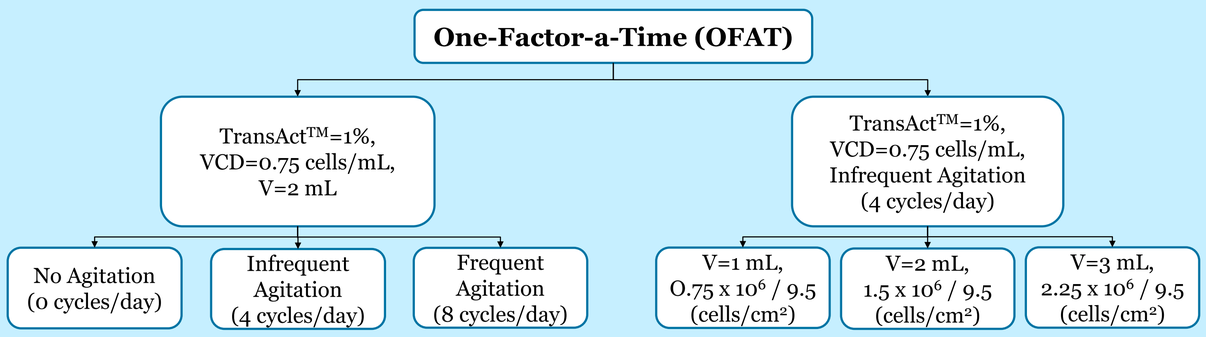


Figure 3. Schematic diagram showing the different conditions investigated in the OFAT.

Multivariate screening profiles

Design of Experiments (DoE)

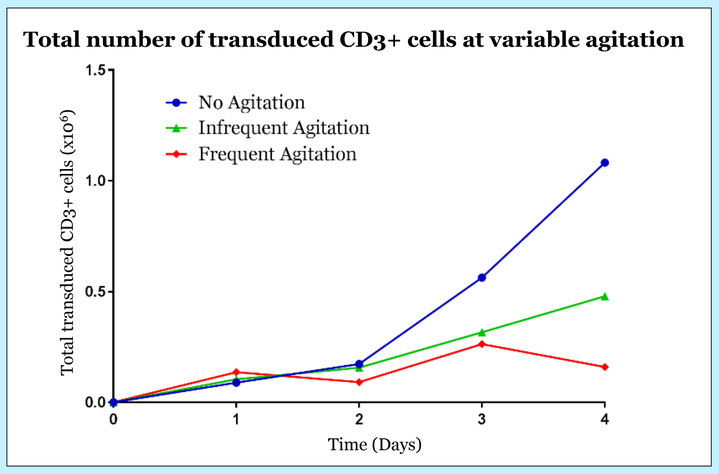
Factors	*Design Space*		
	-1	*0*	*1*
Volume (mL)	1	2	3
Agitation (no 30 min intervals/day)	0	4	8
[TransAct] (% vol.)	0.5	1	1.5
VCD_i (10^6/mL)	0.5	0.75	1

Table 1. Design space for a 2-level 4-factor full factorial DoE screening experiment, in which controlled variation of agitation, VCD_i, volume, and TransAct™ concentration was introduced. All statistical analysis and model generation was performed using Design Expert (Stat-Ease Incorporation; Minneapolis, MN).

3 Results

Time course profiles

Variable Agitation



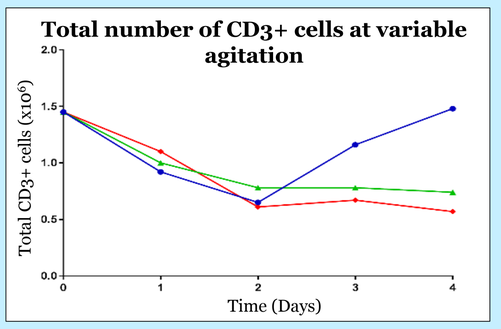
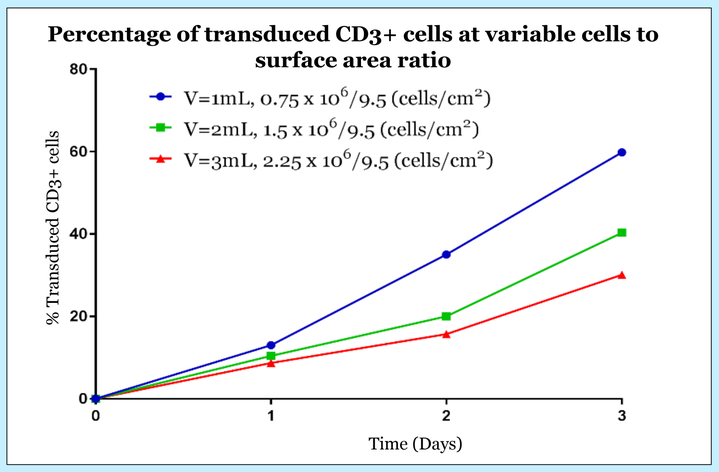


Figure 4. Transient, total number of CD3+ and transduced CD3+ cells through: no agitation; infrequent agitation; and frequent agitation.

- Agitation has a negative effect on transduction efficiency and the total number of transduced cells
- 2.6-fold higher transduction efficiency at static cultures
- 6.9-fold difference of total transduced cells between static and frequently agitated cultures

Cells to surface area ratio



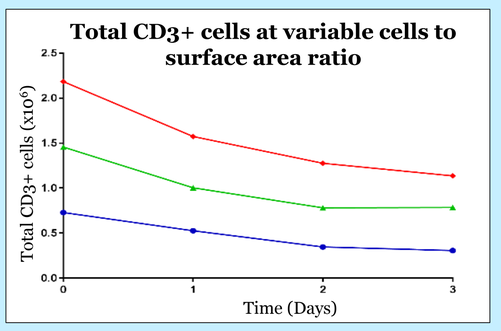


Figure 5. Percentage of transduced CD3+ and total CD3+ cells at variable cell to surface area ratios.

