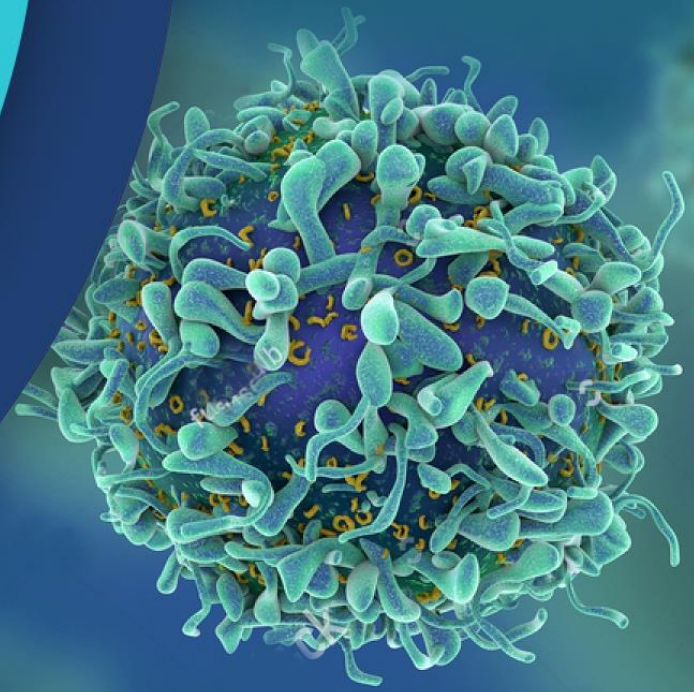
ScaleReady™

G-Rex®: An Integrated Development and Manufacturing Platform for Cell and Gene-Modified Cell Therapy Applications

"Simplicity is the ultimate sophistication"
- Leonardo Da Vinci



History of Cell Culture

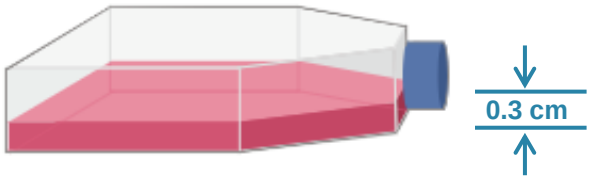
An inverse relationship between oxygen availability and media volume (nutrient availability) necessitated constant interventions

1885 — Onset of cell culture

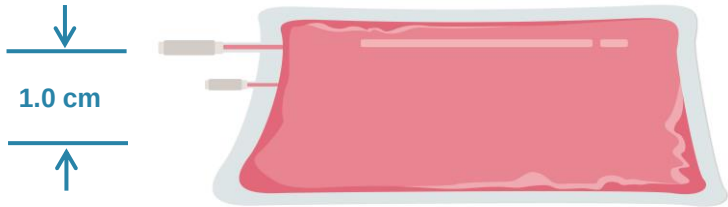
1970 — Introduction of plastic t-flask

1988 — Introduction of gas permeable bags

Non-gas permeable device
maximum media height = 0.3 cm



Gas permeable bags
maximum media height = 1.0 cm



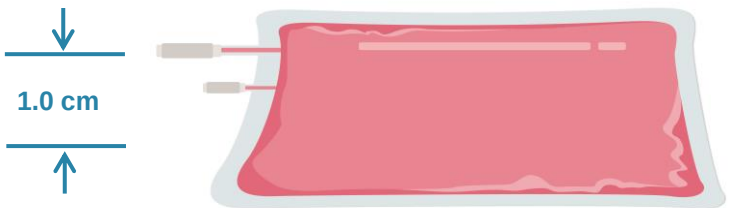
Gas permeable bags corrected the inverse relationship, but continued to necessitate constant interventions

1885 — Onset of cell culture

1970 — Introduction of plastic t-flask

1988 — Introduction of gas permeable bags

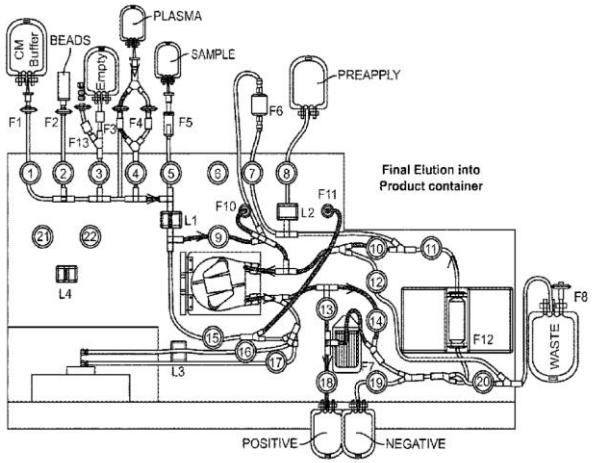
Gas permeable bags
maximum media height = 1.0 cm

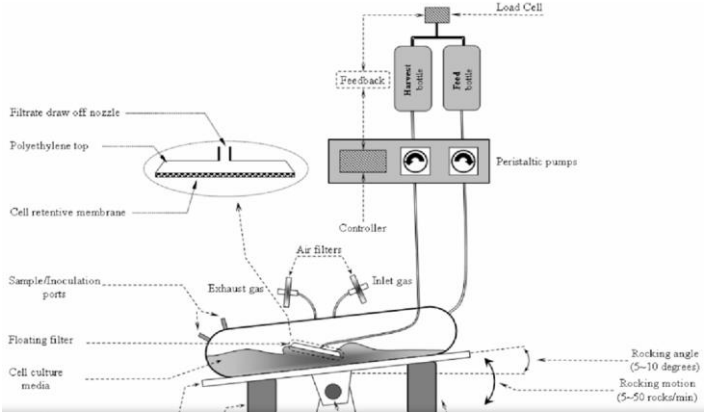


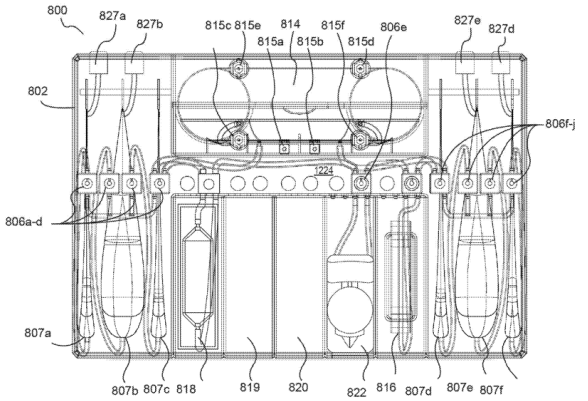
From VueLife Website

WHY ONE CENTIMETER: The selection of one centimeter thickness was based on research which indicated that human lymphocytes would expand at a maximal rate when the media fill was one centimeter thick. Greater thickness provided no greater expansion and is accordingly a waste of media. Lesser fill thickness resulted in lower expansion rates and is accordingly a waste of time.

Complex bioreactors are not a solution to solving scalable manufacturing because…







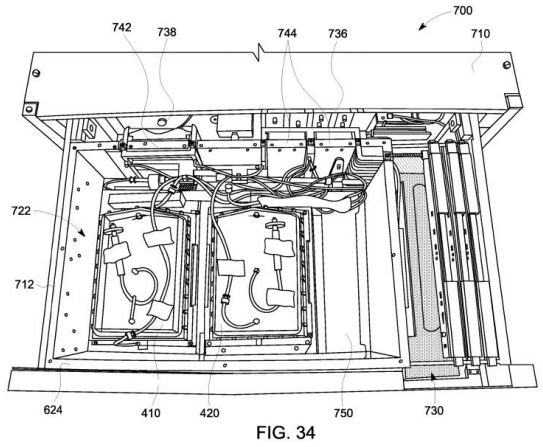


FIG. 34

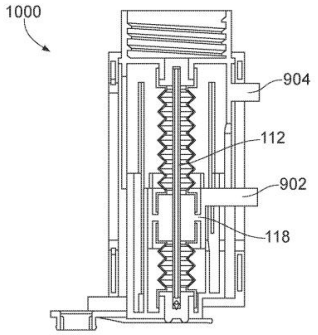
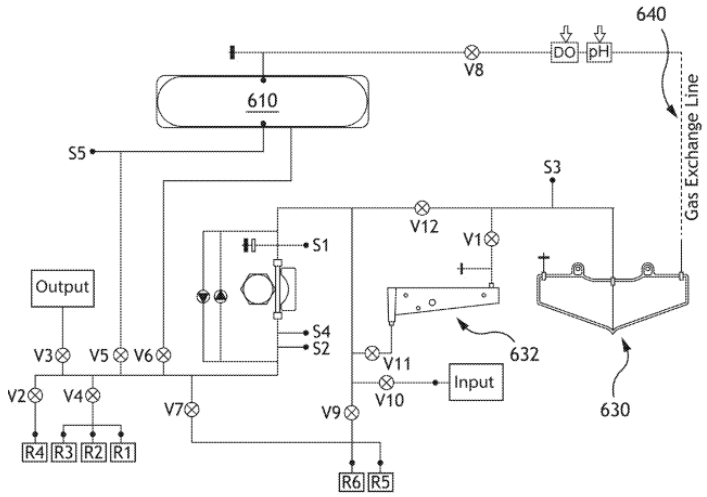


FIG. 37



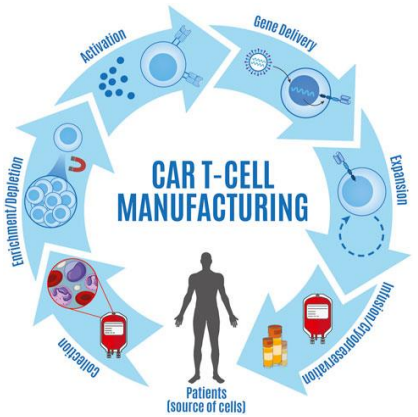
Challenge of scalable manufacturing

Development

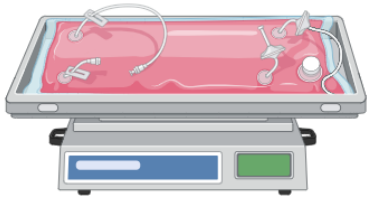
- X No native small-scale model
- ! **Unpredictable translation** of small-scale data
- ! Excessive time and money spent attempting to create poorly predictive scale down models
- ! Excessive time and money spent optimizing CMC at full-scale

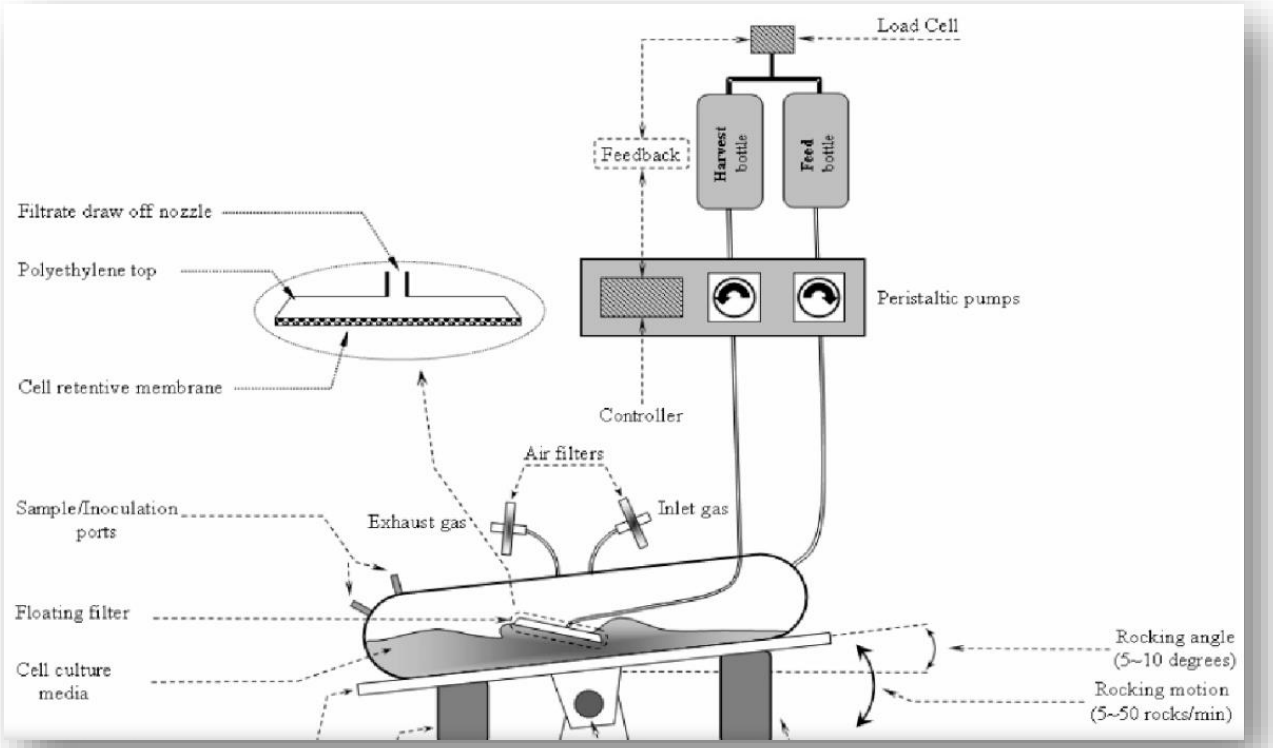
Manufacturing

- X **Continuous fluidic connection** of drug product
- ! Constraint is the manufacturing process itself
- ! Excessive investment in capital equipment for minimal throughput
- ! Limited potential for further innovation



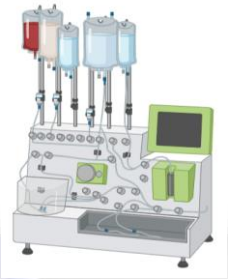
Challenge of Scalable Manufacturing

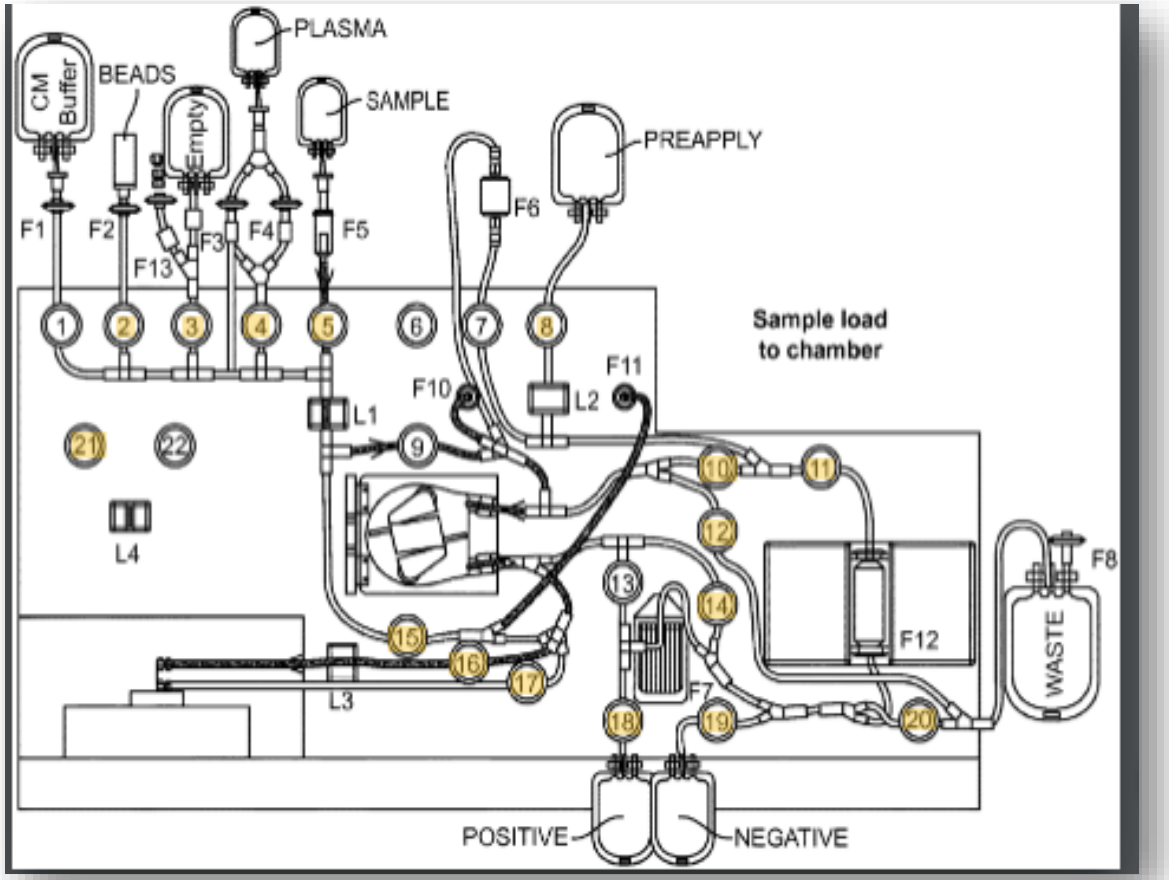




- ! Overly complex movement of fluid
- ! Inefficient use of space
- ! No native small-scale model
- ! No high throughput manufacturing

Challenge of Scalable Manufacturing





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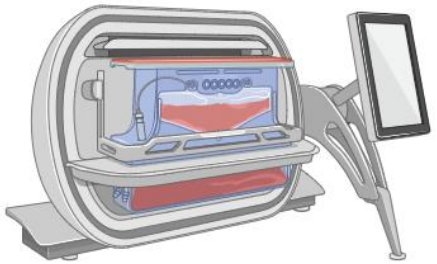
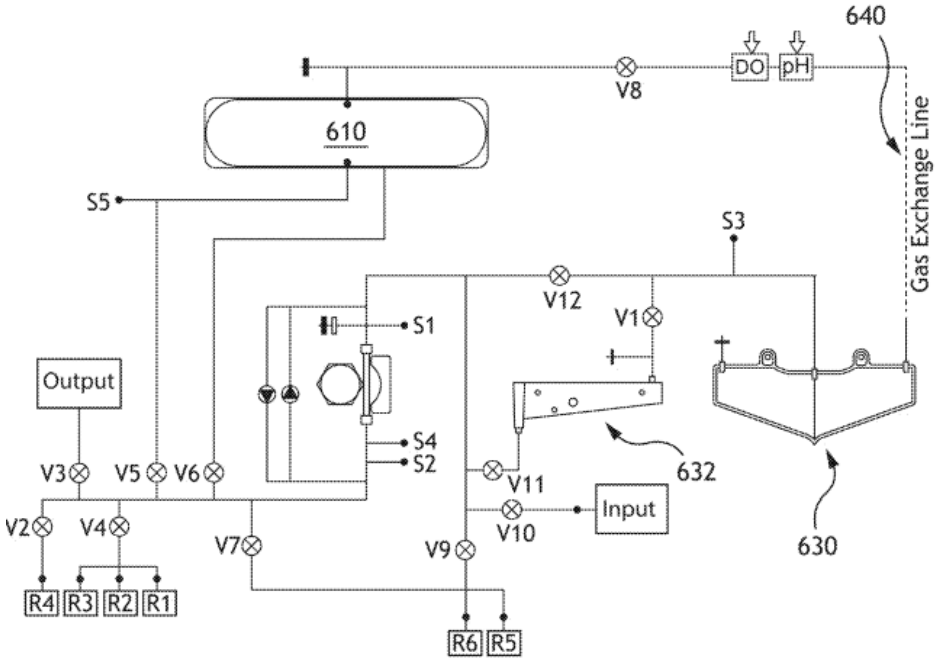
Challenge of Scalable Manufacturing





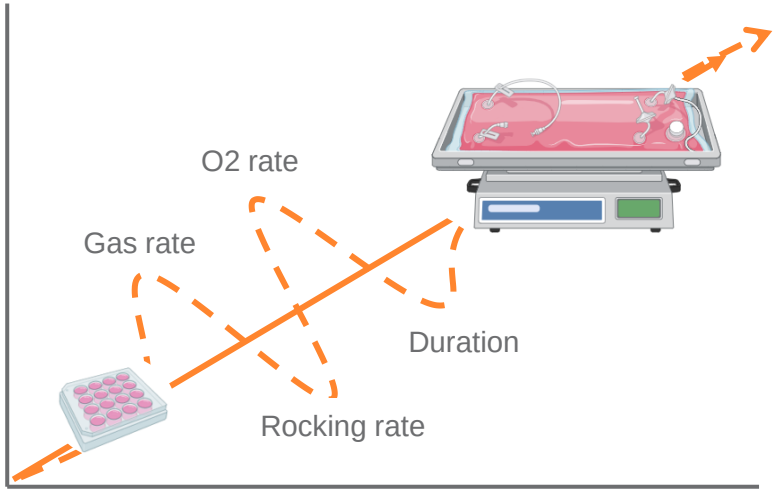
- ! Overly complex movement of fluid
- ! Inefficient use of space
- ! No native small-scale model
- ! No high throughput manufacturing

Automated Instruments Sacrifice Scalability and Practicality

These needlessly complex instruments sacrifice scalability for automation and lack practicality when scaling out for larger patient populations.

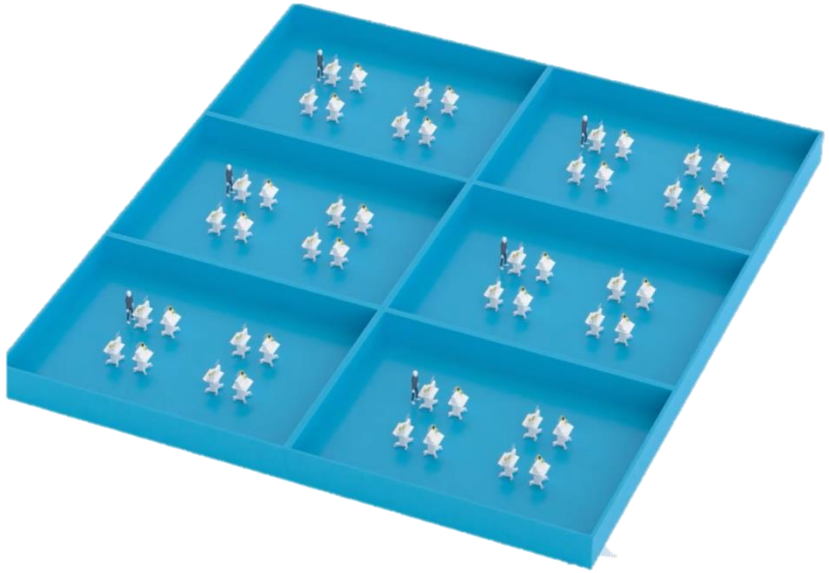
Scale up

Small scale experiments don't easily scale up from benchtop to an instrument or "patient" dose

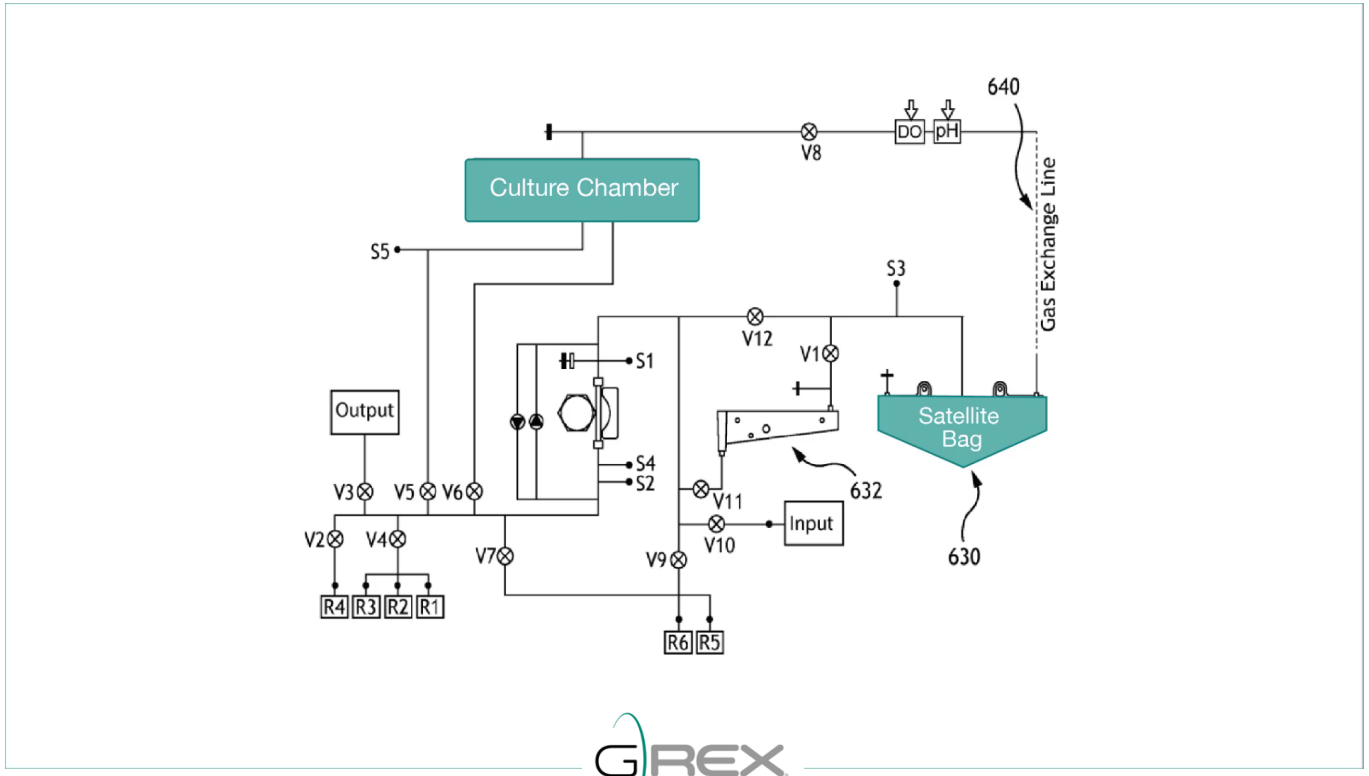


Scale out

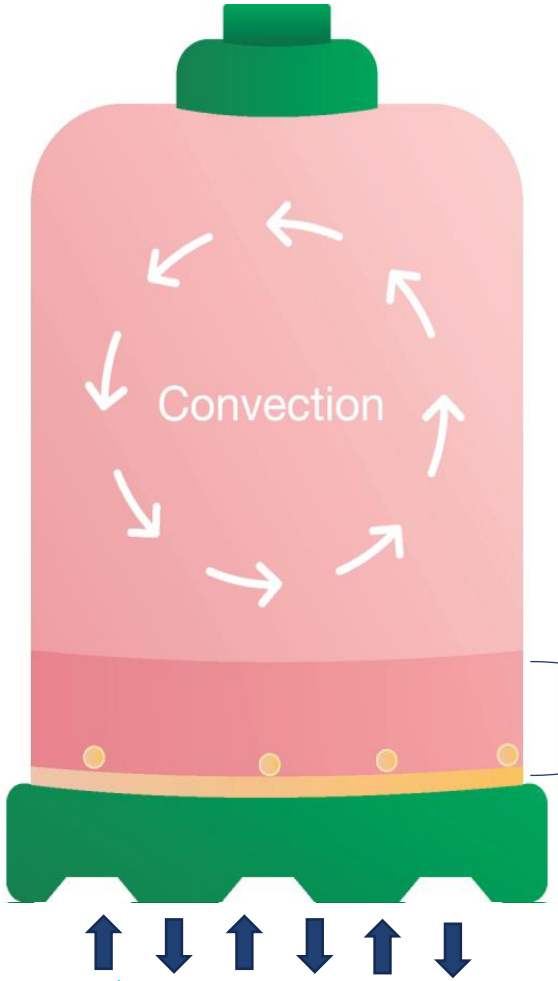
Requiring one instrument per patient means large investment in cleanroom space & capital equipment



Strip away the needless complexity of a complex bioreactor and what do you get? A New Path Forward

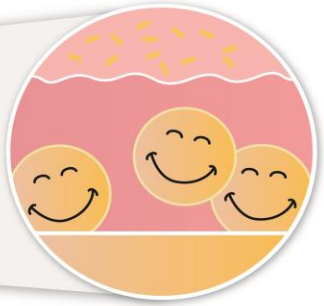


G-Rex: The New Path Forward



1. Unlimited access to oxygen *on demand*
2. Unlimited access to nutrients *on demand*
3. Ideal environment for cell-to-cell communication

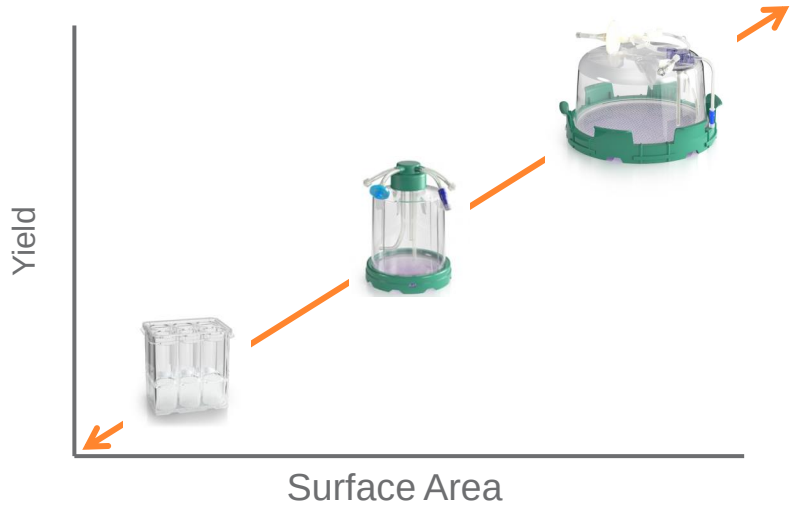
Zone of Diffusion



Where do we go from here?

Development

- ✓ Native small-scale model
- ! Predictable translation
- ! Linear path of least resistance to IND
- ! Cost- and time-efficient CMC improvements



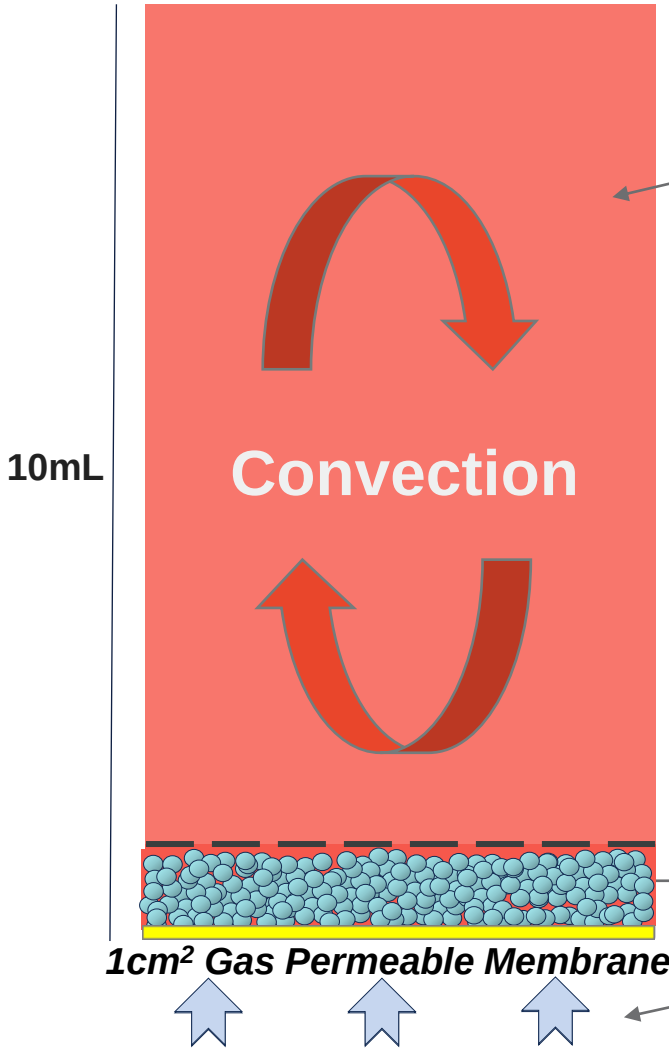
Manufacturing

- ✓ De-couple drug product from instruments
- ! Constraints are individual unit operations
- ! Modest investment for high throughput
- ! Future-proofed

G-Rex® Theory

G-Rex® : How it Works

G-Rex ® = **G**as Permeable **R**apid **Ex**pansion



Zone of Convection

Convection provides cells with nutrients on demand

No mixing or perfusion needed

Easily sample media for indicators of cell quantity

Zone of Diffusion

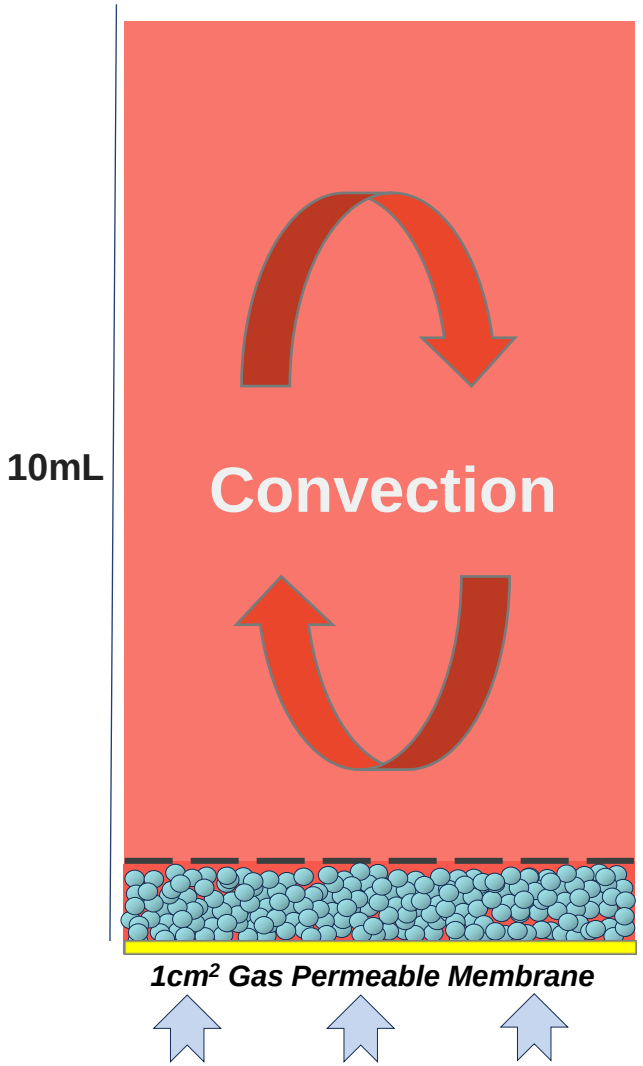
300 µm Boundary Layer of Diffusion

Ideal for cell-to-cell communication

Allows cells to reside in a large volume of media to eliminate additional feeding

Unlimited Oxygen on Demand

G-Rex® : How it Works



G-Rex ® has greatest range of cells/cm² (or cells/mL) in the industry

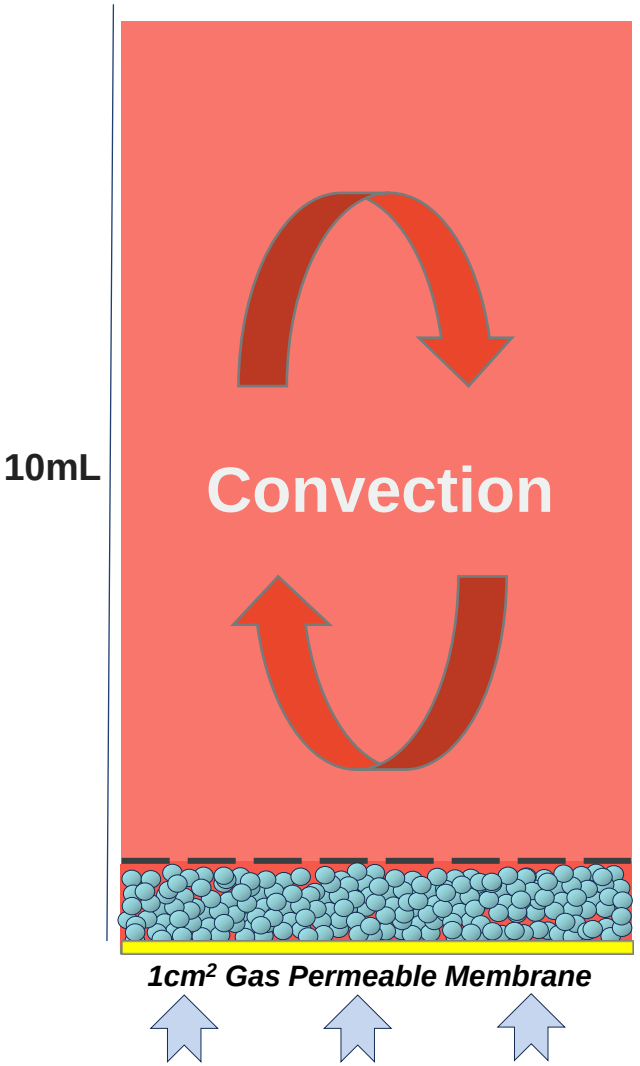
Est. Minimum Seeding Density:

Cell Quality	Min Cells/cm²
Poor/Low	2+ million
Good/High	0.5 million

7-10 Days

Est. Maximum Cell Density:

Cell Type	Max Cells/cm²
T cell	40 million+
NK cell	40 million+



G-Rex® Best Practices

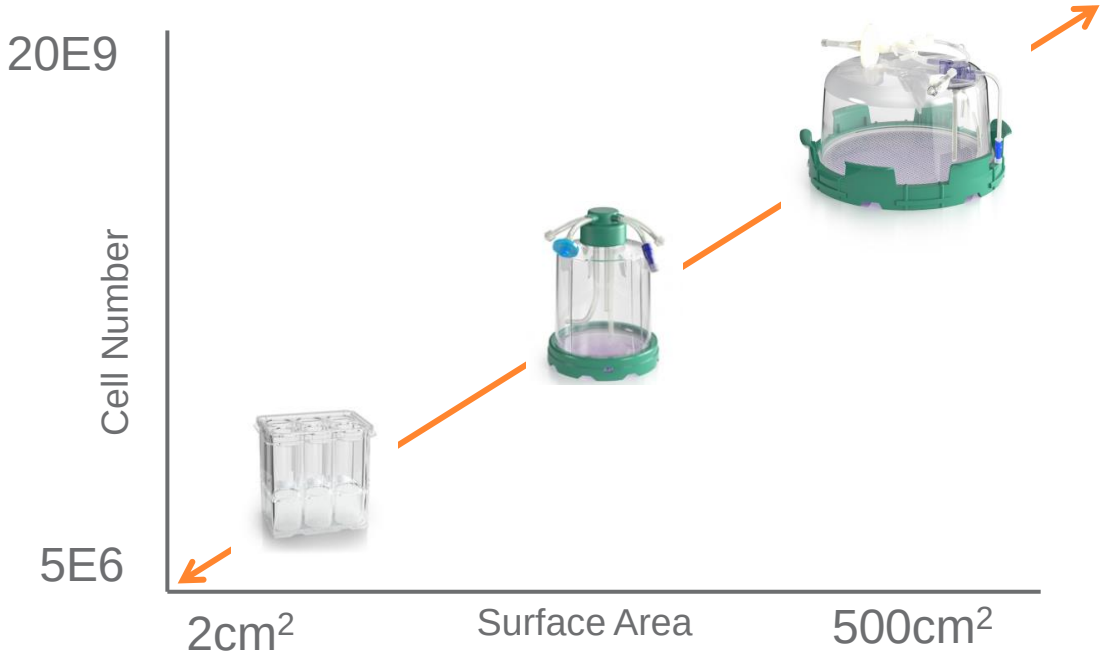
- **Normalize to a Density (cells/cm²)**
  - ✓ *What governs production in G-Rex is space, not cell concentration.*
- **Keep the cells on the G-Rex membrane**
  - ✓ *Avoid resuspending the cells off the membrane, a microenvironment rich in oxygen & nutrients where the cells can secrete and receive the molecules they need to enter and sustain log-phase growth*
- **Use the full media volume capacity (for expansion)**
  - ✓ *10mL/cm² is the proper size nutrient pool and waste sink to facilitate maximum fold expansion without exchanging the media*
- **Trend Lactate**
  - ✓ *Lactate concentrations can be correlated to cell density, trended overtime, and used as a surrogate marker for cell proliferation*

G-Rex®: An integrated development and manufacturing platform

G-Rex® Integrated Development and Manufacturing Platform for CGT

Linear Path of Least Resistance

- Continuity between R&D, PD, and GMP
- Optimize/troubleshoot quickly and cost efficiently with predictability



Modular Scale Out

- High-throughput manufacturing
  - ✓ 500,000 CAR-T doses in 70,000 sq.ft. facility
- Reduced investment in space, capital equipment, specialized labor, etc.

G-Rex “M” Series Integrated Development and Manufacturing Platform

2cm² 5cm² 10cm² 50cm² 100cm² 300cm² 500cm²

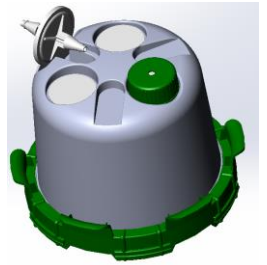
G-Rex6M–2cm²
20mL per well

G-Rex6M–5cm²
50mL per well

G-Rex6M–10cm²
100mL per well

G-Rex50M

G-Rex100M



G-Rex300M
IN DEV

G-Rex500M

G-Rex5M

G-Rex10M

G-Rex50M-CS

G-Rex100M-CS

G-Rex300M-CS
IN DEV

G-Rex500M-CS

G-Rex10M-CS

G-Rex500M-TF

ScaleReady Modular Manufacturing Platform

✓ Simplified **high throughput manufacturing** with a modest investment (low risk, high reward)

Purpose Built Incubators for G-Rex500M-CS®

- Heavy duty shelving for **standard incubators** available today
- **$4x10^{11}$ cells in $<1m^2$**

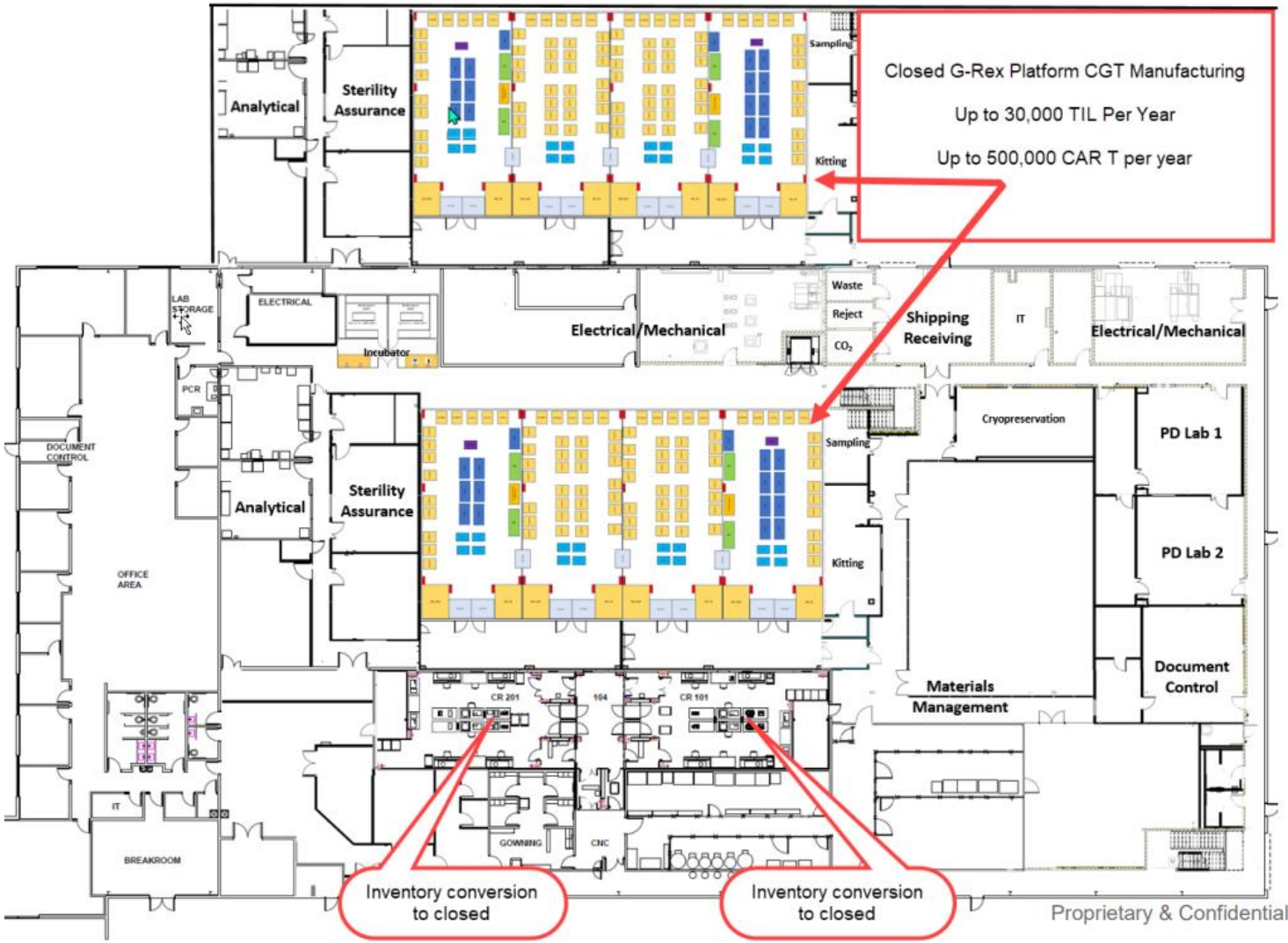
Ordering information

Description	120 V 50/60Hz	230V 50/60 Hz	100V (Japan only)
Heracell VIOS 250i CO_2 Incubator			
Electropolished stainless steel interior with TC CO_2 sensor	51033587	51033589	51033585
Electropolished stainless steel interior with IR CO_2 sensor	51033597	51033599	51033605
Heracell Vios 250i CR (Cleanroom) CO_2 Incubator CTS Series			
Electropolished stainless steel interior with IR CO_2 sensor	51033783	51033784	51033782
Shelving System optimized for G-Rex® 500M-CS*			
VIOS 250i SST Shelving System to support 10 G-Rex® 500M		50164781	

* Only the shelving system, customer-installation (no technician needed). G-Rex bioreactors must be ordered from Wilson Wolf Corporation.

ScaleReady

G-Rex® Open Architecture



Pilot Facility in Houston, TX

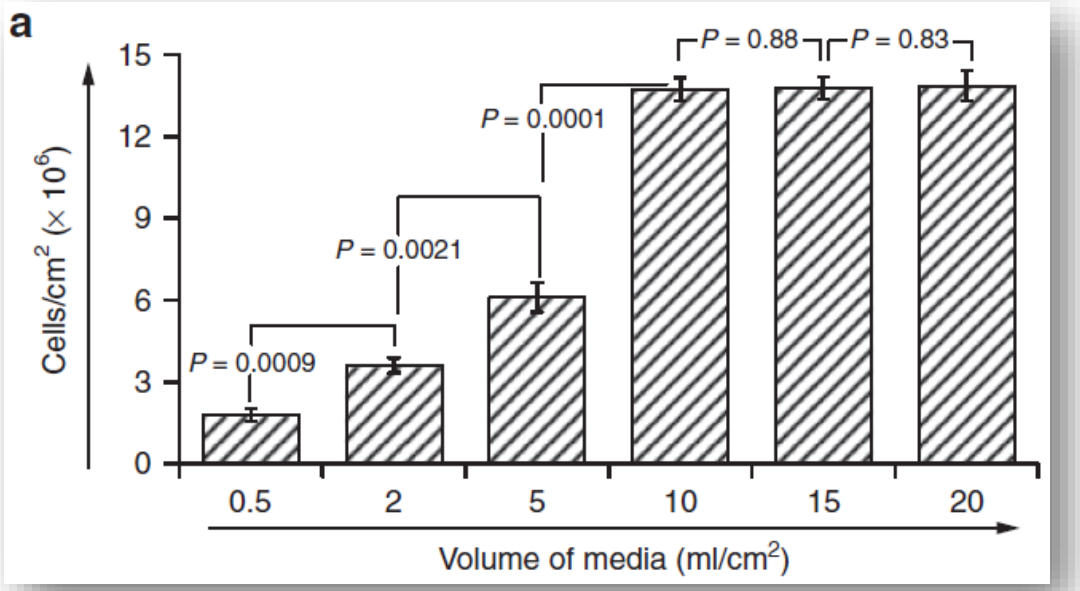
- Approximately 70,000 sq.ft.
- Standardized
- Modular

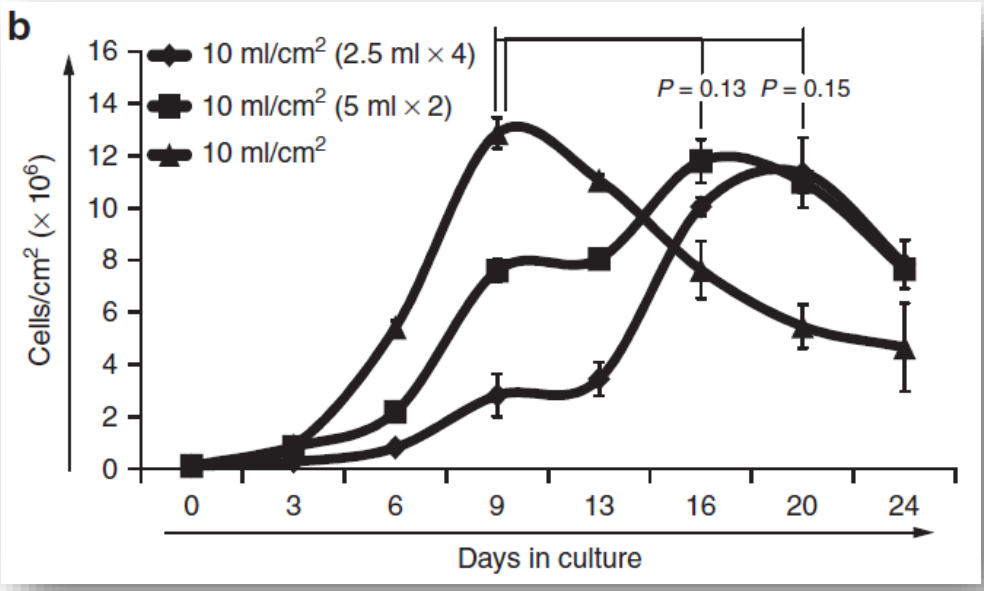
Projected Annual Patient Capacity

- 30,000 TIL @ 80E9 cells/dose
- 500,000 CAR-T @ 4E9 cells/dose
- 1,250,000 NK @ 2E9 cells/dose

G-Rex® : Optimized geometry enables simplified manufacturing

Simplifying Cell Culture with G-Rex®



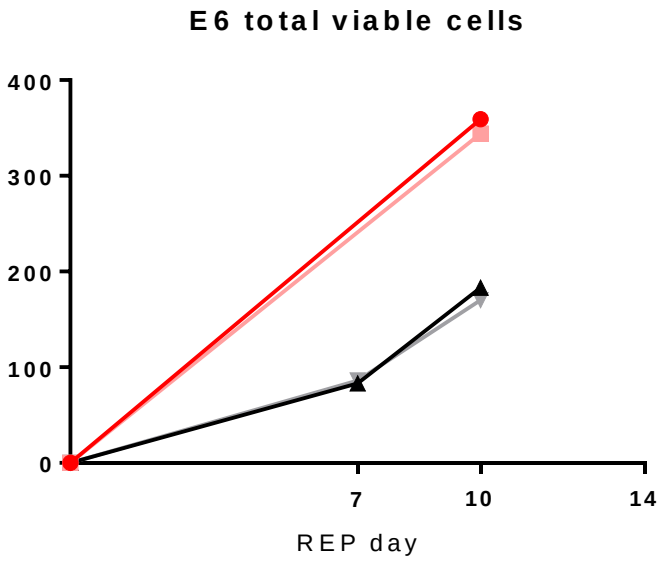


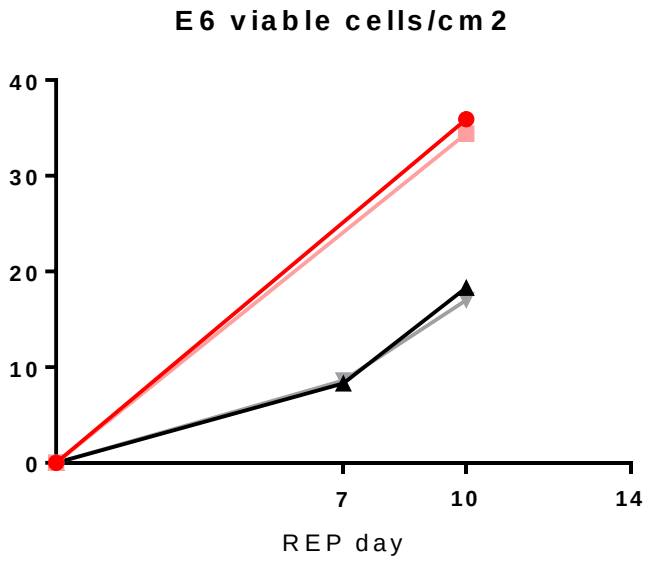
- ✓ **10mL/cm²** is the proper geometry to carry a culture from minimum to maximum density ***without the need to exchange the media***

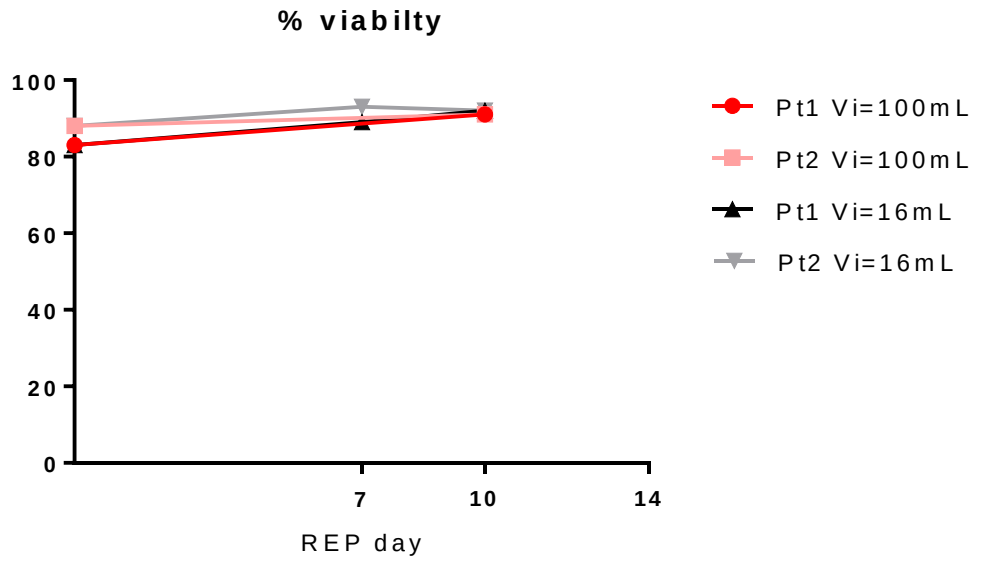
- ✓ Frontload all 10mL/cm² to **avoid resuspending cells** and to **maximize culture kinetics**

Bajgain P, Mucharla R, Wilson J, et al. Optimizing the production of suspension cells using the G-Rex "M" series. *Mol Ther Methods Clin Dev*. 2014;1:14015. Published 2014 May 14. doi:10.1038/mtm.2014.15

Simplifying Cell Culture with G-Rex®







Vi = 100mL Protocol

Day 0: Stimulate 2E5 T cells and 2E7 feeder cells in 100mL cytokine-supplemented media

Days 7, 8, 9, 10: Remove 100uL of media from top of well for lactate testing

Day 10: Re-suspend cells, remove 30uL cell suspension for counts

Vi = 16mL Protocol

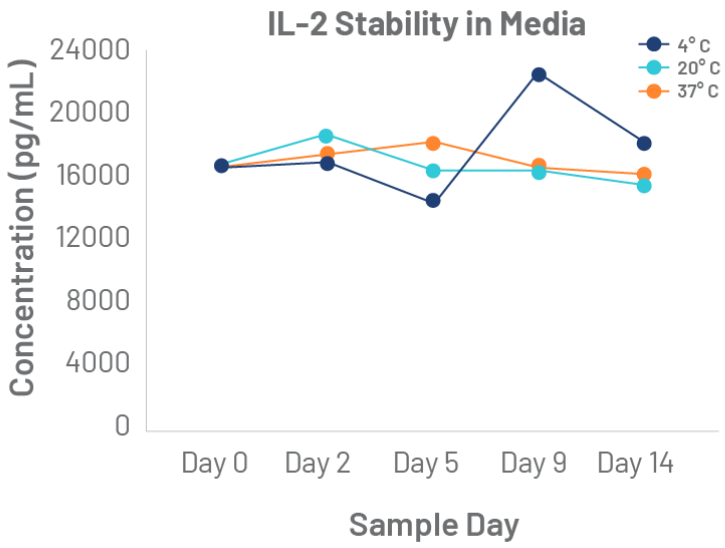
Day 0: Stimulate 2E5 T cells and 2E7 feeder cells in 16mL cytokine-supplemented media

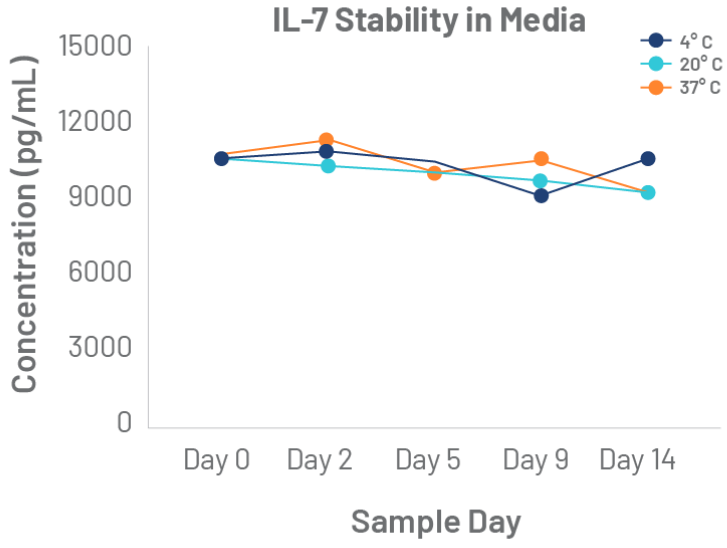
Day 4: Add 16mL cytokine-supplemented media

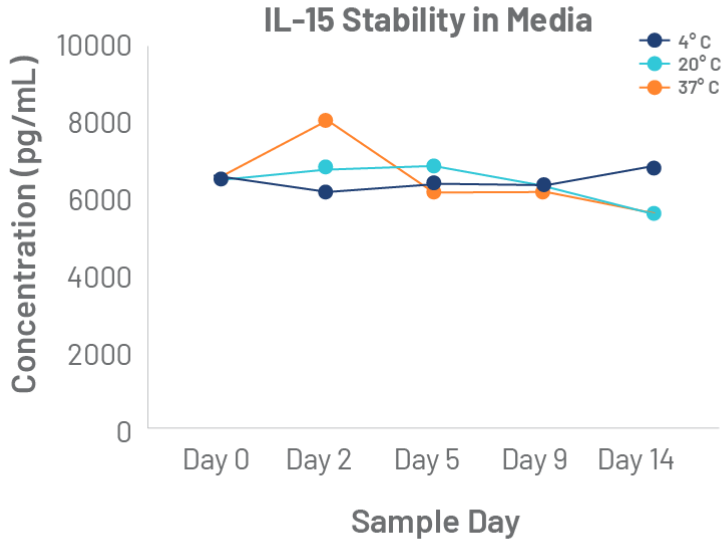
Day 7: Add 24mL cytokine-supplemented media, remove 30uL cell suspension for counts

Day 10: Add 44mL cytokine-supplemented media, remove 30uL cell suspension for counts

Stability of cytokines in media at different temperatures





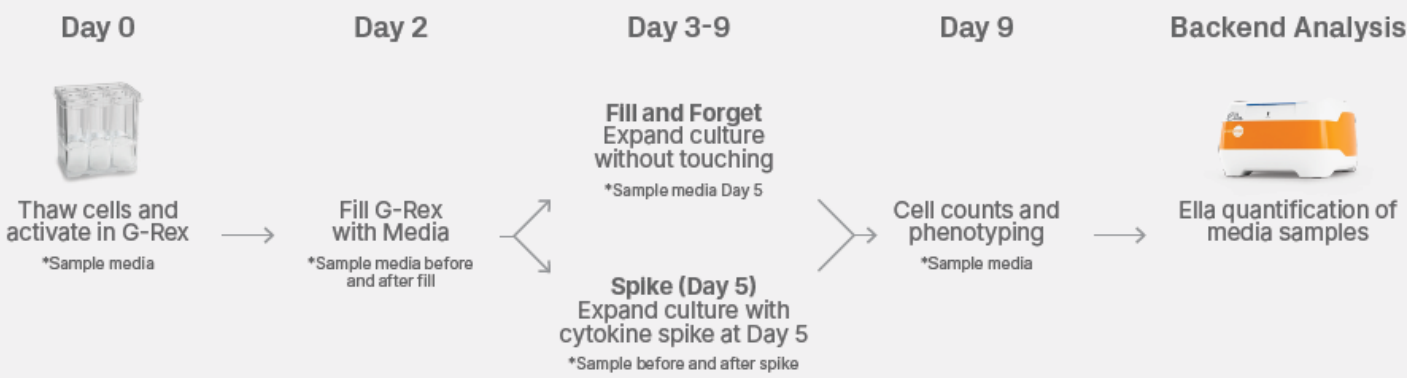


*IL-2 was used at 200IU/mL

Fill & Forget with G-Rex®

Experimental Workflow

Isolated T cells were thawed, plated, and activated in a G-Rex 6M at 0.5e6/mL/cm² using GMP Human T Cell Media supplemented with 5% hAB serum and 10 ng/mL IL-7 and 10 ng/mL IL-15. On Day 2, G-Rex was filled with fresh media to bring the total volume to 10 mL per cm² of G-Rex surface area. Media supernatant was collected for measuring cytokine concentrations on days 0, 2, and 9. Cytokine concentrations were quantified using the Ella Simple Plex automated immunoassay platform. All values shown were averaged across three donors.



Materials and Methods

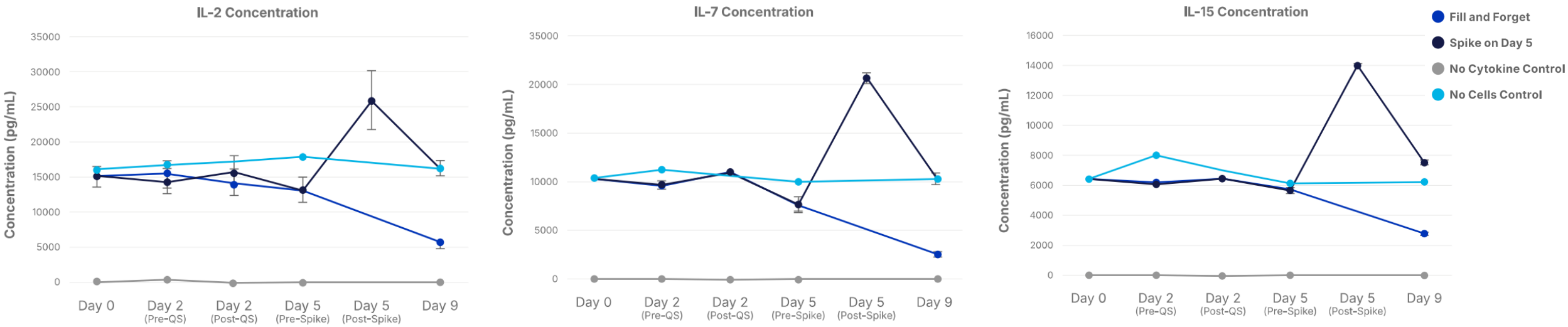
Product	Material	Part #
G-Rex 6M plate*	Culture Vessel	80660M (ScaleReady)
GMP Human T Cell Media	Media	CCM038-GMP
GMP IL-7 (10 ng)**	Cytokine	BT-007-GMP
GMP IL-15 (10 ng)**	Cytokine	BT-015-GMP
Ella	Analytical Instrument	600-100
IL-7 (72x1)	Simple Plex Cartridge	SPCKB-PS-000506
IL-15 (72x1)	Simple Plex Cartridge	SPCKB-PS-000500

* Available exclusively from ScaleReady

** Available from ScaleReady

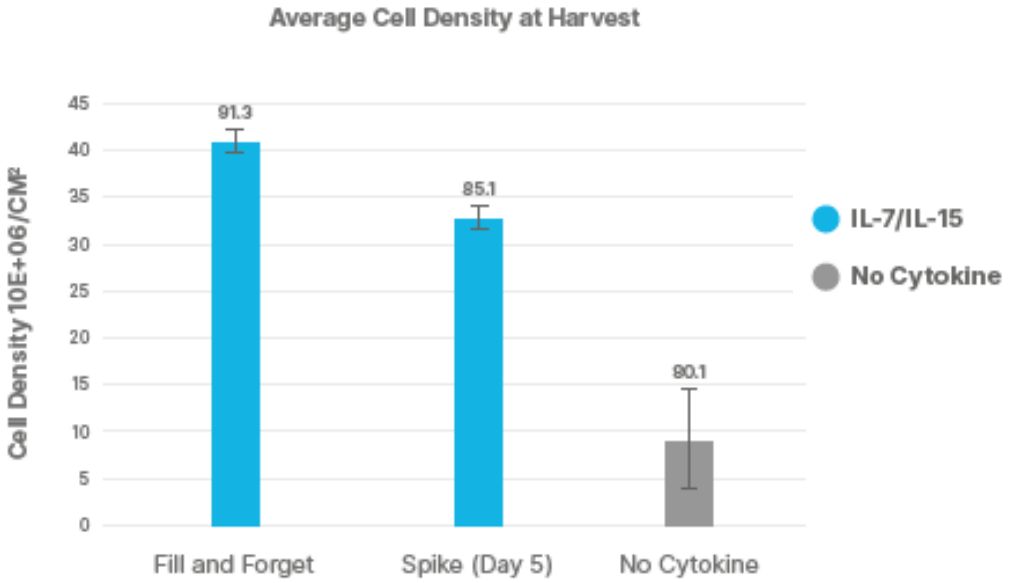
G-Rex is a registered trademark of Wilson Wolf Manufacturing, LLC

- ✓ **G-Rex®** process can be **simplified using R&D Systems reagents** to a single bolus on D0 to achieve max cell density with desirable phenotypes



Fill & Forget with G-Rex®

Fill-and-Forget Workflow Produced Equivalent T Cell Expansion and Viability

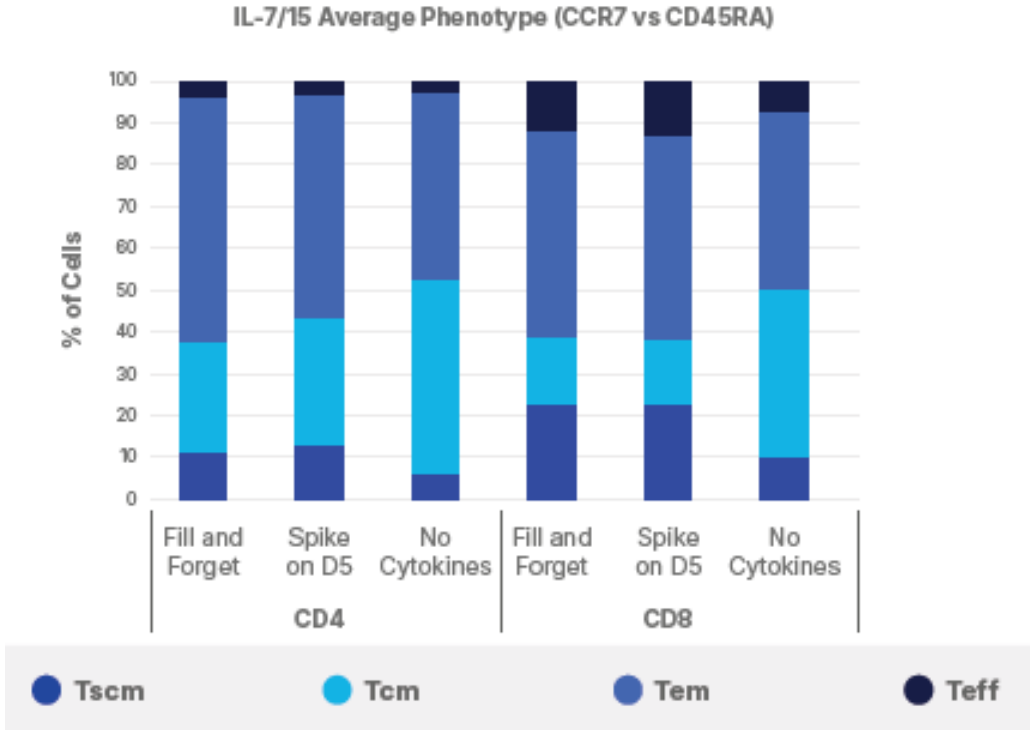


Both workflows supported cell expansion to reach confluent density of 30-40 million cell/cm^2.

No cytokine control showed minimal expansion as expected.

Data labels denote average viability.

Fill-and-Forget Workflow Produced Equivalent Expanded T Cell Phenotypes



G-Rex®: Linear scalability enables continuity

Linear Scalability of G-Rex® (k562)

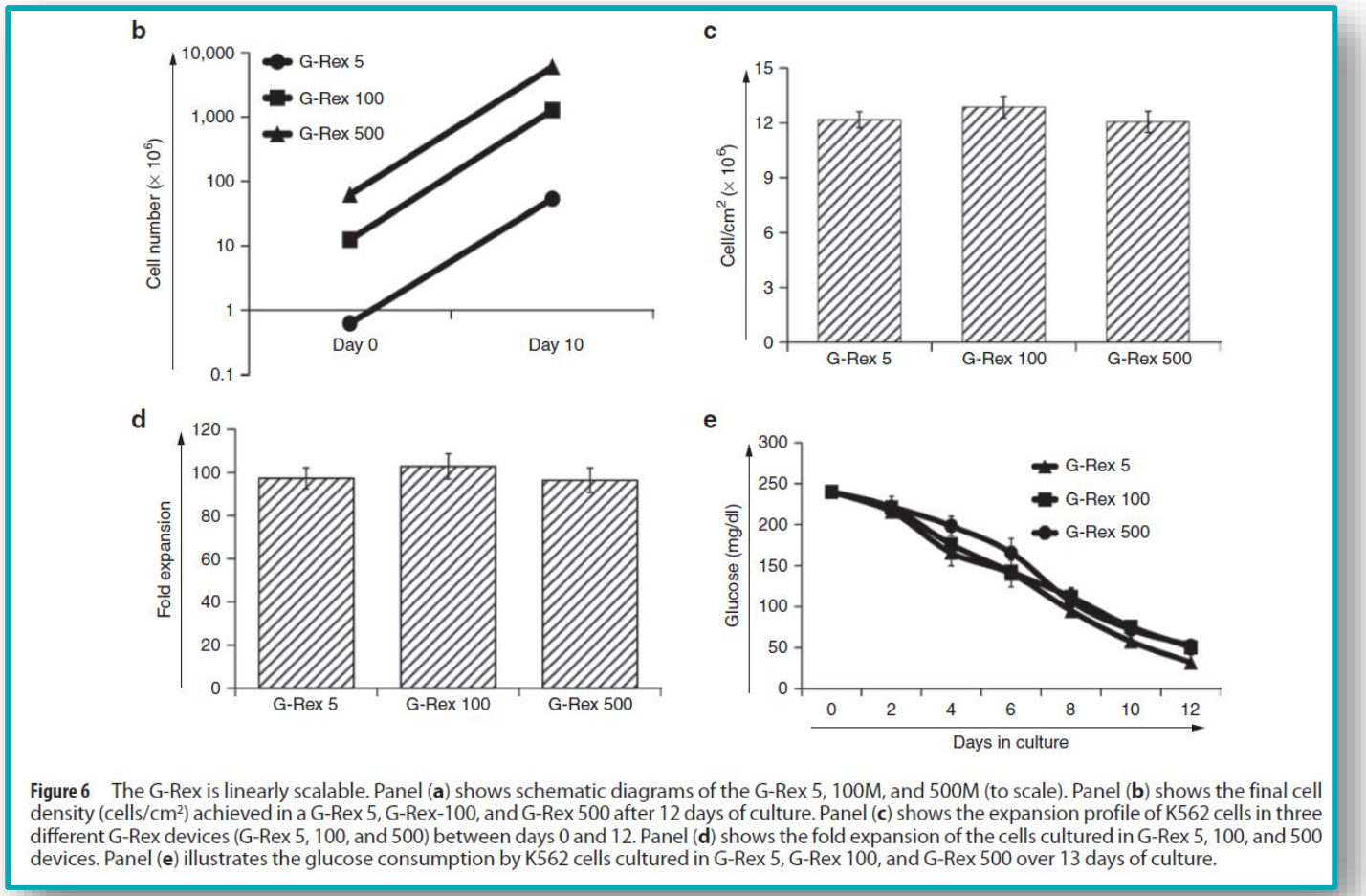
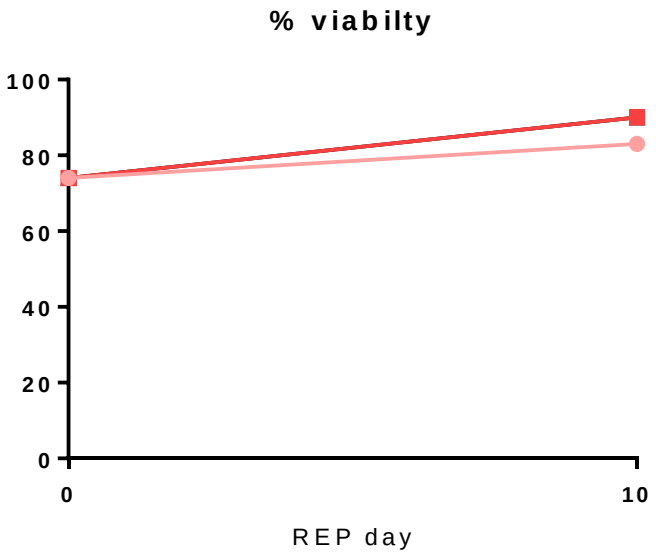


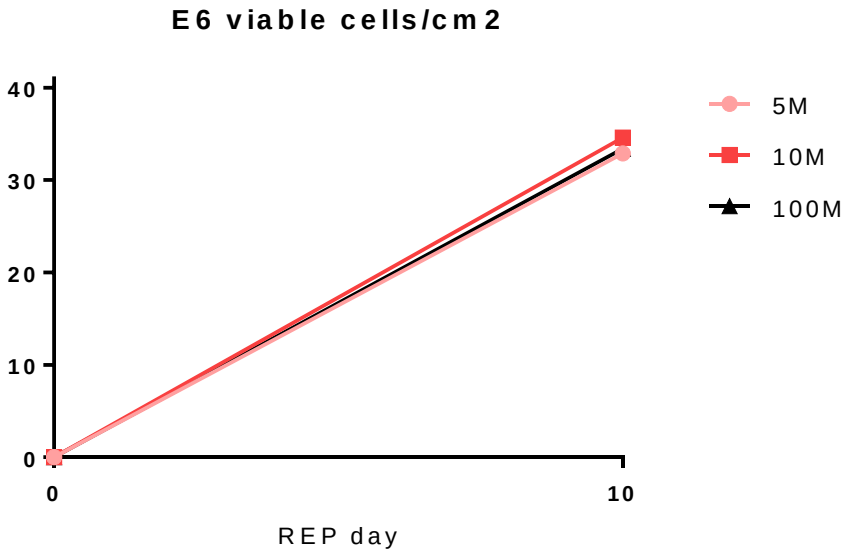
Figure 6 The G-Rex is linearly scalable. Panel (**a**) shows schematic diagrams of the G-Rex 5, 100M, and 500M (to scale). Panel (**b**) shows the final cell density (cells/cm²) achieved in a G-Rex 5, G-Rex-100, and G-Rex 500 after 12 days of culture. Panel (**c**) shows the expansion profile of K562 cells in three different G-Rex devices (G-Rex 5, 100, and 500) between days 0 and 12. Panel (**d**) shows the fold expansion of the cells cultured in G-Rex 5, 100, and 500 devices. Panel (**e**) illustrates the glucose consumption by K562 cells cultured in G-Rex 5, G-Rex 100, and G-Rex 500 over 13 days of culture.

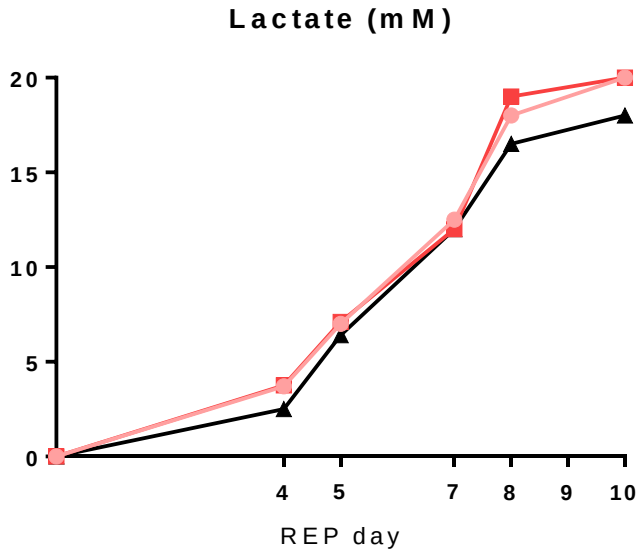
- ✓ **Linear scalability** is unique to the G-Rex platform.
- ✓ Streamline and shorten the lifecycle of a cell therapy by seamlessly **scaling up** from RUO well plates to a GMP bioreactor
- ✓ Take an established process back to the drawing board by **scaling down** from your GMP production vessel to the G-Rex6M well plate.

Bajgain P, Mucharla R, Wilson J, et al. Optimizing the production of suspension cells using the G-Rex "M" series. *Mol Ther Methods Clin Dev*. 2014;1:14015. Published 2014 May 14. doi:10.1038/mtm.2014.15

Linear Scalability of G-Rex® (TCR-T)







*Unpublished data from Dr. Eric Tran Lab. Shared under Data Use Agreement

Linear Scalability of G-Rex® (TCR-T)

Tumor homing

Lymph node homing

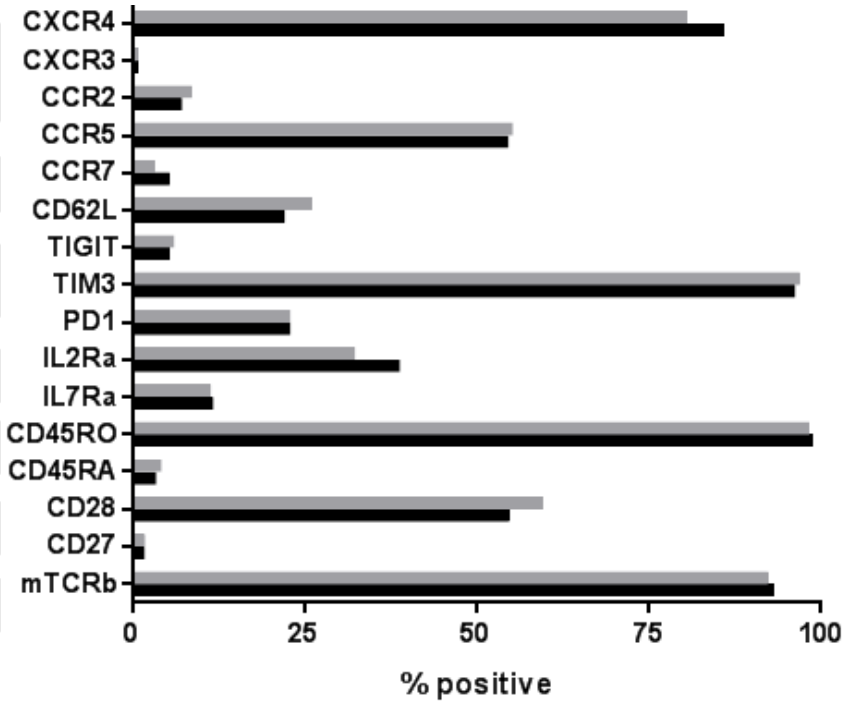
Activation/ exhaustion

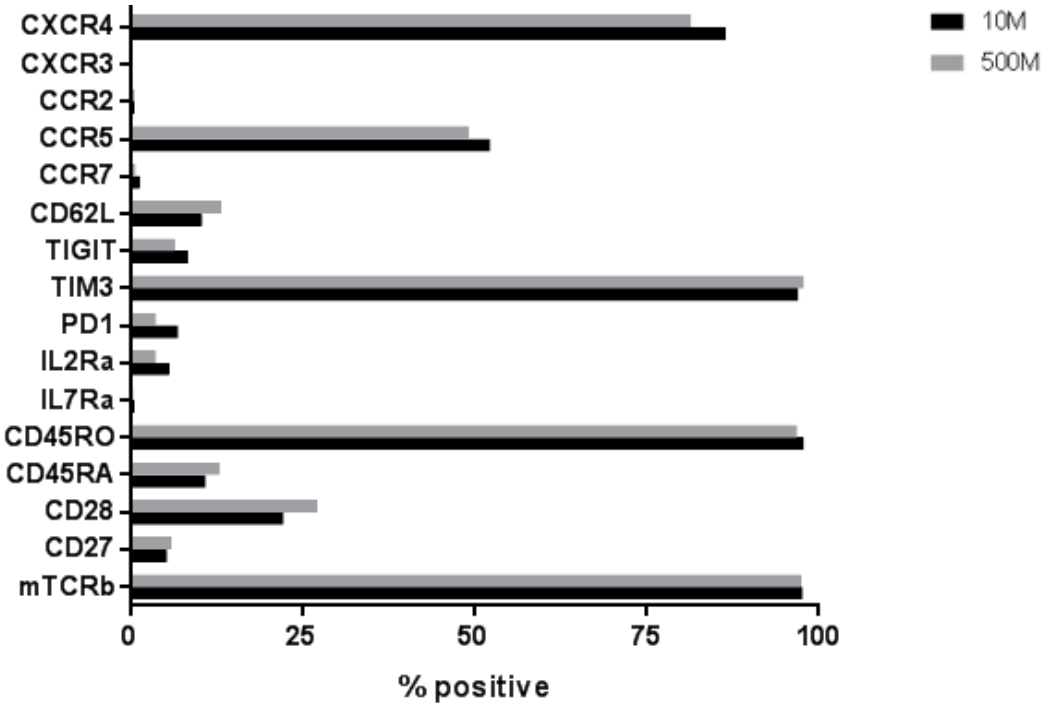
Treg indicators (CD4)

Differentiation status

Co-stimulation

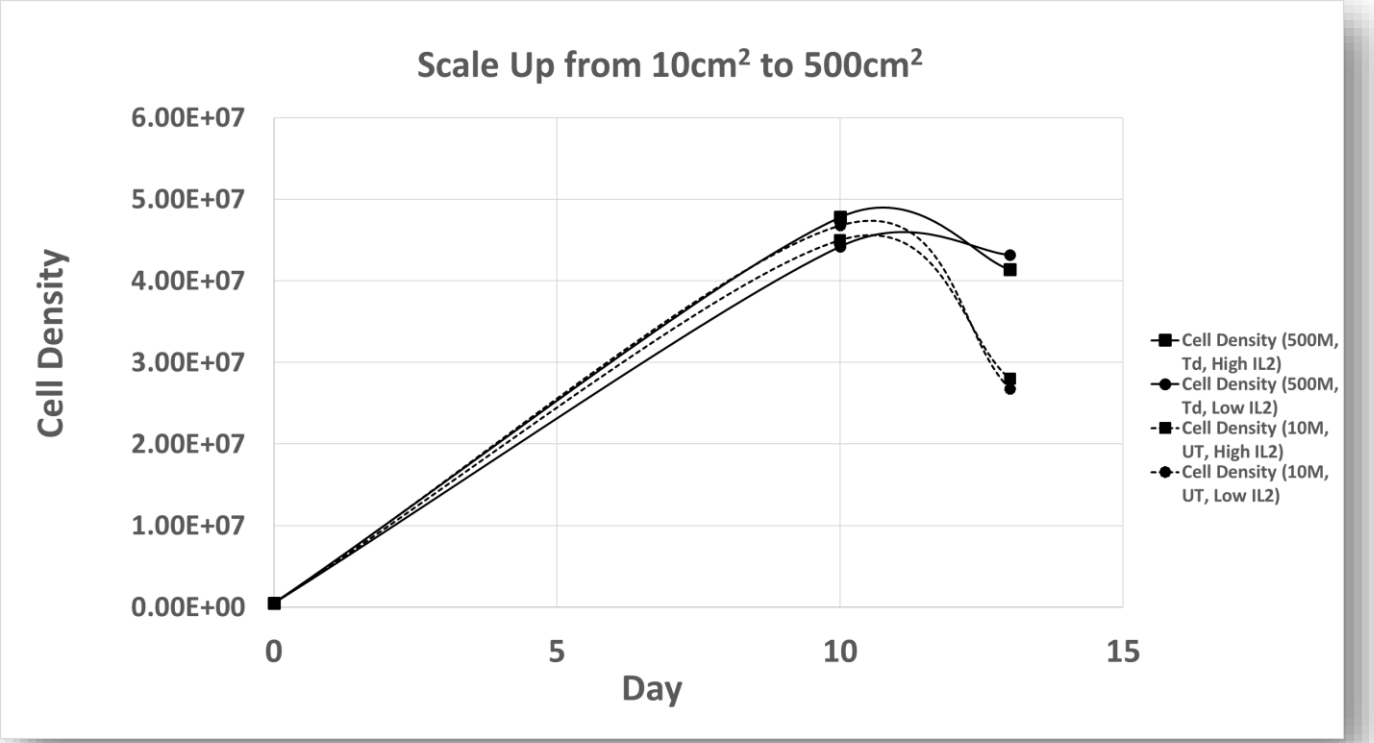
Transduction efficiency





*Unpublished data from Dr. Eric Tran Lab. Shared under Data Use Agreement

Linear Scalability of G-Rex® (TCR-T)



G-Rex10M
(P/N 80110S)

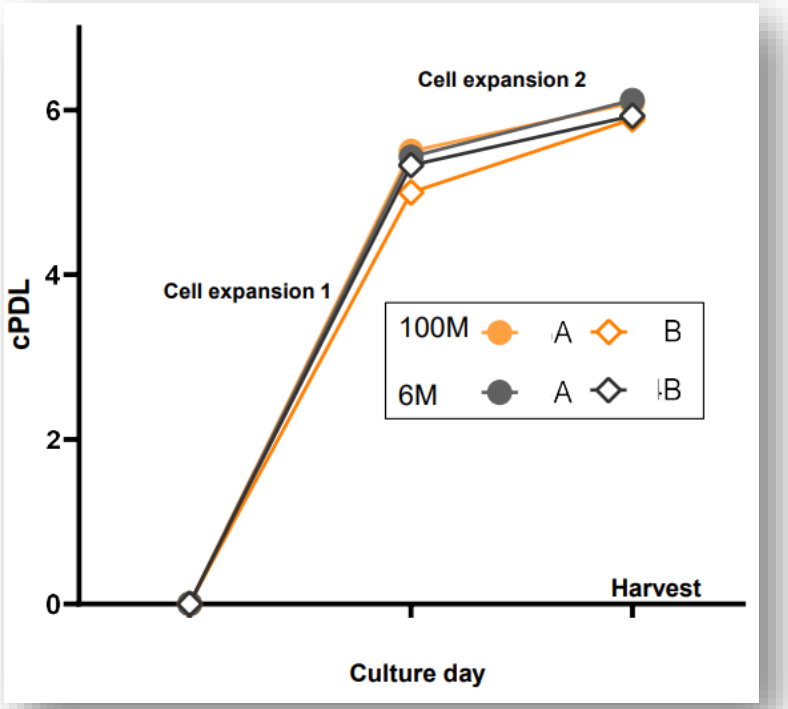
G-Rex500M
(P/N G285500-RU)

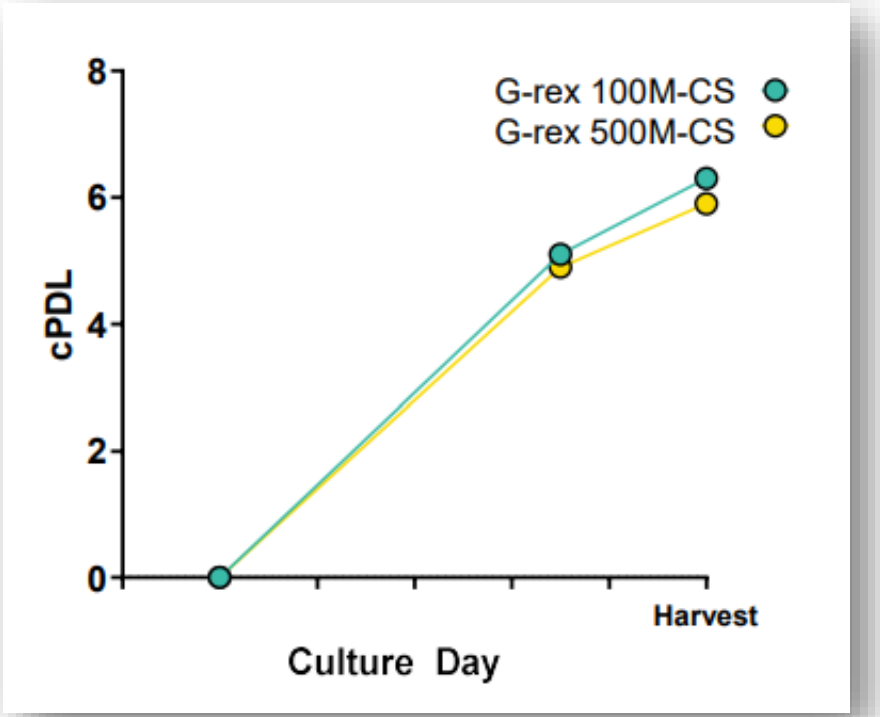
	Day 10 Cell Density (cells/cm²)	Day 10 Total Cells (n)
G-Rex10M	~4.5x10⁷	~4.5x10⁸
G-Rex500M	~4.5x10⁷	**~2.25x10¹⁰**

*Unpublished data from NCI Surgery Branch. Shared with Permission.

Linear Scalability of G-Rex® (CAR-T)

➢ Linear scalability of G-Rex® enables **process continuity**

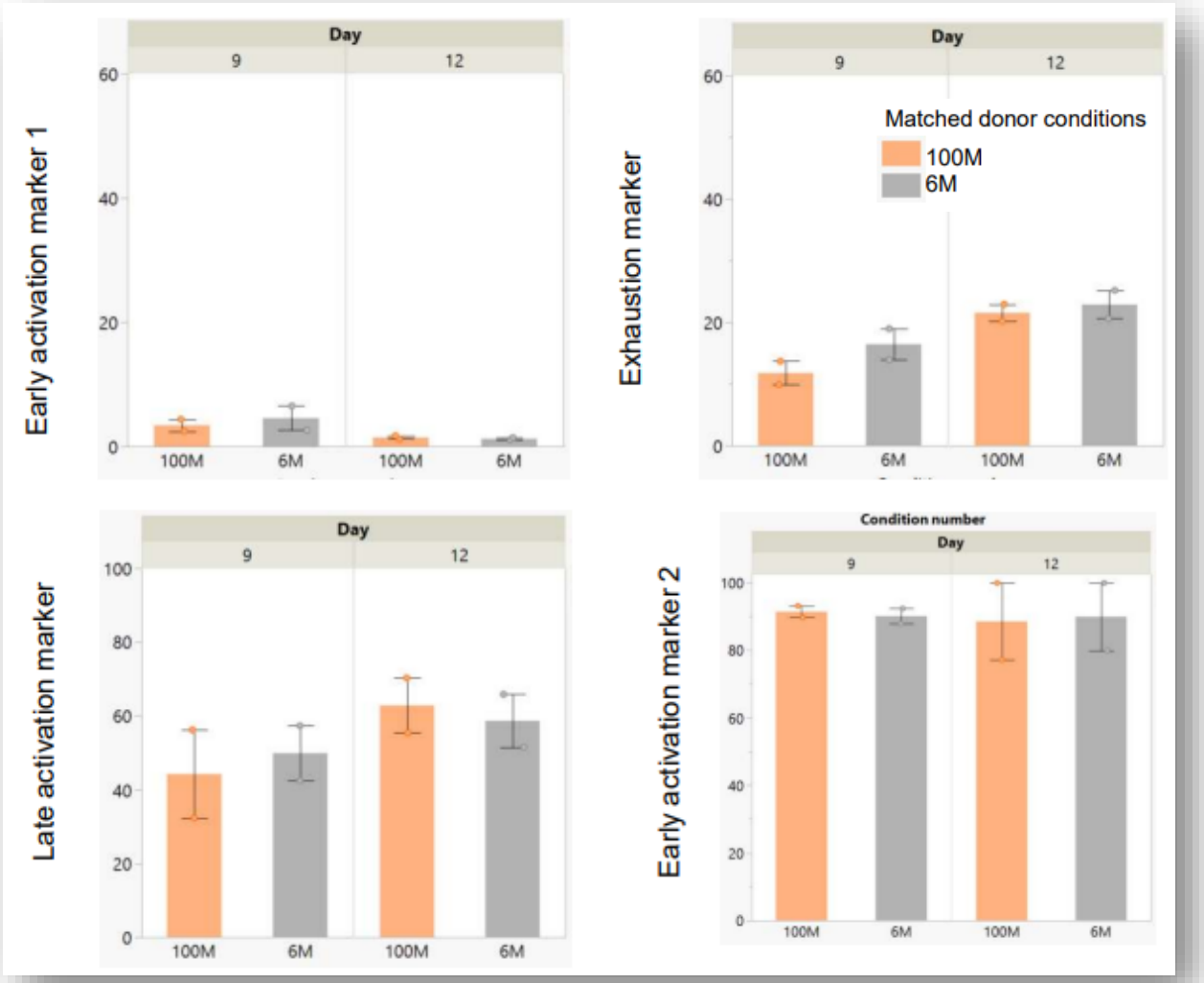




Customer data presented at conference

Linear Scalability of G-Rex® (CAR-T)

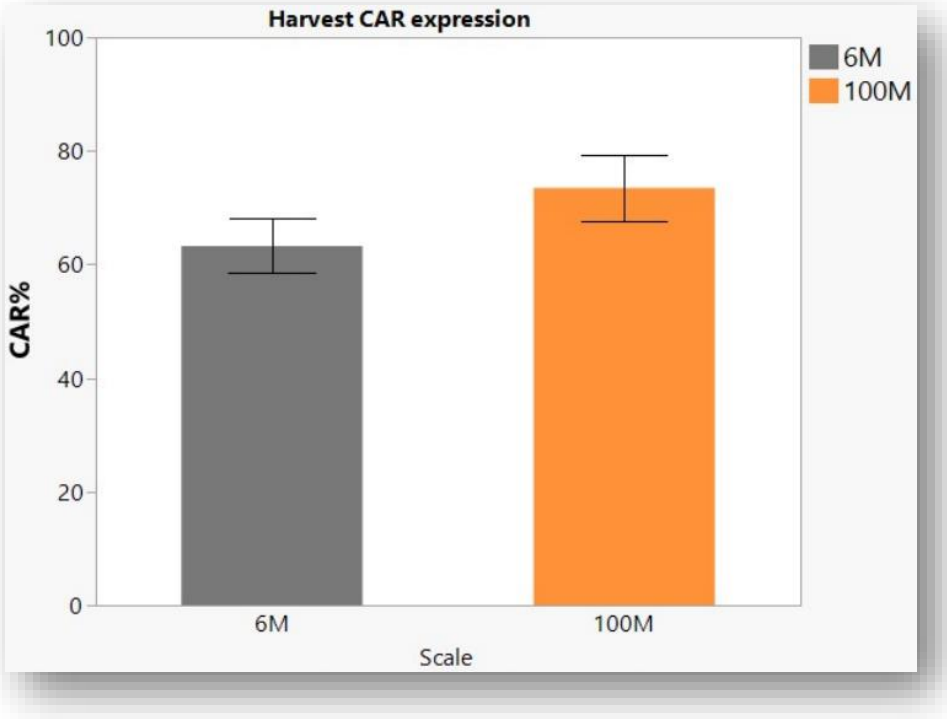
- Linear scalability of G-Rex® enables **product continuity**

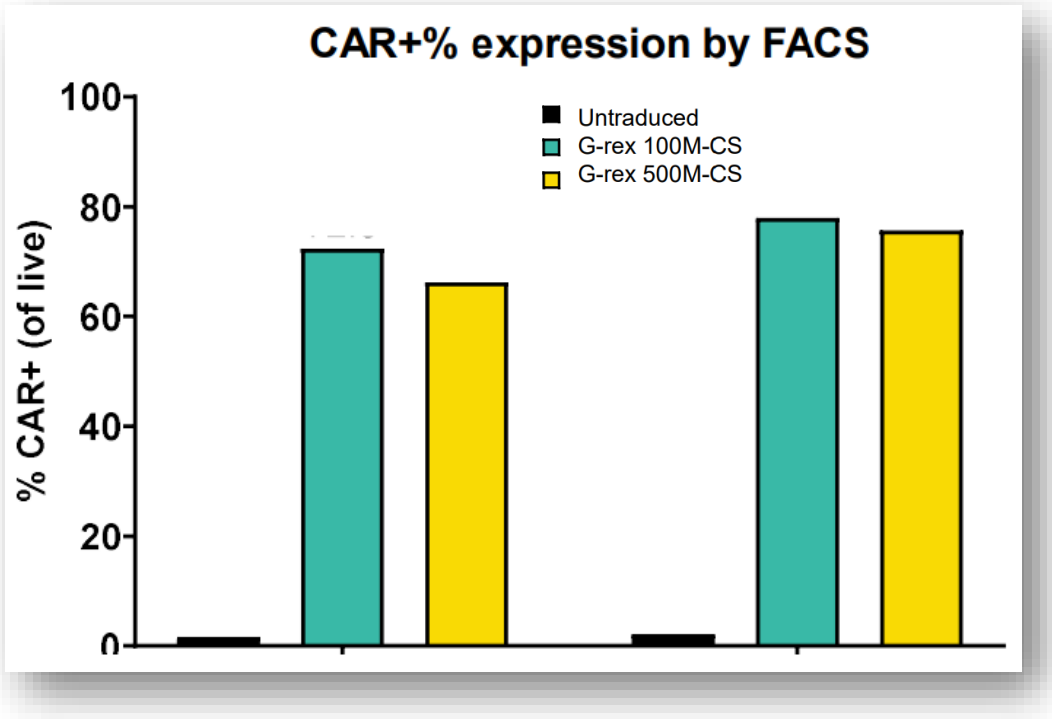


Customer data presented at conference

Linear Scalability of G-Rex® (CAR-T)

- Linear scalability of G-Rex® enables **product continuity**





Customer data presented at conference

Linear Scalability of G-Rex® (NK)

Process Continuity = **Product** Continuity

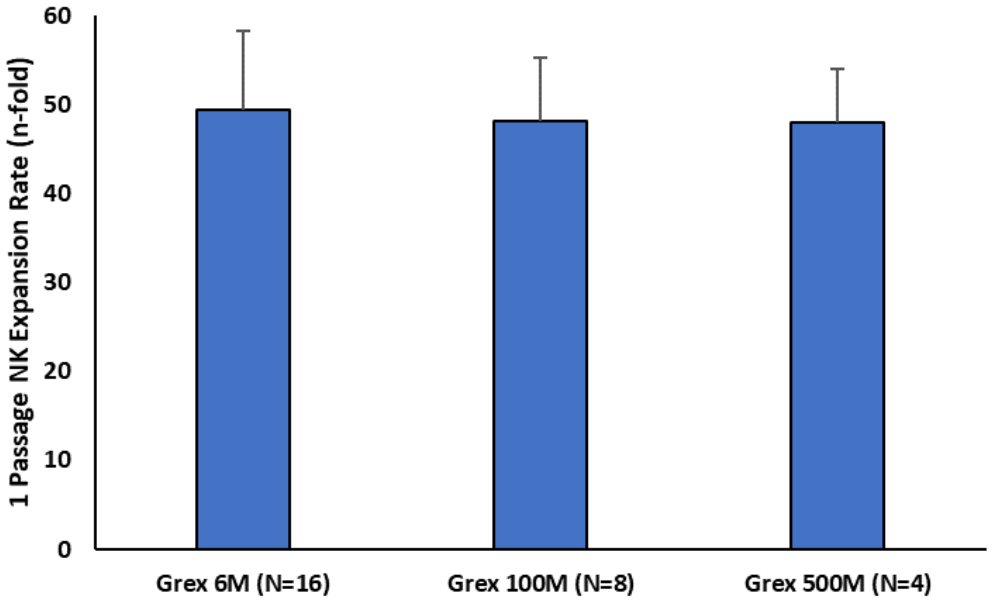


Figure 1. NK-cell expansion rate is comparable between Grex 6M, 100M, and 500M vessels.

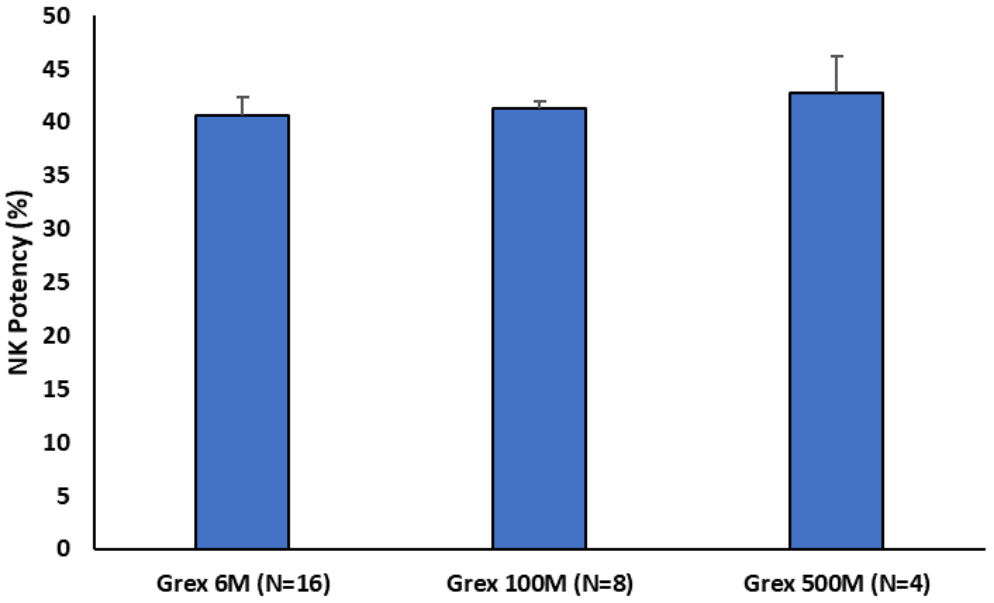
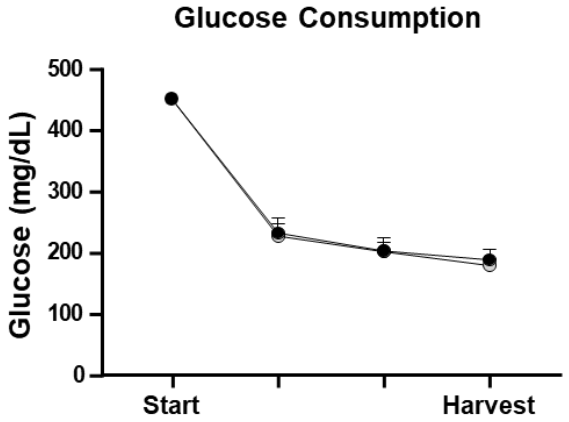


Figure 2. NK-cell potency is comparable between Grex 6M, 100M, and 500M vessels.

Linear Scalability of G-Rex® (NK)

A.



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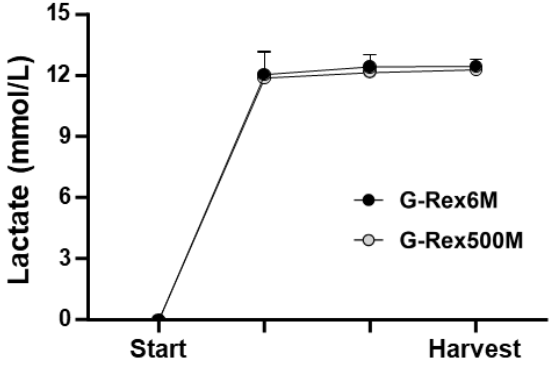
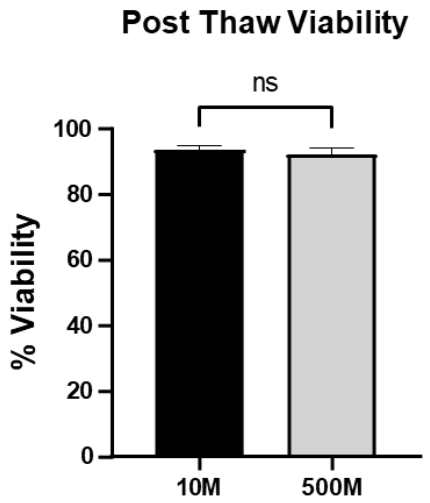
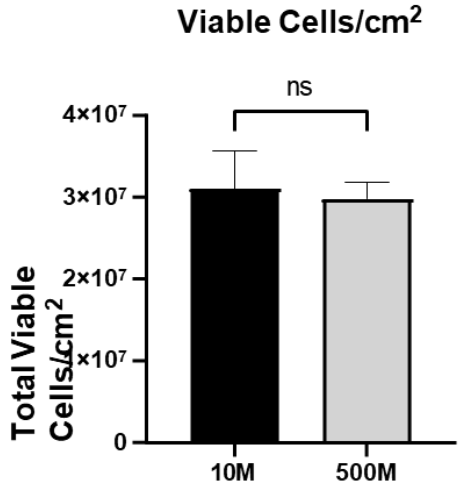


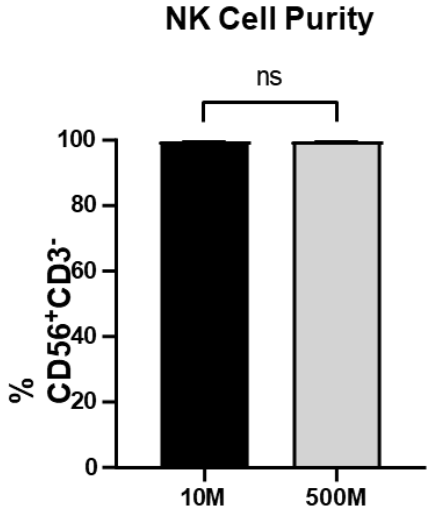
C.