- Increasing the cells to the surface area ratio has a negative effect on transduction efficiency
- 2.0-fold higher levels of transduction efficiency between low and high volume cultures

Multivariate screening profiles

Table 2. Significant terms ($p<0.05$) for screening models 1-7. For each response function, this table shows normalised gradients (∇) to each *significant* model term. Factors: Seeding volume (V_i), agitation frequency (N), viable cell seeding density (VCD_i), and TransAct™ concentration ([TA]) and factor interactions [4].

	Responses		Significant model terms							
			V_i	N	VCD_i	$[TA]$	$V_i N$	$V_i VCD_i$	$N\ VCD_i$	$V_i N{\cdot}VCD_i$
1	$CD3_{TOT}$	∇	**0.4789**	***-0.0895***	**0.2444**			**0.1159**		
		p	< 0.0001	0.0012	< 0.0001			0.0001		
2	CD3/LIVE (%)	∇	**0.0053**			***-0.0057***	**0.0075**	**0.0023**		
		p	< 0.0001			< 0.0001	< 0.0001	0.0349		
3	CD3, $GFP+_{TOT}$	∇	**0.1574**	***-0.0976***	**0.1582**		***-0.0805***	**0.0636**	***-0.0350***	
		p	< 0.0001	< 0.0001	< 0.0001		0.0001	0.0009	0.0320	
4	GFP+/CD3 (%)	∇	***-0.0612***	***-0.0347***	**0.0928**		***-0.0694***			
		p	< 0.0001	0.0014	< 0.0001		< 0.0001			
5	Virus:GFP+	∇		**1.3959**	***-1.5875***		**1.4206**			***-0.9652***
		p		0.0002	< 0.0001		0.0002			0.0038

Table 3. Relative contribution of variance for each individual studied factor.

Responses	Factors			
	V_i	N	VCD_i	$[TA]$
$CD3_{TOT}$	74%	3%	23%	1%
CD3/LIVE (%)	46%	30%	5%	18%
CD3, $GFP+_{TOT}$	42%	20%	36%	1%
GFP+/CD3 (%)	37%	25%	37%	1%
Virus:GFP+	26%	40%	30%	4%

- The lowest virus cost per transduced cell was achieved at (1) no agitation, (2) high initial cell concentration, and (3) high culture volume. This corresponded to a 4.6-fold cost-reduction relative to the highest observed virus cost per transduced cell (3.0 vs. 13.8 viruses per transduced cell).

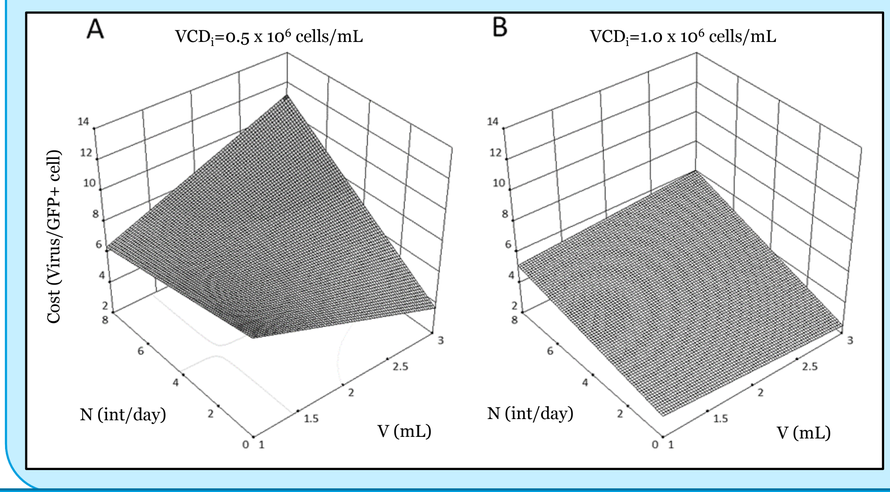


Figure 6. Fitted response surface model for virus cost per transduced cell due to variation of agitation cycles (N) and working volume (V) at (A) low seeding density (VCD_i) and (B) high seeding density.

4 Discussion & Conclusions

- ❑ Transduction efficiency, total transduced cells and cost per transduced cell were significantly sensitive to variations in agitation, culture volumes and seeding density.
- ❑ High cell seeding densities and lower working volumes, with little to no agitation allows maximum transduction of T cells, perhaps due to congregation of cells and virus.
- ❑ Virus cost per transduced cell can be dramatically lowered through bioprocess optimization.

We work with Innovate UK

Cell and Gene Therapy Catapult
12th Floor, Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
+44 (0) 203 728 9500 | info@ct.catapult.org.uk | ct.catapult.org.uk

References
1. Levine BL, Miskin J, Wonnacott K, Keir C. Global Manufacturing of CAR T Cell Therapy. *Mol Ther - Methods Clin Dev*. 2017;4(March):92-101. doi:10.1016/j.omtm.2016.12.006.
2. James D. How short-term gain can lead to long-term pain. *Cell & Gene Therapy Insights. 2017;(April):271-283. doi*: 10.18609/cgti.2017.018.
3. Verhoeyen E, Costa C, Cosset F-L. Lentiviral Vector Gene Transfer into Human T Cells. In: ; 2009:97-114. doi:10.1007/978-1-59745-409-4_8.
4. Anderson M., Whitcomb P. DOE Simplified: Practical Tools for Effective Experimentation, 3rd Edition, CRC Press.

CATAPULT Cell and Gene Therapy