D.



E.



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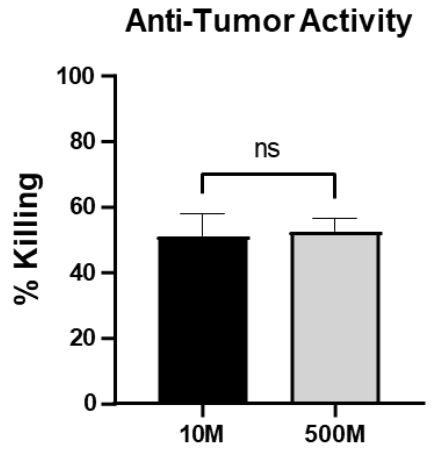
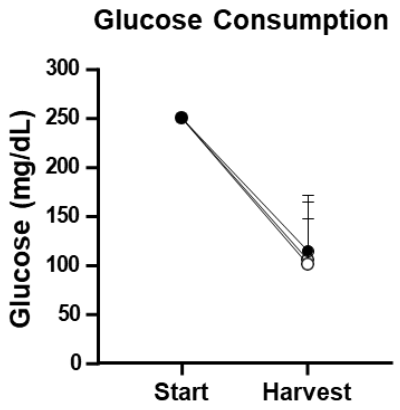


✓ Comparable Expansion Kinetics, Post Thaw Viability and Anti-Tumor Activity of NK Cells When Cultured in a G-Rex6M or G-Rex500M

Linear Scalability of G-Rex® (T cell)

A.



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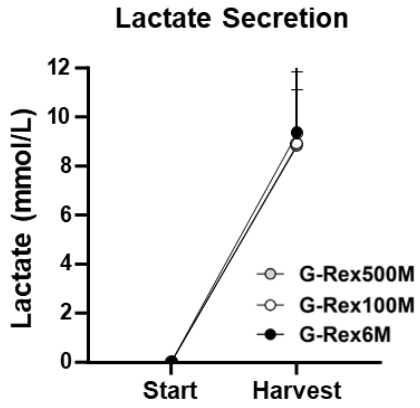

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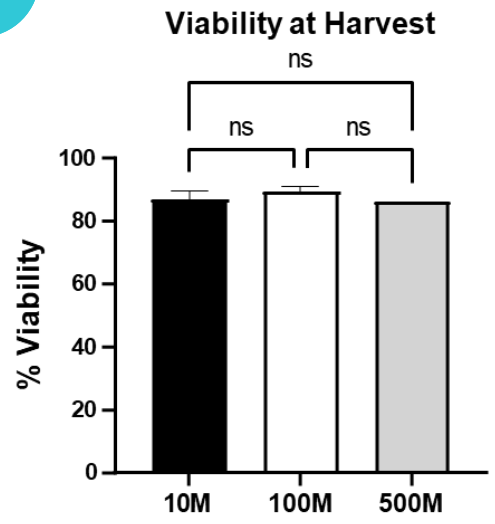

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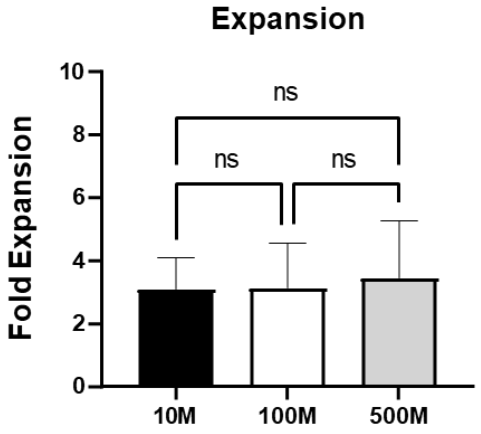

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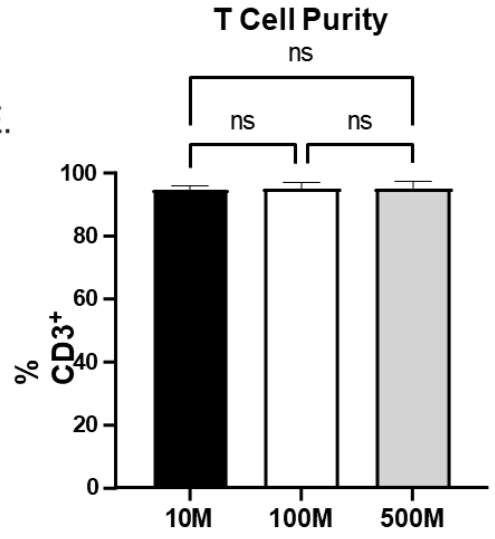

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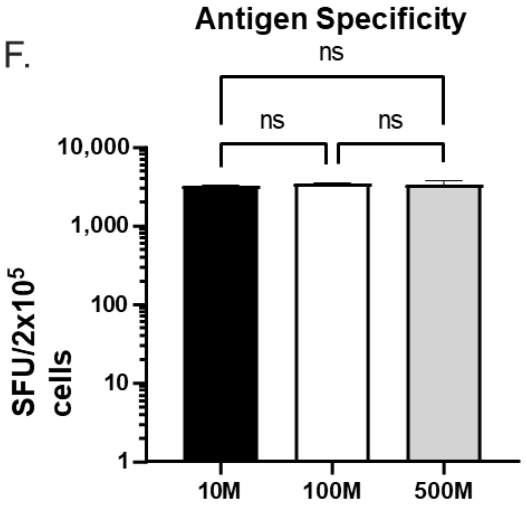
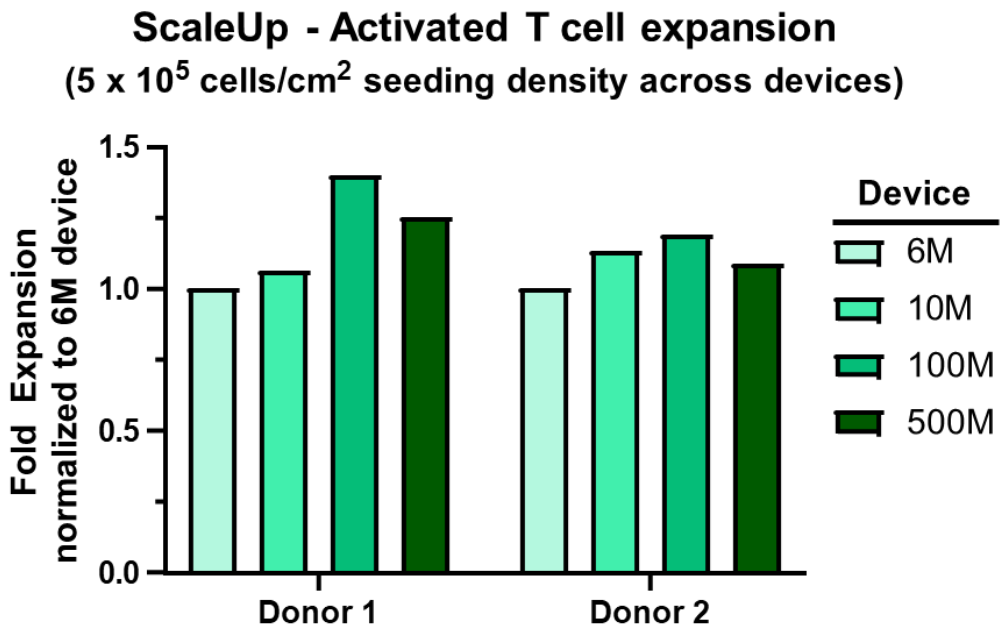
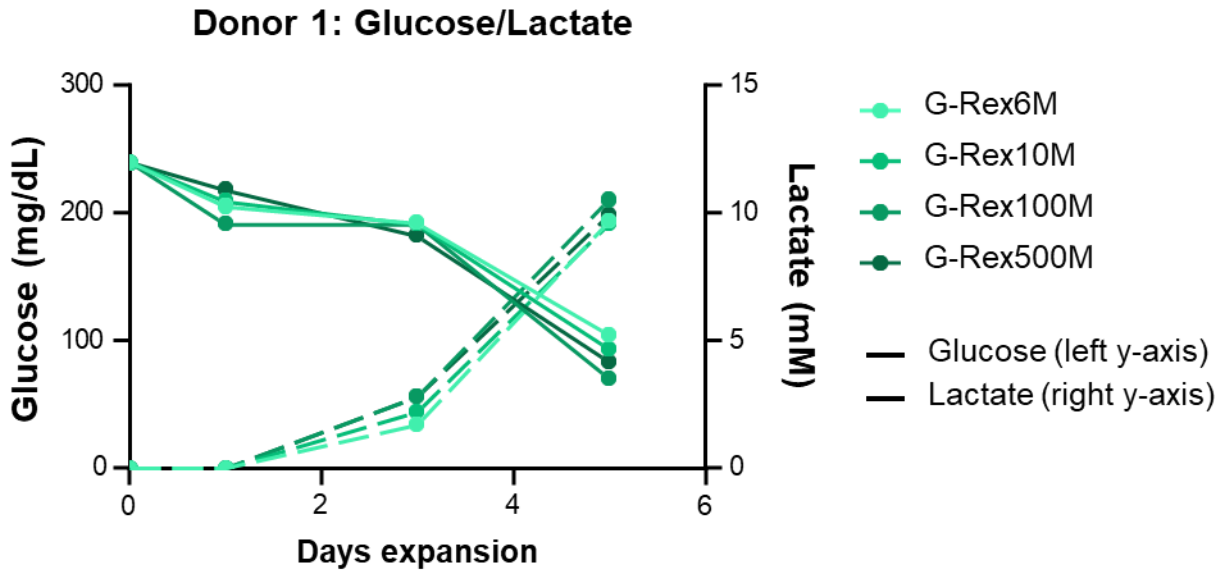
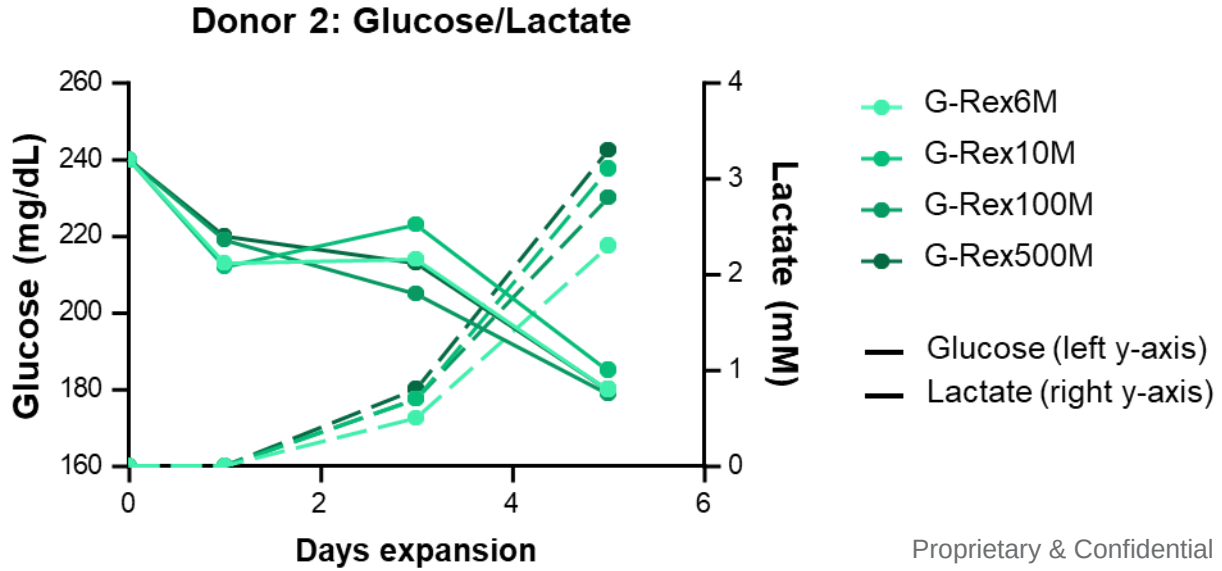


✓ Comparable Expansion Kinetics, Viability and Antigen Specificity of T Cells When Cultured in a G-Rex10M, G-Rex100M or G-Rex500M

Linear Scalability of G-Rex® (CAR-T)







- ✓ Relative to G-Rex6M (10cm^2), non-specific activated T cells display similar expansion kinetics & metabolites at scale-up
- ✓ Despite donor-to-donor variability, the G-Rex® is remarkably scalable from 6M up to 500M

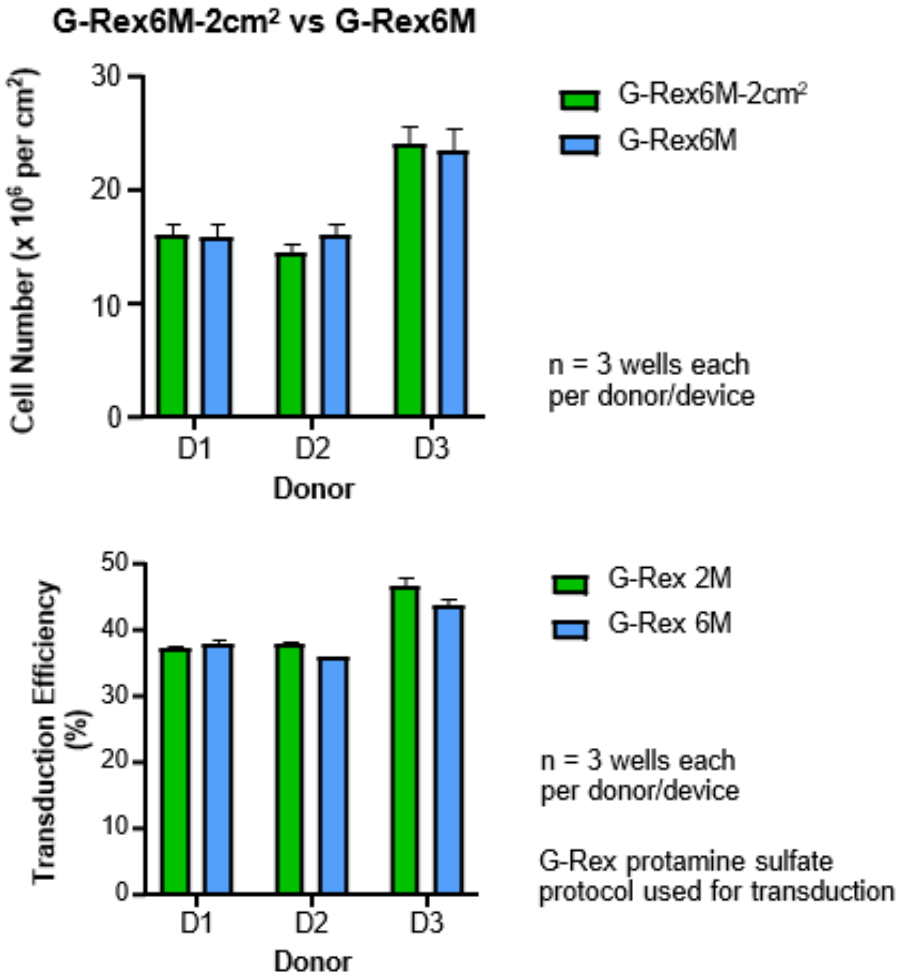
Linear Scalability of G-Rex® (CAR-T)

G-Rex6M-2cm²

Surface Area: 2cm²
Volume: 20mL

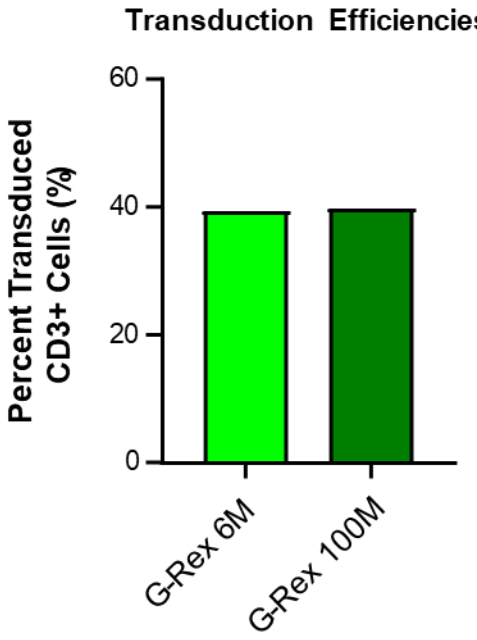
G-Rex6M

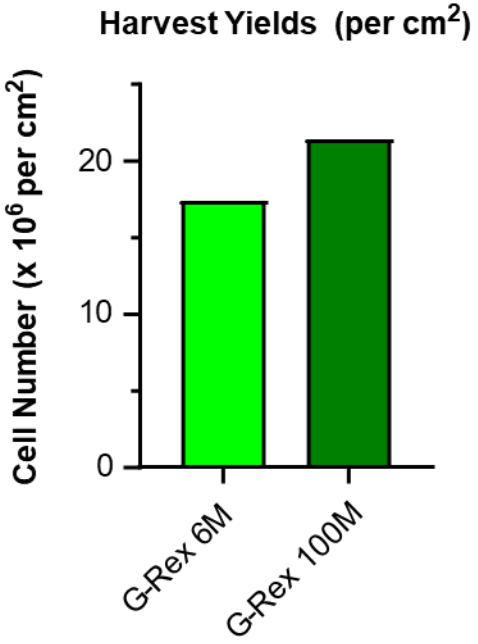
Surface Area: 10cm²
Volume: 100mL

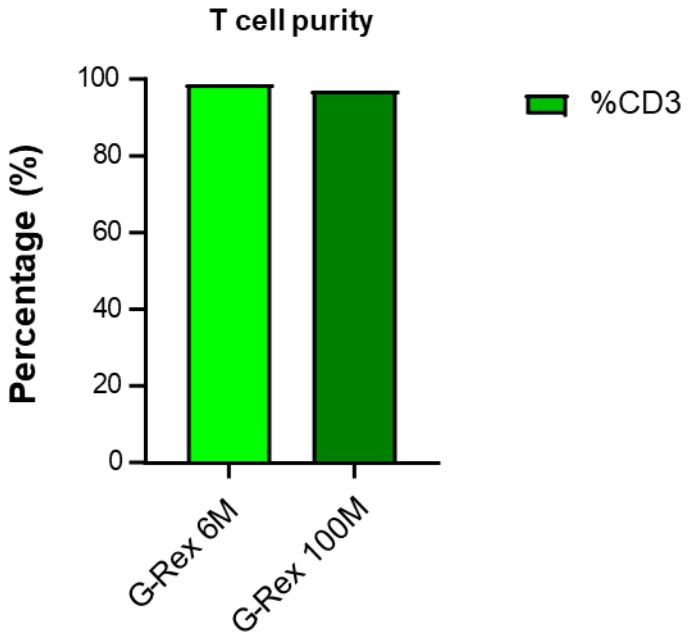


- ✓ Across 3 different healthy donors, lentivirus transduced T cells display similar growth kinetics in the G-Rex6M-2cm² and G-Rex6M. Similar cell densities (cells/cm²) were reached upon harvest.
- ✓ The G-Rex6M-2cm² shows comparability to the G-Rex6M – but requires only 20% of the reagents

Linear Scalability of G-Rex® (CAR-T)

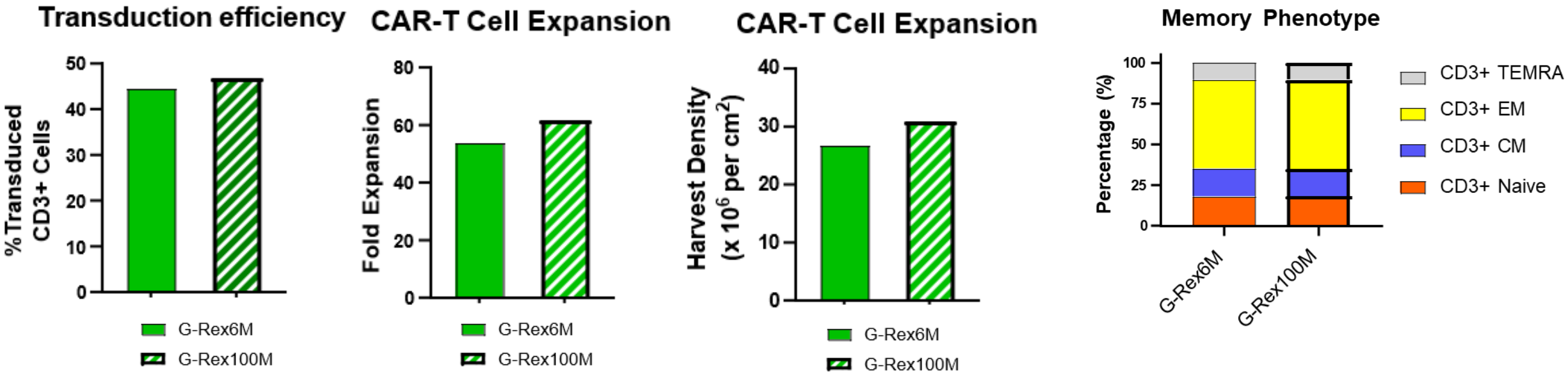






- ✓ Upscaling the process from the G-Rex6M to manufacturing scale in the G-Rex100M does not impact transduction efficiencies, cell yields, or purity
- ✓ Process development in the G-Rex6M can be translated directly to the G-Rex100M for product manufacture

Linear Scalability of G-Rex® (CAR-T)



- ✓ FMC63 anti-CD19 CAR-T manufacturing scalability using retrovirus and RetroNectin-coated G-Rex®
- ✓ Cell Ready compared transduction/expansion in the G-Rex6M to the G-Rex100M (n = 1 donor)
- ✓ 2-day activated PBMCs transduced overnight ($0.5x10^6$ cells/cm^2), expanded until Day 8 for harvest

G-Rex®: Flexibility ~~or~~ **and** Consistency

Flexibility & Consistency with G-Rex® (k562)

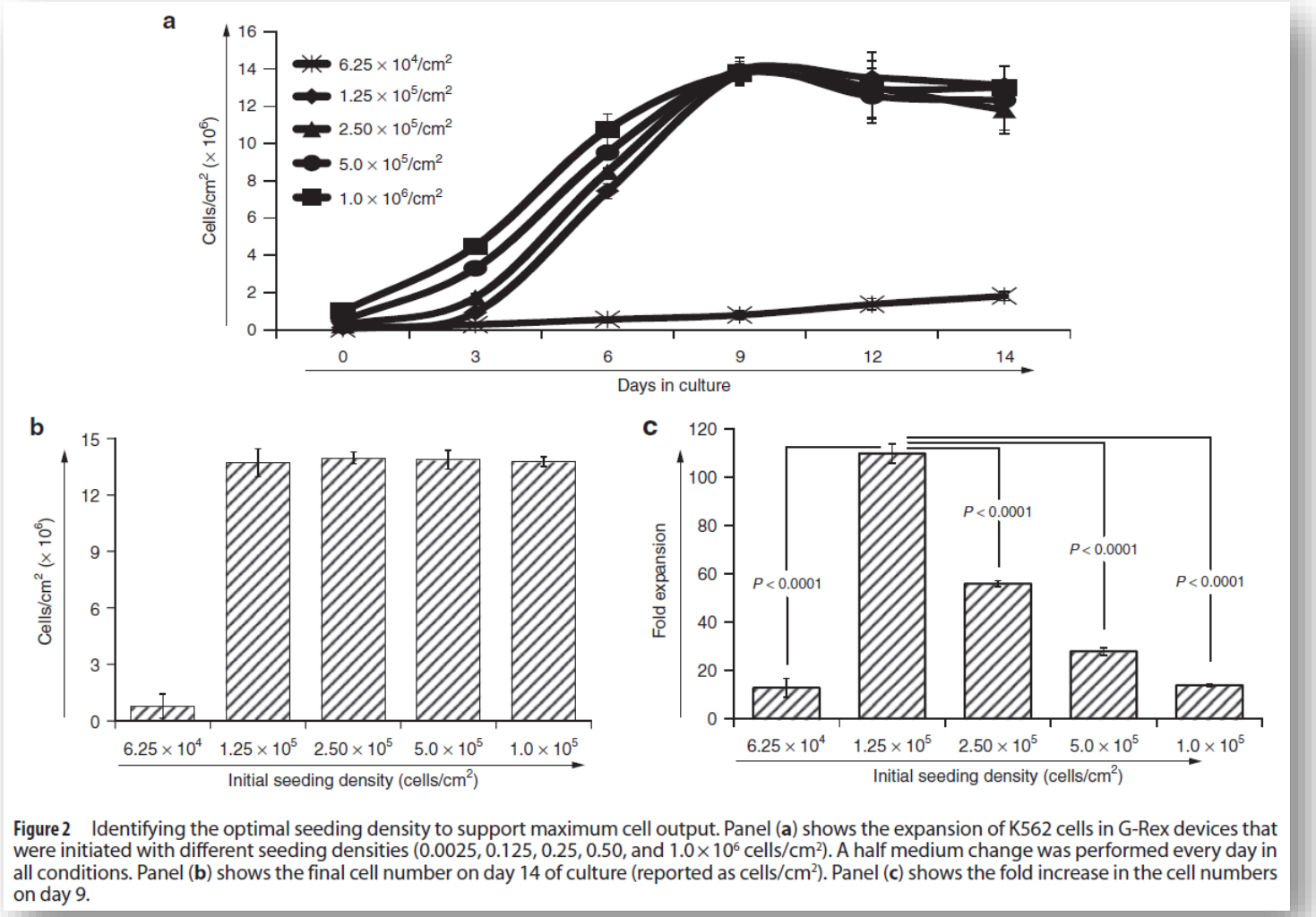
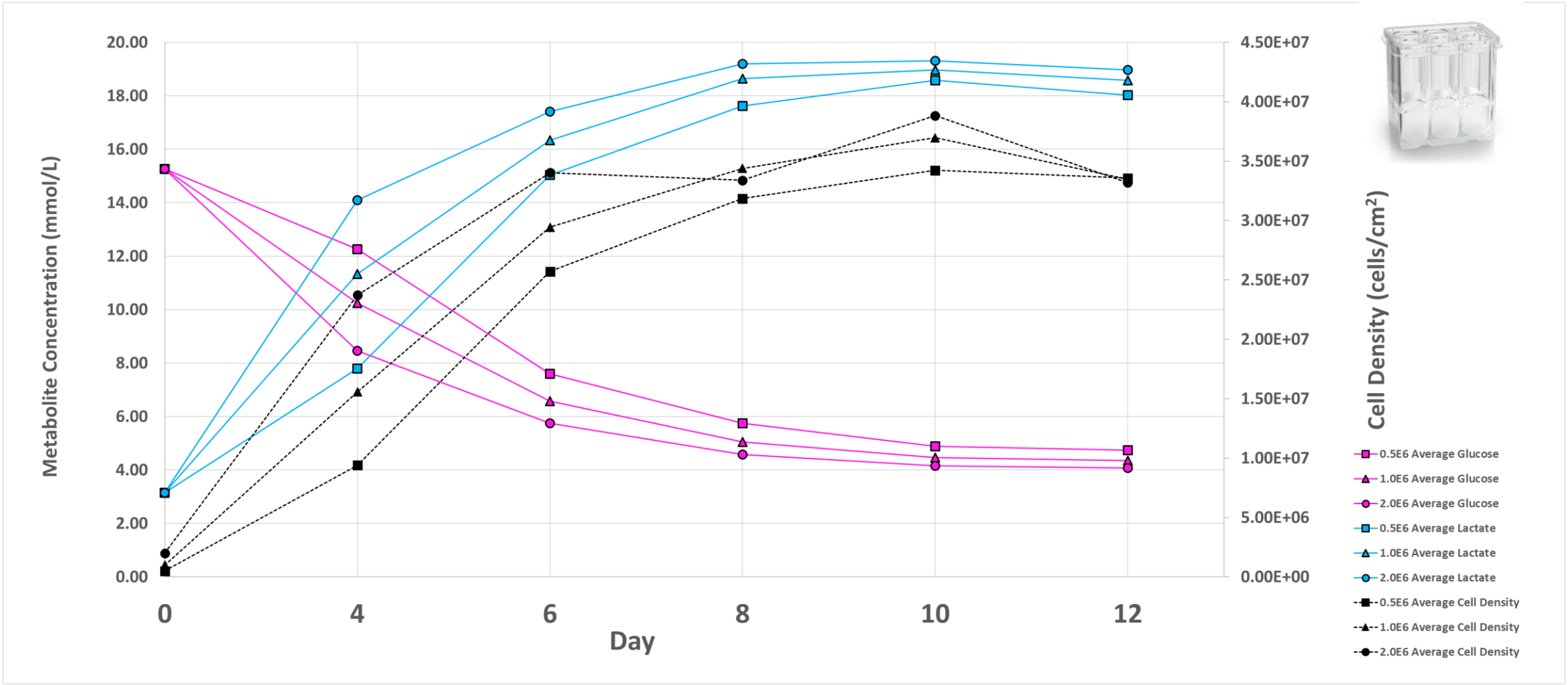


Figure 2 Identifying the optimal seeding density to support maximum cell output. Panel (**a**) shows the expansion of K562 cells in G-Rex devices that were initiated with different seeding densities (0.0025, 0.125, 0.25, 0.50, and 1.0×10^6 cells/cm²). A half medium change was performed every day in all conditions. Panel (**b**) shows the final cell number on day 14 of culture (reported as cells/cm²). Panel (**c**) shows the fold increase in the cell numbers on day 9.

- **Flexible** Seeding Densities (input)
- **Consistent** Max Cell Densities (output)

Bajgain P, Mucharla R, Wilson J, et al. Optimizing the production of suspension cells using the G-Rex "M" series. *Mol Ther Methods Clin Dev*. 2014;1:14015. Published 2014 May 14. doi:10.1038/mtm.2014.15

Flexibility & Consistency with G-Rex® (CAR-T)



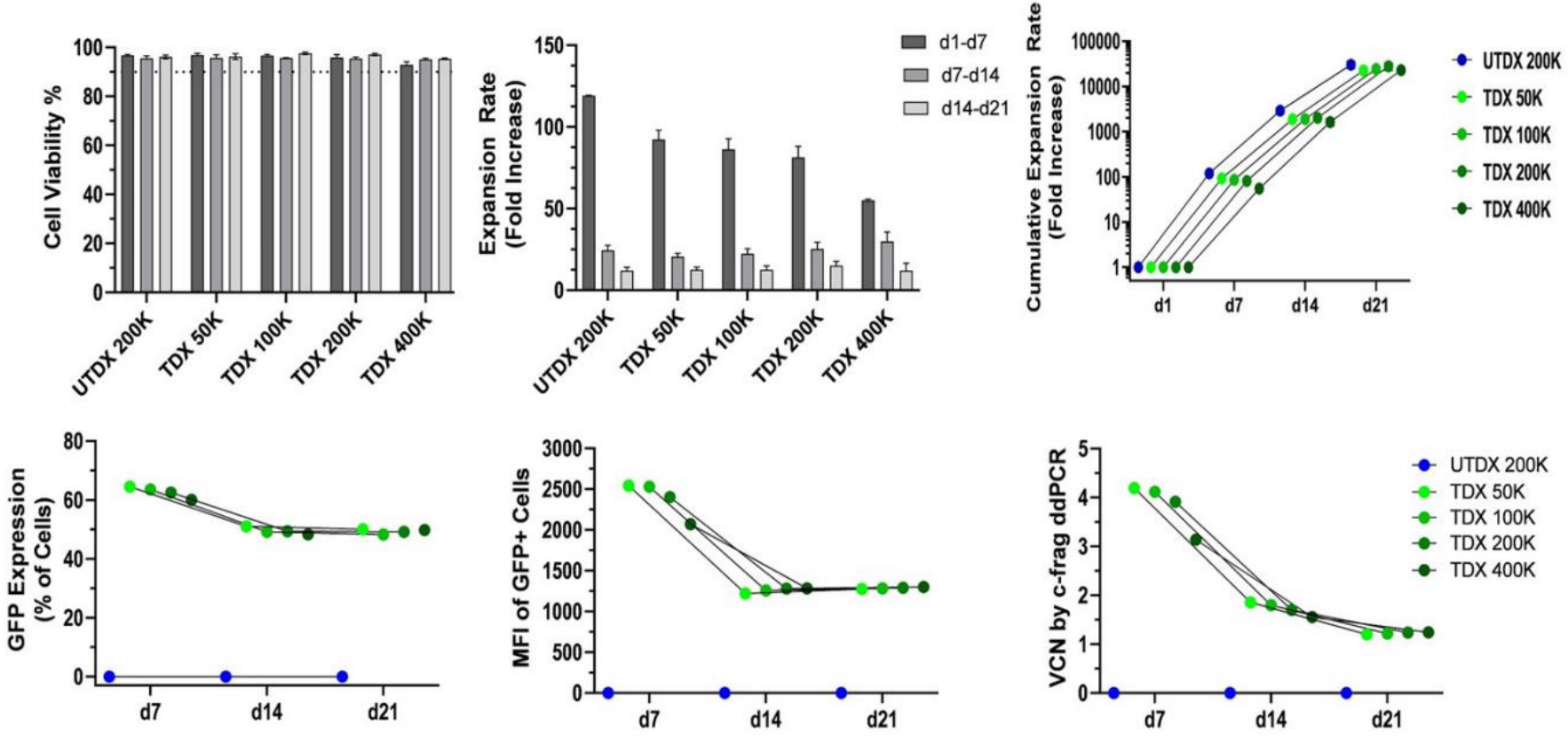
Unpublished customer data. CAR-T seeding density optimization. Shared with permission.

NOVEL, EFFICIENT SYSTEM FOR CULTURE OF TRANSDUCED HEMATOPOIETIC STEM PROGENITOR CELLS (HSPC) FOR VECTOR COPY NUMBER DETERMINATION

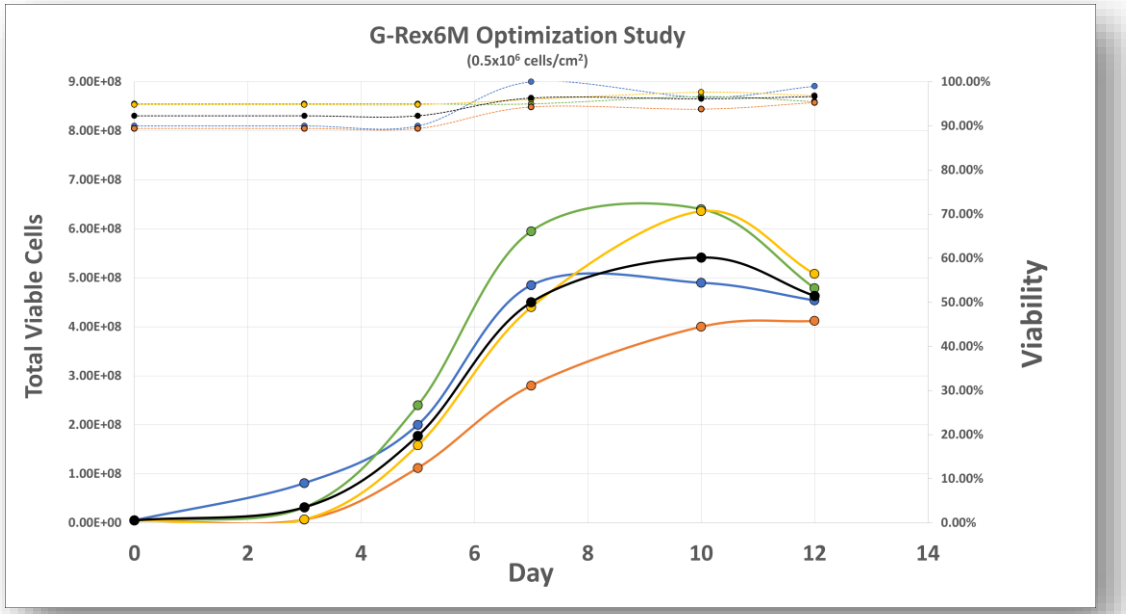
Monica Torres-Coronado[1], Ilse Arciga[1], Diana L. Browning[1], Xiu-Li Li[1], Agnes Gardner[1], Sergio Branciamore[2], John A. Zaia[1], Angelo A. Cardoso[1]

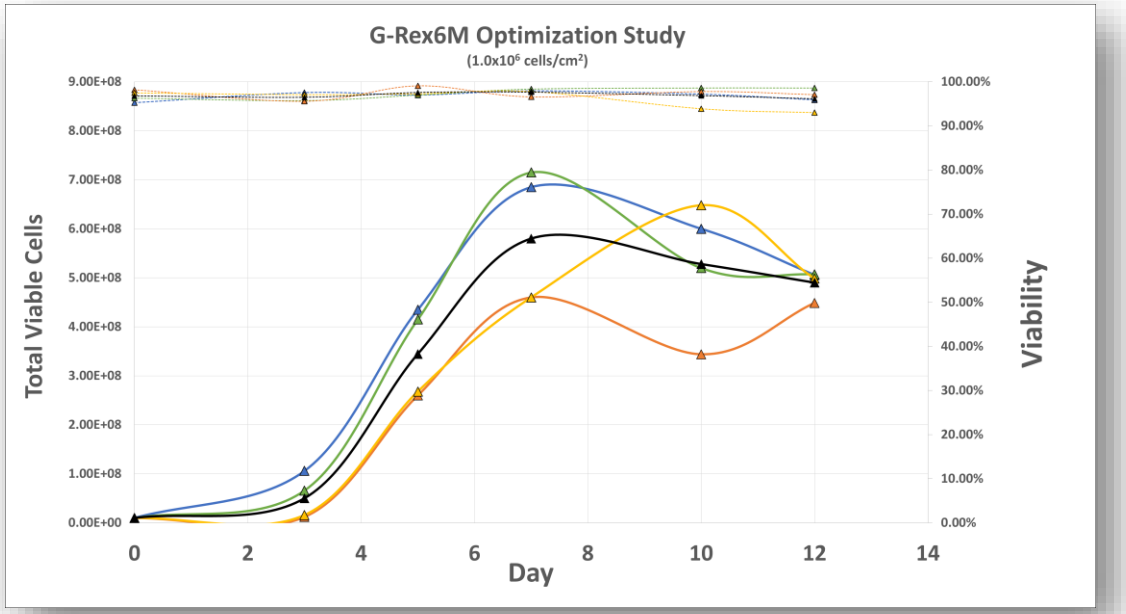
Center for Gene Therapy [1] and Department of Computational and Quantitative Medicine [2] of City of Hope, Duarte, CA

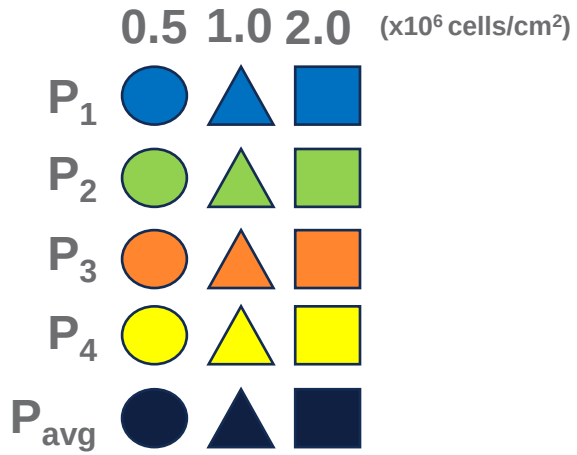
Establishing Seeding Conditions to culture HSPC in G-Rex Plates (TDX with GFP LVV)

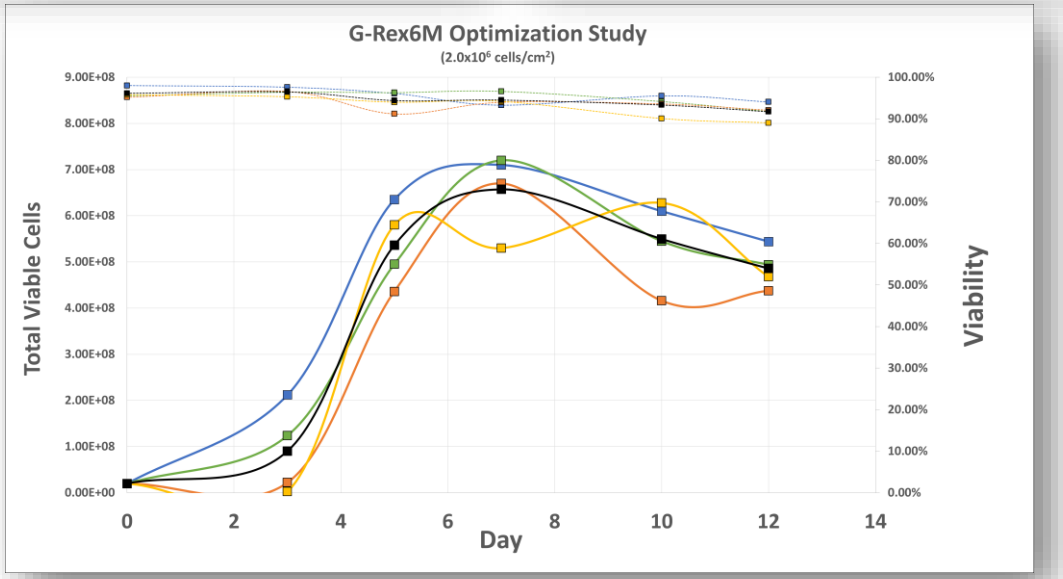


Flexibility & Consistency with G-Rex® (TCR-T)







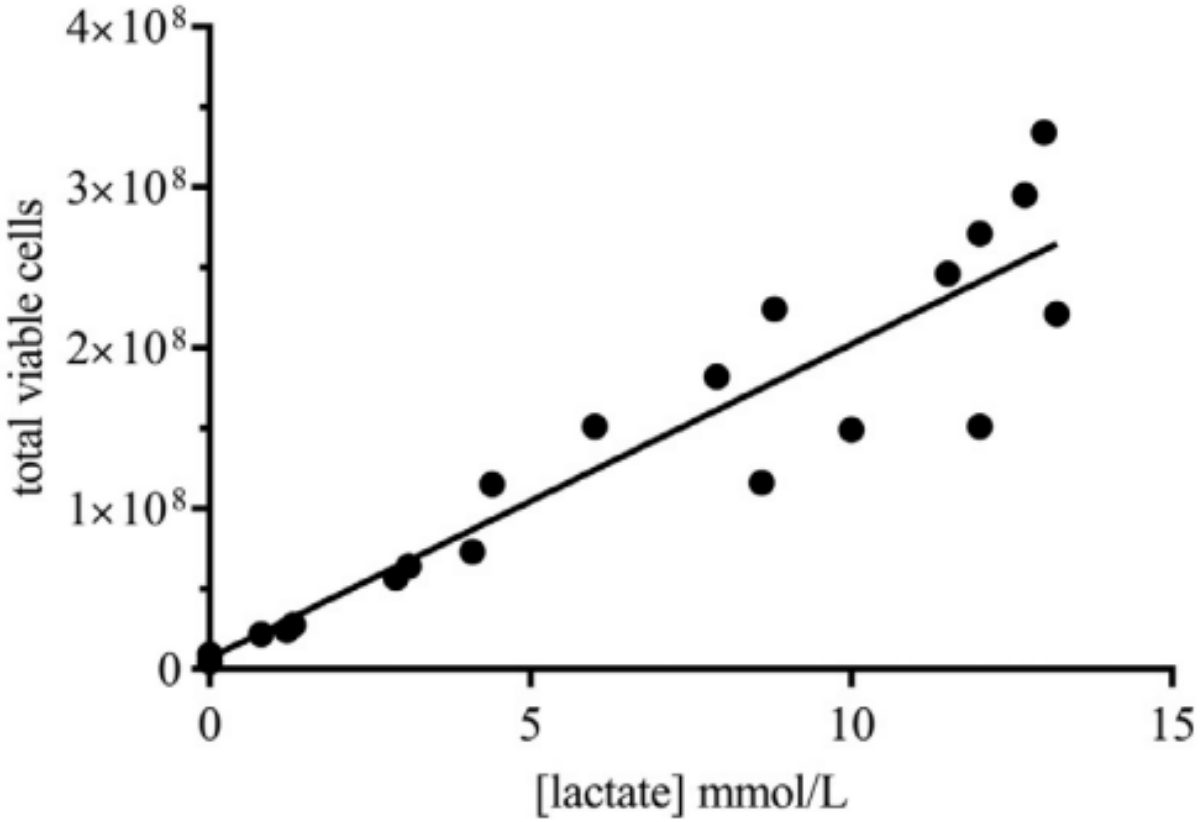


G-Rex6M Well Plate
(P/N 80660M)

ScaleReady

G-Rex®: Trending Metabolites

Measuring Lactate with G-Rex®

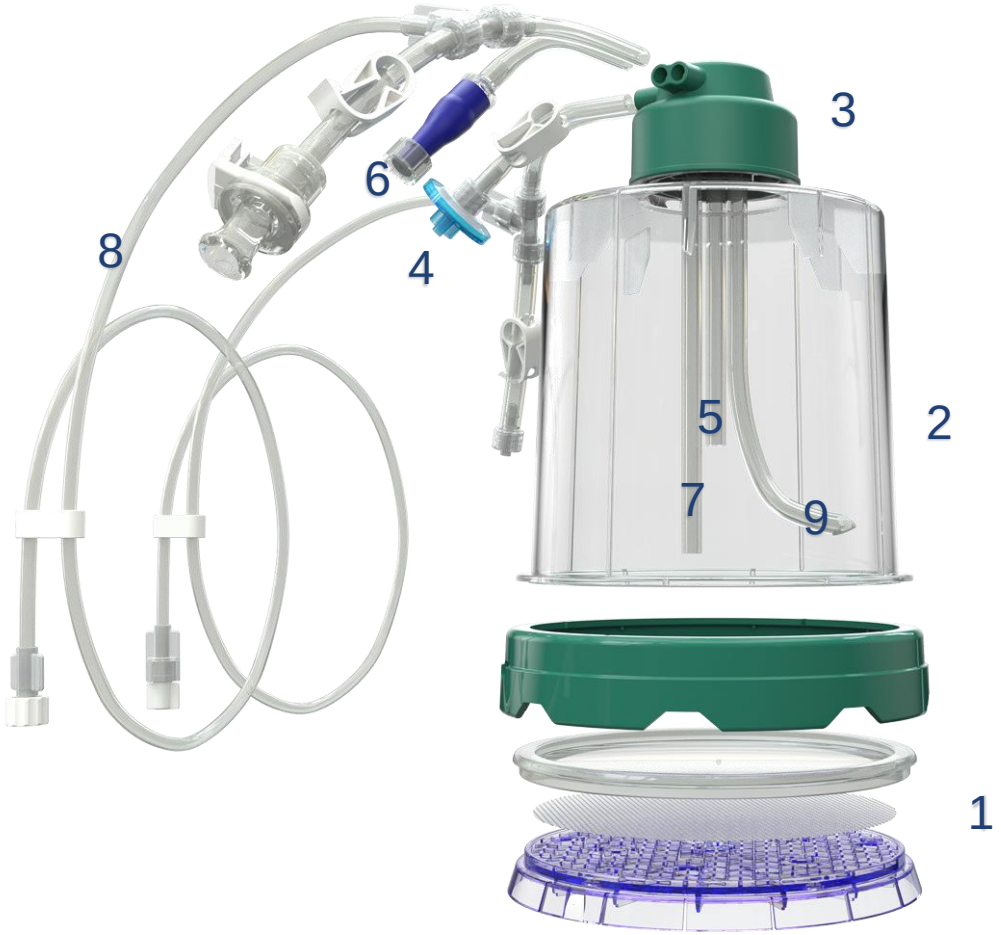


- Lactate concentrations in **G-Rex®** …
  - are useful surrogate measurements and leading indicators
  - correlates nearly perfectly to cell density
  - can inform harvest or passage events

Gagliardi C, Khalil M, Foster AE. Streamlined production of genetically modified T cells with activation, transduction and expansion in closed-system G-Rex bioreactors. Cytotherapy. 2019 Dec;21(12):1246-1257. DOI: 10.1016/j.jcyt.2019.10.006.

G-Rex®: Closed System Overview

G-Rex Closed System Technology



G-Rex100M-CS
(81100-CS, RU81100-CS)

1 – Gas Permeable Membrane

2 – Polycarbonate Shell (Vessel)

3 – Polypropylene Multi-Port Cap

4 – Vent Filter (0.2 µm)

5 – Sample Line Tubing

6 - MicroClave® Connector

7 – Reduction Line Tubing

8 – PVC Weldable Reduction Line

9 – Harvest Line Tubing

10 – PVC Weldable Harvest Line

GatheRex® for efficient, closed system G-Rex® processing

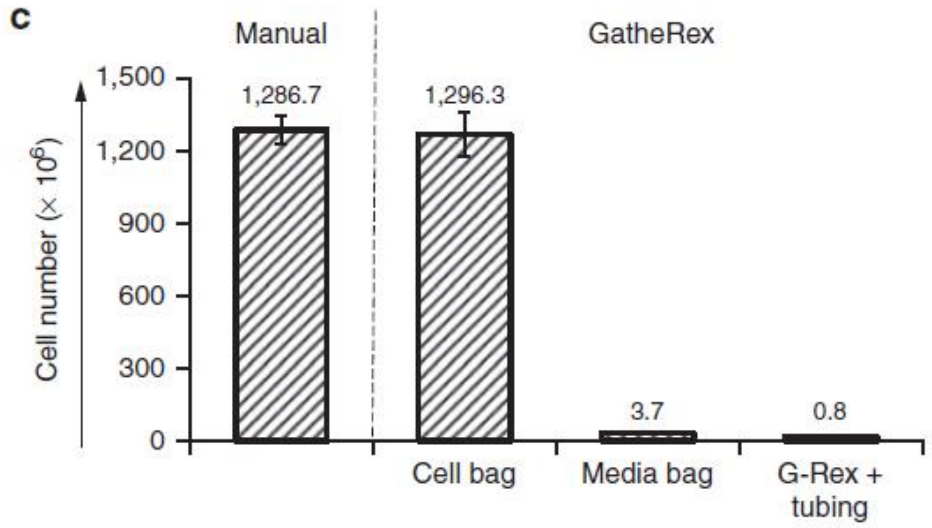
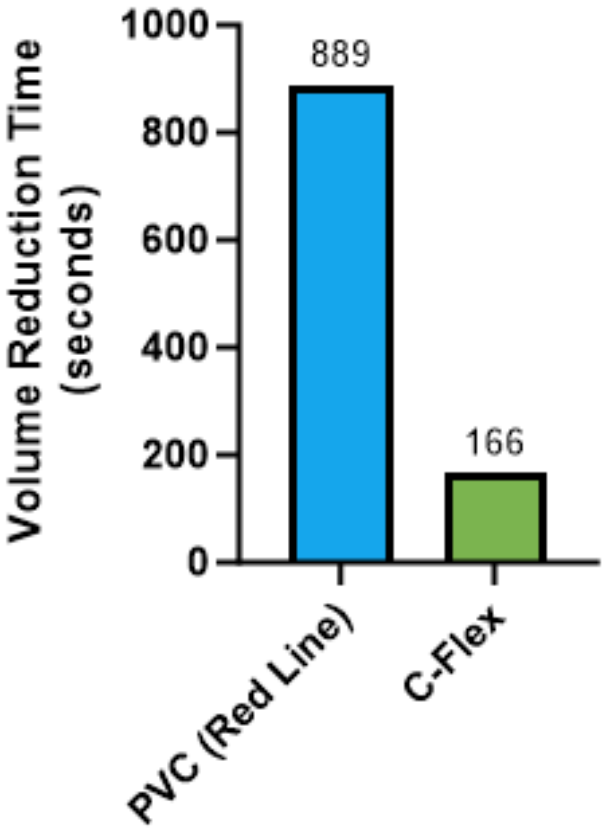


Figure 7 Automated collection of cells in a closed G-Rex system. Panel (**a**) shows the setup of "GatheRex" draining the excess medium to concentrate the cells: (1) pump, (2) 0.2-µm sterile filter, (3) medium collection button, (4) medium line clamp, (5,6) medium/supplements input ports, (7) medium sampling port, (8) medium collection port, (9) medium bag, (10) medium, and (11) medium line optical detector. Panel (**b**) shows the GatheRex harvesting the concentrated cells: (12) resuspended cells, (13) cell collection button, (14) cell line clamp, (15) cell collection port, (16) cell collection bag, (17) harvested cells, and (18) cell line optical detector. Panel (**c**) shows a comparison in cell recovery achieved when cells are collected manually versus by the GatheRex. To confirm the absence of cells in cell collection tubing, the medium collection bag or the G-Rex following cell collection with the GatheRex, each component was washed with media and residual cells were harvested and counted.

- Semi-automated **volume reduction** for concentrated cell product
- Semi-automated **cell harvest** with high recovery

Bajgain P, Mucharla R, Wilson J, et al. Optimizing the production of suspension cells using the G-Rex "M" series. *Mol Ther Methods Clin Dev*. 2014;1:14015. Published 2014 May 14. doi:10.1038/mtm.2014.15

Grex500 Volume Reduction



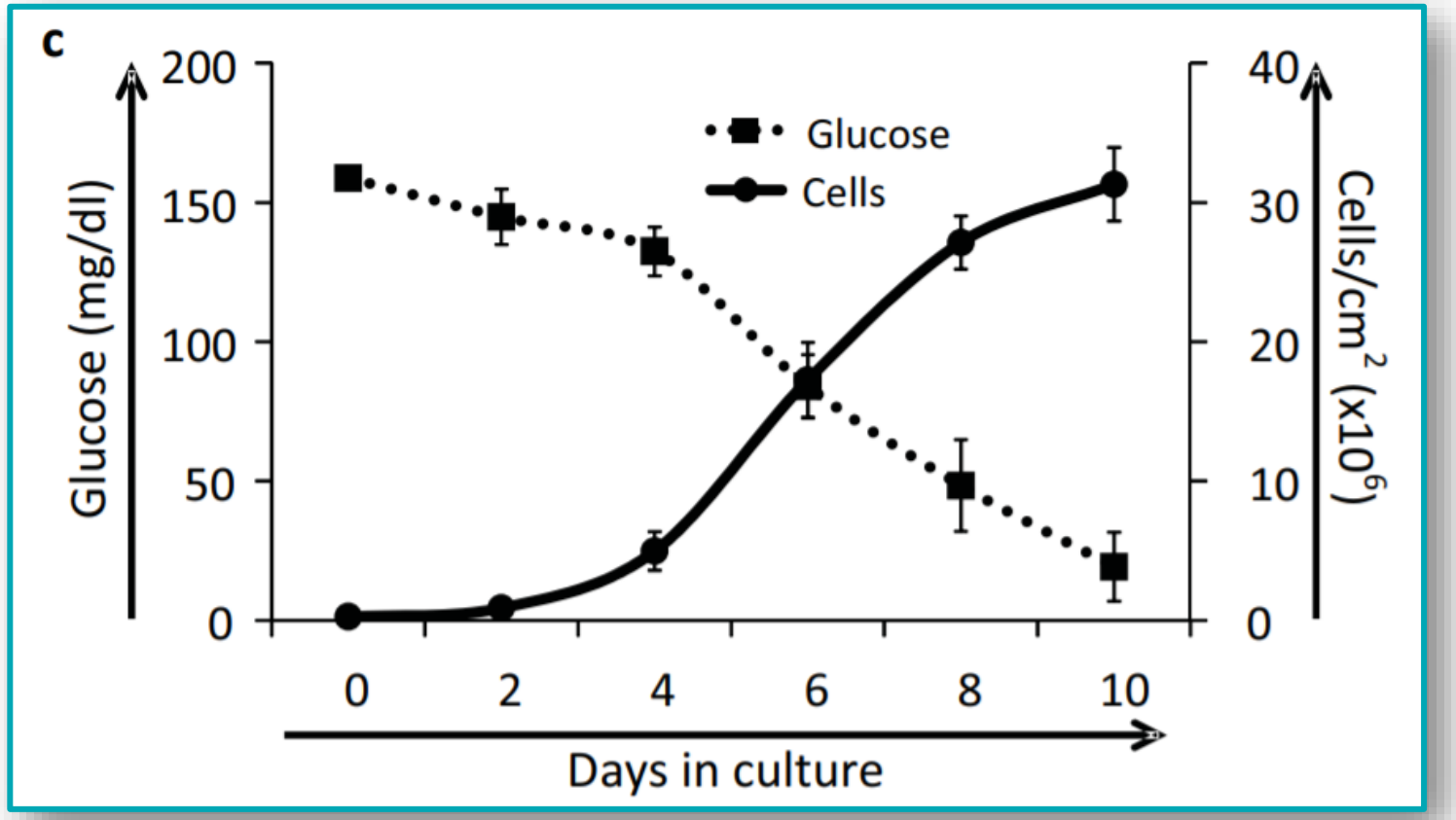
G-Rex500M-CS

G-Rex500M-TF

- ✓ Use large diameter C-Flex tubing to quickly **volume reduce** a G-Rex500M-CS or G-Rex500M-TF
  - ➢ Requires GatheRex model 80000Z
- ✓ Volume reduce 90% (4.5L) of waste media out in **less than 3 minutes**
  - ✓ ~80% faster when compared to small diameter PVC tubing
- ✓ **Concentrate cells** to 40e6 cells/mL in ~500mL for faster downstream processing

Data shared with consent from existing GREX client who prefers to remain anonymous

G-Rex® for CAR-T cell therapy



- ✓ Fill up the G-Rex with the full volume of media (10mL/cm^2) and go from *minimum cell density* to *maximum cell density* in a single REP with **minimal (potentially no) intervention**
- ✓ A single **G-Rex100M-CS** (below) will produce **3+ billion cells**
- ✓ A standard, stackable incubator (like Thermo Fisher's Heracell250i) can hold **~45 G-Rex100M-CS devices per incubator**

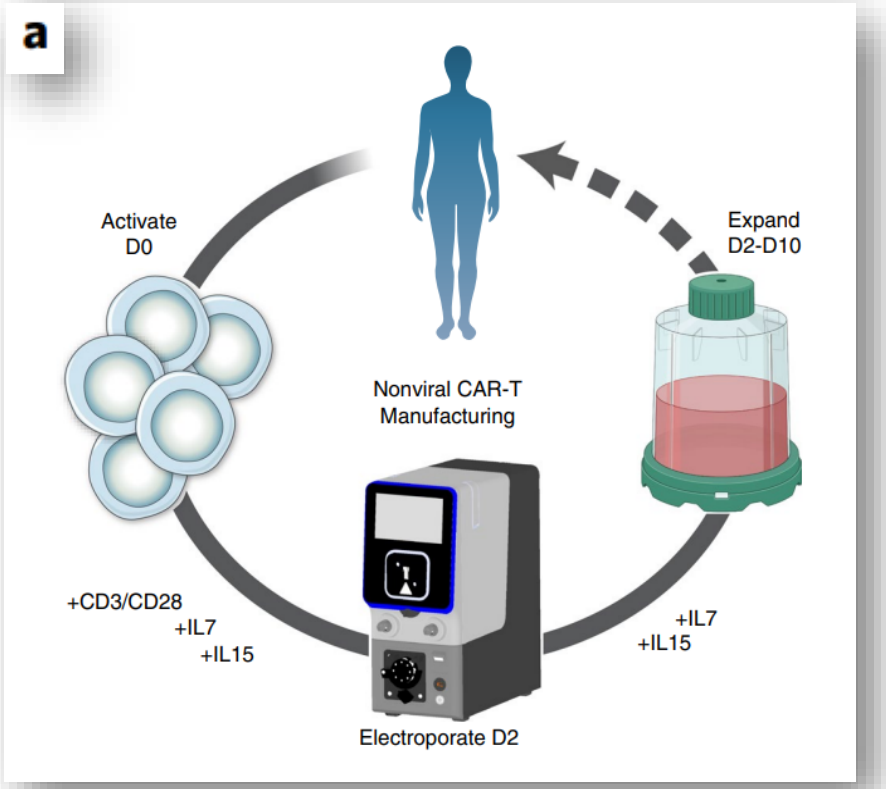
G-Rex100M-CS (P/N 81100-CS)

Bajgain P, Mucharla R, Wilson J, et al. Optimizing the production of suspension cells using the G-Rex "M" series. *Mol Ther Methods Clin Dev*. 2014;1:14015. Published 2014 May 14. doi:10.1038/mtm.2014.15

nature biotechnology
ARTICLES
https://doi.org/10.1038/s41587-022-01418-8
Check for updates

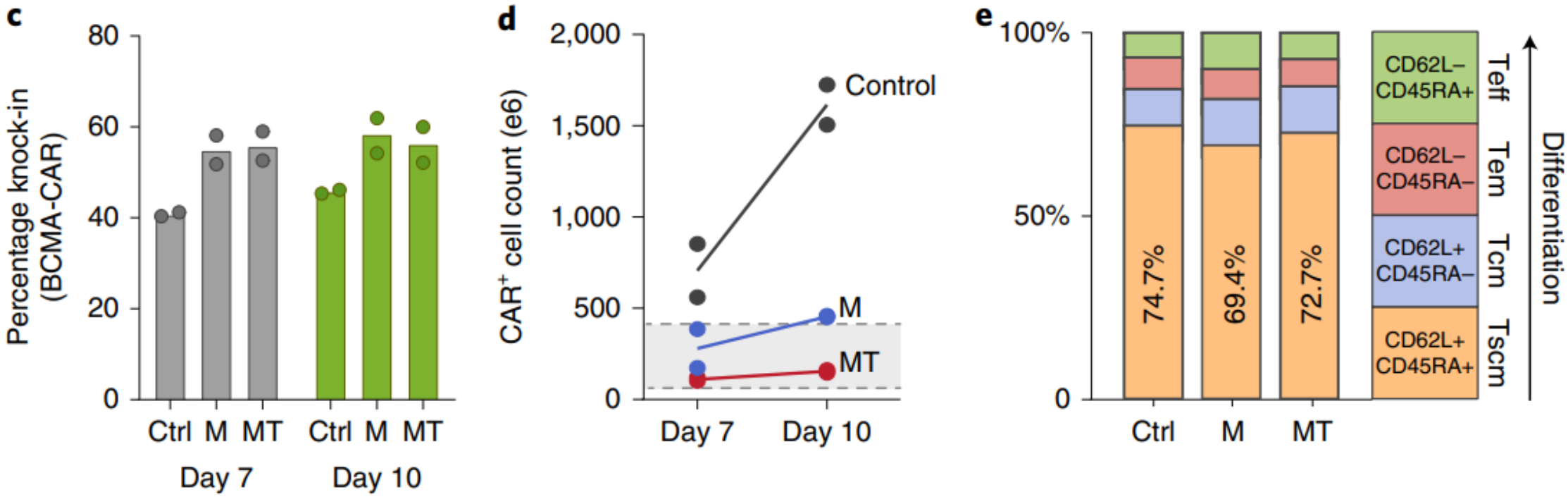
High-yield genome engineering in primary cells using a hybrid ssDNA repair template and small-molecule cocktails

Brian R. Shy[1,2,3,4,17] ✉, Vivasvan S. Vykunta[2,3,17], Alvin Ha[1,2,3], Alexis Talbot[2,3,5,6,7], Theodore L. Roth[2,3,8], David N. Nguyen[2,3,4], Wolfgang G. Pfeifer[9,10], Yan Yi Chen[2,3], Franziska Blaeschke[2,3], Eric Shifrut[2,3], Shane Vedova[2,3], Murad R. Mamedov[2,3], Jing-Yi Jing Chung[2,3,5,6], Hong Li[11], Ruby Yu[2,3], David Wu[8], Jeffrey Wolf[3,12], Thomas G. Martin[3,12], Carlos E. Castro[9,13], Lumeng Ye[11], Jonathan H. Esensten[1,2], Justin Eyquem[2,3,5,6] and Alexander Marson[2,3,4,5,6,12,14,15,16] ✉



➢ **G-REX® centric, cGMP Compliant, Non-Viral CAR-T cell manufacturing**

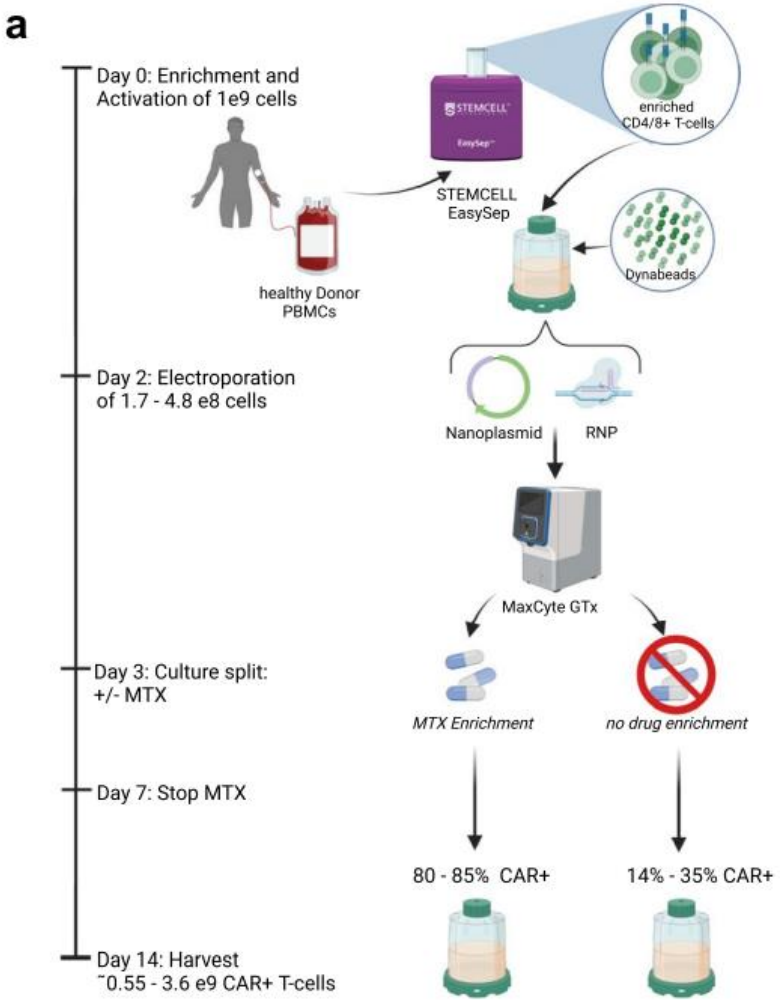
Shy, B. R., Vykunta, V. S., Ha, A., Talbot, A., Roth, T. L., Nguyen, D. N., Pfeifer, W. G., Chen, Y. Y., Blaeschke, F., Shifrut, E., Vedova, S., Mamedov, M. R., Chung, J.-Y. J., Li, H., Yu, R., Wu, D., Wolf, J., Martin, T. G., Castro, C. E., … Marson, A. (2022). High-yield genome engineering in primary cells using a hybrid ssdna repair template and small-molecule cocktails. *Nature Biotechnology*, *41*(4), 521–531. https://doi.org/10.1038/s41587-022-01418-8

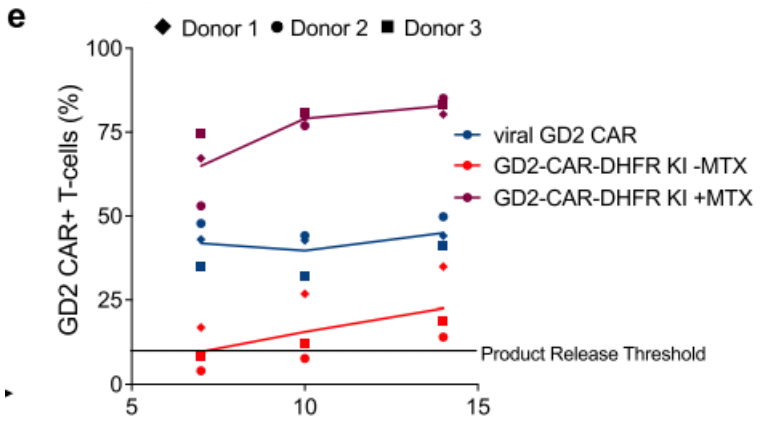


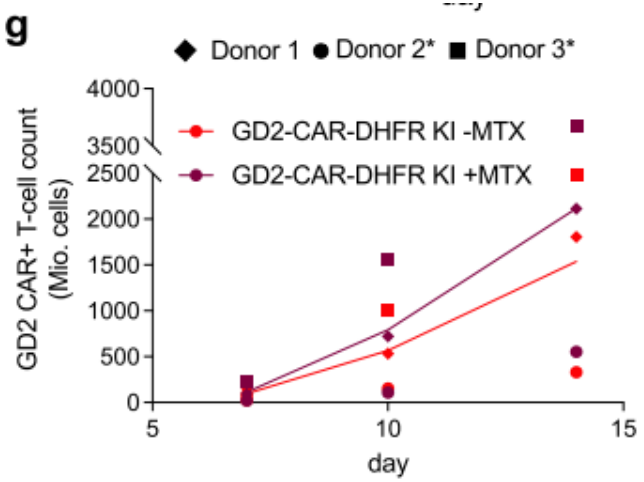
- **G-REX® delivers enough CAR+ cells for patient dose**
- **G-REX® delivers high quality cells with favorable phenotypes**

Shy, B. R., Vykunta, V. S., Ha, A., Talbot, A., Roth, T. L., Nguyen, D. N., Pfeifer, W. G., Chen, Y. Y., Blaeschke, F., Shifrut, E., Vedova, S., Mamedov, M. R., Chung, J.-Y. J., Li, H., Yu, R., Wu, D., Wolf, J., Martin, T. G., Castro, C. E., … Marson, A. (2022). High-yield genome engineering in primary cells using a hybrid ssdna repair template and small-molecule cocktails. *Nature Biotechnology*, *41*(4), 521–531. https://doi.org/10.1038/s41587-022-01418-8

G-Rex® for Autologous CAR-T



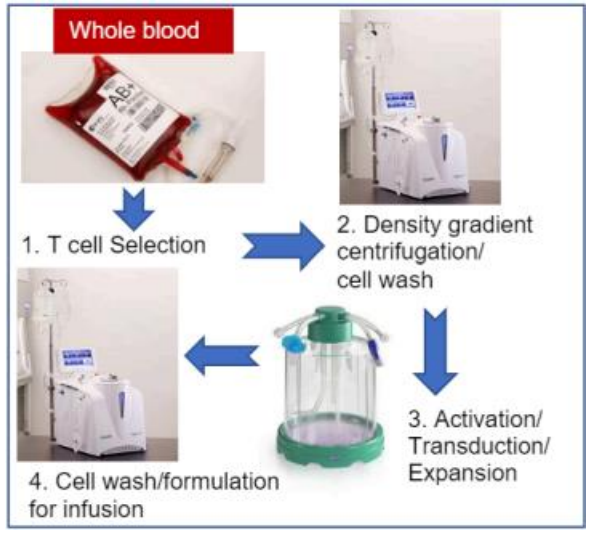




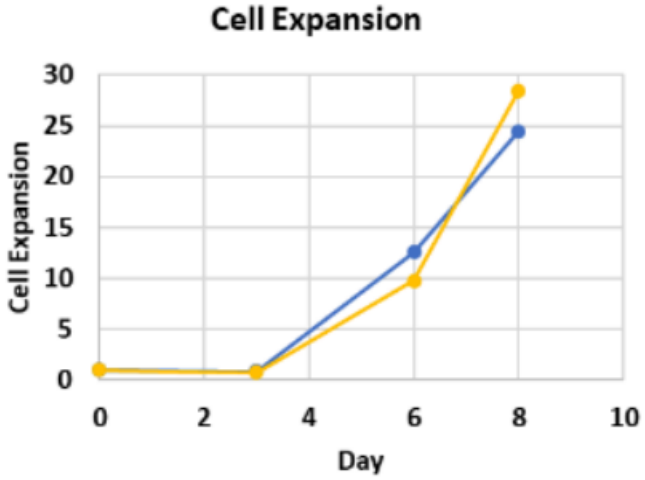
- **G-REX®** is utilized extensively in academic research institutions to streamline the (linear) pathway from benchtop to bedside
- **G-REX®** is ideal for viral and non-viral CAR-T therapies

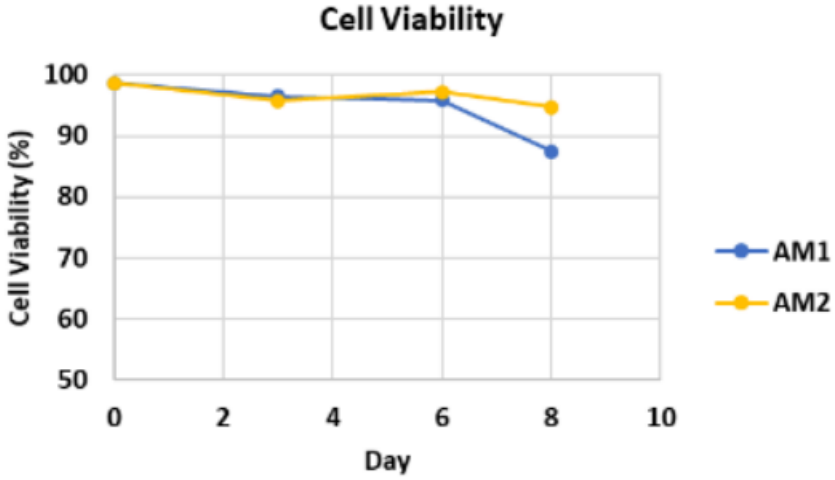
Balke-Want, H., Keerthi, V., Gkitsas, N., Mancini, A., Kurgan, G., Fowler, C., Xu, P., Liu, X., Asano, K., Patel, S., Fisher, C., Brown, A., Tunuguntla, R., Patel, S., Sotillo, E., Mackall, C., & Feldman, S. (2023). Gene editing/gene therapies: Homology-independent targeted insertion (HITI) enables guided car knock-in and efficient clinical scale CAR-T cell manufacturing. Cytotherapy, 25(6). https://doi.org/10.1016/s1465-3249(23)00166-4

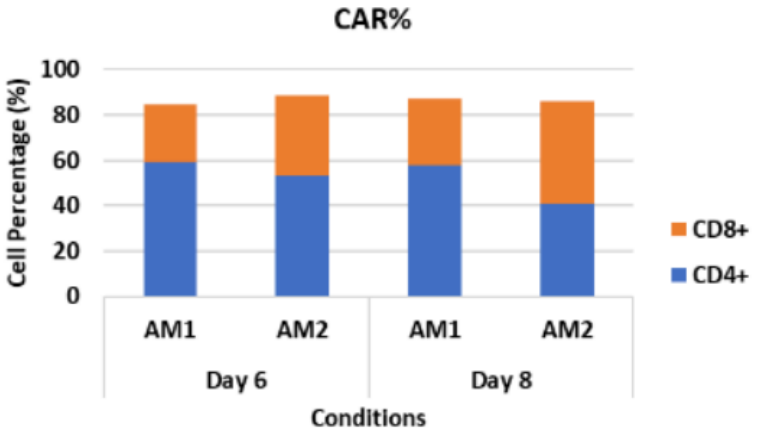
G-Rex® for Autologous CAR-T

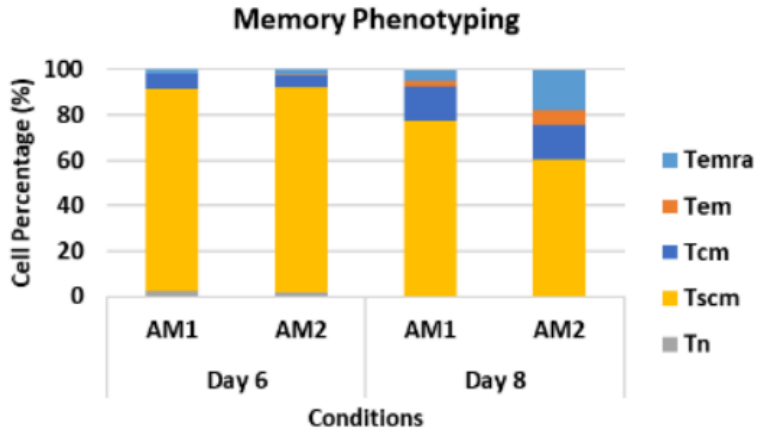


centrifuge. The use of a large format G-Rex device allows for all major steps to be carried out on the day of initiation (Figure 1 and Figure 6), and only sterility and product qualification steps near the end of the culture period would require further technical handling. While a clinical protocol incorporating the advances presented here is yet to implemented in a clinical trial, we present here all the steps required to build a low-cost CAR-T point-of-care production pipeline, at scale, that can be implemented in a global setting either in a dedicated clean-room area or in a mobile clean-room setting.



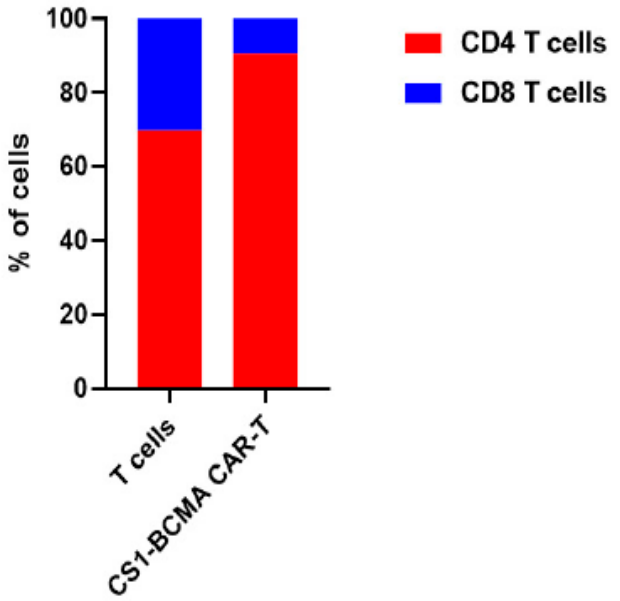


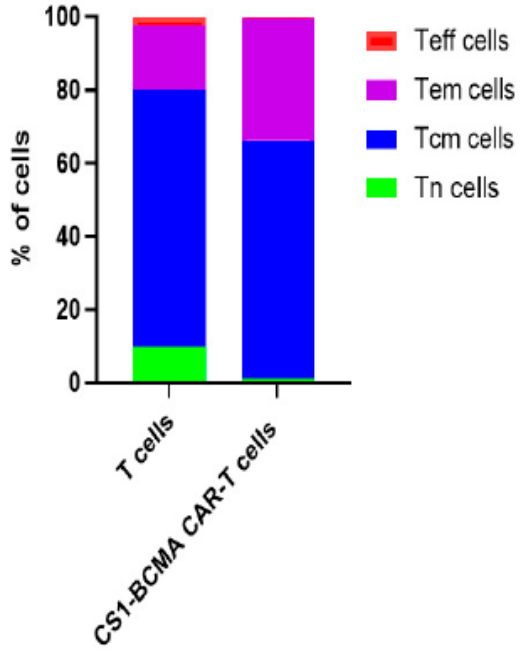


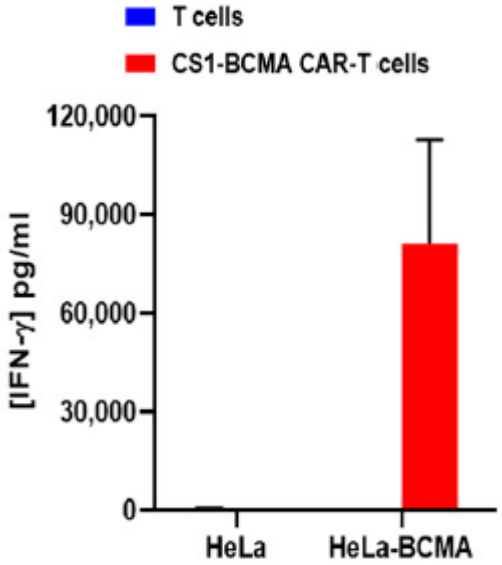


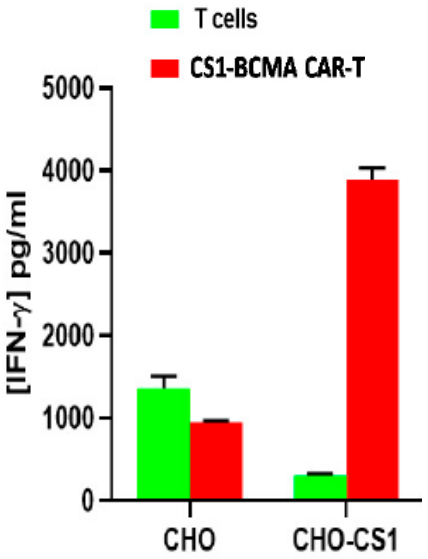
Xiong, Y., Xie, Y., Zhu, Z., Oparaocha, I., Sleesareva, O., Dropulić, B., & Orentas, R. (2023). Technological and Manufacturing Innovation Drive improved access to engineered T cell therapies. Medical Research Archives, 11(11). https://doi.org/10.18103/mra.v11i11.4759

G-Rex® for Autologous CAR-T

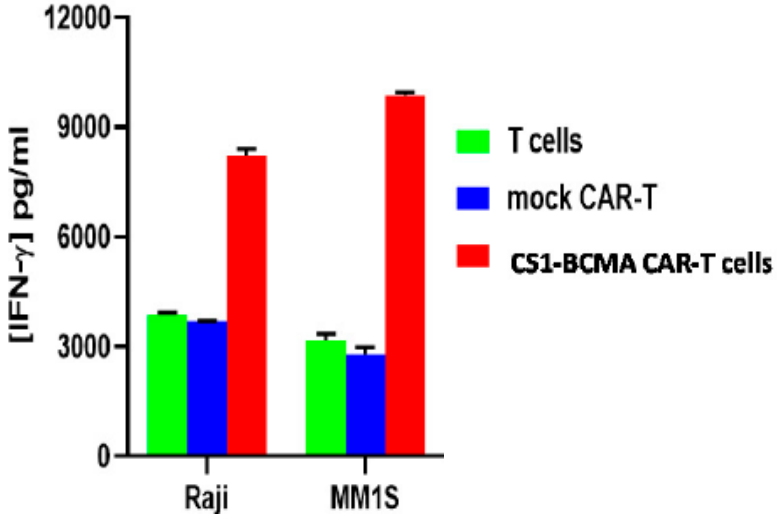








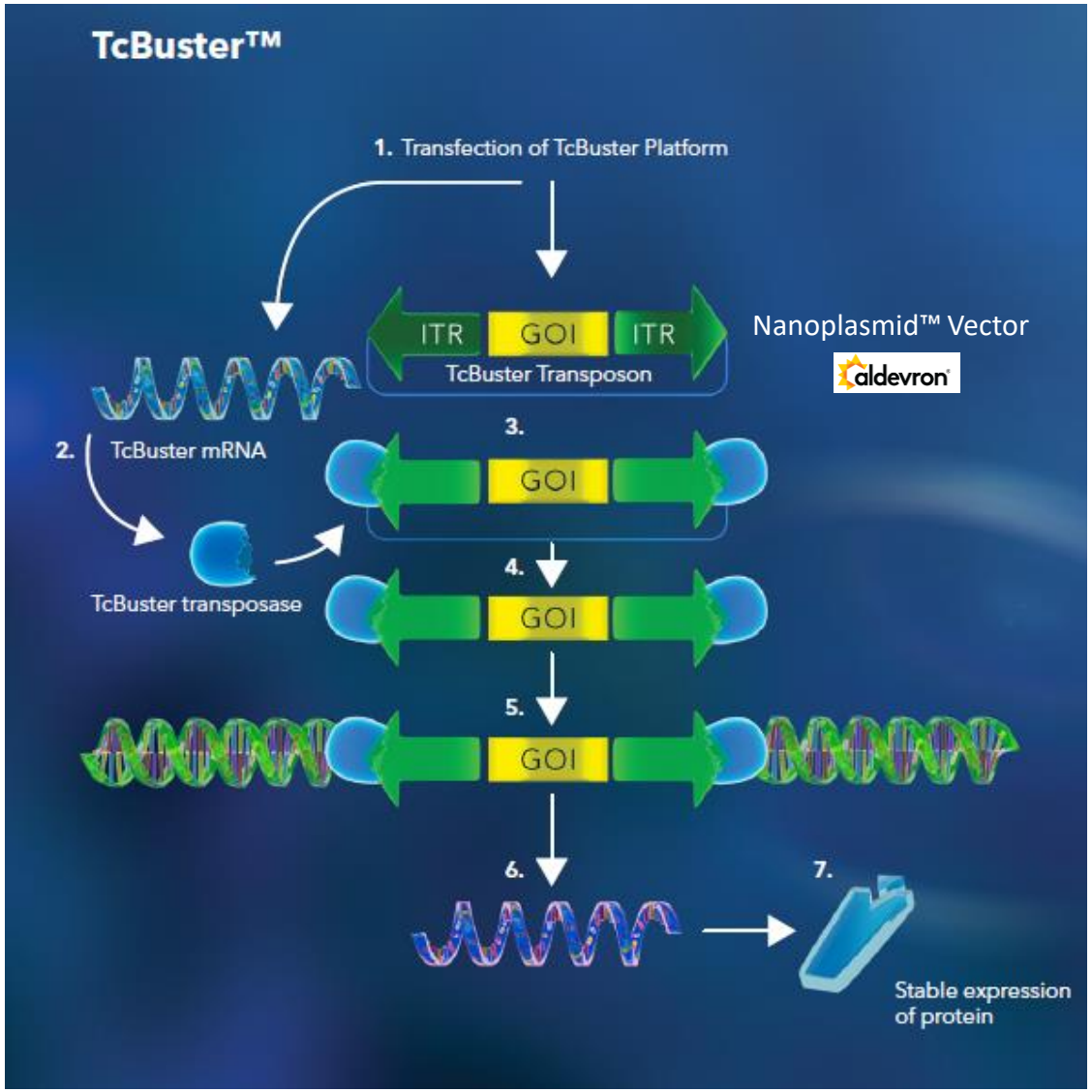
- CAR-T manufactured in **G-REX®** results in high expansion of functional CAR-T cells.
- "The use of **G-REX®** resulted in high expansion of CAR-T cells (>180 fold) with a high number of central memory phenotypes that were shown to be critical for CAR-T cell persistence and increased survival."



Golubovskaya, Vita, et al. "Novel CS1 car-T cells and bispecific CS1-BCMA CAR-T cells effectively target multiple myeloma." *Biomedicines*, vol. 9, no. 10, 9 Oct. 2021, p. 1422, https://doi.org/10.3390/biomedicines9101422.

Our G-Rex® CAR-T platform that is ready-to-scale

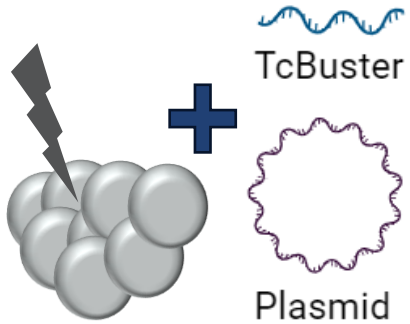
Non-viral Manufacturing of CAR T cells in G-Rex®



1. TcBuster reagents are introduced into the cells via electroporation
   - Transposase is mRNA to limit DNA toxicity and ensure safety
   - Transposon is the Nanoplasmid™ vector with minimal backbone to improve transposition rates and eliminate encoded antibiotic marker
2. TcBuster mRNA is translated into transposase enzyme
3. TcBuster transposase binds to ITR regions on transposon Nanoplasmid™ vector
4. TcBuster cuts gene of interest (GOI) out of transposon
5. TcBuster inserts GOI into genome
6. GOI transcribed and translated
7. GOI stably expressed in cells

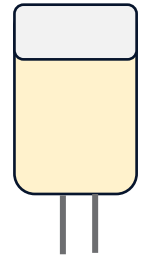
Non-viral Manufacturing of CAR T cells in G-Rex®

Day 0: Activation
- ✓CD4 & CD8 isolated T cells from apheresis
- ✓TransAct
- ✓XF GMP T cell Media
- ✓Liquid IL-7 and IL-15 (10ng/mL each)
- ✓G-Rex100M



Day 2: Gene Edit
- ✓TcBuster™
  - ✓Transposon
  - ✓Transposase
- ✓Electroporator
  - ✓MaxCyte
  - ✓CTS Xenon
  - ✓Nucleofector

Day 2-9: Expansion
- ✓XF GMP T cell Media
- ✓Liquid IL-7 and IL-15 (10ng/mL each)
- ✓G-Rex100M

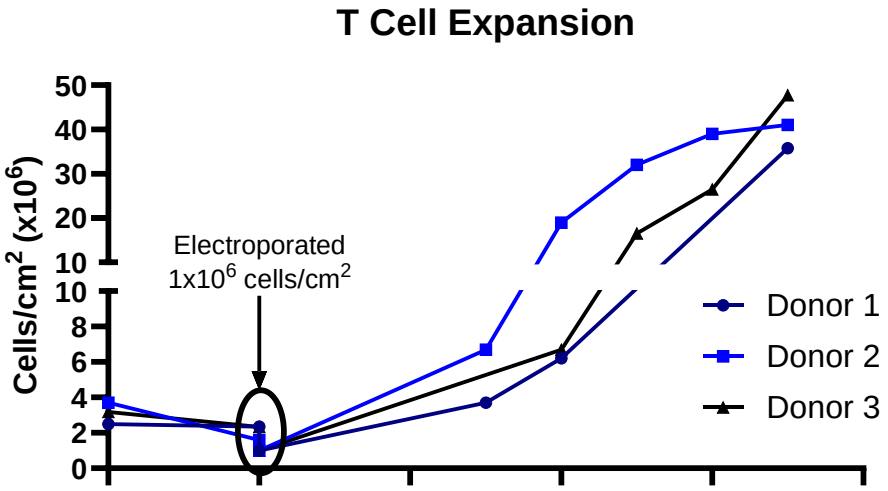


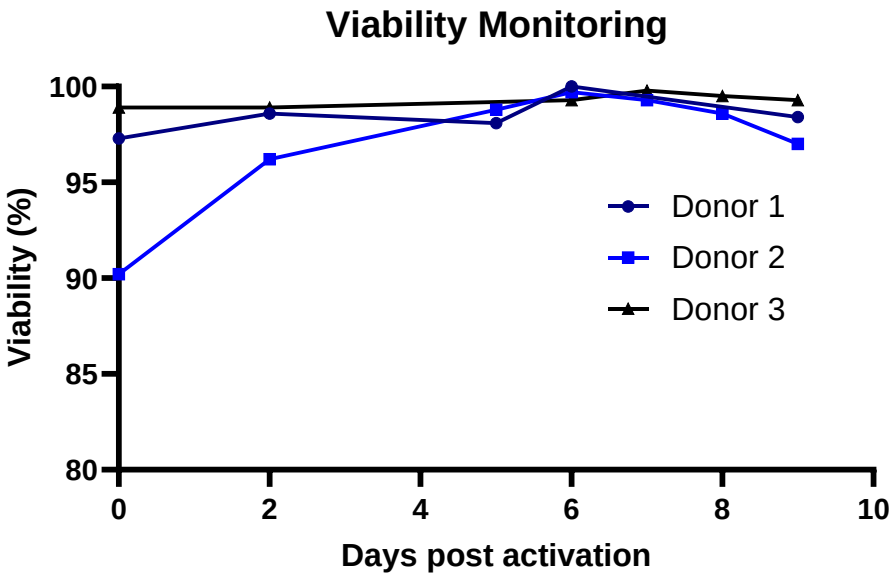
Day 9: Cryopreservation
- ✓CryoStor CS10

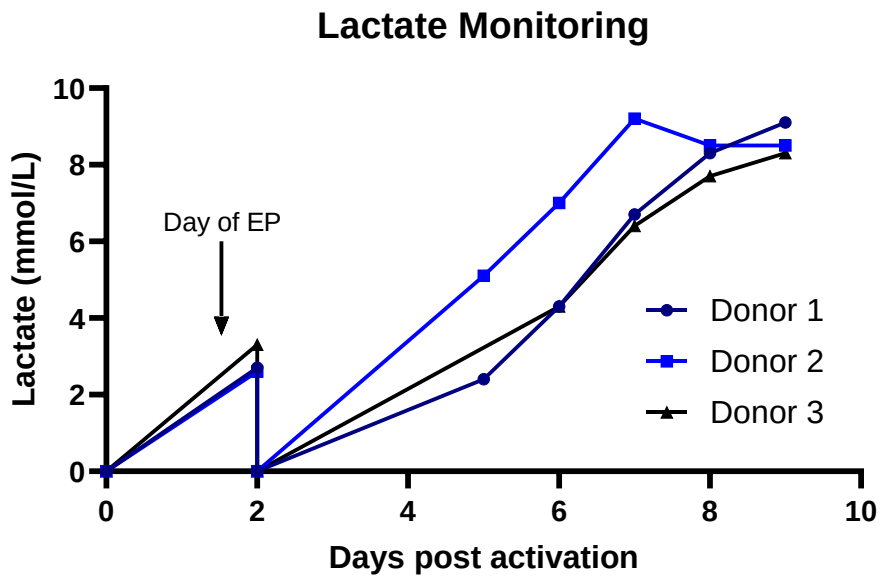
Thaw and Assay
- ✓Cell Viability
- ✓Cell Count (% Recovery)
- ✓Identity
- ✓Phenotype
- ✓Transgene Expression
- ✓Adjusted Copy Number
- ✓Potency

Non-viral Manufacturing of CAR T cells in G-Rex®

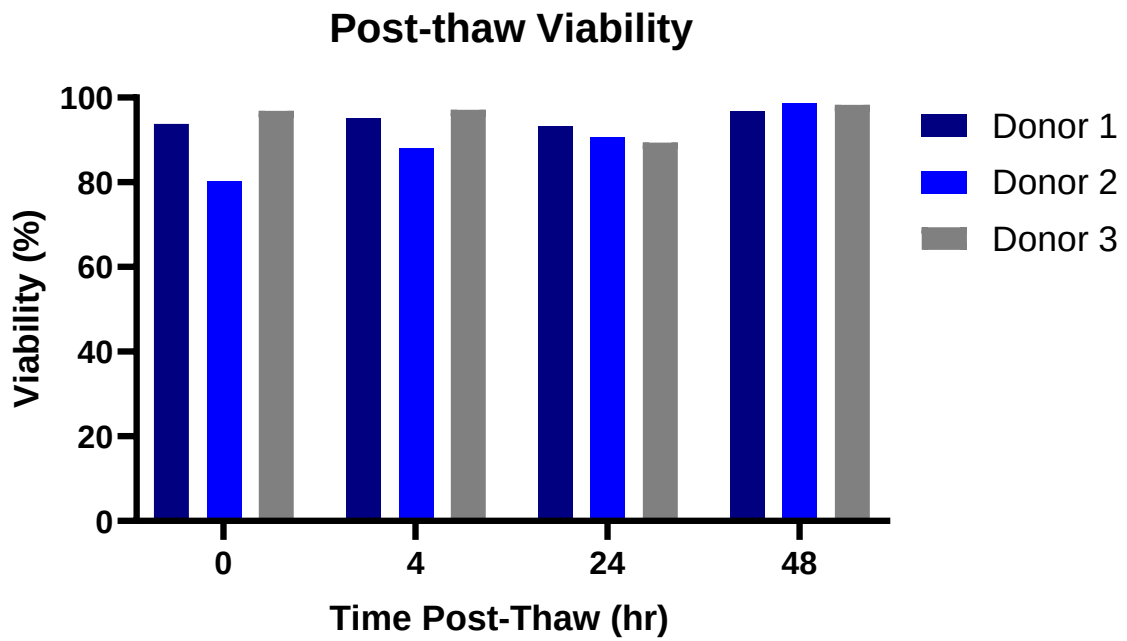
- Sacrificial wells in G-Rex 6M well plate
- Leaves the G-Rex 100M left untouched

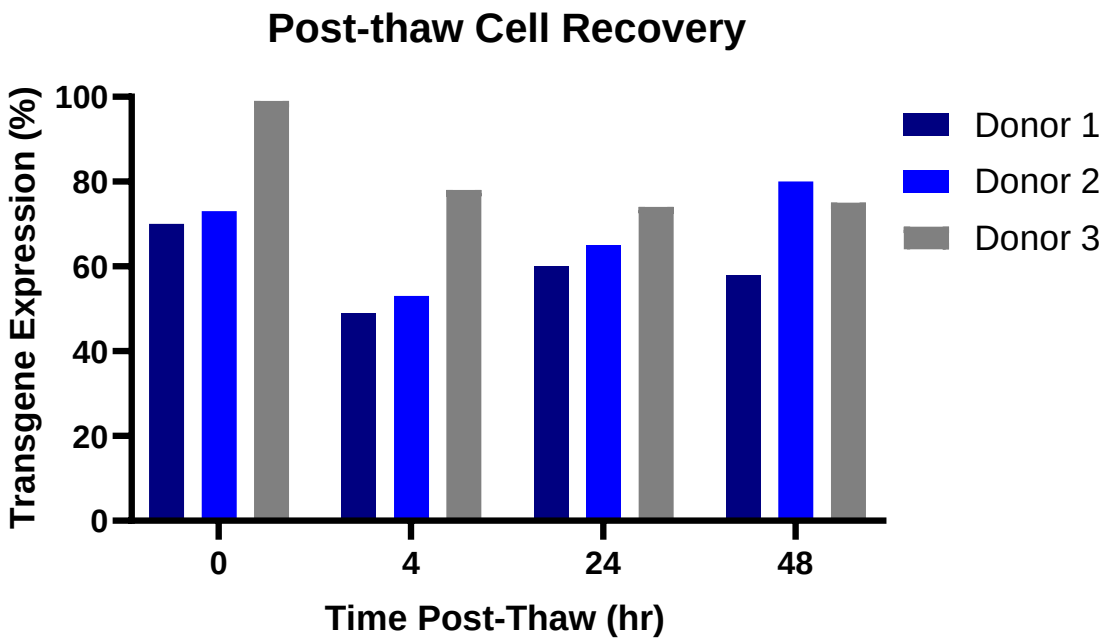






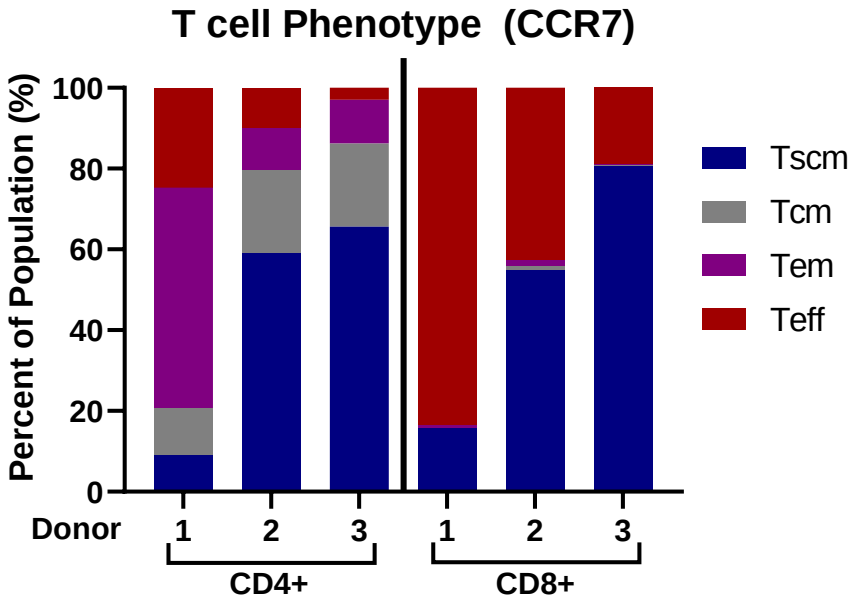
Non-viral Manufacturing of CAR T cells in G-Rex®

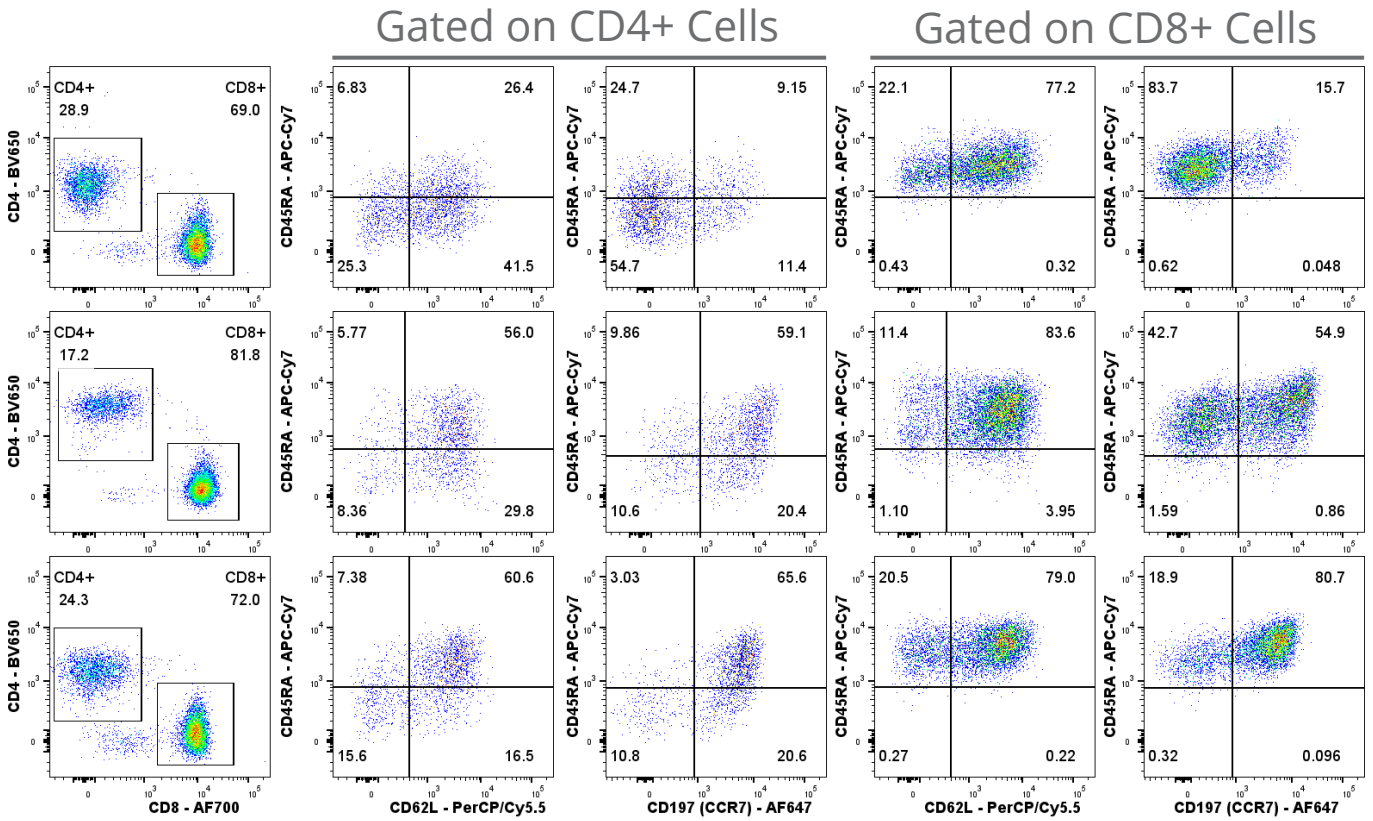




Test (%)	Donor 1 Results	Donor 2 Results	Donor 3 Results
Post-thaw Viability	93.8	80.2	96.9
Cell Count (% recovery at thaw)	70	73	99

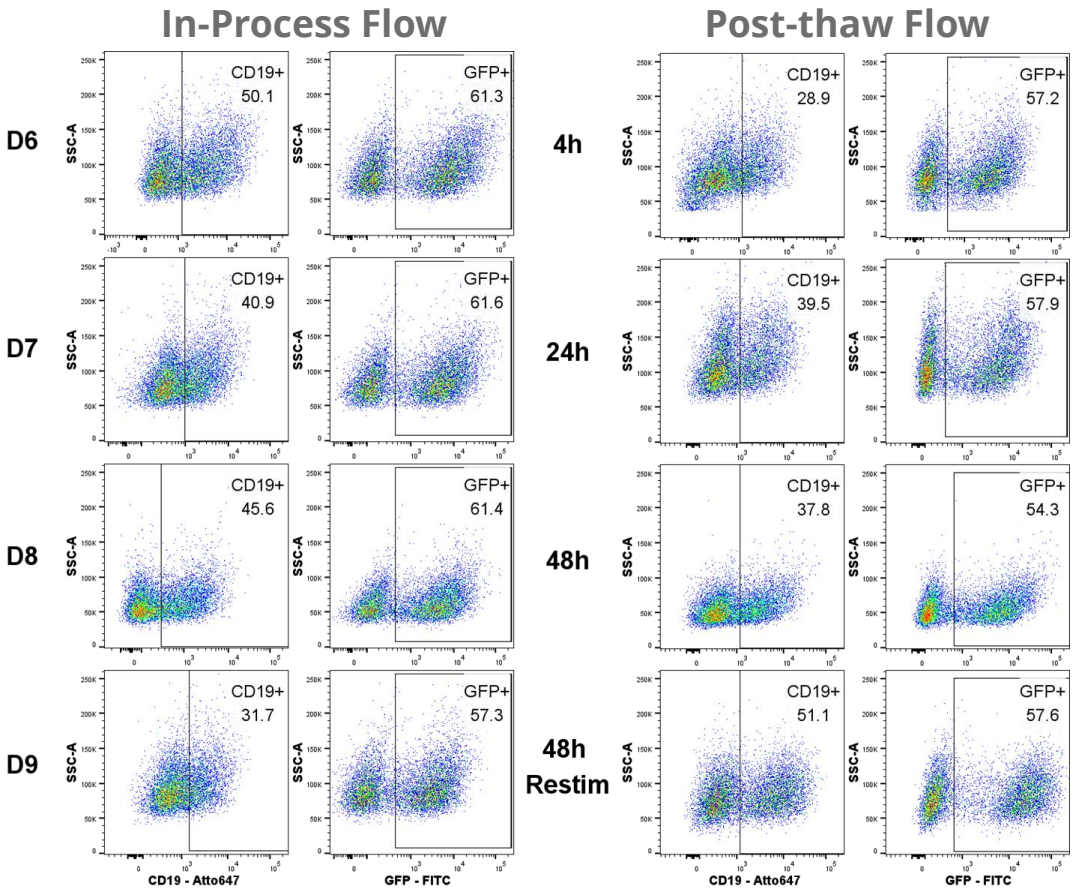
Non-viral Manufacturing of CAR T cells in G-Rex®

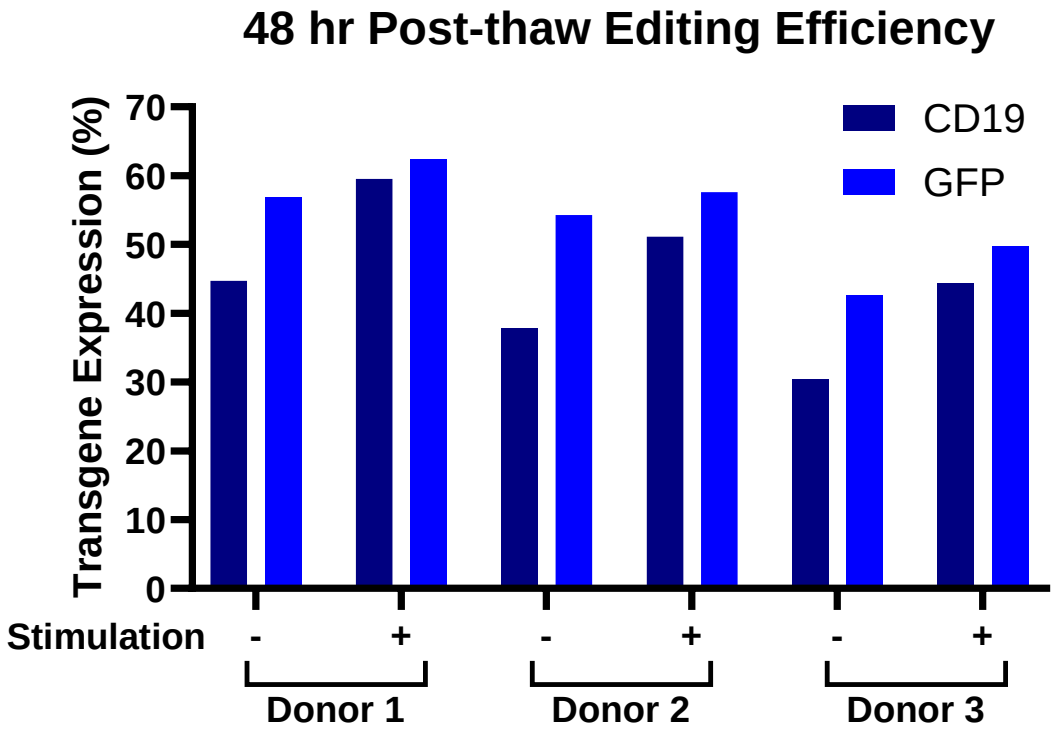




Test (%)	Donor 1 Results	Donor 2 Results	Donor 3 Results
T cell Purity (CD4 + CD8)	97.9%	99.0%	96.3%

Non-viral Manufacturing of CAR T cells in G-Rex®





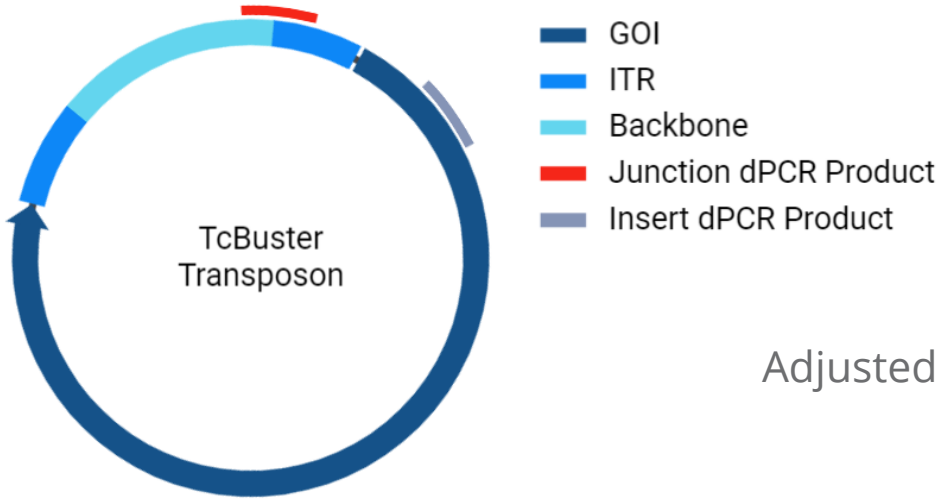
Test (%)	Donor 1 Results	Donor 2 Results	Donor 3 Results
CD19/GFP Expression (No Stim)	44.7/**56.9**	37.8/**54.3**	30.4/**42.6**
CD19/GFP Expression (w/ Stim)	59.5/62.4	51.1/57.6	44.4/49.8

Bolded Values used for T cell potency assay normalization and copy number assay

ScaleReady

Non-viral Manufacturing of CAR T cells in G-Rex®

Transposon copy number determination by dPCR



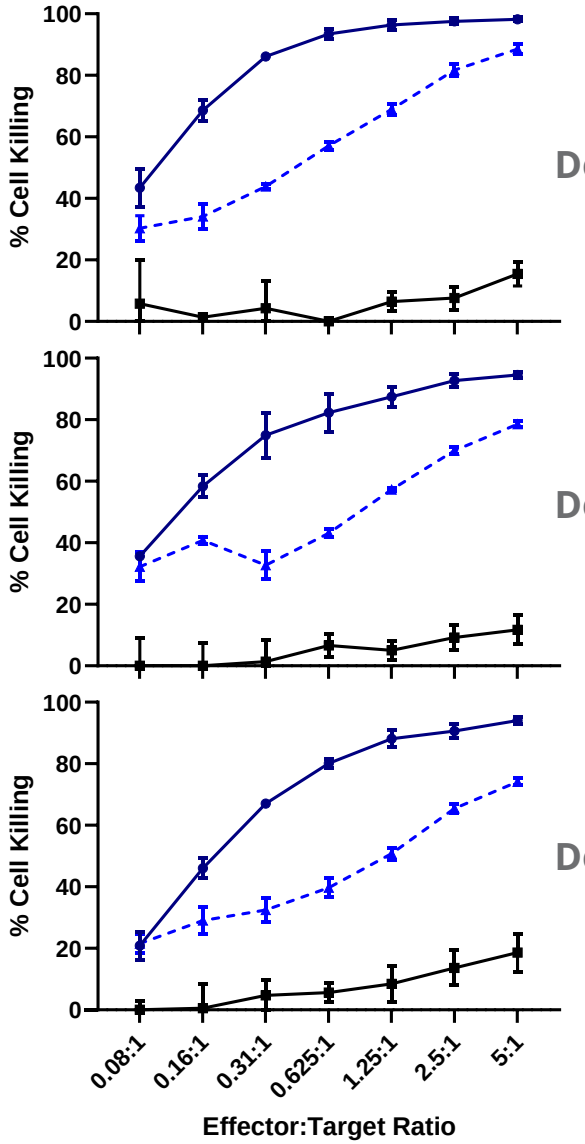
$$\text{Adjusted Copy Number} = \frac{\left[\begin{array}{c}\text{Insert}\\(\text{all construct reads})\end{array}\right] - \left[\begin{array}{c}\text{Junction}\\(\text{episomal reads})\end{array}\right]}{\text{Transposition Efficiency}}$$

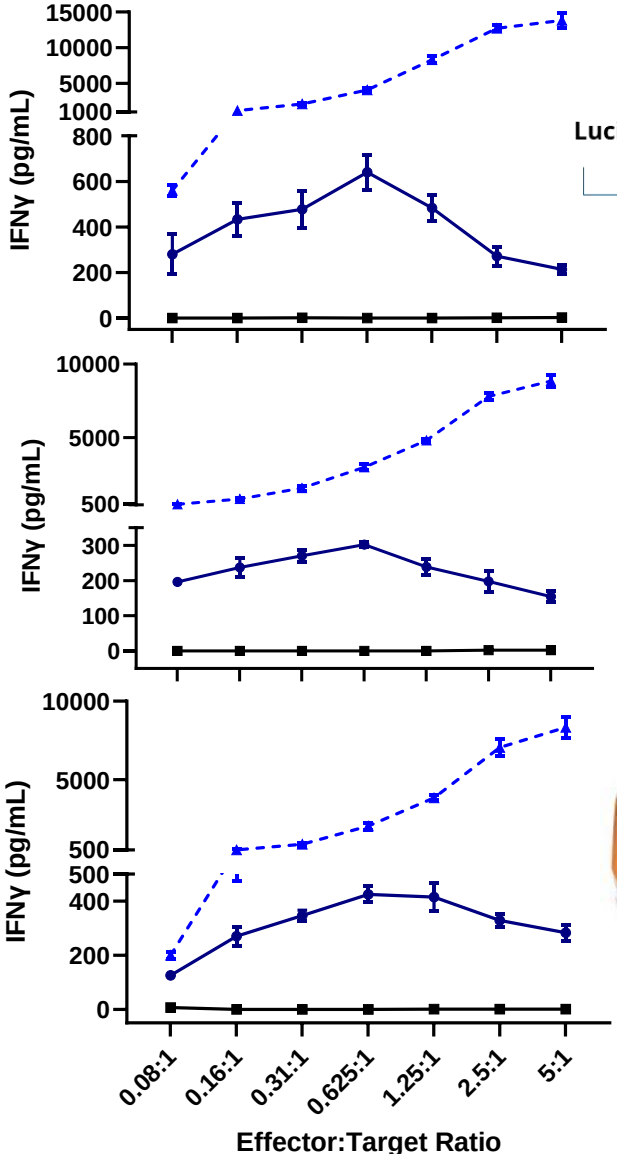
Test (%)	Donor 1 Results	Donor 2 Results	Donor 3 Results
Copy Number (Adjusted to CAR+)	5.6	5.5	5.8

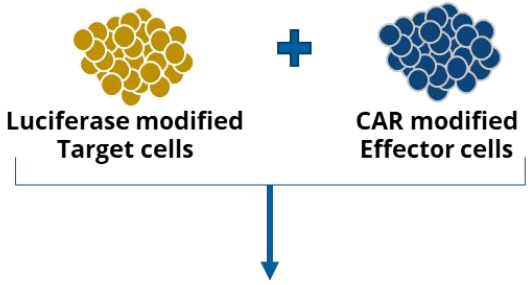
Non-viral Manufacturing of CAR T cells in G-Rex®

Incubate
O/N

Read out Luciferase



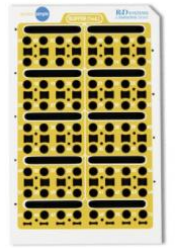




Incubate
O/N

Collect Supernatant

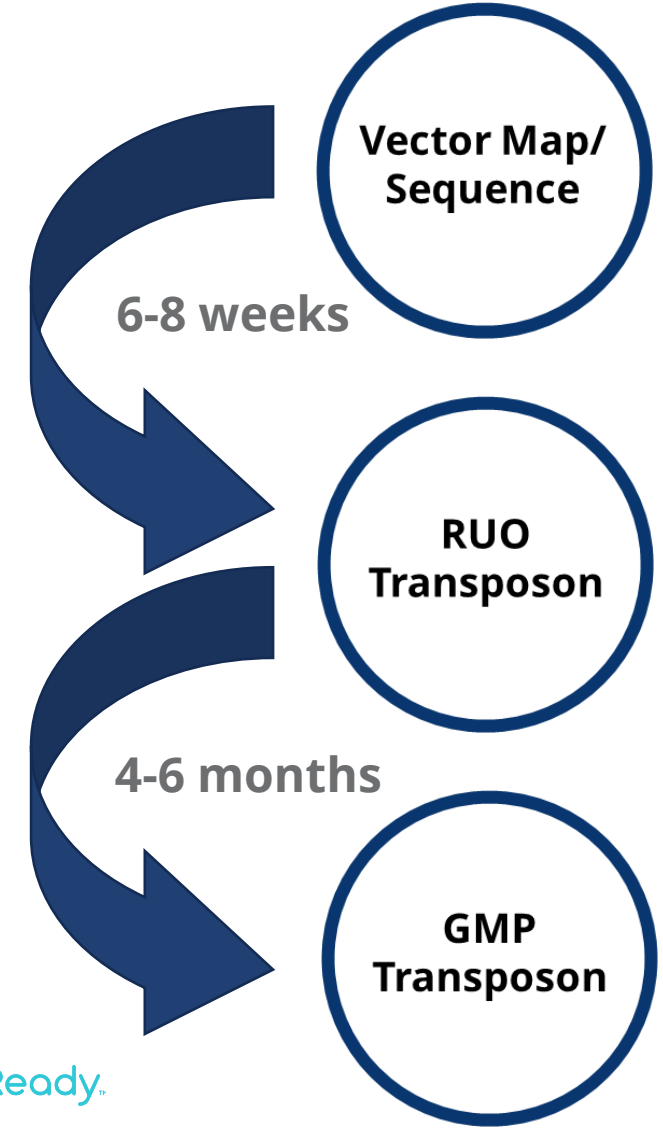
Single Plex



72 x 1 analyte

Nalm-6 K562/CD19+ K562

Non-viral Manufacturing of CAR T cells in G-Rex®



Transposon Batch Size	Patients Treated at 50 ug/mL EP	Patients Treated at 150 ug/mL EP
100 mg	400	133
250 mg	1,000	333
500 mg	2,000	666
1 g	4,000	1,333

*Assumes 2E8 cells/mL and 1E9 total cells processed (5 x 1 mL electroporation reactions)

TcBuster-M™ Advantages

- ✓ **Larger cargo** delivery allows for more efficacious products
- ✓ **Improved timelines** for discovery and manufacturing
- ✓ **Integrates** genes in **safer locations** than lentiviral methods
- ✓ **Scalable** from research to commercial scale
- ✓ **Reduced COGS** versus viral editing
- ✓ **Stronger supply chain** for manufacturing of nucleic acids

G-Rex® for Tumor Infiltrating Lymphocyte (TIL) therapy

G-Rex® in world's first TIL Therapy

Dr. Steven Rosenberg in his National Cancer Institute lab with the legacy G-Rex100

Simplified Method of the Growth of Human Tumor Infiltrating Lymphocytes (TIL) in Gas-Permeable Flasks to Numbers Needed for Patient Treatment

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JOURNAL OF Immunotherapy

G-Rex vs T-flasks & Gas Permeable Bags

- ✓ Far fewer vessels are needed because G-Rex can support much greater cell densities/concentrations
- ✓ 3-4 fold reduction in the use of media, cytokines, activating reagents, serum, etc.
- ✓ Reduced culture/harvest volume reduces need for specialized cell process equipment
- ✓ Less incubator space required
- ✓ Less labor is required because laboratory staff maintain fewer vessels, prepare less media, vessels do not require media exchanges, less volume is processed post REP

ScaleReady

Jin, J., Sabatino, M., Somerville, R., Wilson, J. R., Dudley, M. E., Stroncek, D. F., & Rosenberg, S. A. (2012). Simplified method of the growth of human tumor infiltrating lymphocytes in gas-permeable flasks to numbers needed for patient treatment. Journal of Immunotherapy, 35(3), 283–292. https://doi.org/10.1097/cji.0b013e31824e801f

G-Rex® in world's first TIL Therapy

G-Rex100M-CS

G-Rex500M-CS

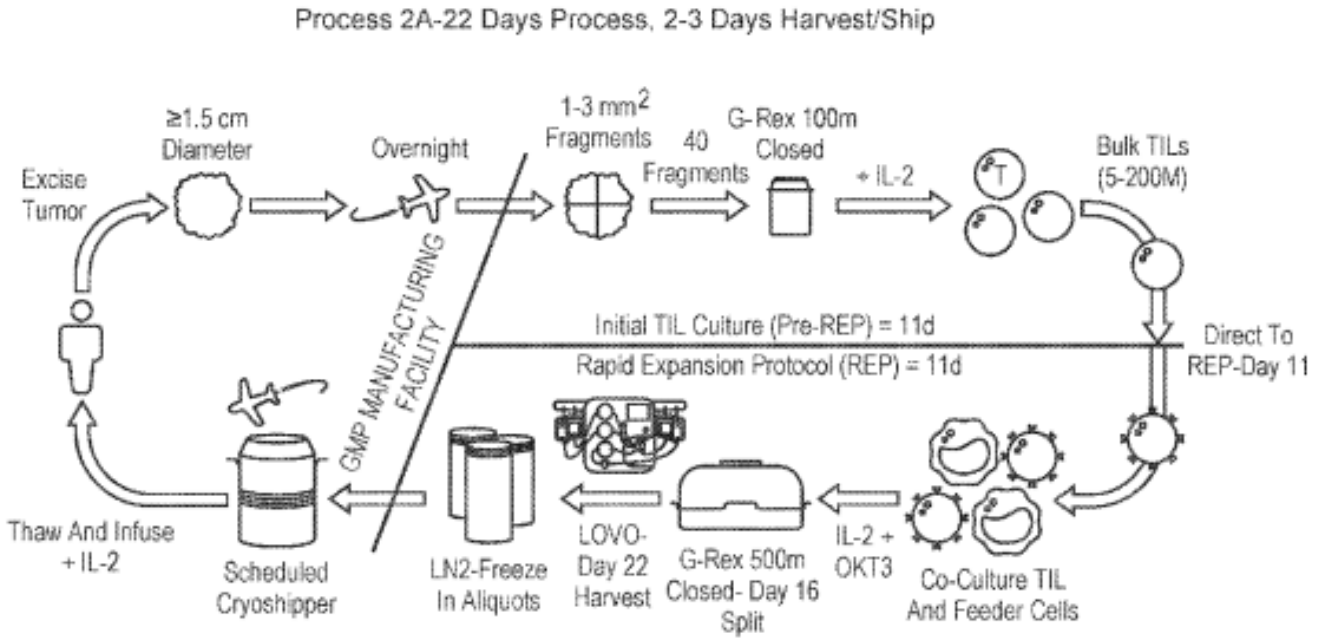


Figure 1

(57) Abstract: The present invention provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs, including novel methods for expanding TIL populations in a closed system that lead to improved efficacy, improved phenotype, and increased metabolic health of the TILs in a shorter time period, while allowing for reduced microbial contamination as well as decreased costs. Such TILs find use in therapeutic treatment regimens.

- ✓ **G-Rex®** is a core technology in the world's first approved TIL therapy
- ✓ **G-Rex®** is the gold standard for TIL therapy

G-Rex® Rescues Poor Growing Patients

The beneficial effects of a gas-permeable flask for expansion of Tumor-Infiltrating lymphocytes as reflected in their mitochondrial function and respiration capacity

Marie-Andrée Forget[1,†], Cara Haymaker[1,†], Jennifer B Dennison[2], Christopher Toth[1], Sourindra Maiti[3], Orenthial J Fulbright[1], Laurence J N Cooper[3], Patrick Hwu[1], Laszlo G Radvanyi[1,4,5], and Chantale Bernatchez[1,*]

[1]Department of Melanoma Medical Oncology; The University of Texas MD Anderson Cancer Center (MDACC); Houston, TX USA; [2]Department of Systems Biology; The University of Texas MDACC; Houston, TX USA; [3]Division of Pediatrics; The University of Texas MDACC; Houston, TX USA; [4]Lion Biotechnologies; Tampa, FL USA; [5]Department of Immunology; H. Lee Moffitt Cancer Center; Tampa, FL USA

Interestingly, we observed significantly better growth when using the G-Rex flask for TIL lines that did poorly (lower range) in the traditional devices (**Fig. 1B**, n = 4, $p = 0.007$). This observation explains the "trend" of better growth observed in the G-Rex flask across all samples since good growing TIL lines were not affected by the type of device used (**Fig. 1**). Neither device negatively affected the post-REP viability (>85 %, data not shown). The differential growth of poor growing TIL lines in the two systems used prompted us to explore potential underlying explanations.

Figure S1

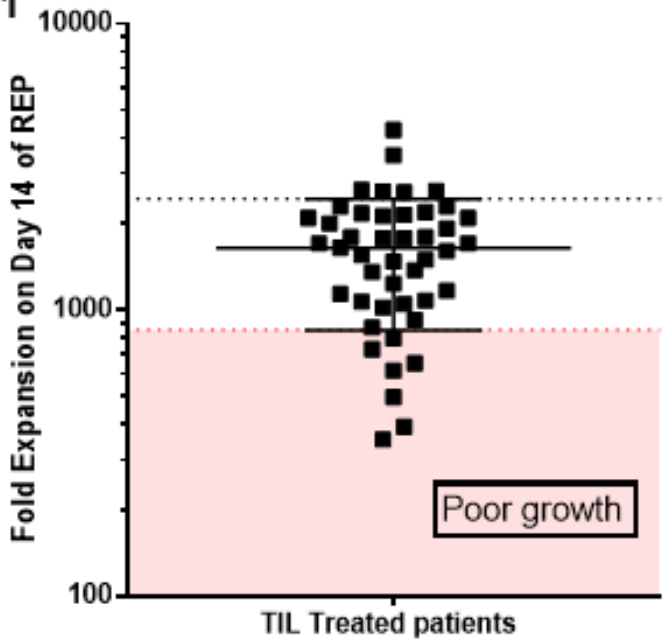


Figure S1. TIL fold expansion on day 14 of the Rapid Expansion Protocol. TIL fold expansion obtained for the first 45 patients treated with TIL ACT at MDACC. TIL were expanded in T175 flasks for the first week and transferred to 3L cell culture bags for the last 7 days. The mean fold expansion was 1651 with a standard deviation of 799. Poor growth was established as TIL expansion not reaching the mean minus one standard deviation (1651 - 799 = 852).

Forget, M.-A., Haymaker, C., Dennison, J. B., Toth, C., Maiti, S., Fulbright, O. J., Cooper, L. J., Hwu, P., Radvanyi, L. G., & Bernatchez, C. (2015). The beneficial effects of a gas-permeable flask for expansion of tumor-infiltrating lymphocytes as reflected in their mitochondrial function and respiration capacity. *OncoImmunology*, *5*(2). https://doi.org/10.1080/2162402x.2015.1057386

G-Rex® Preserves Clonal Diversity

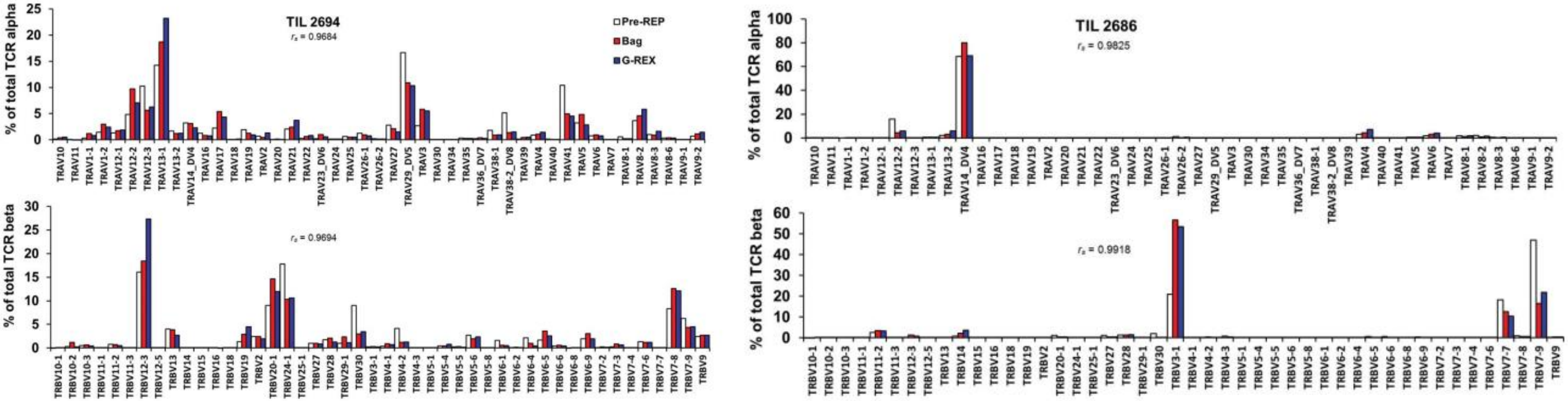


Figure 2. Rapid expansion of TIL in the Gas-permeable flask (G-Rex) does not favor selection and expansion of specific T cell clones. Clonal diversity was evaluated by measurement of the expression of major TCR α and β chain gene by the NanoString nCounter® technology. The analysis of the level of expression of 45 Vα and 46 Vβ genes in RNA isolated from the pre-REP TIL lines in comparison with the different expansion devices demonstrated no difference in the clonal diversity obtained post REP. Two out of 4 TIL lines from 4 melanoma patients are shown. Statistical analysis using a Spearman correlation comparing cells grown in traditional flask and bag (red) vs. Gas-permeable flask (blue) is shown for both Vα and Vβ genes.

Forget, M.-A., Haymaker, C., Dennison, J. B., Toth, C., Maiti, S., Fulbright, O. J., Cooper, L. J., Hwu, P., Radvanyi, L. G., & Bernatchez, C. (2015). The beneficial effects of a gas-permeable flask for expansion of tumor-infiltrating lymphocytes as reflected in their mitochondrial function and respiration capacity. *OncoImmunology*, *5*(2). https://doi.org/10.1080/2162402x.2015.1057386

The utility of G-Rex® on TIL cell quality

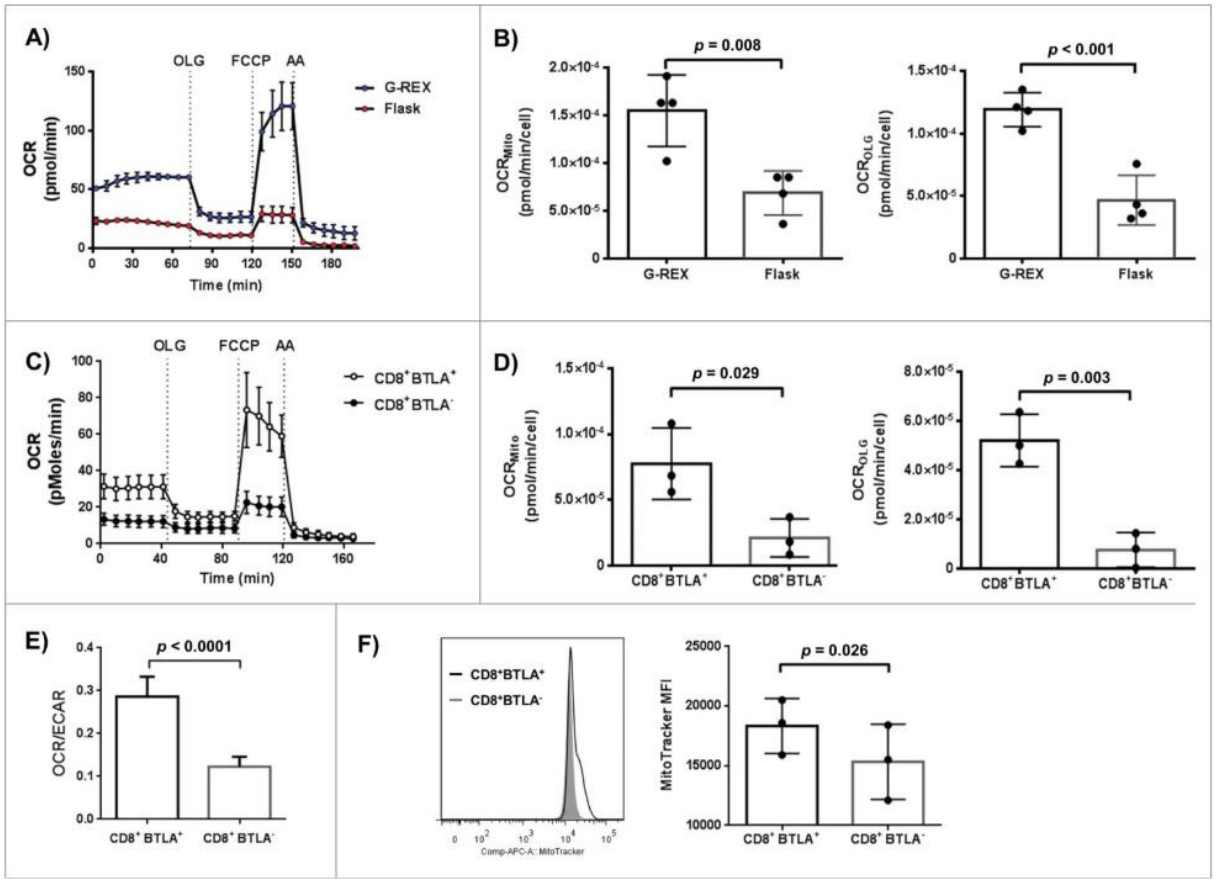
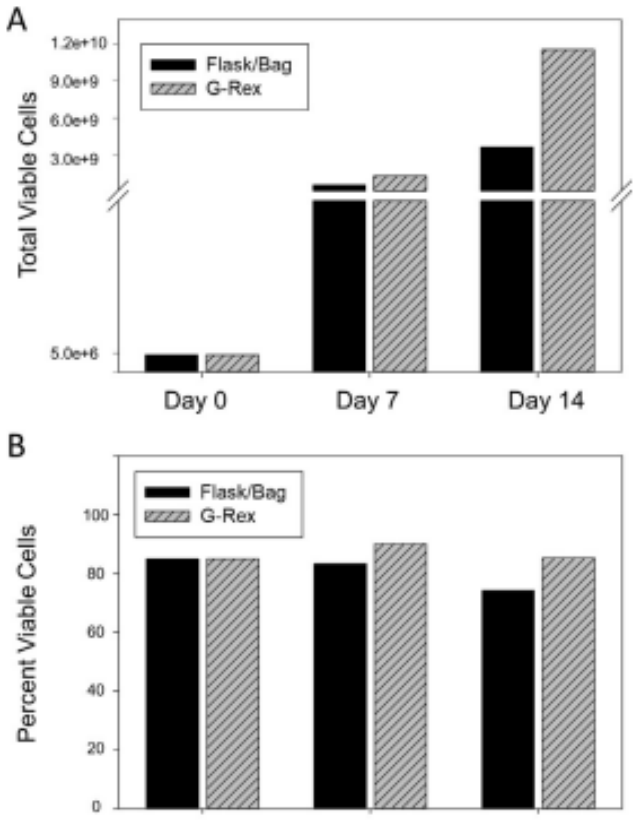


Figure 3. Bioenergetic analysis of melanoma TIL lines to evaluate mitochondrial function. (**A–D**) OCR of 4 TIL lines on day 7 of the REP (**A and B**) or sorted, pre-REP $CD8^+BTLA^+$ and $CD8^+BTLA^-$ TIL line from 3 patients (**C and D**) were determined using a Seahorse XP96 Bioanalyzer. OCR were calculated after 3 min of mix time and 4 min of measurement time. OCR[Mito] is the total mitochondrial OCR, the value just prior to the Oligomycin (OLG) injection minus the non-mitochonddrial OCR component determine by Antimycin (AA) treatment. OCR[OLG] is the component of the OCR that is sensitive to Oligomycin treatment, the rate used by ATP-syntase. The OCR on a per cell basis was determined by dividing the OCR by the seeded TIL count of 250000. (**E**) OCR/ECAR ratio is the OCR[OLG] divided by the ECAR basal which is reflextive of a cell dependence on glycolysis. (**F**) pre-REP TIL were stained using a MitoTracker dye. The MFI of the MitoTracker staining of the $CD8^+BTLA^+$ and $CD8^+BTLA^-$ subset from 3 TIL lines is shown. The histogram shows staining of a representative TIL line. Statistical significance was determined by using a paired *t*-test.

- ✓ The G-Rex® grown cells consistently had a **higher respiratory capacity** than the corresponding T175 flask cells. All together, the metabolic phenotype of melanoma TIL propagated in G-Rex® was **consistently more oxidative**.
- ✓ High spare respiratory capacity is believed to be a feature of memory T cells that imparts them with **better in vivo persistence**, as opposed to effector T cells. We are thus postulating that the high spare respiratory capacity and the high OCR that we observed in TIL grown in high oxygen environment, more specifically the $CD8^+BTLA^+$ cells, could therefore result in **enhanced survival and persistence** after transfer, which could confer **better tumor control.**

Forget, M.-A., Haymaker, C., Dennison, J. B., Toth, C., Maiti, S., Fulbright, O. J., Cooper, L. J., Hwu, P., Radvanyi, L. G., & Bernatchez, C. (2015). The beneficial effects of a gas-permeable flask for expansion of tumor-infiltrating lymphocytes as reflected in their mitochondrial function and respiration capacity. *OncoImmunology*, *5*(2). https://doi.org/10.1080/2162402x.2015.1057386

G-Rex® Simplifies TIL Manufacturing



unavailable. In summary, in addition to production of greater numbers of viable TILs, transition to G-Rex 100MCS flasks has resulted in fewer total vessels, less media, less incubator space and less labor than traditional culture methods using flasks and bags.

Given the benefits shown by addition of anti-4-1BB antibody during preREP and culturing TILs in G-Rex 100MCS during REP, we designed a phase 1 clinical trial to incorporate both process changes. When taken together, the optimization decreased the culture period from 51–37 days and limited the technical intervention to 14 days. Most importantly, optimizing the TIL culture expansion allowed for consistent achievement of the optimal therapeutic dose of 60×10^9 cells for all patients compared with achievement of optimal dose in only 40% of patients using traditional culture methods. This clinical trial

G-Rex10M-CS

Pre-REP

G-Rex100M-CS

G-Rex500M-CS

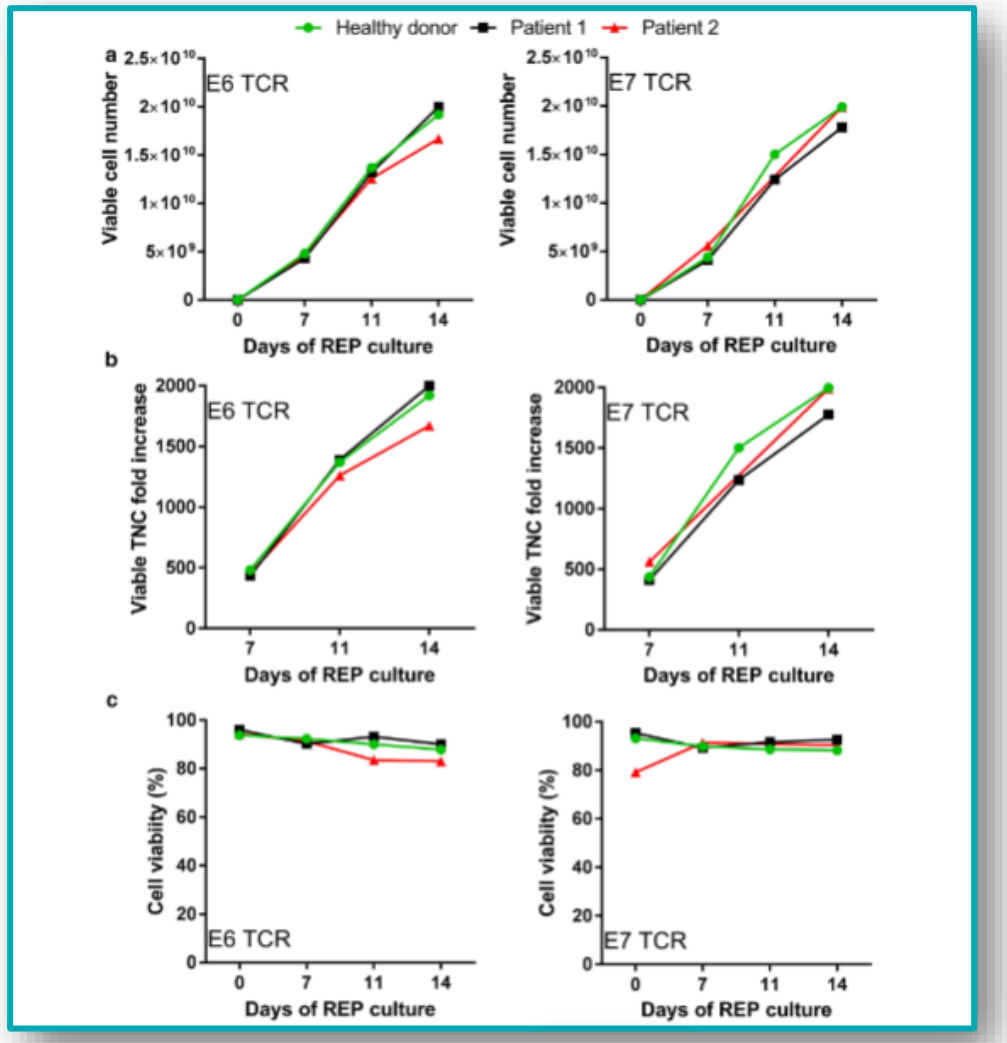
REP

ScaleReady

Hopewell, E. L., Cox, C., Pilon-Thomas, S., & Kelley, L. L. (2019). Tumor-infiltrating lymphocytes: Streamlining a complex manufacturing process. *Cytotherapy*, *21*(3), 307–314. https://doi.org/10.1016/j.jcyt.2018.11.004

G-Rex® for TCR-T cell therapy

G-Rex® for Autologous TCR-T



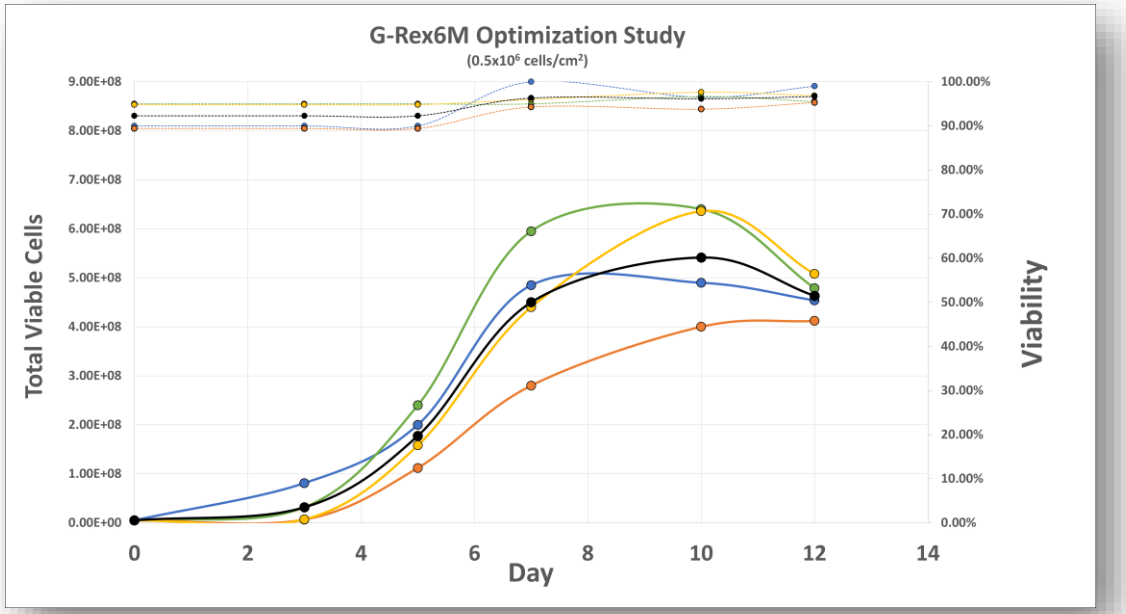
Central memory T cells seem to be required for long-term in vivo repopulation, and there are concerns that excessive in vitro T-cell proliferation before infusion may lead to terminal differentiation and exhaustion. To determine whether the G-Rex would favor the unwanted production of exhausted, terminally differentiated effector cells,[40] we measured both cell proliferation and cell death. We found that the increased cell numbers were a result of improved survival rather than increased proliferation. In confirmation, phenotypic analysis of G-Rex-grown CTL revealed no apparent detrimental effects on the ratio of effector:memory phenotype.

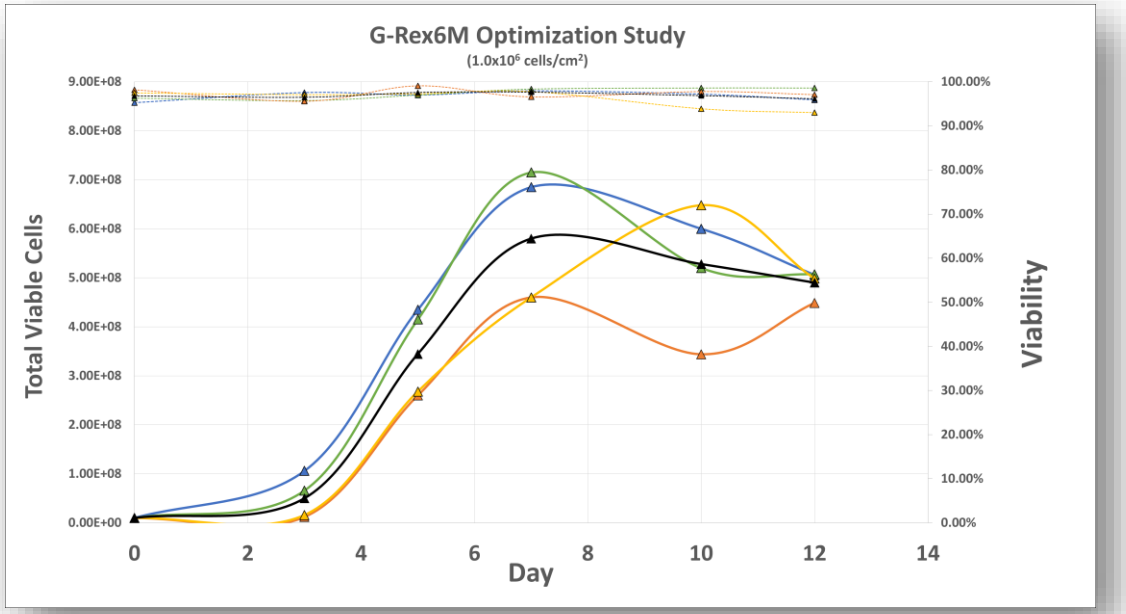
- ✓ A single G-Rex500M-CS (above) produces **15-20 billion cells**
- ✓ Standard, stackable incubators hold ~8 devices. This equals **240-320 billion cells in only two stacked incubators!**
- ✓ **High viability rates** means less population doublings are required to reach final cell numbers
- ✓ Less population doublings yield **move favorable, less differentiated, phenotypes**

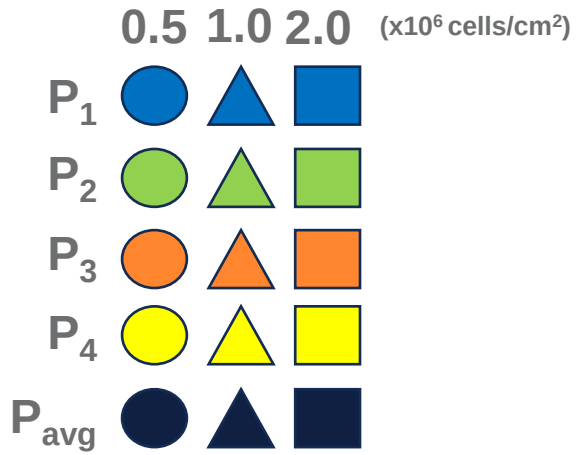
Vera JF, Brenner LJ, Gerdemann U, Ngo MC, Sili U, Liu H, Wilson J, Dotti G, Heslop HE, Leen AM, Rooney CM. Accelerated production of antigen-specific T cells for preclinical and clinical applications using gas-permeable rapid expansion cultureware (G-Rex). J Immunother. 2010 Apr;33(3):305-15. doi: 10.1097/CJI.0b013e3181c0c3cb. PMID: 20445351; PMCID: PMC2946348.

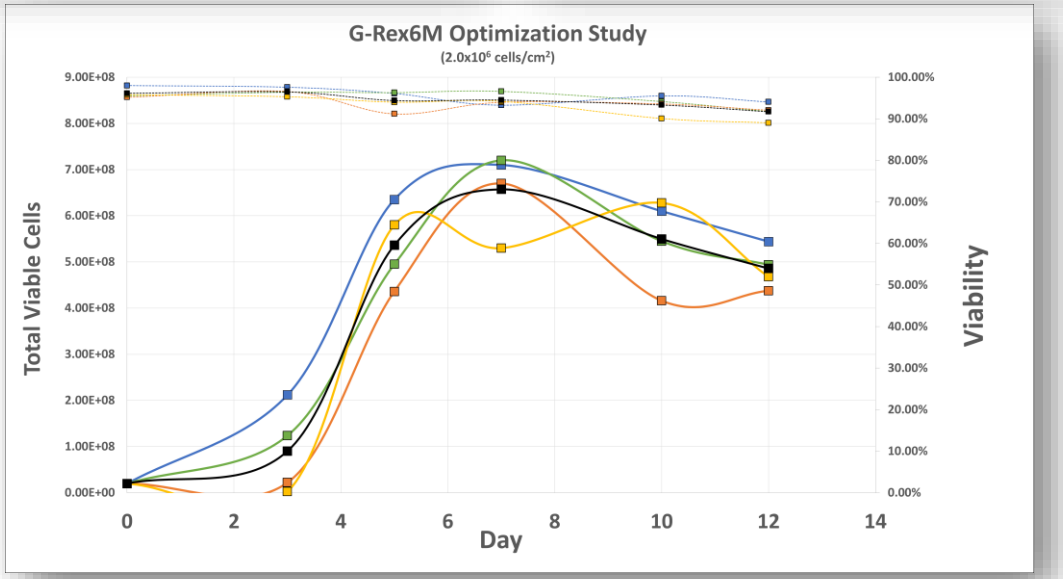
Jin, J., Gkitsas, N., Fellowes, V.S. *et al.* Enhanced clinical-scale manufacturing of TCR transduced T-cells using closed culture system modules. *J Transl Med* **16,** 13 (2018). https://doi.org/10.1186/s12967-018-1384-z

Flexibility & Consistency with G-Rex® (TCR-T)





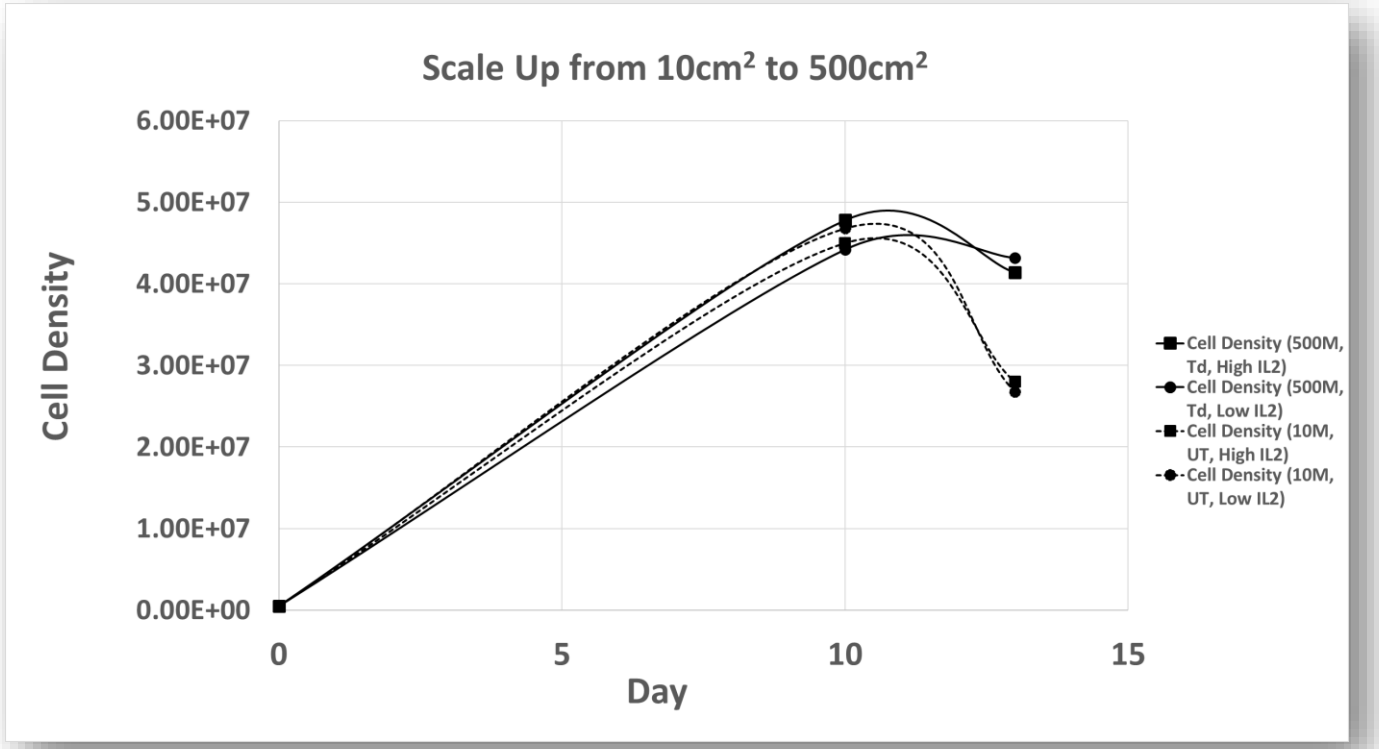




G-Rex6M Well Plate
(P/N 80660M)

ScaleReady

Linear Scalability of G-Rex® (TCR-T)



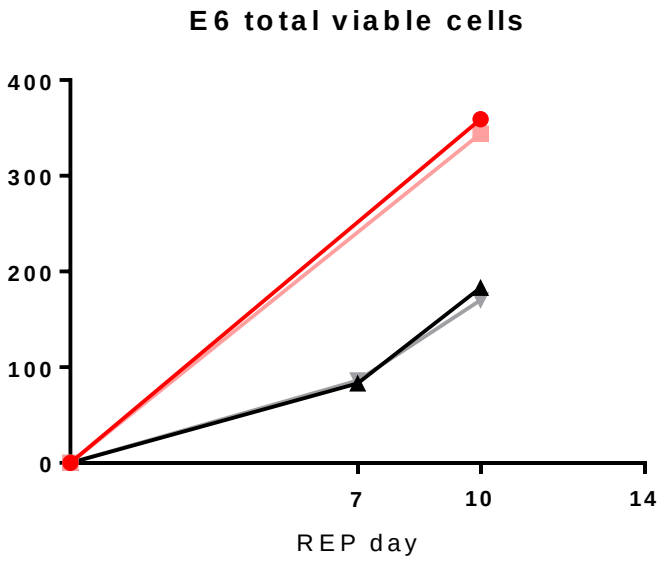
G-Rex10M
(P/N 80110S)

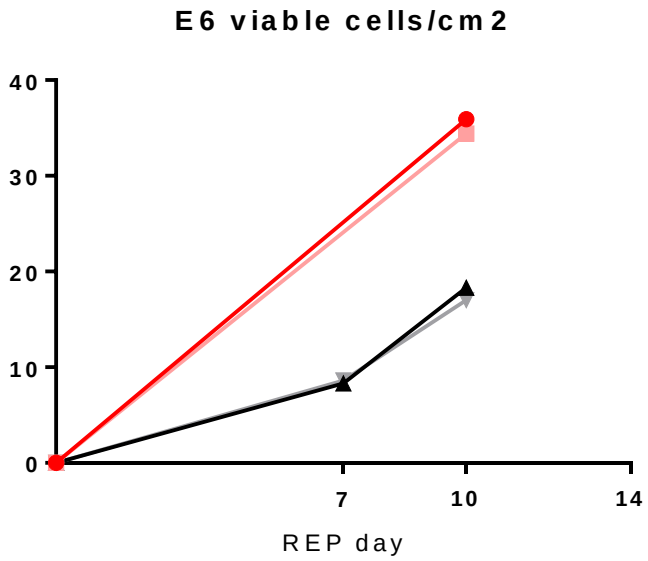
G-Rex500M
(P/N G285500-RU)

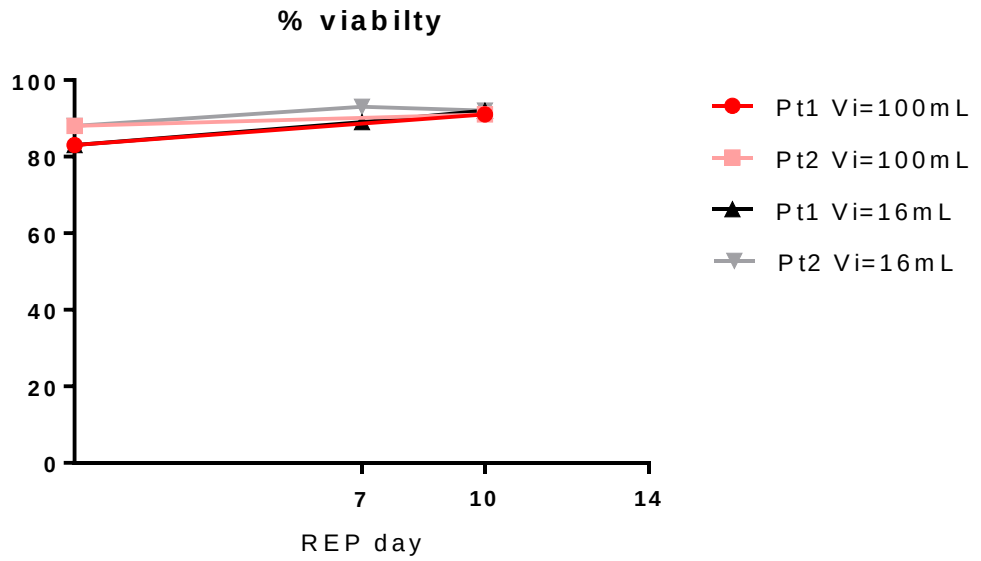
	Day 10 Cell Density (cells/cm²)	Day 10 Total Cells (n)
G-Rex10M	~4.5x10⁷	~4.5x10⁸
G-Rex500M	~4.5x10⁷	**~2.25x10¹⁰**

*Unpublished data from NCI Surgery Branch. Shared with Permission.

Simplifying Cell Culture with G-Rex®







Vi = 100mL Protocol

Day 0: Stimulate 2E5 T cells and 2E7 feeder cells in 100mL cytokine-supplemented media

Days 7, 8, 9, 10: Remove 100uL of media from top of well for lactate testing

Day 10: Re-suspend cells, remove 30uL cell suspension for counts

Vi = 16mL Protocol

Day 0: Stimulate 2E5 T cells and 2E7 feeder cells in 16mL cytokine-supplemented media

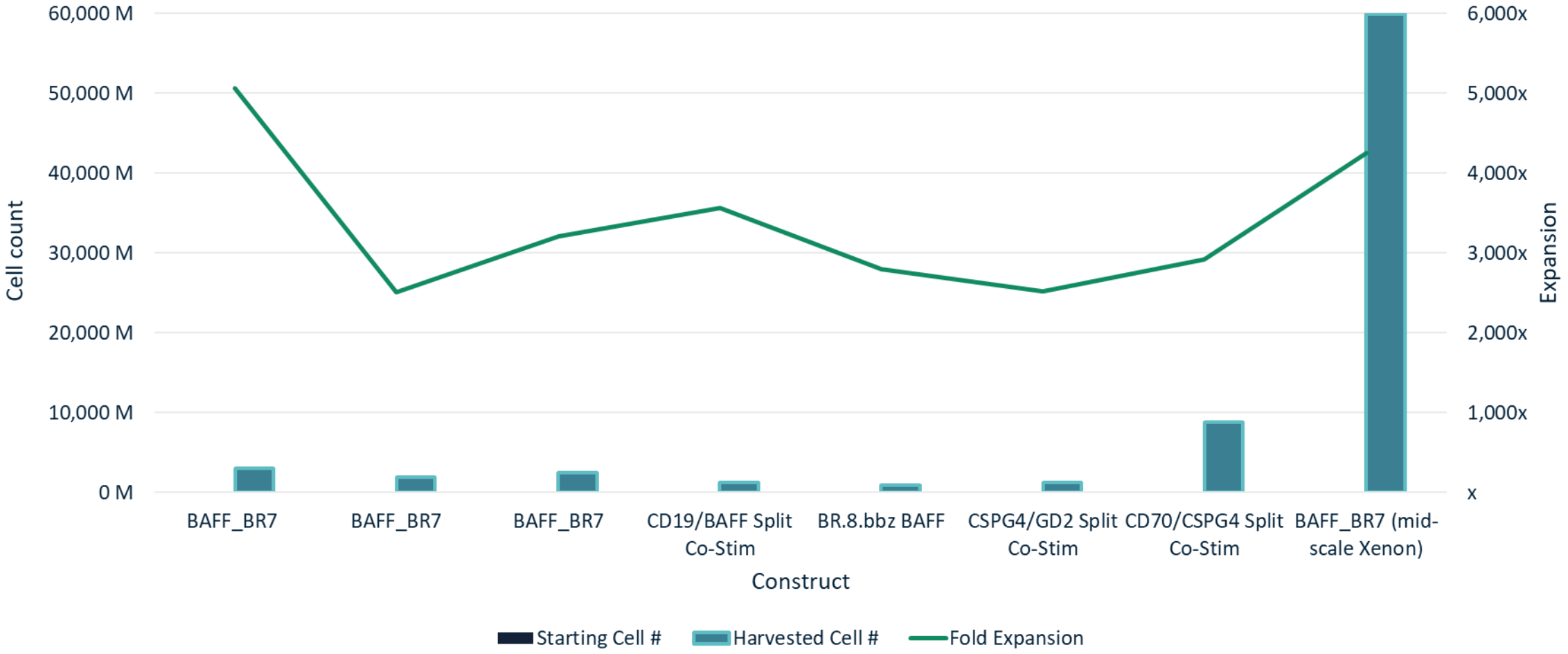
Day 4: Add 16mL cytokine-supplemented media

Day 7: Add 24mL cytokine-supplemented media, remove 30uL cell suspension for counts

Day 10: Add 44mL cytokine-supplemented media, remove 30uL cell suspension for counts

G-Rex® for γδ T cell therapy

Luminary Therapeutics' Gamma 2.0 Manufacturing achieves Enhanced Expansion



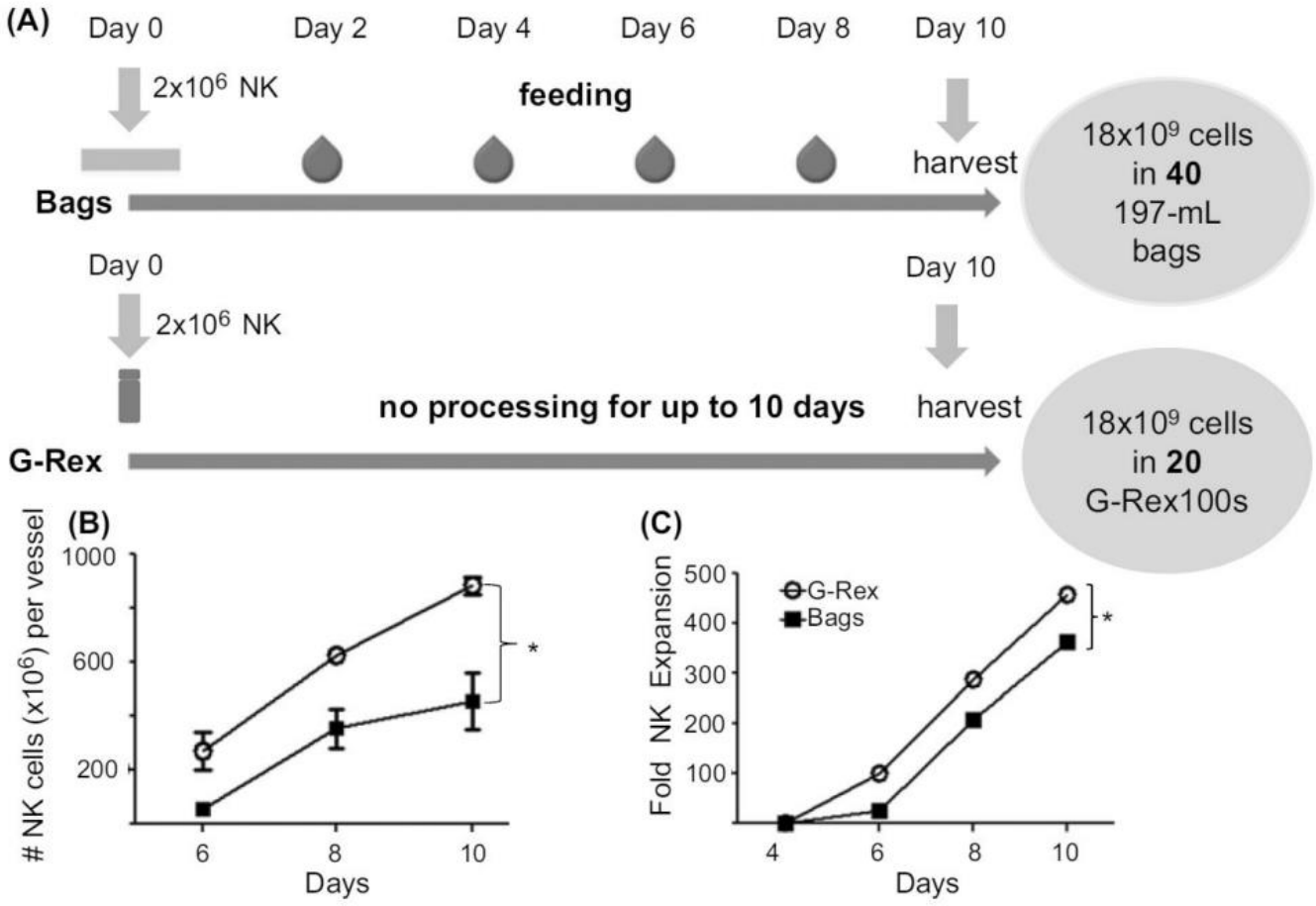
Client Testimony

- Luminary is utilizing the G-Rex500M-CS and starting from ~20% of a full leukopak has achieved 194 patient doses of 65% CAR+ engineered gamma delta cells
- Patient Dose size calculated at 350M cells
- Luminary is driving new levels of patient access in cell therapy

- *"Our manufacturing platform when coupled with the G-Rex will allow us to make **50,000 patient products** per year in two conventional clean rooms" – Luminary CEO*
- *"We are confident that we are currently operating with the lowest COGS for any cell therapy product in the entire industry" – Luminary CEO*

G-Rex® for Natural Killer (NK) cell therapy

G-Rex® for Donor-Derived Allo NK



Cytotherapy. 2012 October ; 14(9): 1131–1143. doi:10.3109/14653249.2012.700767.

Large-scale *ex vivo* expansion and characterization of natural killer cells for clinical applications

NATALIA LAPTEVA[1], APRIL G. DURETT[1], JIALI SUN[1], LISA A. ROLLINS[1], LESLIE L. HUYE[1], JIAN FANG[1], VARADA DANDEKAR[1], ZHUYONG MEI[1], KIMBERLEY JACKSON[1], JUAN VERA[1], JUN ANDO[1], MINHTRAN C. NGO[1], ELAINE COUSTAN-SMITH[3], DARIO CAMPANA[3], SUSANN SZMANIA[4], TARUN GARG[4], AMBERLY MORENO-BOST[4], FRITS VANRHEE[4], ADRIAN P. GEE[1,2], and CLIONA M. ROONEY[1,2]

[1]Center for Cell and Gene Therapy, The Methodist Hospital, Texas Children's Hospital, Houston, TX

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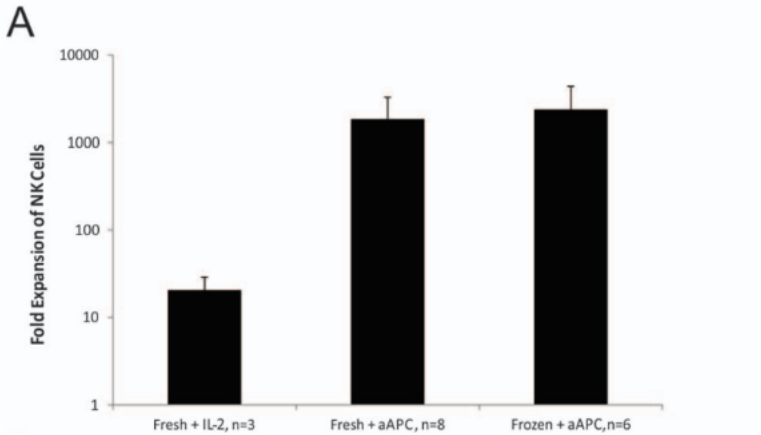
[4]University of Arkansas Medical Center, Little Rock, Arkansas, USA

Figure 2.

G-Rex yields higher numbers of NK cells and requires fewer culture manipulations than cultures in bags. (A) Schematic view of NK cell expansion in bags and G-Rexes. Less cell processing is required to grow a similar number of cells in G-Rexes. (B) NK cells from three different donors were seeded in the 197-mL bags and G-Rex100s. Bags were seeded with PBMC containing 2×10^6 NK cells and 2×10^7 K562-mb15-41BBL cells in a 40-mL starting volume and cultures required feedings with 40 mL fresh media and cytokine every other day, i.e. days 2, 4, 6 and 8, resulting in 200 mL of total volume by day 8. G-Rex100s were seeded with PBMC containing 2×10^6 NK cells and 2×10^7 K562-mbIL15-41BBL in 400 mL medium and were not subsequently fed. Cells were counted and analyzed by flow cytometry for percentage $CD56^+$ $CD3^-$ cells ($n = 3$). (C) Fold expansion of $CD56^+$ $CD3^-$ cells in G-Rexes and bags ($n = 3$). *$P < 0.5$.

G-Rex® for Umbilical Cord Blood derived NK



Antigen Presenting Cell-Mediated Expansion of Human Umbilical Cord Blood Yields Log-Scale Expansion of Natural Killer Cells with Anti-Myeloma Activity

Nina Shah[1]*, Beatriz Martin-Antonio[1], Hong Yang[1], Stephanie Ku[2], Dean A. Lee[3], Laurence J. N. Cooper[3], William K. Decker[2,4], Sufang Li[1], Simon N. Robinson[1], Takuya Sekine[1], Simrit Parmar[1], John Gribben[5], Michael Wang[6], Katy Rezvani[1], Eric Yvon[1], Amer Najjar[7], Jared Burks[8], Indreshpal Kaur[1], Richard E. Champlin[1], Catherine M. Bollard[2], Elizabeth J. Shpall[1]

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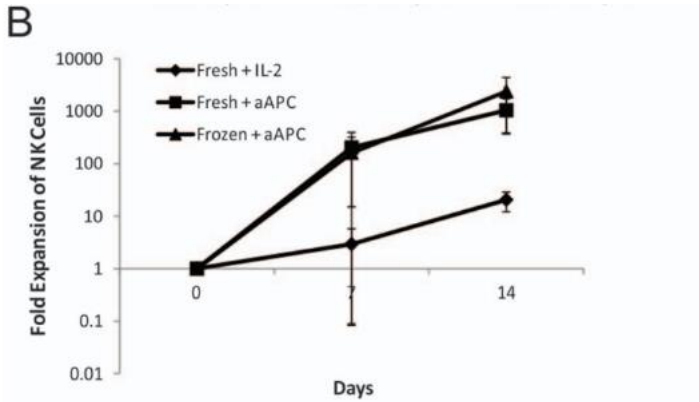


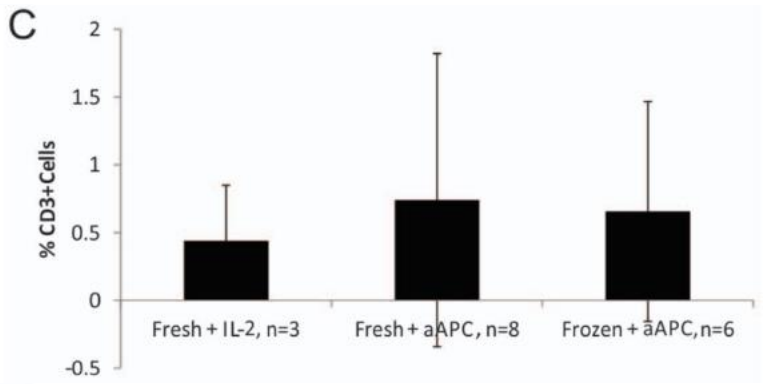


Figure 2. Co-culture of CB MNCs with IL-2 and aAPCs yields significantly greater expansion of NK cells than culture with IL-2 alone. A. Mean fold growth of $CD56^+/CD3^-$ NK cells from 8 fresh and 6 frozen cord blood expansions with aAPCs and IL-2 versus 3 expansions with IL-2 alone (14 day culture). B. Time course of NK cell growth over 14 day culture between all 3 conditions. By day 7, the fresh CB aAPC-containing culture demonstrated greater NK cell growth than culture with IL-2 alone ($p<0.05$). The frozen CB showed a similar trend at day 7, which did not reach statistical significance ($p=0.06$). C. All three culture conditions yielded comparable, low percentages of $CD3^+$ cells:. 0.44%, 0.74% and 0.66% $CD3^+$ cells from the culture with IL-2 alone, fresh CB MNCs with aAPC feeders or frozen CB MNCs with aAPC feeders respectively ($p>0.5$ for all comparisons). Mean +/− SD is shown for each figure. $P<0.05$ where indicated (*).
doi:10.1371/journal.pone.0076781.g002

Shah, N., Martin-Antonio, B., Yang, H., Ku, S., Lee, D. A., Cooper, L. J., Decker, W. K., Li, S., Robinson, S. N., Sekine, T., Parmar, S., Gribben, J., Wang, M., Rezvani, K., Yvon, E., Najjar, A., Burks, J., Kaur, I., Champlin, R. E., … Shpall, E. J. (2013). Antigen presenting cell-mediated expansion of human umbilical cord blood yields log-scale expansion of natural killer cells with anti-myeloma activity. PLoS ONE, 8(10). https://doi.org/10.1371/journal.pone.0076781

G-Rex® vs. WAVE for NK cells

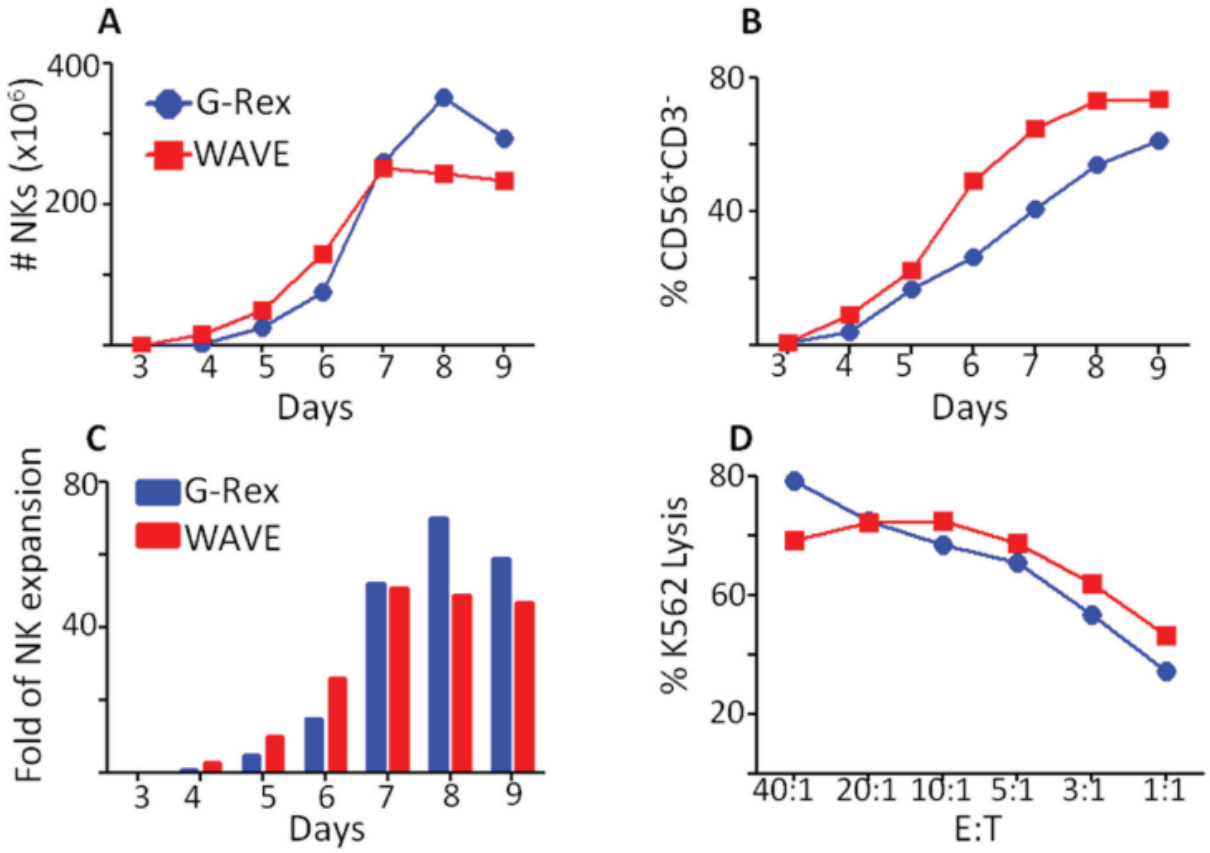


FIG. 4: Comparison of NK cell expansion in G-Rex flasks and WAVE bioreactor. PBMCs from healthy donor containing 5×10^6 NK cells were co-cultured with K562-41BBL-mbIL-15 cells for 9 days. Cells were moved to WAVE on day 4 of culture. (A, B, C) Similar numbers and fold of NK expansion were observed in G-Rex and WAVE cultures. (D) Potency (killing K562 cells) of both products was also comparable. Similar expansion was obtained for three donors and the representative data for one donor is shown.

Critical Reviews™ in Oncogenesis, 19(1-2):121–132 (2014)

Clinical Grade Purification and Expansion of Natural Killer Cells

Natalia Lapteva[1*], Susann M. Szmania[2], Frits van Rhee[2], & Cliona M. Rooney[1*]

[1] Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX 77030; [2]University of Arkansas Medical Center, Little Rock, AK 72205

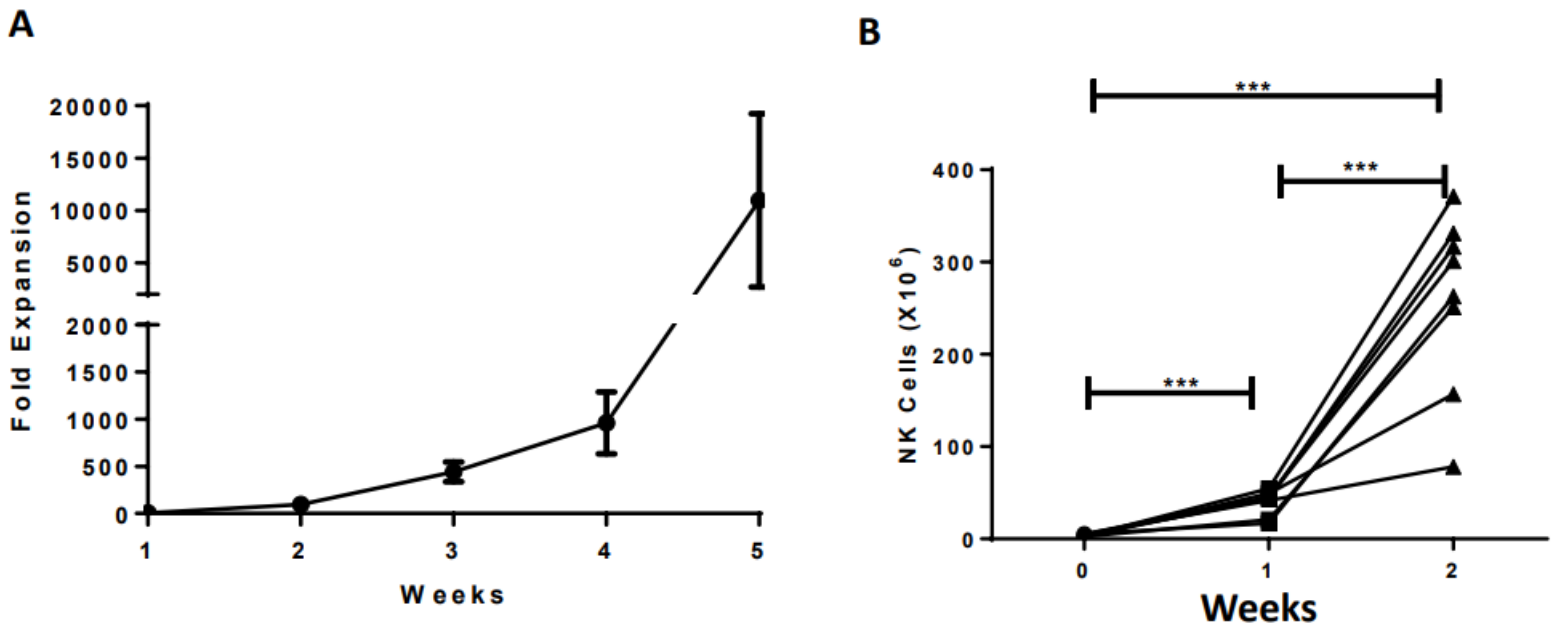
toxicity assays). Interestingly, the WAVE-generated products contain fewer $CD3^+$ T cells and a higher frequency of $CD56^+CD3^-$ NK cells, perhaps because T cells prefer static culture (Fig. 4). The clinical trial in multiple myeloma demands both autologous and allogeneic NK cells. In the case of allogeneic products, $CD3^+$ T cells are efficiently removed (as well as CD3+56+ cells) by depletion with the CliniMACS CD3 reagent, reducing the CD3+ cell frequency from 19±9% to 0.4±0.5% (N=5). This depletion allows less than 5×10^5 $CD3^+$ $CD56^-$ T cells kg^{-1} for infusion, as mandated by the clinical protocol.

Lapteva, N., Szmania, S. M., van Rhee, F., & Rooney, C. M. (2014). Clinical grade purification and expansion of Natural Killer Cells. Critical Reviews in Oncogenesis, 19(1–2), 121–132. https://doi.org/10.1615/critrevoncog.2014010931

SCIENTIFIC REPORTS
nature research

Membrane bound IL-21 based NK cell feeder cells drive robust expansion and metabolic activation of NK cells

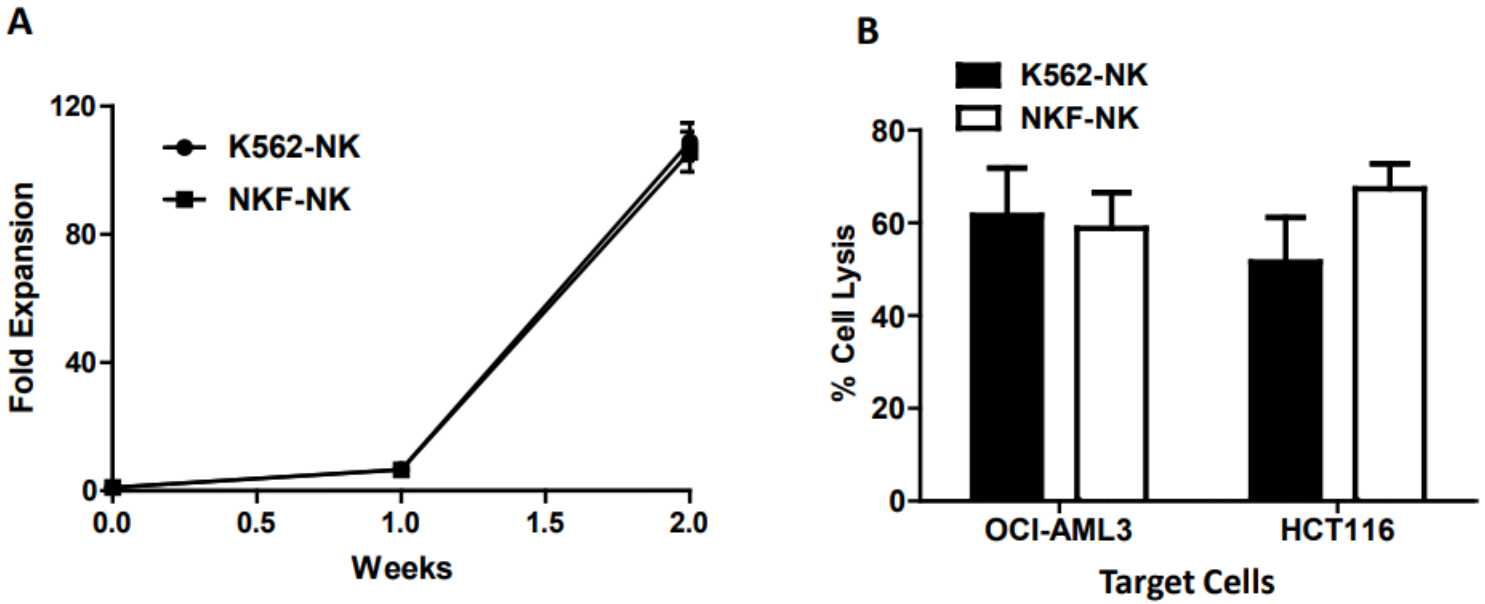
Evelyn O. Ojo[1], Ashish Arunkumar Sharma[1], Ruifu Liu[1], Stephen Moreton[1], Mary-Ann Checkley-Luttge[2], Kalpana Gupta[1], Grace Lee[1], Dean A. Lee[3], Folashade Otegbeye[4], Rafick-Pierre Sekaly[1], Marcos de Lima[4] & David N. Wald[1,5*]



Supplemental Figure 5. A. Fold expansion of NKF-NK cells at 5:1 feeder-to-NK ratio and 200U/mL IL-2 after 5 weeks, n=6. B, Expansion of NK cells using NKF cells at 5:1 feeder-to-NK ratio and 200U/mL IL-2 for 2 weeks, using G-Rex flasks, n=8. ***p<0.001. Data represent mean +/- SEM.

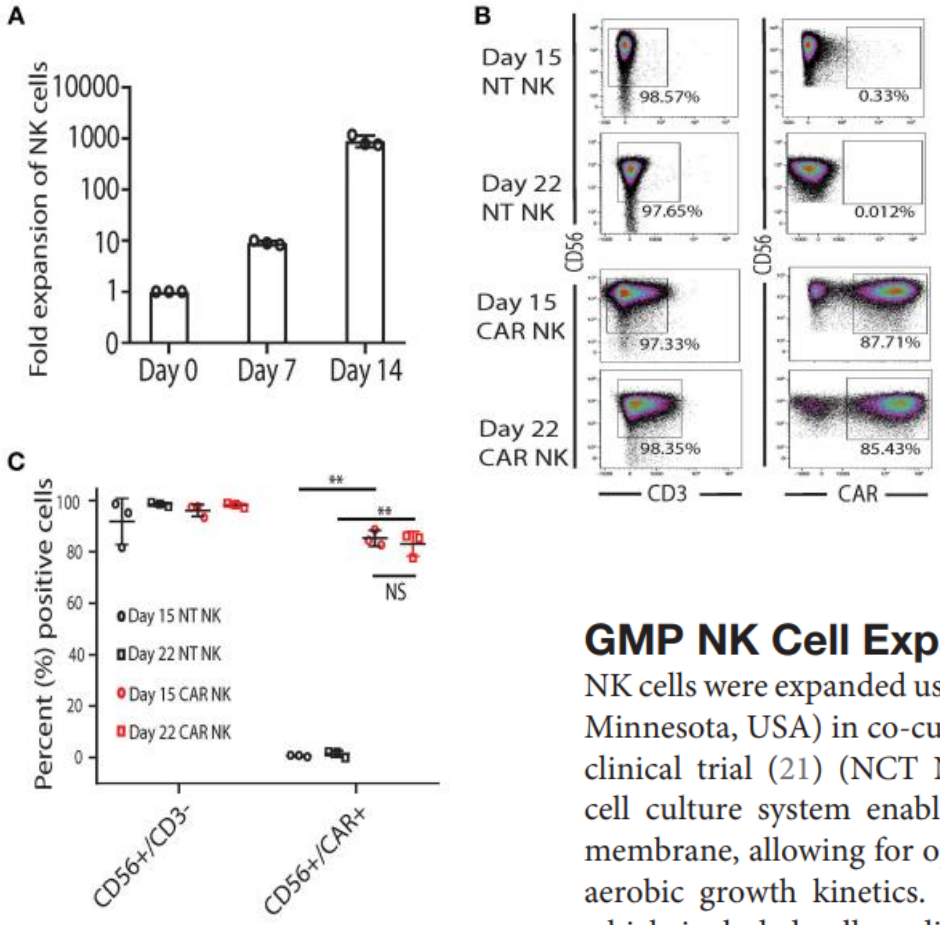
✓ G-Rex® enables robust fold expansion of Natural Killer cells

G-Rex® for Donor-Derived Allo NK



Supplemental Figure 2. A. Fold expansion of K562-NK and NKF-NK cells at 5:1 feeder-to-NK ratio and 100U/mL IL-2 after 2 weeks, n=1. B, The cytotoxic activity of 2 weeks-expanded NKF-NK and K562-NK was assessed against OCI-AML3 and HCT116 cells after 4hr co-culture, n=3. The NK cell-to-target ratio was 1-1. Data represent mean +/- SEM.

- ✓ G-Rex® can be used with **multiple feeder** cell lines to produce large amounts of NK cells with high tumor cytotoxicity



GMP-Compliant Universal Antigen Presenting Cells (uAPC) Promote the Metabolic Fitness and Antitumor Activity of Armored Cord Blood CAR-NK Cells

Enli Liu[1†], Sonny O. T. Ang[1†], Lucila Kerbauy[1,2,3], Rafet Basar[1], Indreshpal Kaur[1], Mecit Kaplan[1], Li Li[1], Yijiu Tong[1], May Daher[1], Emily L. Ensley[1], Nadima Uprety[1], Ana Karen Nunez Cortes[1], Ryan Z. Yang[1], Ye Li[1], Hila Shaim[1], Francia Reyes Silva[1], Paul Lin[1], Vakul Mohanty[4], Sunil Acharya[1], Mayra Shanley[1], Luis Muniz-Feliciano[1], Pinaki P. Banerjee[1], Ken Chen[4], Richard E. Champlin[1], Elizabeth J. Shpall[1] and Katayoun Rezvani[1]*

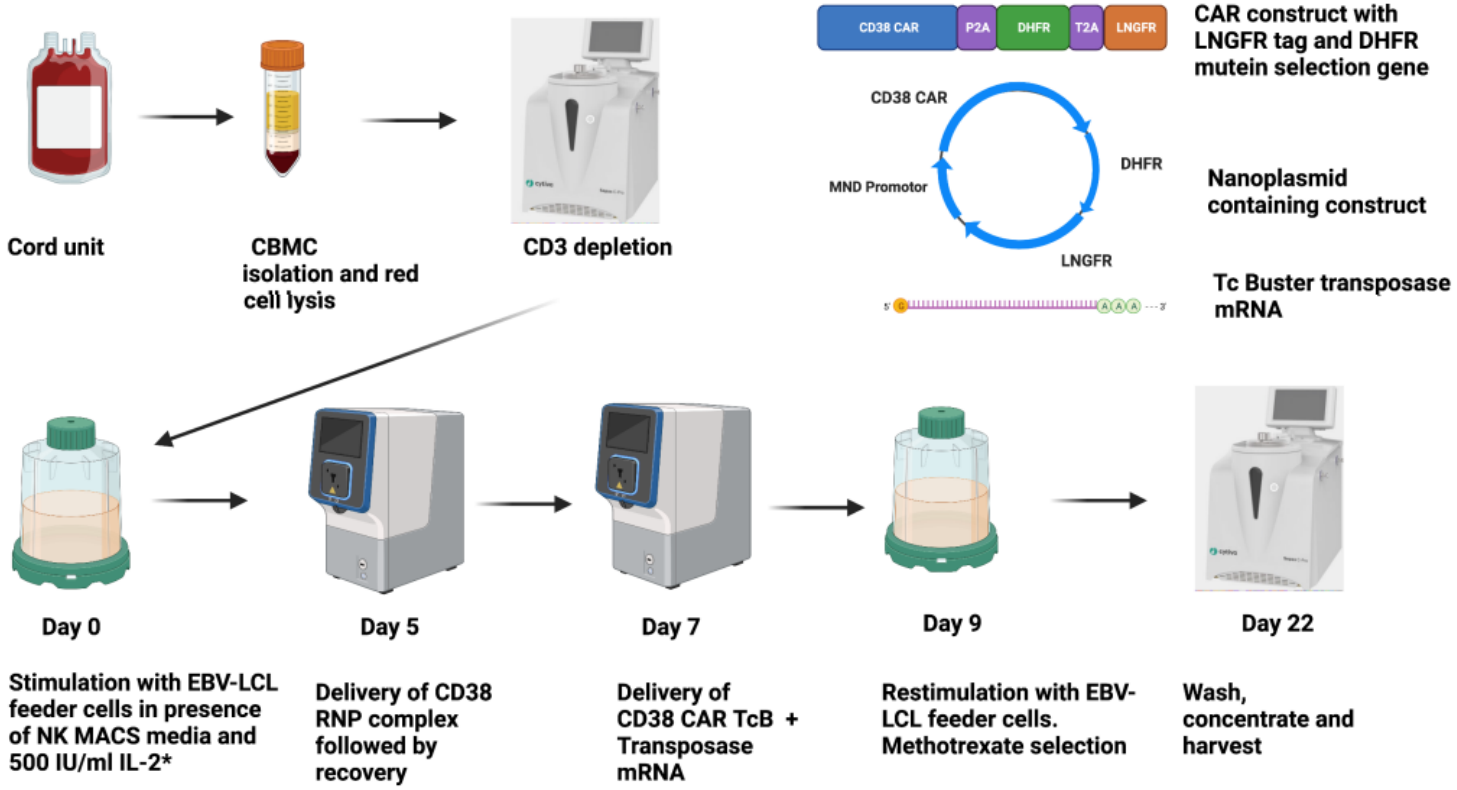
[1] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, United States, [2] Departments of Stem Cell Transplantation and Hemotherapy/Cellular Therapy, Hospital Israelita Albert Einstein, Saõ Paulo, Brazil, [3] Department of Genetics and Evolutionary Biology, Human Genome and Stem Cell Research Center, Biosciences Institute, University of Saõ Paulo, Saõ Paulo, Brazil, [4] Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, United States

GMP NK Cell Expansion With uAPC

NK cells were expanded using the G-Rex® bioreactor (Saint Paul, Minnesota, USA) in co-cultures with uAPC for the CB-NK CAR clinical trial (21) (NCT Number: NCT03056339). This closed cell culture system enables using flasks with a gas-permeable membrane, allowing for optimal gaseous exchange and ensuring aerobic growth kinetics. Using previously optimized variables which included cell seeding density, calibrated media volume, and media formulation, we were able to adapt the G-Rex M100 series from pre-clinical protocols to translate and linearly scale the procedure to the desired yield in the GMP setting.

CB derived, optimized affinity CD38 CAR-NK cells with CD38 KO show promising *in-vivo* activity in a Multiple Myeloma model

S Brophy[1], L Kirkham McCarthy[1], M Köylijärvi[1], D Hardwicke[1], H Hintz[2], N Otto[2], B Ettestad[2], D Hermanson[2], E Shevlin[1], M Reilly[1] and M O'Dwyer[1]
Bio-Techne[2] and ONK Therapeutics[1]



- ✓ The process resulted in **successful expansion** and enrichment of CD56 positive, CD3 negative NK cells (>96%) with minimal T cell contamination.
- ✓ Using G-Rex6M plates, CD38 CAR-NK cells with CD38 KO expanded approximately **400 fold** by D22.
- ✓ Extending cell expansion until D27 increased expansion 4-8 fold*
  - ✓ **1,600-3,200 fold expansion* by D27**

Process Continuity = **Product** Continuity

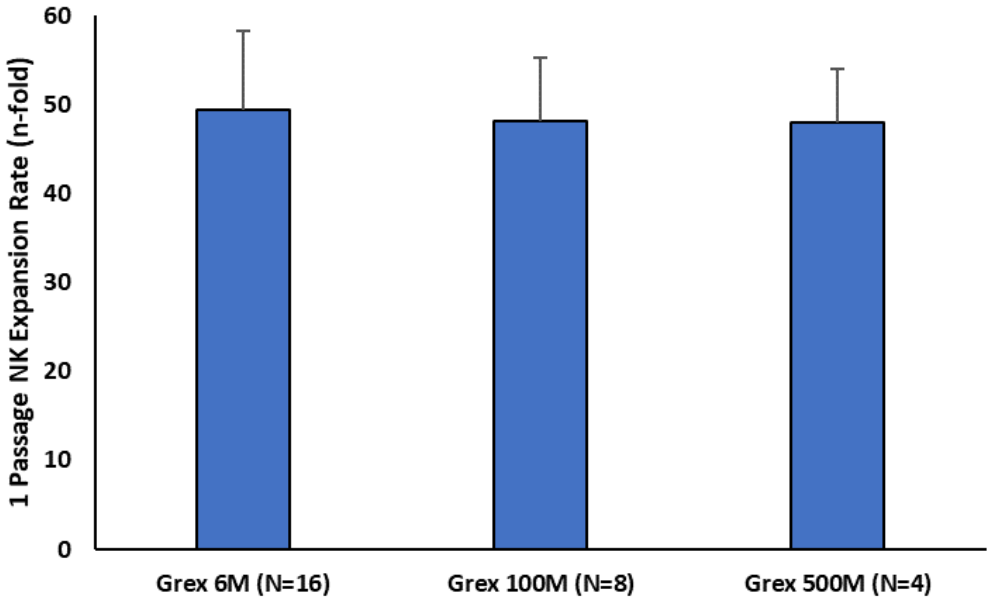


Figure 1. NK-cell expansion rate is comparable between Grex 6M, 100M, and 500M vessels.

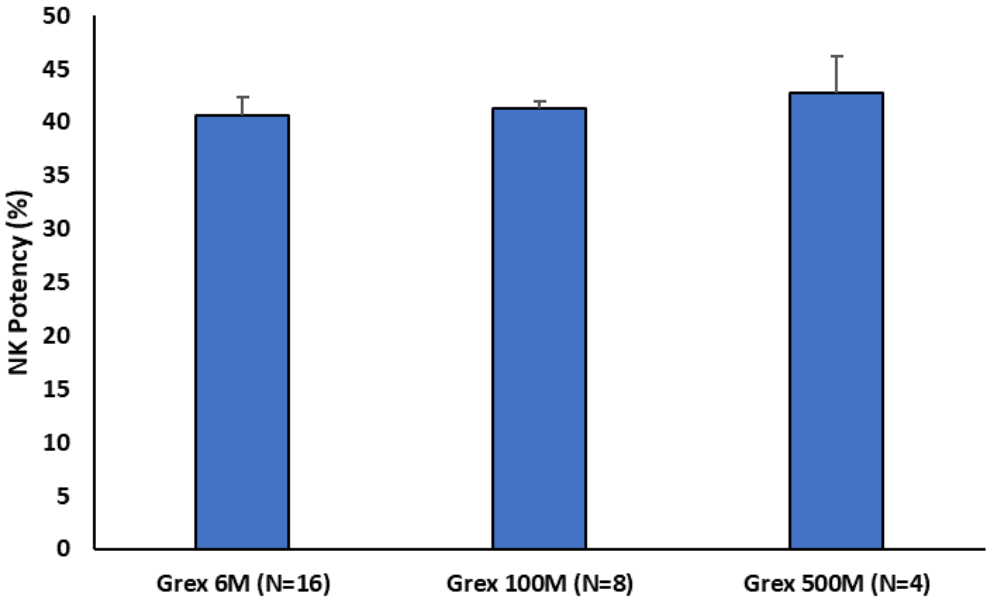
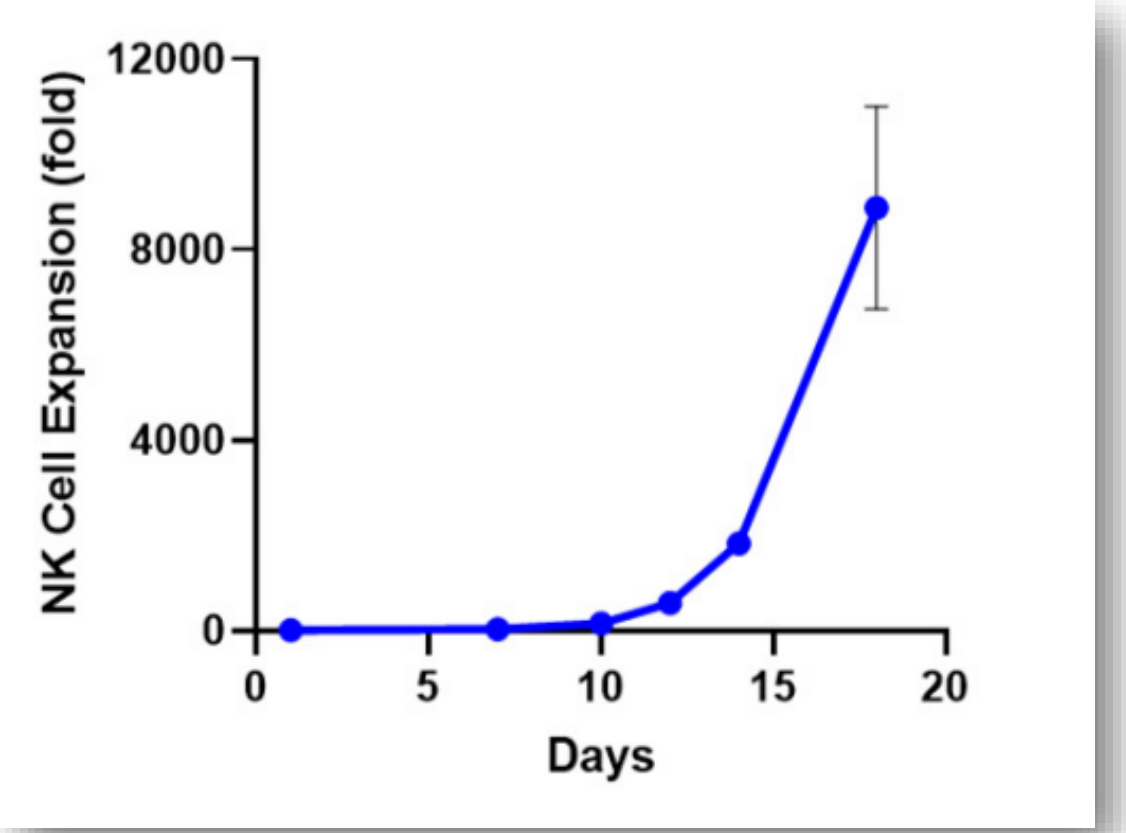
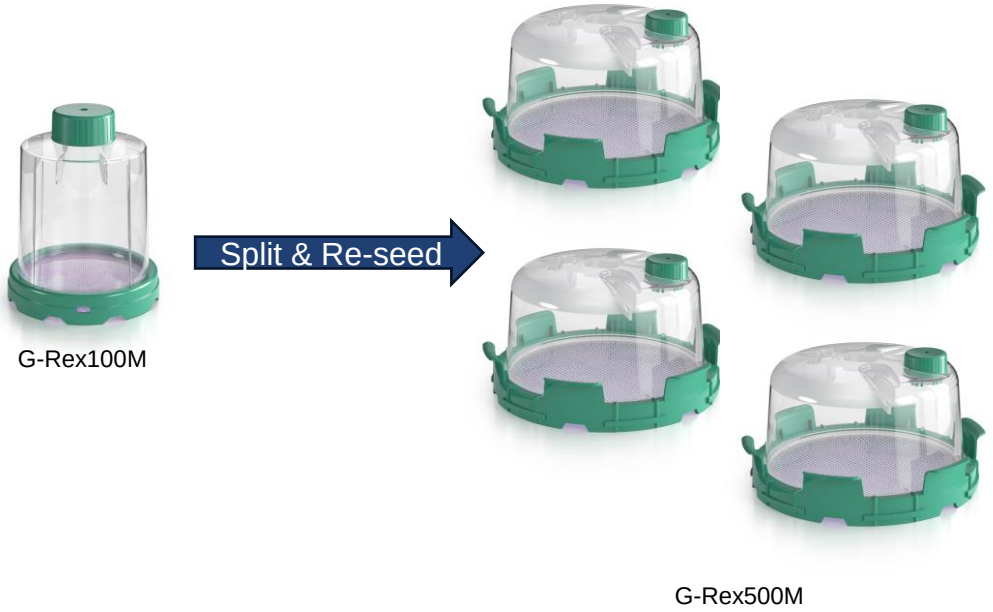


Figure 2. NK-cell potency is comparable between Grex 6M, 100M, and 500M vessels.

G-Rex® for Donor-Derived Allo NK





✓ **Maximize fold expansion** and **minimize cell processing burden**

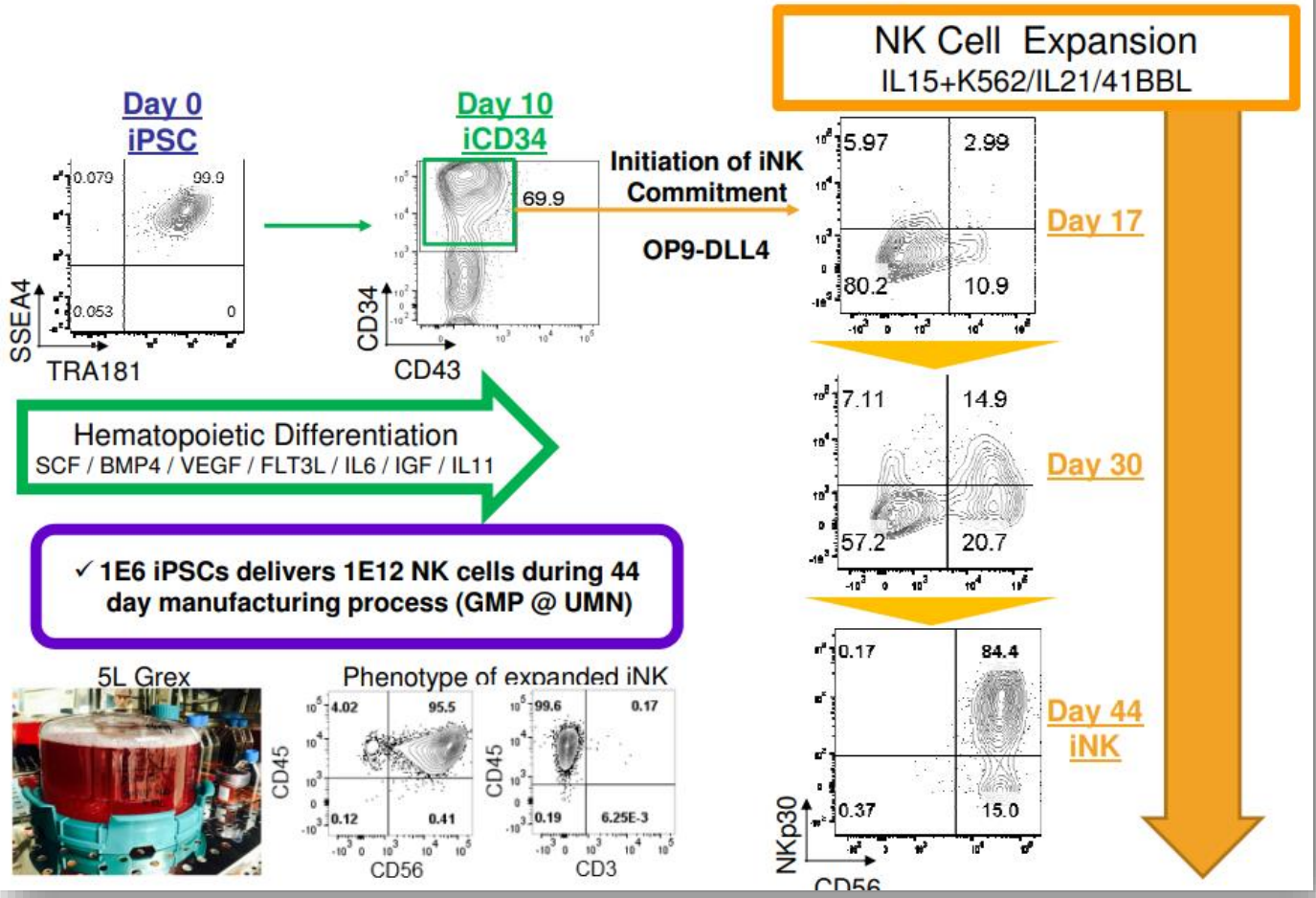
Golubovskaya, V., Sienkiewicz, J., Sun, J., Zhang, S., Huang, Y., Zhou, H., Harto, H., Xu, S., Berahovich, R., & Wu, L. (2023). Car-NK cells generated with mrna-lnps kill tumor target cells in vitro and in vivo. *International Journal of Molecular Sciences*, *24*(17), 13364. https://doi.org/10.3390/ijms241713364

Clinical Translation of Pluripotent Cell-Derived Off-the-Shelf Natural Killer Cell Cancer Immunotherapy

Frank Cichocki[1], Ryan Bjordahl[2], Svetlana Gaidarova[2], Paul Rogers[2], Raedun Clarke[2], Brian Groff[2], Stacey Moreno[2], Ramzey Abujarour[2], Megan Robinson[2], Greg Bonello[2], Tom Lee[2], Weijie Lan[2], Betsy Rezner[2], Stewart Abbot[2], Darin Sumstad[1], Bruce Blazar[1], Daniel Shoemaker[2], Scott Wolchko[2], Dan Kaufman[3], David McKenna[1], Bahram Valamehr[2], Sarah Cooley[1] and Jeffrey S. Miller[1]

[1]University of Minnesota Cancer Center, Minneapolis, MN
[2] Fate Therapeutics, San Diego, CA
[3]University of California San Diego, CA

iPSC → iNK Manufacturing Process



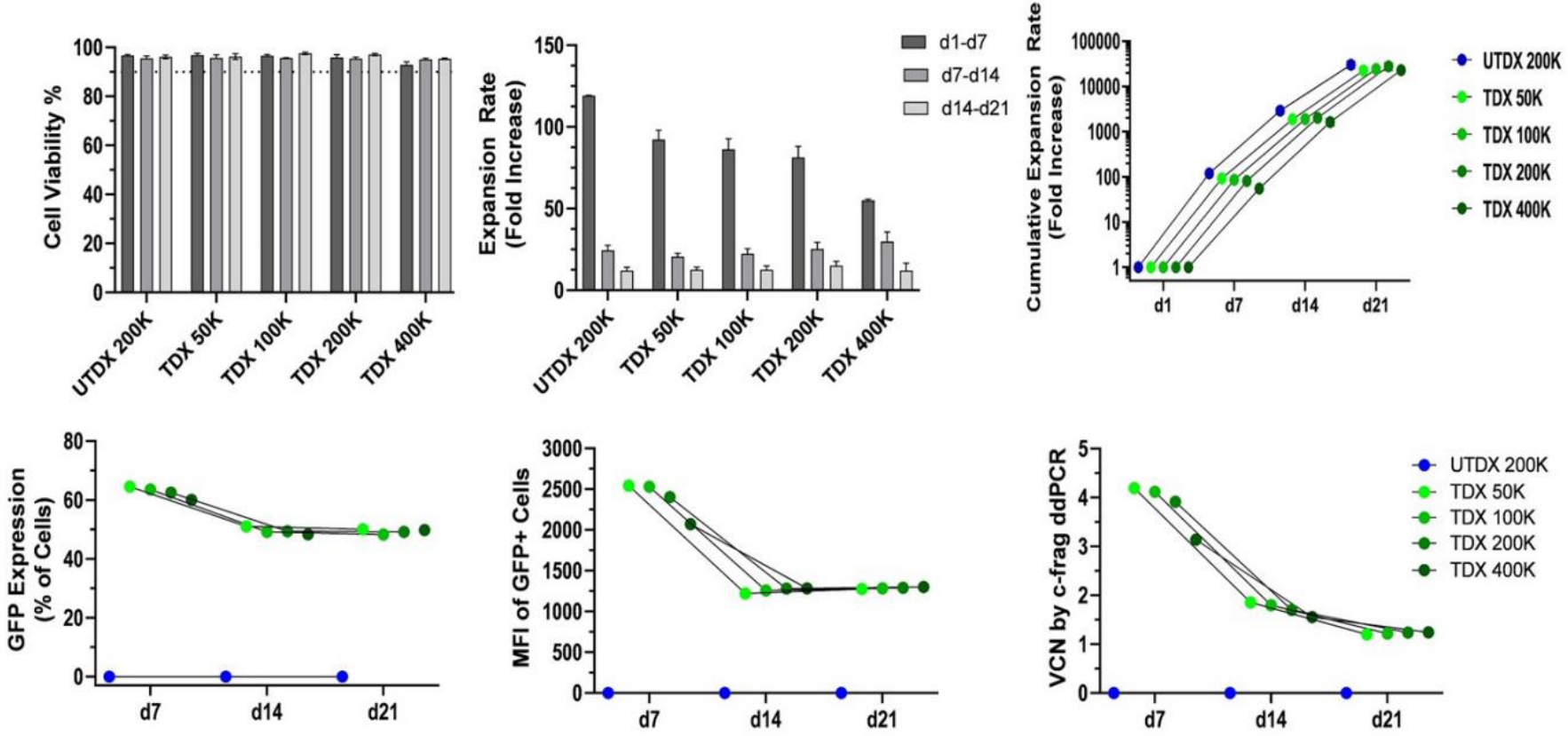
G-Rex® for Hematopoietic Stem Cell (HSC) therapy

NOVEL, EFFICIENT SYSTEM FOR CULTURE OF TRANSDUCED HEMATOPOIETIC STEM PROGENITOR CELLS (HSPC) FOR VECTOR COPY NUMBER DETERMINATION

Monica Torres-Coronado[1], Ilse Arciga[1], Diana L. Browning[1], Xiu-Li Li[1], Agnes Gardner[1], Sergio Branciamore[2], John A. Zaia[1], Angelo A. Cardoso[1]

Center for Gene Therapy [1] and Department of Computational and Quantitative Medicine [2] of City of Hope, Duarte, CA

Establishing Seeding Conditions to culture HSPC in G-Rex Plates (TDX with GFP LVV)

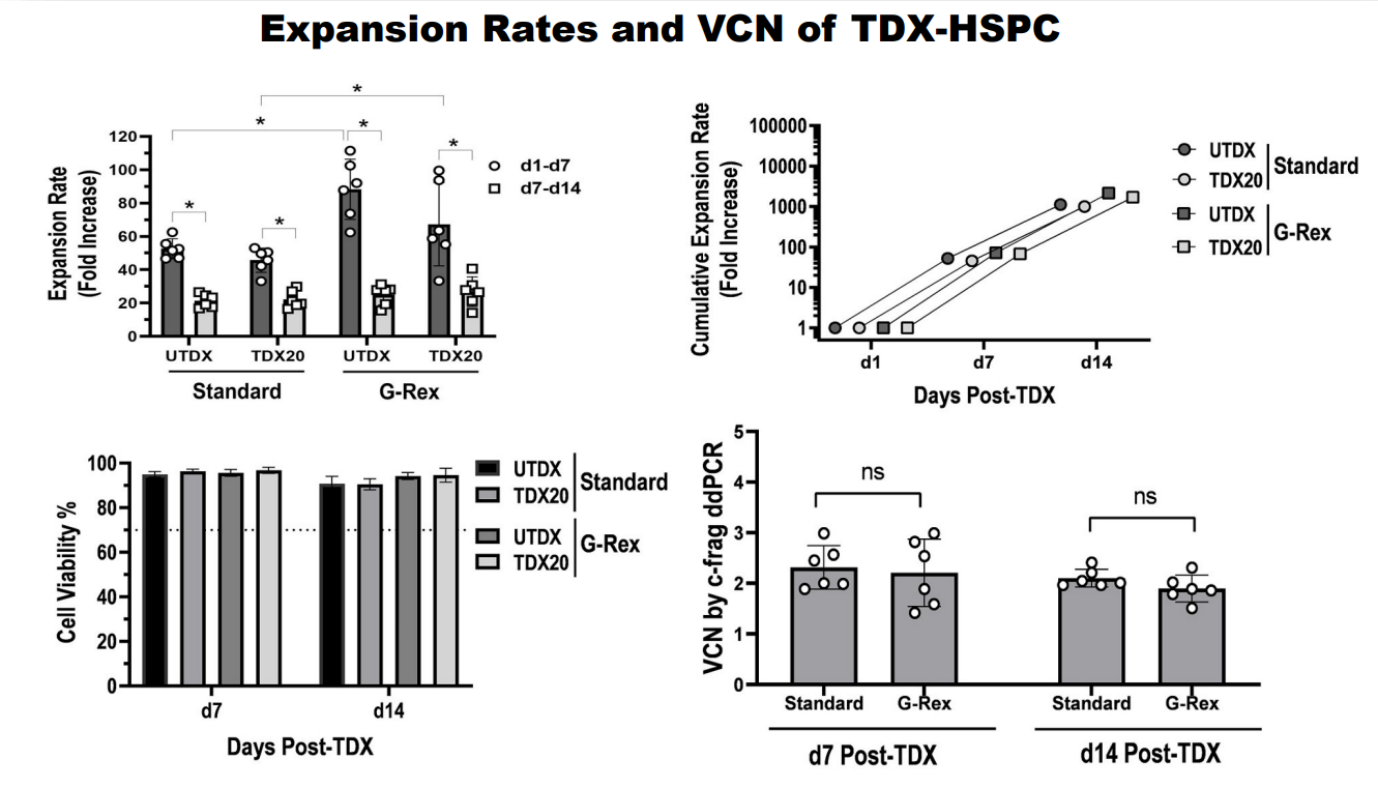


G-Rex® for CD34+ HSCs

NOVEL, EFFICIENT SYSTEM FOR CULTURE OF TRANSDUCED HEMATOPOIETIC STEM PROGENITOR CELLS (HSPC) FOR VECTOR COPY NUMBER DETERMINATION

Monica Torres-Coronado[1], Ilse Arciga[1], Diana L. Browning[1], Xiu-Li Li[1], Agnes Gardner[1], Sergio Branciamore[2], John A. Zaia[1], Angelo A. Cardoso[1]

Center for Gene Therapy [1] and Department of Computational and Quantitative Medicine [2] of City of Hope, Duarte, CA



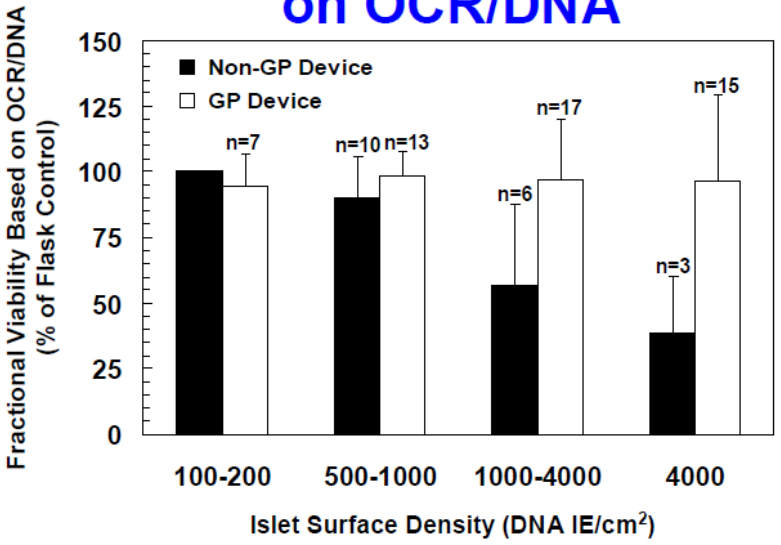
- ✓ The **Cell Viability of G-Rex cultures was consistently high during culture** (d7, 96.8%; d14, 94.6%) and comparable to that seen in Standard cultures.
- ✓ **Higher cell expansion rate was observed in G-Rex** method during the first week, with similar expansion rates in the second week of culture.
- ✓ This novel, **G-Rex-based system of TDX-HSPC expansion/differentiation** for VCN determination is **simple and robust, providing marked labor and cost savings**.
- ✓ This system is being integrated in the QC testing for release of TDX-HSPC Drug Products for clinical manufacturing. This method is **suitable for automation** using a robotic platform integrating genomic DNA preparation and ddPCR processing.

G-Rex® for Pancreatic Islet Cells

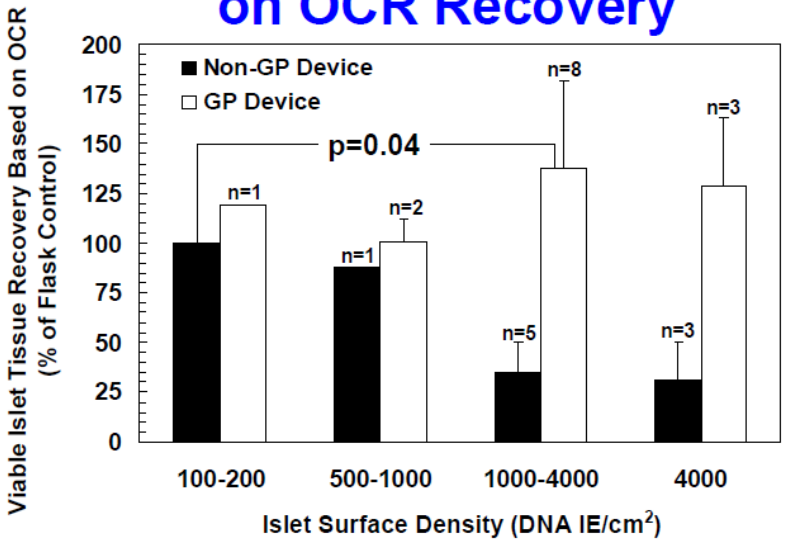
Improved Islet Culture on Top of Gas-Permeable Membranes

Klearchos K Papas[1], Bernhard J Hering[1], Phillip R Rozak[1], John Wilson[2], and Efstathios S Avgoustiniatos[1]
[1]Diabetes Institute for Immunology and Transplantation, Department of Surgery, University of Minnesota, Minneapolis, MN, USA
[2]Wilson Wolf Manufacturing, New Brighton, MN, USA

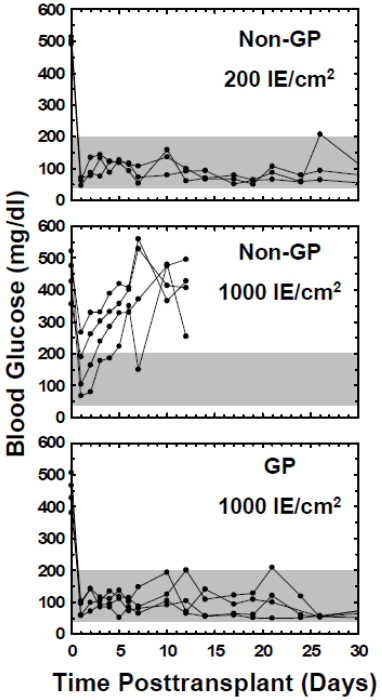
Effect of Islet Surface Density on OCR/DNA



Effect of Islet Surface Density on OCR Recovery



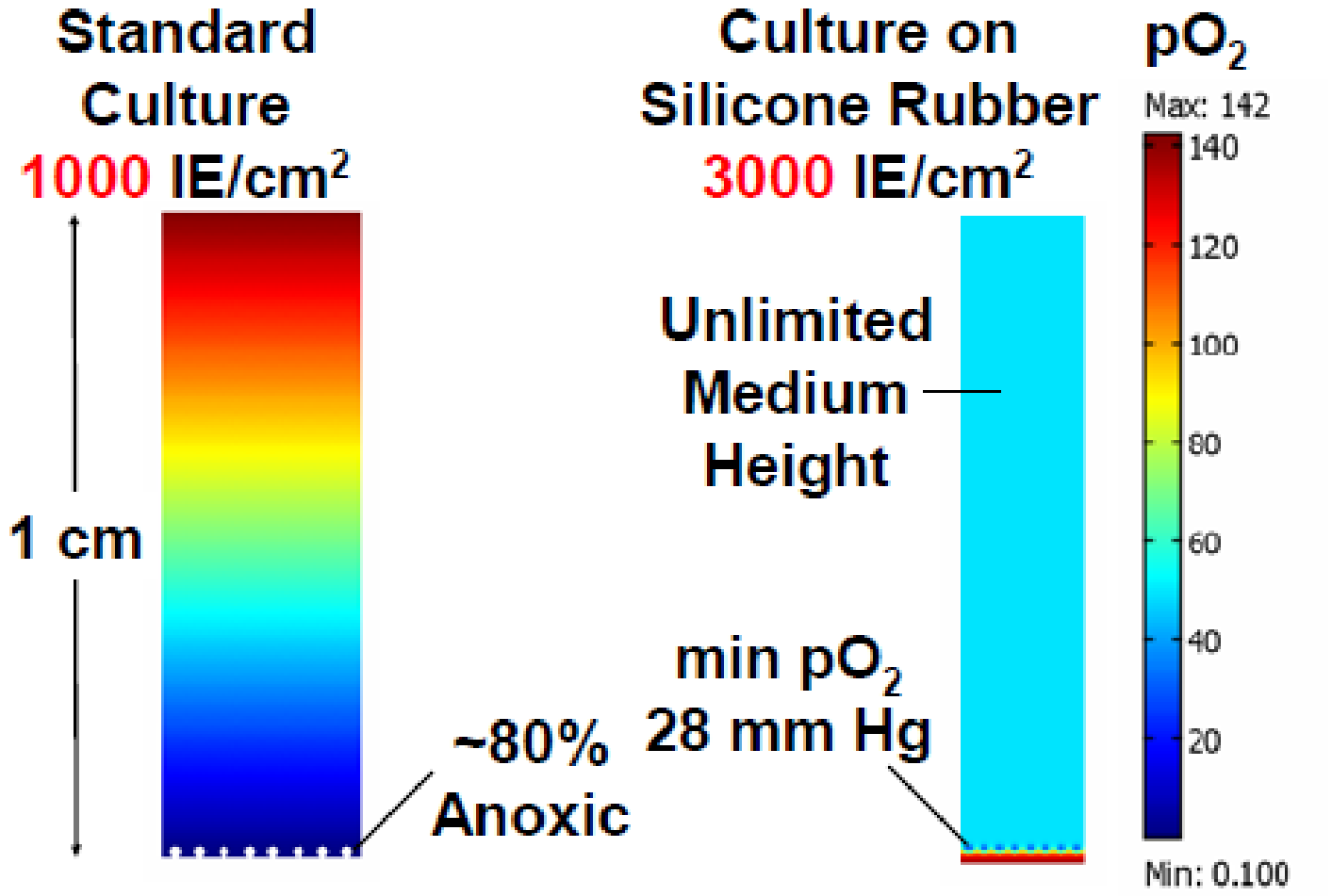
Nude Mouse Bioassay



Improved Islet Culture on Top of Gas-Permeable Membranes

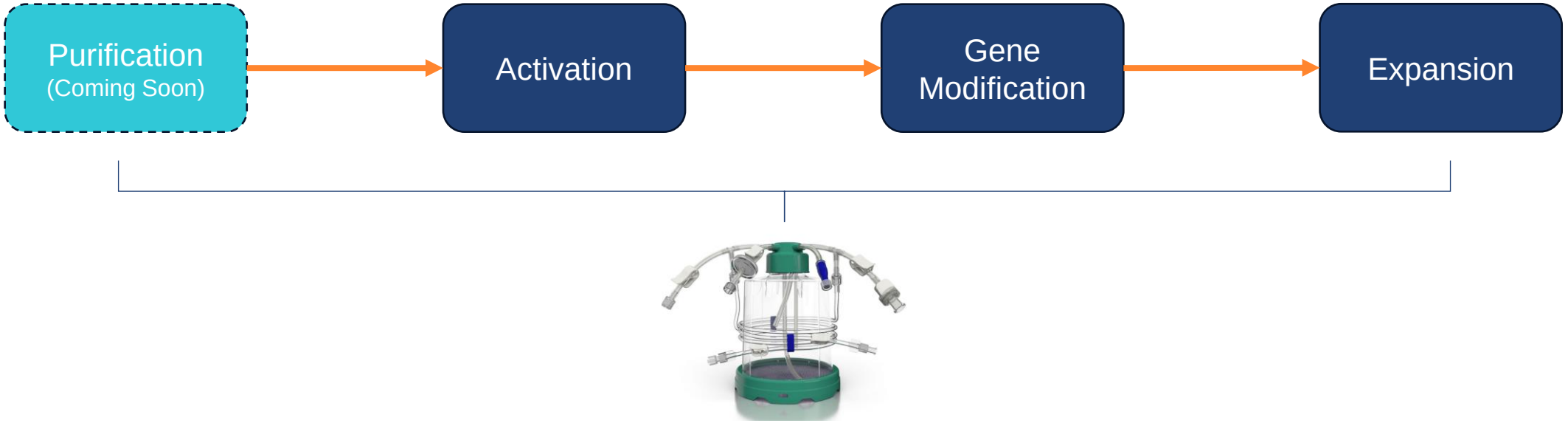
Klearchos K Papas[1], Bernhard J Hering[1], Phillip R Rozak[1], John Wilson[2], and Efstathios S Avgoustiniatos[1]
[1]Diabetes Institute for Immunology and Transplantation, Department of Surgery, University of Minnesota, Minneapolis, MN, USA
[2]Wilson Wolf Manufacturing, New Brighton, MN, USA

Simulation Results

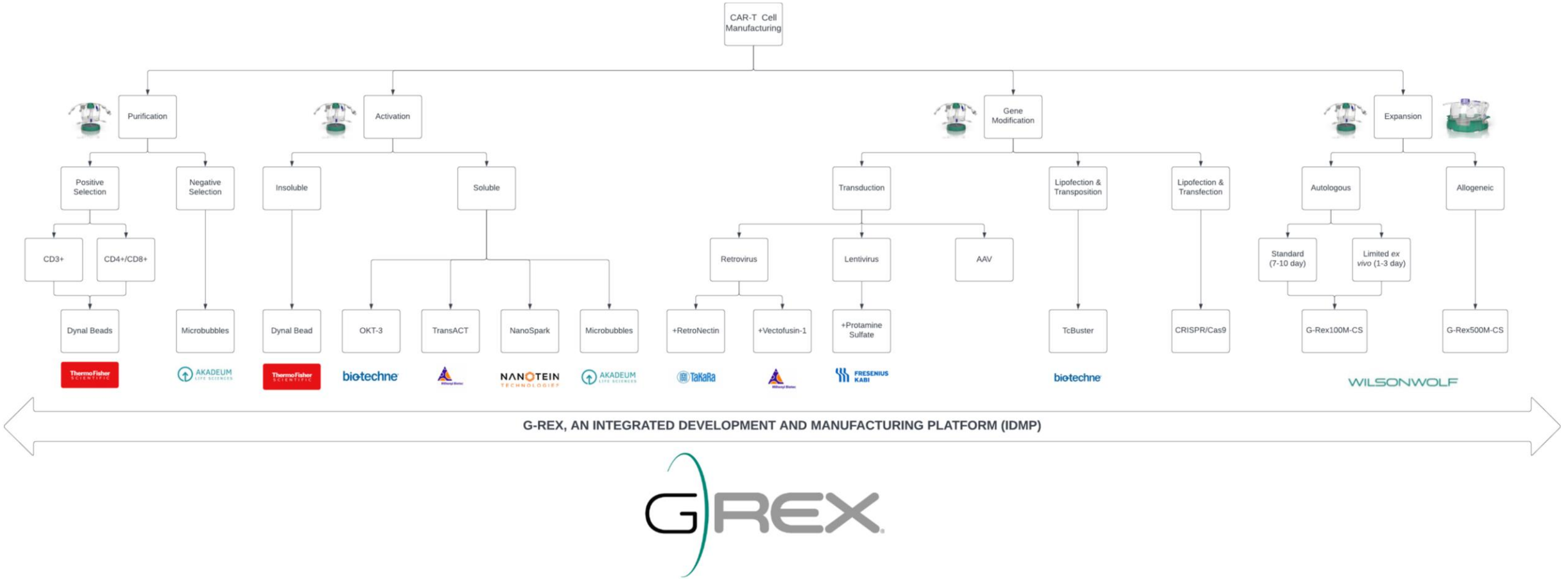


G-Rex® is an integrated development and manufacturing platform

G-REX: An Integrated Manufacturing Device



G-Rex® Integrated Development and Manufacturing Platform for CGT



G-Rex® Integrated Manufacturing Device

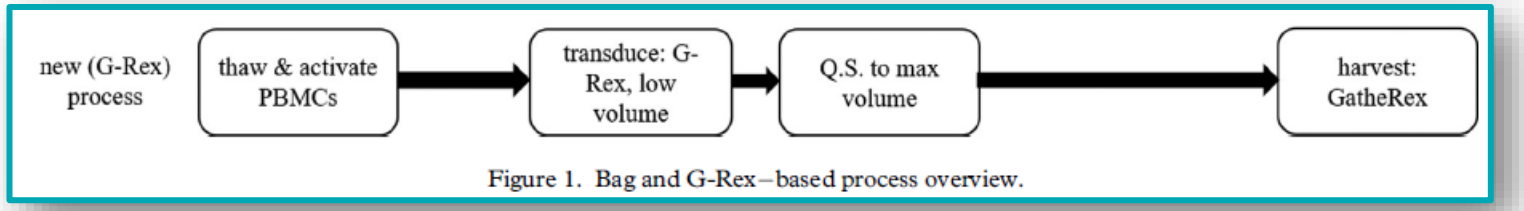
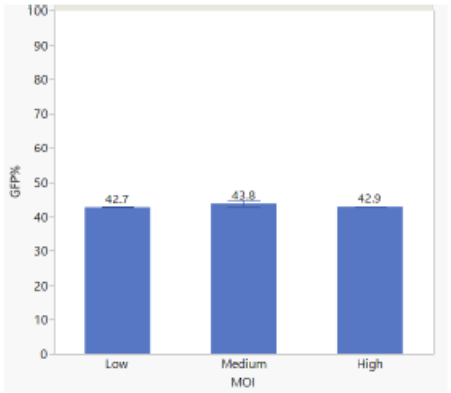


Figure 1. Bag and G-Rex–based process overview.

Transduction Model

- Lentivirus transduction timing was optimized using a CAR-GFP lentivector
- Showed sufficient transduction efficiency (day7) with low MOI in scaled down model for transduction in G-Rex
- However, highly dependent on vector, GOI, and patient cells
- Target range 2-50% CAR+ cells.

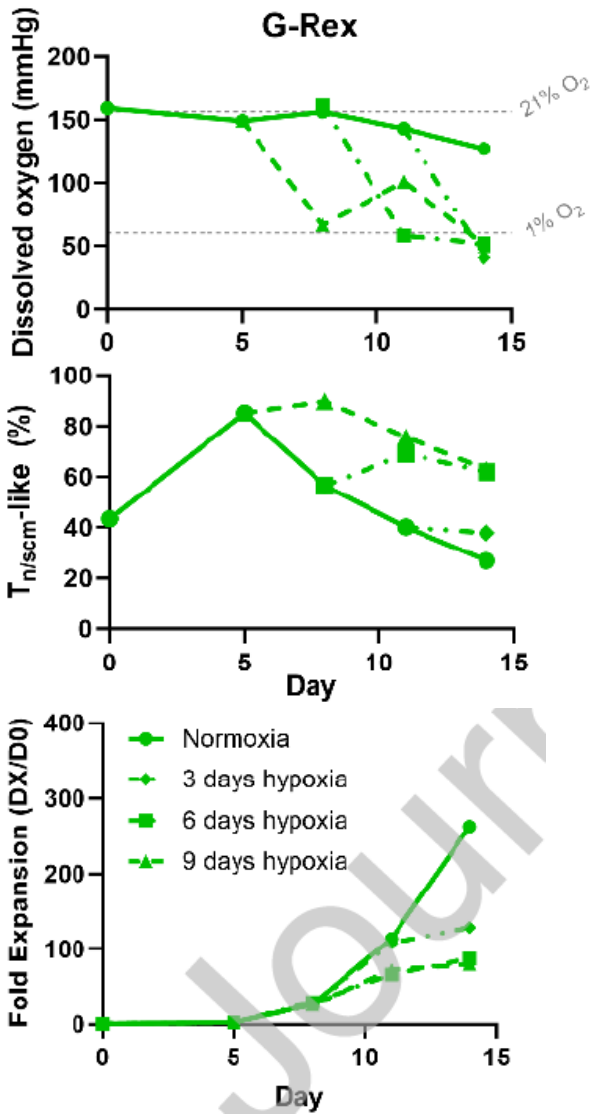


- ✓ G-Rex is a **"factory in a jar"**
- ✓ Incorporate multiple unit operations within the G-Rex to **streamline manufacturing** process and **reduce risk of contamination**

Gagliardi C, Khalil M, Foster AE. Streamlined production of genetically modified T cells with activation, transduction and expansion in closed-system G-Rex bioreactors. Cytotherapy. 2019 Dec;21(12):1246-1257. DOI: 10.1016/j.jcyt.2019.10.006.

Joseph, Gil, et al. *Innovative Development of Closed CAR-T Platform.* WuXi Advanced Therapies. Accessed 12 Jan. 2021.

G-Rex® = Highly Controllable Process



CAR-T cell expansion platforms yield distinct T cell differentiation states

Hannah W. Song • Michaela Prochazkova • Lipei Shao • ... David F. Stroncek • Javed Khan • Steven L. Highfill • Show all authors

Open Access • Published: March 14, 2024 • DOI: https://doi.org/10.1016/j.jcyt.2024.03.003

- ✓ **G-Rex®** is the **most controllable** C&GT platform in the industry
- ✓ **G-Rex®** enables **tight control** over critical process parameters to deliver proper critical quality attributes
- ✓ **G-Rex®** is **only platform** that can be leveraged **to deliver the proper cell quality** in the **proper cell quantity** **at any scale**

Getting started in G-Rex®

Optimize expansion and read-outs at small-scale.

G-Rex “M” series well plates available in $2cm^2$, $5cm^2$, and $10cm^2$

G-Rex “M” Series Well Plate Experiment:

1. Correlate glucose consumption and lactate production trends to cell density
2. Understand how culture duration affects the quality cell output
3. Confirm optimal minimum and maximum cell densities
4. Perfect cytokine supplementation strategy

Linear Scale Up

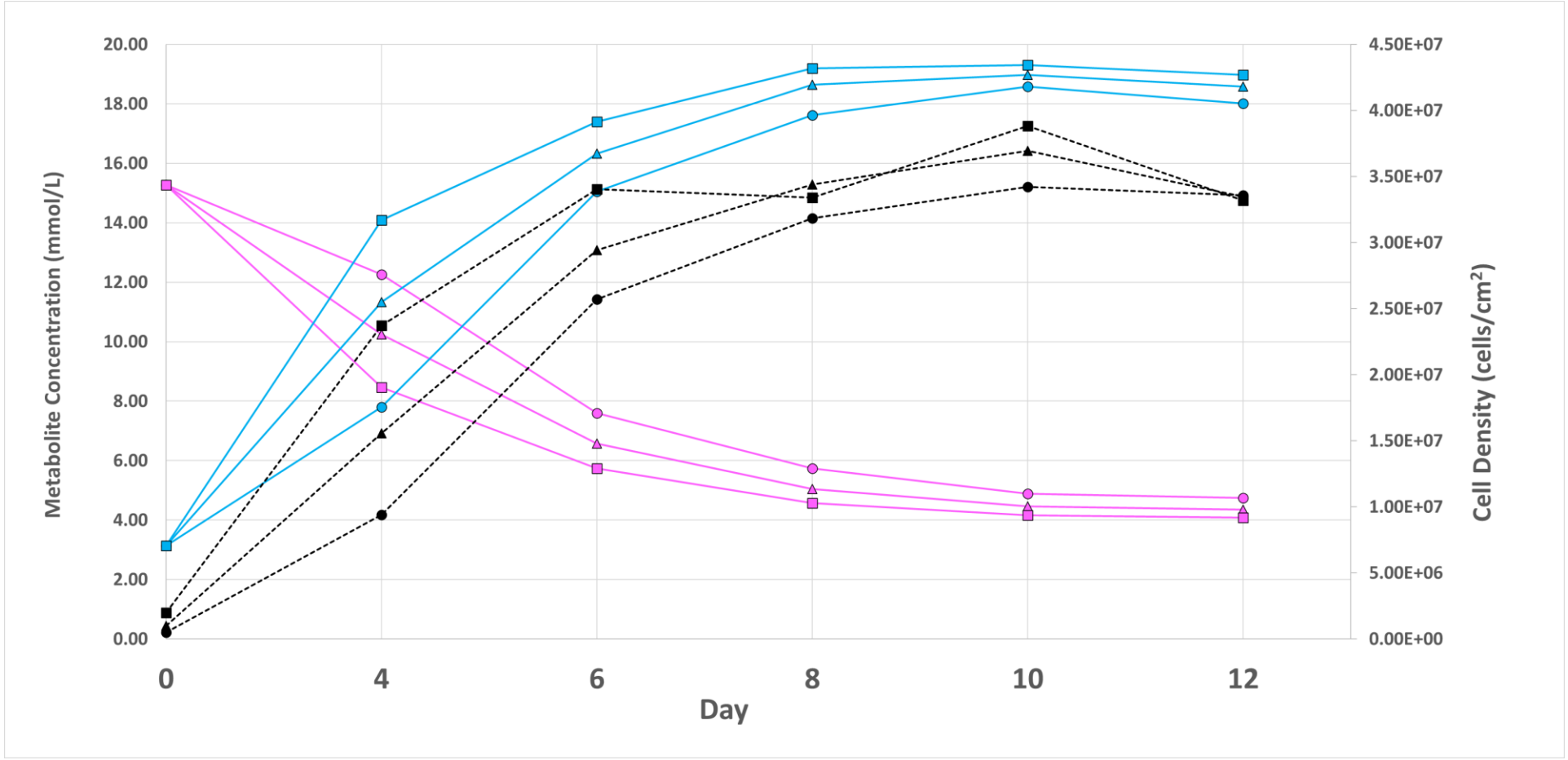
G-Rex10M-CS

G-Rex50M-CS

G-Rex100M-CS

G-Rex500M-CS

G-Rex6M Well Plate® Optimization Study



Legend

Pink = Glucose [mmol/L]

Blue = Lactate [mmol/L]

Black = Cell Density (cells/cm^2)

Circle Icon= 0.5x10^6 cells/cm^2 seeding density

Triangle Icon = 1.0x10^6 cells/cm^2 seeding density

Square Icon = 2.0x10^6 cells/cm^2 seeding density

G-Rex® vs. Static Gas Permeable Bags

G-Rex® vs. Gas Permeable Bags

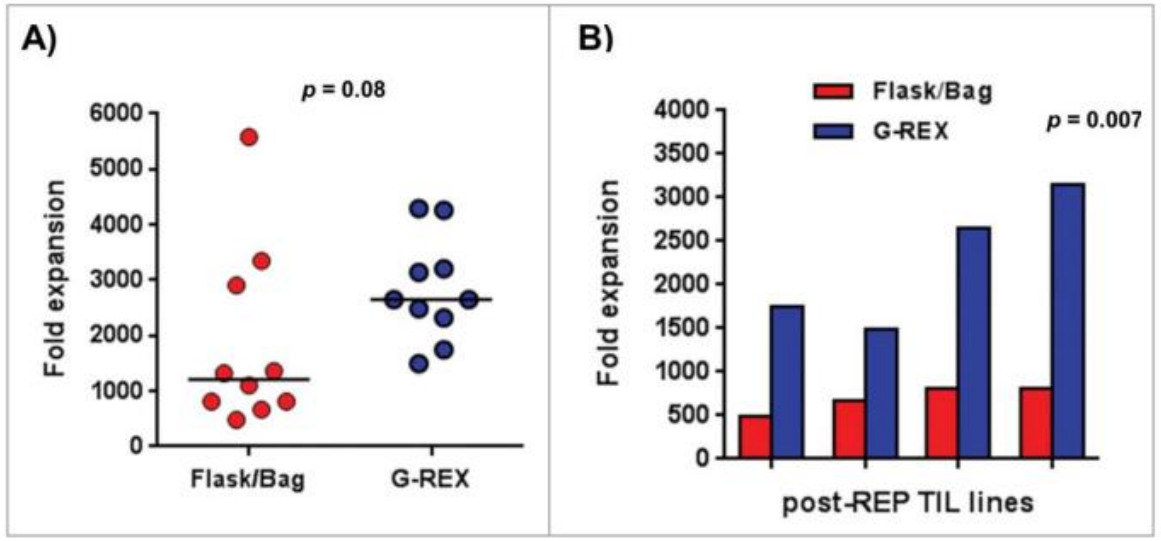


Figure 1. Assessment of TIL growth after rapid expansion in the traditional flask and bag vs. Gas-permeable flask (G-Rex) reveals a trend toward improved TIL expansion when using the G-Rex flask. (**A**) Fold expansion of post-REP TIL lines expanded shows a trend toward a better expansion when using the G-Rex. N = 10. (**B**) The G-Rex flask facilitates the expansion of TIL lines from which the growth is impaired in the REP using the traditional flask and bag devices N = 4. Statistical significance was determined by using a paired *t*-test.

Table 1. Bag and G-Rex process cost comparison.

					Bag-based process		G-Rex–based process	
Item	Manufacturer	Catalog no.	Size	List price	Required per run	Cost per run	Required per run	Cost per run
TexMACS GMP medium	Miltenyi Biotec	170-075-306	2 L	$ 305.00	1.00	$ 305.00	1.00	$ 305.00
MACS GMP Vectofusin-1	Miltenyi Biotec	170-076-165	1 mg	$ 875.00	0.00	$ -	1.00	$ 875.00
G-Rex 100MCS	Wilson Wolf Corp	81100-CS	3 pack	$ 729.84	0.00	$ -	0.33	$ 243.28
MACS GMP CD3 pure	Miltenyi Biotec	170-076-116	1 mg	$ 1750.00	0.58	$ 1015.00	0.08	$ 140.00
MACS GMP CD28 pure	Miltenyi Biotec	170-076-117	0.5 mg	$ 895.00	1.45	$ 1297.75	0.20	$ 179.00
hIL-7, premium grade	Miltenyi Biotec	130-095-363	100 μg	$ 1150.00	0.13	$ 149.50	0.45	$ 517.50
hIL-15, premium grade	Miltenyi Biotec	130-095-765	100 μg	$ 1150.00	0.04	$ 46.00	0.15	$ 172.50
Retronectin, GMP grade	Takara	T202	2.5 mL	$ 1442.00	0.50	$ 721.00	0.00	$ -
Cell culture bags	Saint Gobain	290-C	10 pack	$ 993.51	0.30	$ 298.05	0.00	$ -
Transduction bag	Saint Gobain	290-AC	10 pack	$ 993.51	0.10	$ 99.35	0.00	$ -
						$3931.65		$2432.28

The cost of generating 1.4×10^9 transduced cells in the bag- and G-Rex–based process. Cost for the bag process was calculated based on scaling up to a process that could generate as many cells as the G-Rex–based process.

Table 2. Bag and G-Rex process time comparison.

Operation	Bag-based process		G-Rex-based process	
	Method	Time (h)	Method	Time (h)
Activation	Anti-CD3/CD28 in solution	1.00	Anti-CD3/CD28 in solution	1.00
Transduction	Culture wash, transfer to Retronectin-coated bags	4.00	Vectofusin-1 in solution	0.50
Transduction stop	Culture wash, transfer bag	2.00	Medium addition	0.50
Expansion	Feed and transfer, as needed	4.00	NA	0.00
Harvest	Centrifuge	2.00	GatheRex	0.50
In-process monitoring	Mix cell suspension, sample for count (x6 d)	3.00	Sample supernatant for lactate (x6 d)	1.50
		16.00		4.00

The estimated time, in hours, required for each operation in the bag- and G-Rex–based process. Estimates are based on a single operator working in a non-GMP setting.
NA, not applicable.

Gagliardi, C., Khalil, M., & Foster, A. E. (2019). Streamlined production of genetically modified T cells with activation, transduction and expansion in closed-system G-rex bioreactors. *Cytotherapy*, *21*(12), 1246–1257. https://doi.org/10.1016/j.jcyt.2019.10.006

Forget, M.-A., Haymaker, C., Dennison, J. B., Toth, C., Maiti, S., Fulbright, O. J., Cooper, L. J., Hwu, P., Radvanyi, L. G., & Bernatchez, C. (2015). The beneficial effects of a gas-permeable flask for expansion of tumor-infiltrating lymphocytes as reflected in their mitochondrial function and respiration capacity. *OncoImmunology*, *5*(2). https://doi.org/10.1080/2162402x.2015.1057386

G-Rex® vs. Gas Permeable Bags

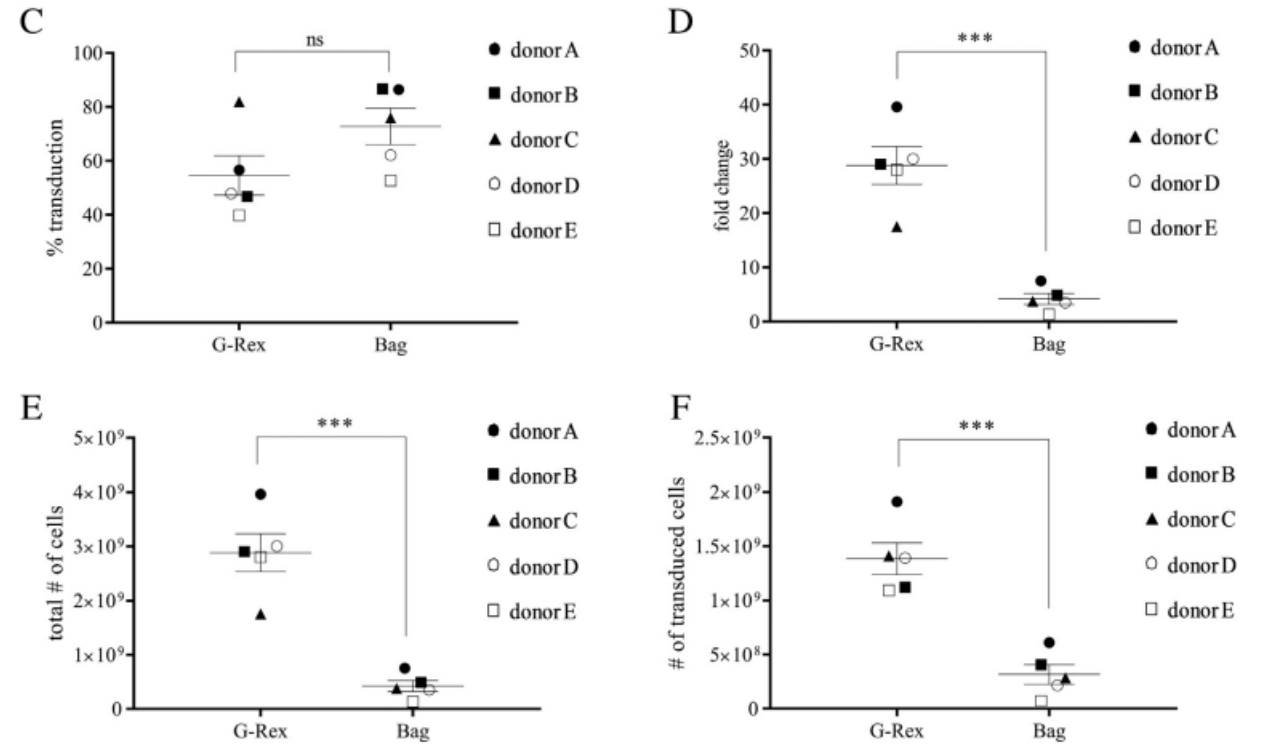


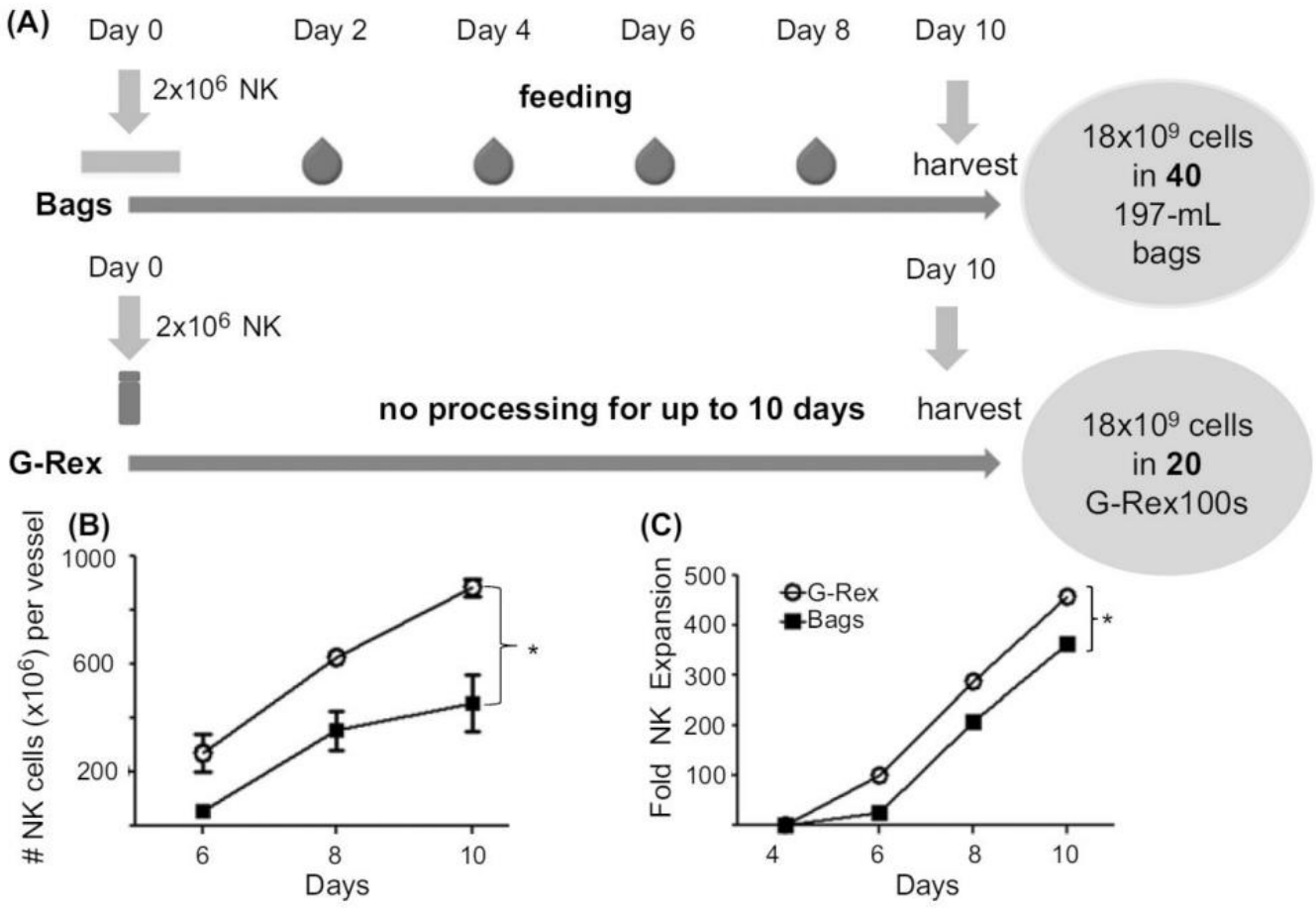
Figure 6. Large-scale G-Rex process compared with bag process lactate, transduction and expansion. (A, B) Lactate concentration (y-axis) plotted against process day (x-axis) for large-scale G-Rex100MCS (A) and bag cultures (B). (C–F) Comparison of transduction percentage (A), fold change (B), total cells at harvest (C) and total transduced cells at harvest (D) of cells generated in the G-Rex and bag-based processes. Each marker represents 1 donor. ***$P \leq 0.001$, not significant $P > 0.05$.

- Equivalent tdx %
- Significantly greater fold expansion in G-REX®
- Significantly greater total cell yield in G-REX®
- Significantly greater CAR+ cell yield in G-REX®

cant. Expansion in the G-Rex bioreactor resulted in significantly more total viable cells and transgenic cells at harvest. The average harvest from the G-Rex process was $1.4 \pm 0.1 \times 10^9$ transgenic cells. A hypothetical 80-kg patient who needs a dose of 5.0×10^6 cells/kg could receive >3 doses from one manufacturing process with the G-Rex. In comparison, a bag process, starting with the same number of cells, results in barely enough transgenic cells for a single dose of the same size. Further, the G-Rex–based process reduced the cost of materials by 38% and hand-on time by 75% to generate a batch of 1.4×10^9 transgenic cells.

Gagliardi, C., Khalil, M., & Foster, A. E. (2019). Streamlined production of genetically modified T cells with activation, transduction and expansion in closed-system G-rex bioreactors. *Cytotherapy*, *21*(12), 1246–1257. https://doi.org/10.1016/j.jcyt.2019.10.006

G-Rex® for Donor-Derived Allo NK



Cytotherapy. 2012 October ; 14(9): 1131–1143. doi:10.3109/14653249.2012.700767.

Large-scale *ex vivo* expansion and characterization of natural killer cells for clinical applications

NATALIA LAPTEVA[1], APRIL G. DURETT[1], JIALI SUN[1], LISA A. ROLLINS[1], LESLIE L. HUYE[1], JIAN FANG[1], VARADA DANDEKAR[1], ZHUYONG MEI[1], KIMBERLEY JACKSON[1], JUAN VERA[1], JUN ANDO[1], MINHTRAN C. NGO[1], ELAINE COUSTAN-SMITH[3], DARIO CAMPANA[3], SUSANN SZMANIA[4], TARUN GARG[4], AMBERLY MORENO-BOST[4], FRITS VANRHEE[4], ADRIAN P. GEE[1,2], and **CLIONA M. ROONEY[1,2]**

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[3]National University of Singapore, Singapore

[4]University of Arkansas Medical Center, Little Rock, Arkansas, USA

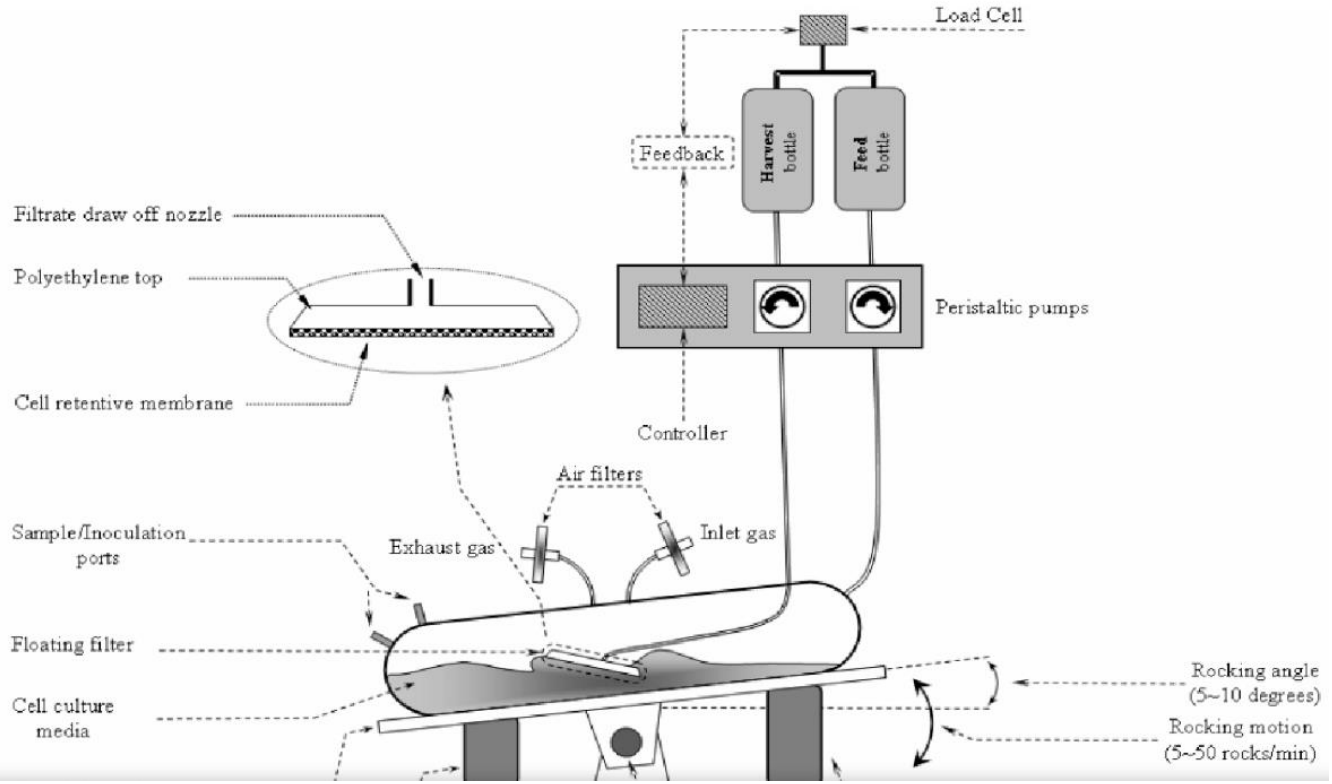
Figure 2.

G-Rex yields higher numbers of NK cells and requires fewer culture manipulations than cultures in bags. (A) Schematic view of NK cell expansion in bags and G-Rexes. Less cell processing is required to grow a similar number of cells in G-Rexes. (B) NK cells from three different donors were seeded in the 197-mL bags and G-Rex100s. Bags were seeded with PBMC containing 2×10^6 NK cells and 2×10^7 K562-mb15-41BBL cells in a 40-mL starting volume and cultures required feedings with 40 mL fresh media and cytokine every other day, i.e. days 2, 4, 6 and 8, resulting in 200 mL of total volume by day 8. G-Rex100s were seeded with PBMC containing 2×10^6 NK cells and 2×10^7 K562-mbIL15-41BBL in 400 mL medium and were not subsequently fed. Cells were counted and analyzed by flow cytometry for percentage $CD56^+$ $CD3^-$ cells ($n = 3$). (C) Fold expansion of $CD56^+$ $CD3^-$ cells in G-Rexes and bags ($n = 3$). *$P < 0.5$.

G-Rex® vs. “Automated” Perfusion Bags

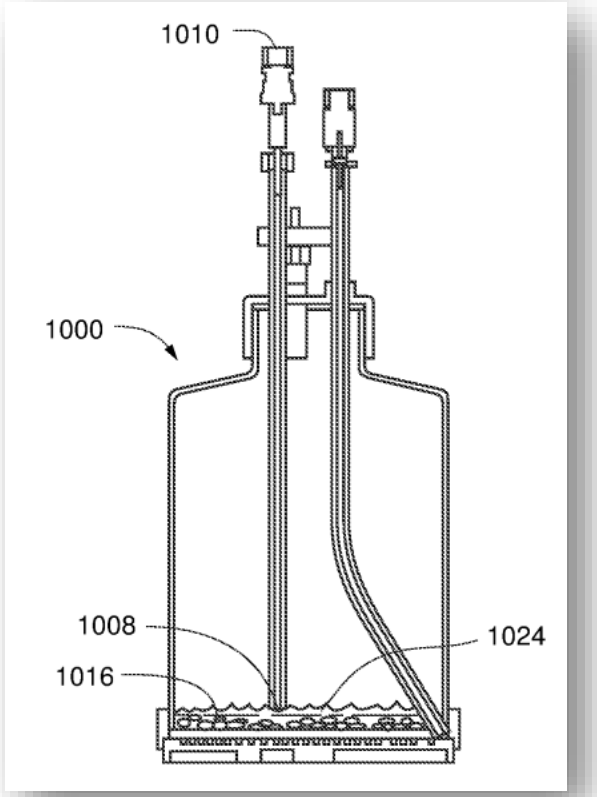
G-Rex® vs. Automated Perfusion Bag

cytiva



! Unnecessary complexity

GREX



✓ **Simplicity**

G-Rex® vs. Automated Perfusion Bag

Supplementary Table 4: Rocking rate settings (rocks per minute) used for Xuri W25 manufacturing process.

Day	Donor 1	Donor 2	Donor 3	Donor 4
5	6	6	6	6
6	12	6	6	6
7	12	8	6	6
8	12	8	8	6
9	12	8	8	6
10	12	8	10	6
11	12	8	10	6
12	12	8	12	6
13	12	8	12	6
14	12	8	12	6

Supplementary Table 3: Media feed volumes and perfusion rate settings used for Xuri W25 manufacturing process.
Innoc = inoculation.

Day	Donor 1	Donor 2	Donor 3	Donor 4
5	400 mL innoc.	400 mL innoc.	400 mL innoc.	400 mL innoc.
6	Add 600 mL, batch feed (=1 L total)	Add 600 mL, batch feed (=1 L total)	Add 600 mL, batch feed (=1 L total)	Add 350 mL, batch feed (=750 mL total)
7	1 L/day	0.5 L/day	1 L/day	Add 250 mL batch feed (=1L total)
8	1.5 L/day	0.5 L/day	1.5 L/day	no feed
9	1.5 L/day	0.75 L/day	1.5 L/day	0.25 L/day
10	2 L/day	0.75 L/day	2 L/day	0.25 L/day
11	2 L/day	1 L/day	2 L/day	0.25 L/day
12	2 L/day	1 L/day	2 L/day	0.25 L/day
13	2 L/day	1 L/day	2 L/day	0.25 L/day
14	Harvest	Harvest	Harvest	Harvest

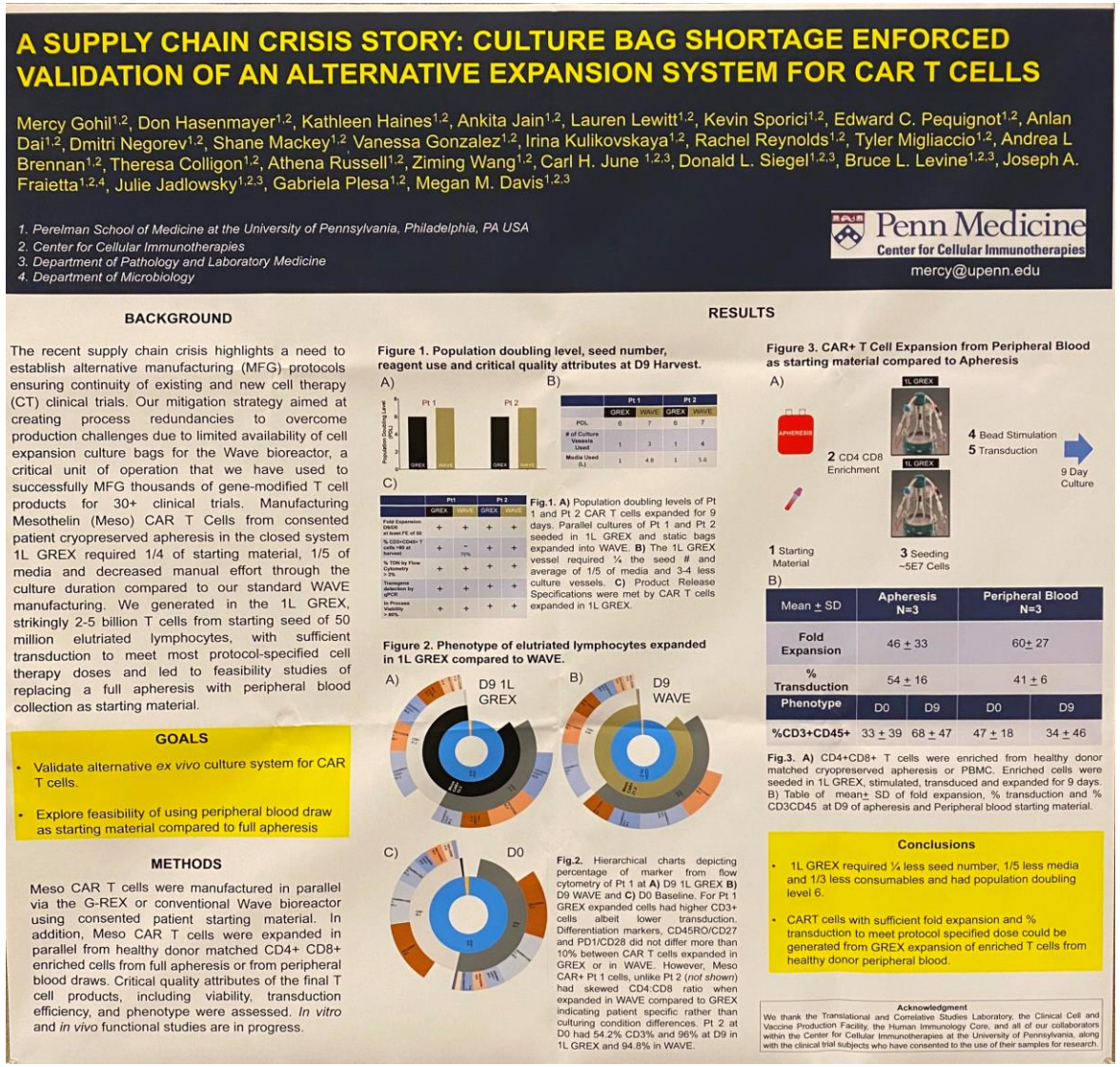
- ! **Xuri cannot deliver consistency** because of inherent design flaws
  - ! **Variability** in rocking settings, feed volumes, and perfusion rates **across donors**
  - ! **Variability** in rocking settings, feed volumes, and perfusion rates **within donors**

Total cell number (× 10^9), Day 14

Donor	Bag	G-Rex	Xuri	Prodigy
1	54.04	17.10	21.40	4.81
2	37.76	8.64	6.86	3.74
3	15.04	11.88	3.61	3.58
4	6.44	7.83	1.26	5.21
Avg	**28.32**	**9.45**	**8.28**	**4.18**
SD	21.65	4.21	9.04	0.90

Hannah W. Song, Michaela Prochazkova, Lipei Shao,Roshini Traynor, Sarah Underwood, Mary Black, Vicki Fellowes, Rongye Shi, Marie Pouzolles, Hsien-Chao Chou, Adam T. Cheuk, Naomi Taylor, Ping Jin, Robert P. Somerville, David F. Stroncek, Javed Khan, Steven L. Highfill, CAR-T cell expansion platforms yield distinct T cell differentiation states, *Cytotherapy* (2024), doi; https://doi.org/10.1016/j.jcyt.2024.03.003

G-Rex® vs. Automated Perfusion Bag



A SUPPLY CHAIN CRISIS STORY: CULTURE BAG SHORTAGE ENFORCED VALIDATION OF AN ALTERNATIVE EXPANSION SYSTEM FOR CAR T CELLS

Mercy Gohil[1,2], Don Hasenmayer[1,2], Kathleen Haines[1,2], Ankita Jain[1,2], Lauren Lewitt[1,2], Kevin Sporici[1,2], Edward C. Pequignot[1,2], Anlan Dai[1,2], Dmitri Negorev[1,2], Shane Mackey[1,2] Vanessa Gonzalez[1,2], Irina Kulikovskaya[1,2], Rachel Reynolds[1,2], Tyler Migliaccio[1,2], Andrea L Brennan[1,2], Theresa Colligon[1,2], Athena Russell[1,2], Ziming Wang[1,2], Carl H. June [1,2,3], Donald L. Siegel[1,2,3], Bruce L. Levine[1,2,3], Joseph A. Fraietta[1,2,4], Julie Jadlowsky[1,2,3], Gabriela Plesa[1,2], Megan M. Davis[1,2,3]

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BACKGROUND

The recent supply chain crisis highlights a need to establish alternative manufacturing (MFG) protocols ensuring continuity of existing and new cell therapy (CT) clinical trials. Our mitigation strategy aimed at creating process redundancies to overcome production challenges due to limited availability of cell expansion culture bags for the Wave bioreactor, a critical unit of operation that we have used to successfully MFG thousands of gene-modified T cell products for 30+ clinical trials. Manufacturing Mesothelin (Meso) CAR T Cells from consented patient cryopreserved apheresis in the closed system 1L GREX required 1/4 of starting material, 1/5 of media and decreased manual effort through the culture duration compared to our standard WAVE manufacturing. We generated in the 1L GREX, strikingly 2-5 billion T cells from starting seed of 50 million elutriated lymphocytes, with sufficient transduction to meet most protocol-specified cell therapy doses and led to feasibility studies of replacing a full apheresis with peripheral blood collection as starting material.

GOALS

- Validate alternative *ex vivo* culture system for CAR T cells.
- Explore feasibility of using peripheral blood draw as starting material compared to full apheresis

METHODS

Meso CAR T cells were manufactured in parallel via the G-REX or conventional Wave bioreactor using consented patient starting material. In addition, Meso CAR T cells were expanded in parallel from healthy donor matched CD4+ CD8+ enriched cells from full apheresis or from peripheral blood draws. Critical quality attributes of the final T cell products, including viability, transduction efficiency, and phenotype were assessed. *In vitro* and *in vivo* functional studies are in progress.

RESULTS

Figure 1. Population doubling level, seed number, reagent use and critical quality attributes at D9 Harvest.

A)

B)

	Pt 1		Pt 2	
	GREX	WAVE	GREX	WAVE
PDL	6	7	6	7
# of Culture Vessels Used	1	3	1	4
Media Used (L)	1	4.8	1	5.6

C)

	Pt 1		Pt 2	
	GREX	WAVE	GREX	WAVE
Fold Expansion ONCE at least FE of 50	+	+	+	+
% CD3+CD45+ T cells >80 at harvest	+	– 70%	+	+
% TDN by Flow Cytometry > 7%	+	+	+	+
Transgene detection by qPCR	+	+	+	+
In Process Viability > 60%	+	+	+	+

Fig.1. A) Population doubling levels of Pt 1 and Pt 2 CAR T cells expanded for 9 days. Parallel cultures of Pt 1 and Pt 2 seeded in 1L GREX and static bags expanded into WAVE. **B)** The 1L GREX vessel required ¼ the seed # and average of 1/5 of media and 3-4 less culture vessels. **C)** Product Release Specifications were met by CAR T cells expanded in 1L GREX.

Figure 2. Phenotype of elutriated lymphocytes expanded in 1L GREX compared to WAVE.

Fig.2. Hierarchical charts depicting percentage of marker from flow cytometry of Pt 1 at **A)** D9 1L GREX **B)** D9 WAVE and **C)** D0 Baseline. For Pt 1 GREX expanded cells had higher CD3+ cells albeit lower transduction. Differentiation markers, CD45RO/CD27 and PD1/CD28 did not differ more than 10% between CAR T cells expanded in GREX or in WAVE. However, Meso CAR+ Pt 1 cells, unlike Pt 2 (*not shown*) had skewed CD4:CD8 ratio when expanded in WAVE compared to GREX indicating patient specific rather than culturing condition differences. Pt 2 at D0 had 54.2% CD3% and 96% at D9 in 1L GREX and 94.8% in WAVE.

Figure 3. CAR+ T Cell Expansion from Peripheral Blood as starting material compared to Apheresis

A)

B)

Mean ± SD	Apheresis N=3		Peripheral Blood N=3	
Fold Expansion	46 ± 33		60± 27	
% Transduction	54 ± 16		41 ± 6	
Phenotype	D0	D9	D0	D9
%CD3+CD45+	33 ± 39	68 ± 47	47 ± 18	34 ± 46

Fig.3. A) CD4+CD8+ T cells were enriched from healthy donor matched cryopreserved apheresis or PBMC. Enriched cells were seeded in 1L GREX, stimulated, transduced and expanded for 9 days. B) Table of mean± SD of fold expansion, % transduction and % CD3CD45 at D9 of apheresis and Peripheral blood starting material.

Conclusions

- 1L GREX required ¼ less seed number, 1/5 less media and 1/3 less consumables and had population doubling level 6.
- CART cells with sufficient fold expansion and % transduction to meet protocol specified dose could be generated from GREX expansion of enriched T cells from healthy donor peripheral blood.

Acknowledgment
We thank the Translational and Correlative Studies Laboratory, the Clinical Cell and Vaccine Production Facility, the Human Immunology Core, and all of our collaborators within the Center for Cellular Immunotherapies at the University of Pennsylvania, along with the clinical trial subjects who have consented to the use of their samples for research.

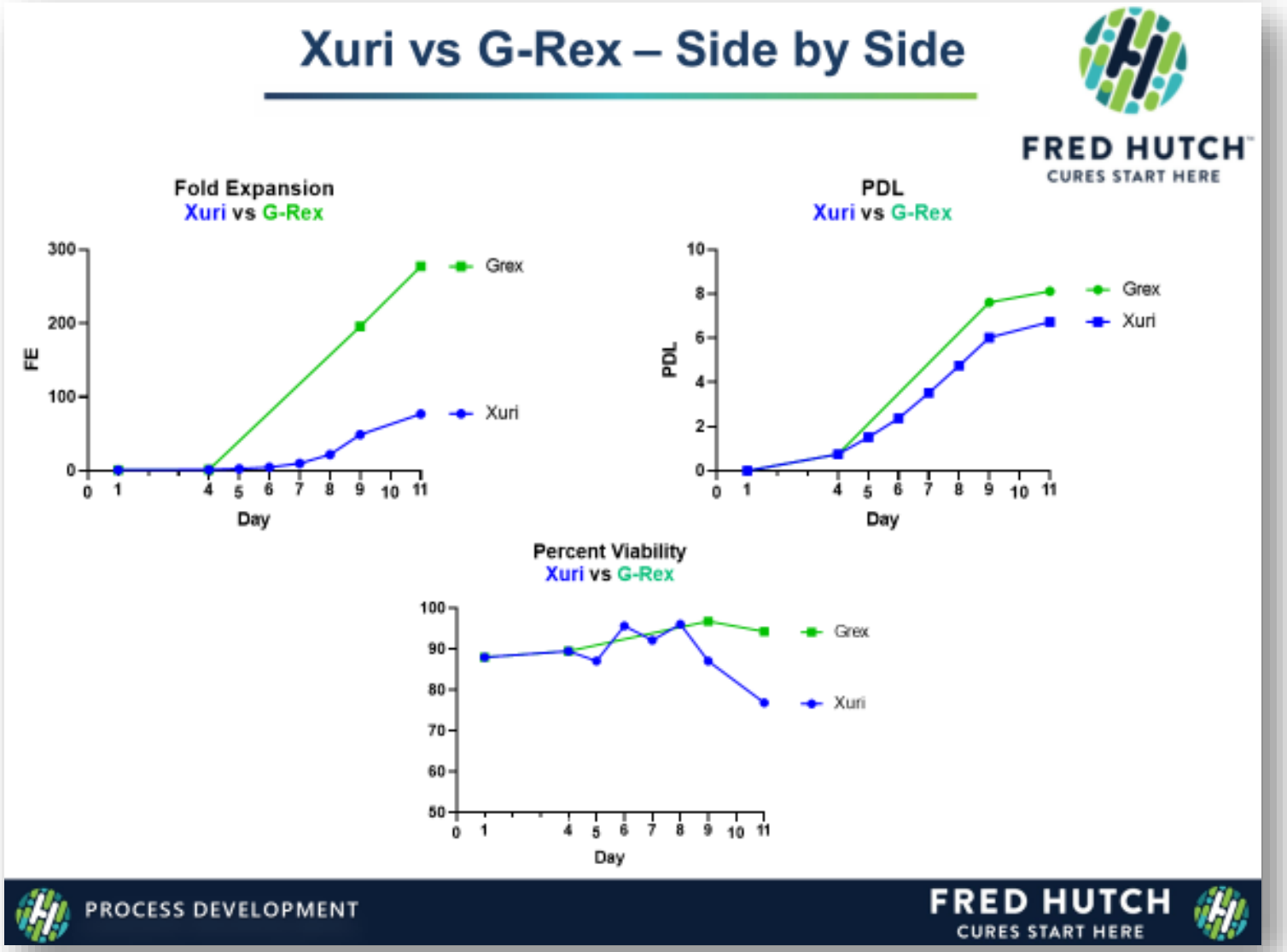
A SUPPLY CHAIN CRISIS STORY: CULTURE BAG SHORTAGE ENFORCED VALIDATION OF AN ALTERNATIVE EXPANSION SYSTEM FOR CAR T CELLS

M. Gohil[1], D. Hasenmayer[1], K. M. Haines[1], A. Jain[1], L. Lewitt[1], K. Sporici[1], A. Dai[1], D. Negorev[1], V. E. Gonzalez[1], I. Kulikovskaya[1], R. Reynolds[1], T. Migliaccio[1], A. L. Brennan[1], T. A. Colligon[1], A. L. Russell[1], Z. Wang[1], C. June[1], D. Siegel[1], B. L. Levine[1], J. A. Fraietta[1], J. Jadlowsky[1], G. Plesa[1], M. M. Davis[1]
[1]*Center for Cellular Immunotherapies, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.*

Using G-Rex® vs. WAVE…

- Reduces media consumption by 80%
- Reduces starting material requirements by 66%
- Reduces manual effort
- Reduces reliance on specialized capital equipment
- Reduces footprint
- Increases throughput (via enabling simultaneous manufacturing of products)

G-Rex® vs. Automated Perfusion Bag



G-Rex® vs. Automated Perfusion Bag

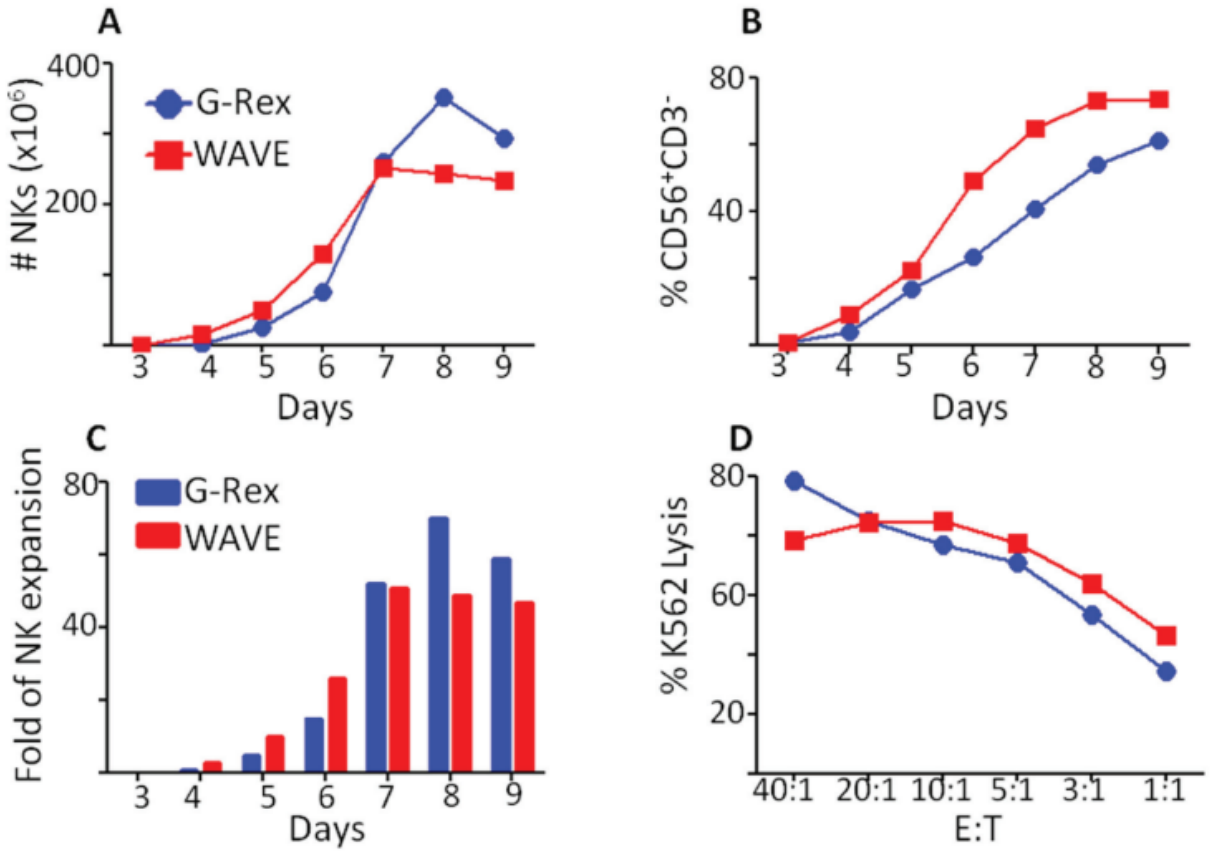


FIG. 4: Comparison of NK cell expansion in G-Rex flasks and WAVE bioreactor. PBMCs from healthy donor containing 5×10^6 NK cells were co-cultured with K562-41BBL-mbIL-15 cells for 9 days. Cells were moved to WAVE on day 4 of culture. (A, B, C) Similar numbers and fold of NK expansion were observed in G-Rex and WAVE cultures. (D) Potency (killing K562 cells) of both products was also comparable. Similar expansion was obtained for three donors and the representative data for one donor is shown.

Critical Reviews™ in Oncogenesis, 19(1-2):121–132 (2014)

Clinical Grade Purification and Expansion of Natural Killer Cells

Natalia Lapteva[1*], Susann M. Szmania[2], Frits van Rhee[2], & Cliona M. Rooney[1*]

[1] Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX 77030; [2]University of Arkansas Medical Center, Little Rock, AK 72205

toxicity assays). Interestingly, the WAVE-generated products contain fewer $CD3^+$ T cells and a higher frequency of $CD56^+CD3^-$ NK cells, perhaps because T cells prefer static culture (Fig. 4). The clinical trial in multiple myeloma demands both autologous and allogeneic NK cells. In the case of allogeneic products, $CD3^+$ T cells are efficiently removed (as well as CD3+56+ cells) by depletion with the CliniMACS CD3 reagent, reducing the CD3+ cell frequency from 19±9% to 0.4±0.5% (N=5). This depletion allows less than 5×10^5 $CD3^+$ $CD56^-$ T cells kg^{-1} for infusion, as mandated by the clinical protocol.

Lapteva, N., Szmania, S. M., van Rhee, F., & Rooney, C. M. (2014). Clinical grade purification and expansion of Natural Killer Cells. Critical Reviews in Oncogenesis, 19(1–2), 121–132. https://doi.org/10.1615/critrevoncog.2014010931

Static Culture vs. Perfusion for TIL

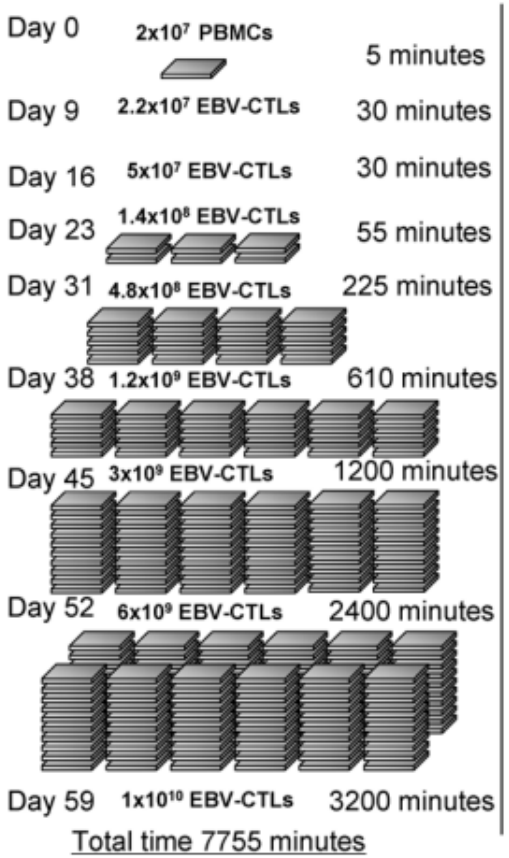
Due to the fact that CD8+ effector cells are our desired cell population the increased representation of CD4+ cells in rapid expansions carried out in the WAVE bioreactor compared to static bags is problematic. The underlying cause of this skewed expansion could relate to differences in the culture microenvironment generated in the two styles of bioreactor. In static bags the cells rest on the lower surface of the bag and there is minimal mixing of the bag contents. In this situation there is extensive cell to cell contact and any secreted factors essential to cell expansion will exist as gradients, with the highest concentration surrounding the cells. Contrast this with the WAVE bioreactor, where the coupling of rocking with media perfusion creates a more homogenous suspension of cells and oxygenated nutrient rich media. In this situation any secreted factors will be diluted by the continual perfusion, while the constant motion will disrupt any gradient formation and reduce the overall time that cells spend in contact with each other. A second

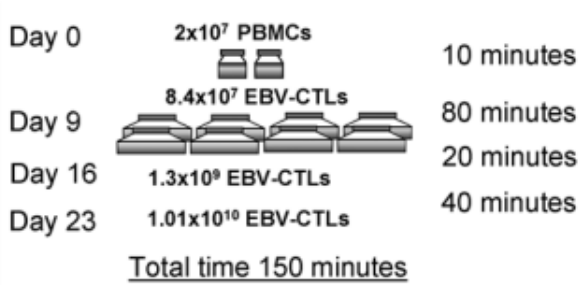
There are some disadvantages with the WAVE bioreactor including a difficult transition from research scale expansions, where the initial cell number and seeding density are determined, along with the day of WAVE inoculation and rocking speed, to full scale clinical expansions. In addition to the WAVE bioreactors themselves it is necessary to purchase additional ancillary equipment. This, along with the inherent complexities of a system that depends on constant motion, such as a predisposition to electrical and mechanical failure, means that there is a need for multiple bioreactors to expand a single patient's cells. In order to exclusively adopt the WAVE bioreactor as the sole platform for rapid expansions, therefore requires a substantial initial investment, which may be beyond the means of a small cell production facility that produces only a minimal number of cell products; however, the WAVE bioreactor is ideal for larger scale manufacturing facilities, where multiple cell products need to manufactured concurrently. We have identified alternative bioreactors which don't require an additional expenditure on ancillary equipment, as they utilize equipment and monitoring systems that are standard to most cell production facilities [34].

Somerville, R. P., Devillier, L., Parkhurst, M. R., Rosenberg, S. A., & Dudley, M. E. (2012). Clinical scale rapid expansion of lymphocytes for adoptive cell transfer therapy in the WAVE® bioreactor. *Journal of Translational Medicine*, *10*(1). https://doi.org/10.1186/1479-5876-10-69

G-Rex® vs. T-Flask

G-Rex® vs. T-flask





	24 well plate		G-Rex	
Starting cell number	2x10⁷ PBMCs		2x10⁷ PBMCs	
Final cell number	1x10¹⁰ CTLs		1x10¹⁰ CTLs	
Technician time	129 hours	$ 3,096	2.5 hours	$ 60
GMP facility charge		$ 2,280		$ 300
Cultureware	310 plates	$ 2,325	2 (G-Rex40) 8 to 16 (G-Rex500)	$ 2200
Media / cytokines	11160 ml	$ 1,674	3280 ml	$ 492
Regular pipette use	618	$ 309	20	$ 10
product Testing		$ 1,843		$ 1,843
Final cost	$ 11,527($1.15 x 1x10⁸ cells)		$ 4,905 ($0.49 x 1x10⁸ cells)	

	24 well plate	G-Rex
Number of interventions	222720	34
Antigen stimulation	9	2
Risk of contamination	++++	+
Biohazard	++++	+
Time to generate the product	59 days	23 days

FIGURE 6. Bioprocess optimization for CTLs production decreases the complexity, risk of contamination, and cost of manufacture. The conventional method of CTL culture using 24-well plates is convoluted, demanding frequent manipulation to sustain culture growth. This increases the manufacture cost and risk of contamination. In contrast, CTL culture using the optimized APC:CTL ratio in the G-Rex can produce the same number of cells in a shorter period of time thus decreasing the cost and complexity of manufacture.

The G-Rex Reduces the Time, Complexity, and Cost Associated With CTL Production for Clinical Use

The broader implementation of T-cell immunotherapy is limited by (i) the cost of production; (ii) the complexity of production, including repeat feeding of open culture systems, and multiple skilled "judgment calls," thereby limiting scalability, and (iii) the time required for activation and expansion. Figure 6 shows the effects of the G-Rex on these obstacles. The "hands on" time for CTL manufacture is reduced because fewer cell manipulations are required, which consequently reduces the labor costs, decreases the complexity of manufacture, and diminishes the risk of contamination. The duration of CTL manufacture is also reduced by the increased rate of expansion. Hence production of 1×10^{10} CTLs, which would typically require approximately 60 days and 129 hours of technician time by conventional culture, is reduced to 23 days with 3 hours of labor and a reduction in the number of interventions from >200,000 to just 34, translating to a saving of >50% in CTL production costs (Fig. 6).

Vera, J. F., Brenner, L. J., Gerdemann, U., Ngo, M. C., Sili, U., Liu, H., Wilson, J., Dotti, G., Heslop, H. E., Leen, A. M., & Rooney, C. M. (2010). Accelerated production of antigen-specific T cells for preclinical and clinical applications using gas-permeable rapid expansion cultureware (G-Rex). Journal of Immunotherapy, 33(3), 305–315. https://doi.org/10.1097/cji.0b013e3181c0c3cb

G-Rex® vs. T-flask

Optimization of therapeutic T cell expansion in G-Rex device and applicability to large-scale production for clinical use

Elisa Gotti[1], Sarah Tettamanti[2], Silvia Zaninelli[1], Carolina Cuofano[1], Irene Cattaneo[1], Maria Caterina Rotiroti[2,†], Sabrina Cribioli[3], Rachele Alzani[4], Alessandro Rambaldi[1], Martino Introna[1,*], Josée Golay[1,5]

[1] *Center of Cellular Therapy "G. Lanzani," Division of Hematology, Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Italy*
[2] *Tettamanti Research Center, Department of Pediatrics, University of Milano-Bicocca/Fondazione MBBM, Monza, Italy*
[3] *Accelera srl, Nerviano, Italy*
[4] *Nerviano Medical Sciences, Nerviano, Italy*
[5] *Fondazione per la Ricerca Ospedale Maggiore, Bergamo, Italy*

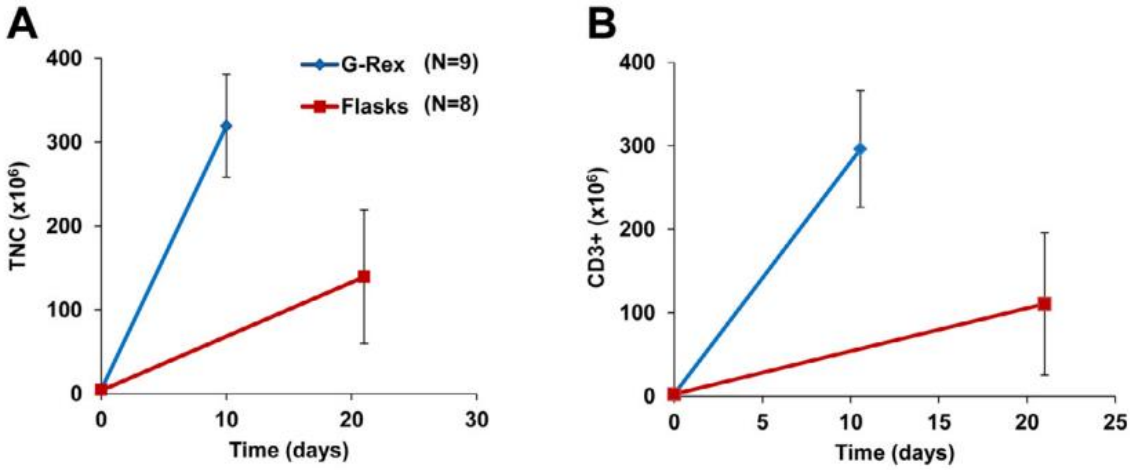


Figure 1. Expansion of CIKs from PB is more efficient in G-Rex devices compared with T-flasks. PBMCs were plated at 0.5 × 10^6/cm^2 in G-Rex vessels (G-Rex-6M, -10M or -100M) or at 3 × 10^6/mL in T-flasks in CIK conditions. After 10 to 11 days for G-Rex or 21 days for flasks, cells were collected and counted (A). The number of live $CD3^+$ was measured by immunophenotyping and flow cytometry (B). Lactate levels in the supernatant of G-Rex cultures were measured at different times to follow cell growth. The results of six representative cultures are shown (C). (Color version of figure is available online.)

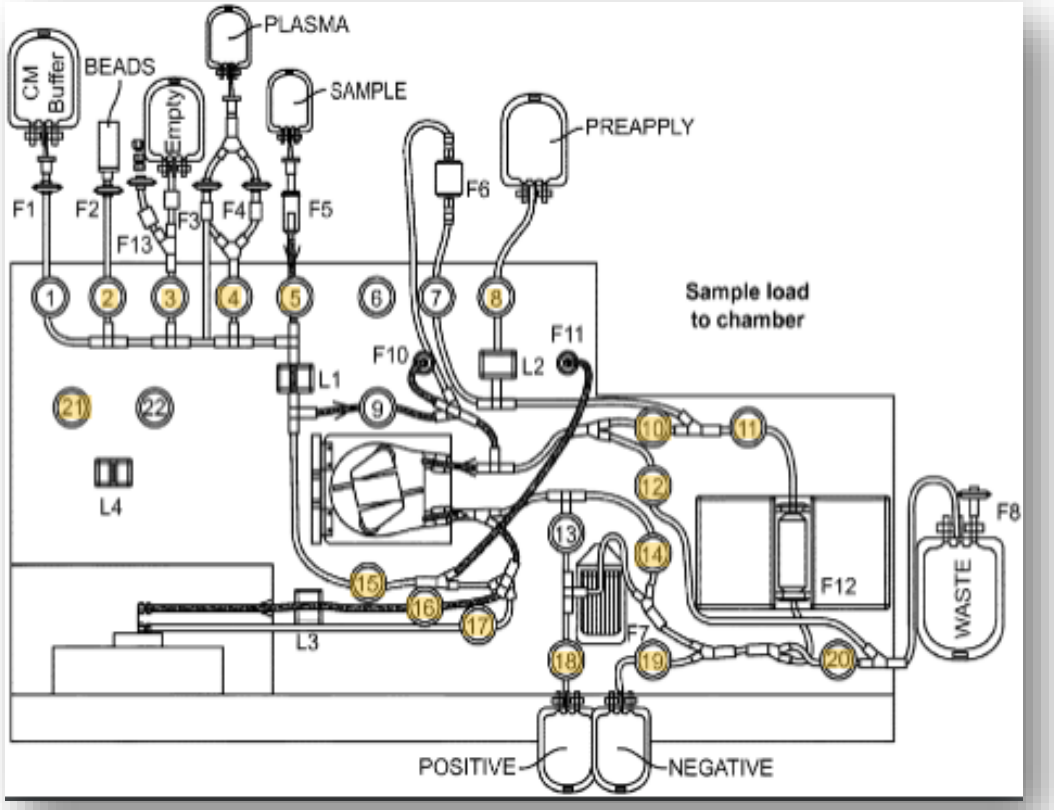
➢ **G-Rex®** produces **more cells** in **less time**

We observed that culture in G-Rex vessels in these conditions allowed more efficient and rapid CIK expansion compared with T-flasks, starting from the same number of cells. Indeed, using 5 × 10^6 PBMCs, a mean of 320 × 10^6 total cells and 300 × 10^6 $CD3^+$ T cells were reproducibly obtained in only 10 to 11 days of culture using the G-Rex 10M vessel (100 mL), compared with a mean of 140 × 10^6 total cells and 110 × 10^6 T cells obtained in 21 days in standard flasks (Fig. 1A and B). The rate of CIK expansion in G-Rex was quite reproducible, as shown by measured lactate levels over time in different batches (Fig. 1C) and cell yields on days 10 and 11 (Fig. 1A and B).

G-Rex® vs. “Automated” T-Flask

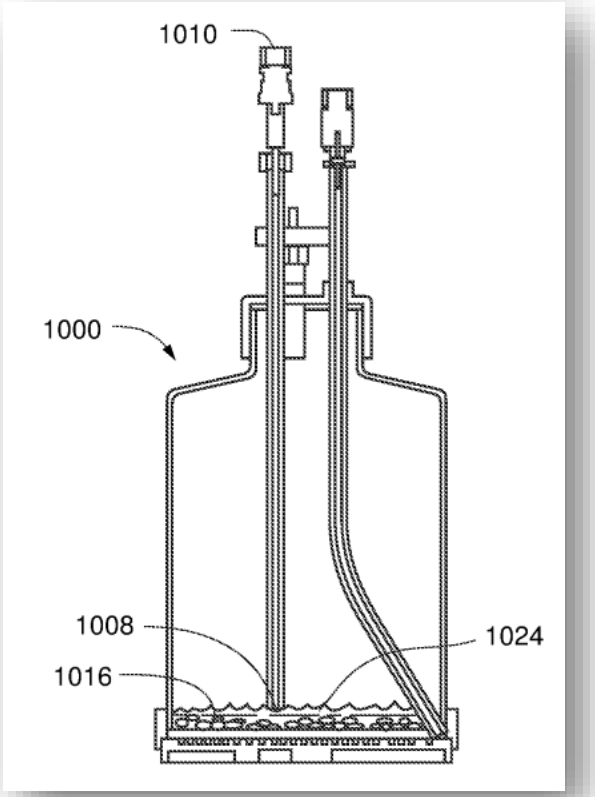
G-Rex® vs. Automated T-Flask

Miltenyi Biotec



! Unnecessary complexity

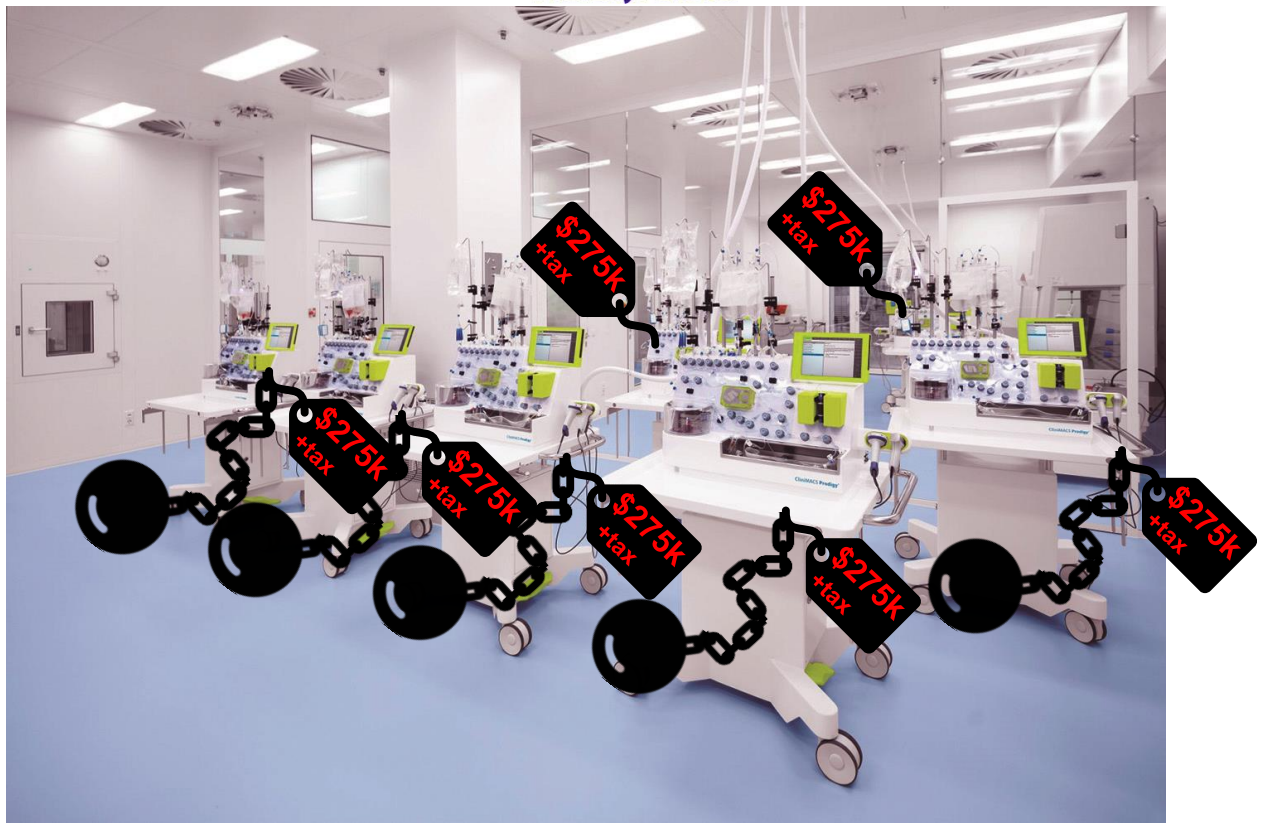
GREX



✓ **Simplicity**

G-Rex® vs. Automated T-Flask

Miltenyi Biotec



! Inefficient use of space
! No high throughput manufacturing

GREX

✓ **Efficient use of space**
✓ **High throughput manufacturing**

G-Rex® vs. Automated T-Flask

Lonza

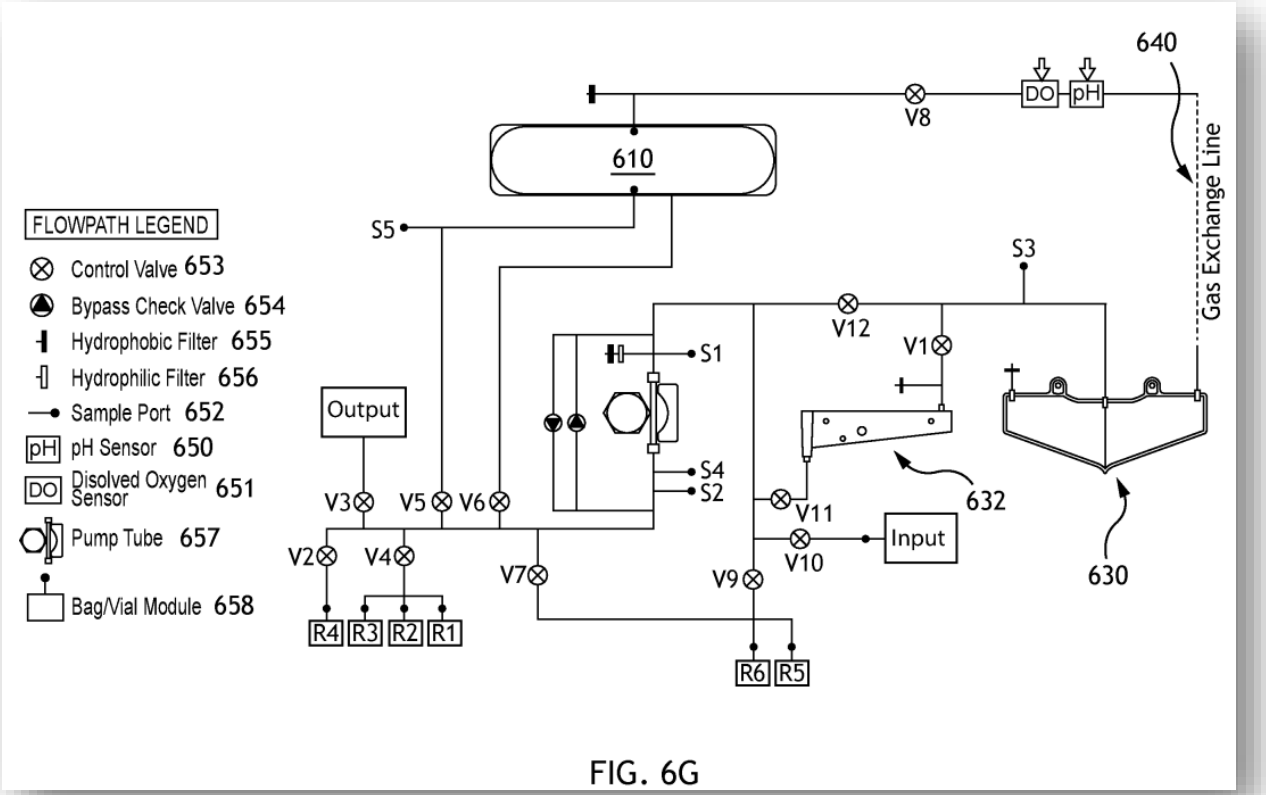
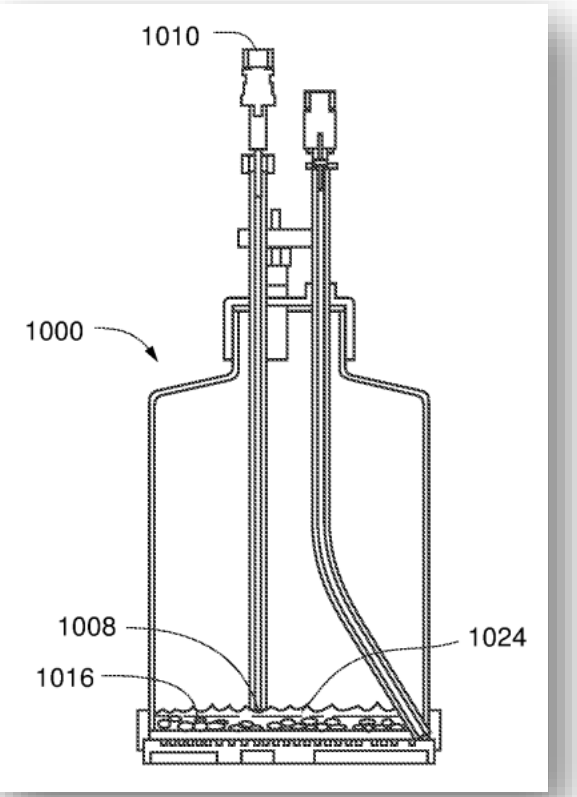


FIG. 6G

! Needlessly Complex
! Unproven and Risky



✓ Simple and sophisticated
✓ Proven and De-risked

Lonza

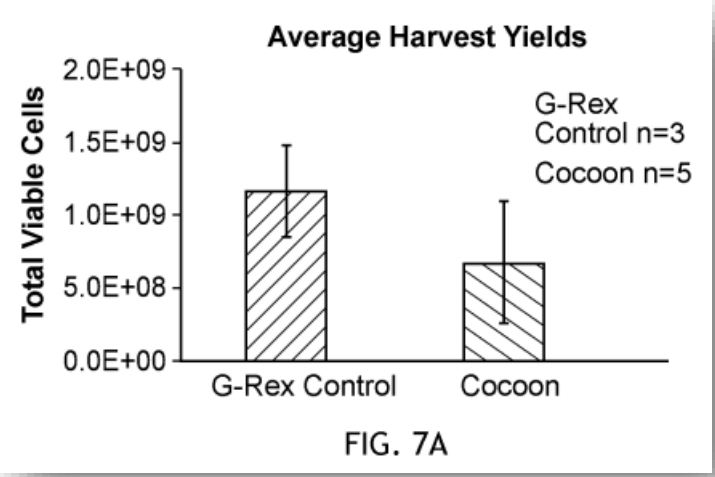


FIG. 7A

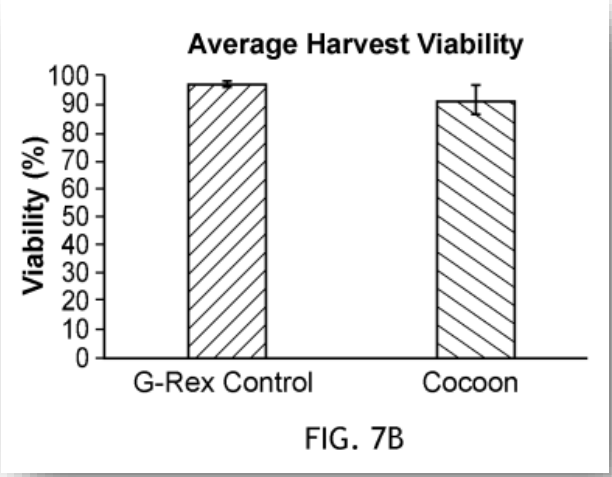


FIG. 7B

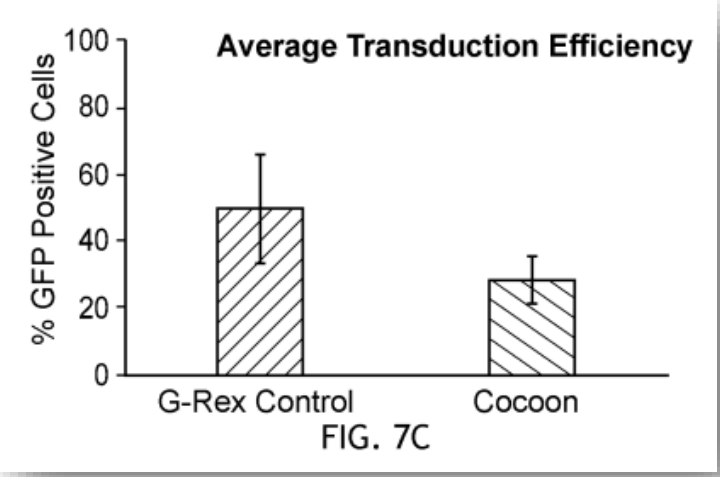


FIG. 7C

- G-Rex® produces **more cells** than “automated” T-Flasks
- G-Rex® produces cells with **higher viability** than “automated” T-Flasks
- G-Rex® produces **more CAR+ cells** than “automated” T-Flasks

G-Rex® Throughput

What drives cost?

$$\boldsymbol{Cost}(\$) = \frac{\boldsymbol{Overhead}\ (\$)}{\boldsymbol{Drug\ Products}\ (\boldsymbol{n})} = \frac{\boldsymbol{Facility + Materials + Labor}\ (\$)}{\boldsymbol{Throughput}\ (\boldsymbol{n})}$$

Facility

- ❑ Small (modest) footprint
- ❑ Standard equipment
- ❑ Modular and flexible

Materials

- ❑ Availability of supply
- ❑ Limited (reduced) consumption
- ❑ High quality

Labor

- ❑ Limited (reduced) application
- ❑ Repeatable and consistent tasks
- ❑ Non-expert (democratized) skill

Throughput

- ❑ In parallel processing with limited capital investment

G-Rex® is the **only** commercially viable platform

Input Process Parameters	
Manufacturing Lines (n)	1
Selection (hr)	2
Activation (hr)	24
Transduction (hr)	24
Expansion (d)	5

Drug Name	Est. Incident Rate (US)	Est. Eligible Patient Pop (US)
Carvykti®	35,000	**14,000**
Kymriah®	60,000	8,000
Yescarta®	60,000	8,000
Breyanzi®	60,000	8,000
Abecma®	35,000	5,000
Yescarta®	20,000	2,000
Breyanzi®	20,000	2,000
Tecartus®	5,000	1,000
Kymriah®	6,000	800
Tecartus®	1,500	300
Yescarta®	2,400	240

Miltenyi Biotec · cytiva · Adva Biotechnology · Oribiotech · TERUMO · Lonza Cell & Gene

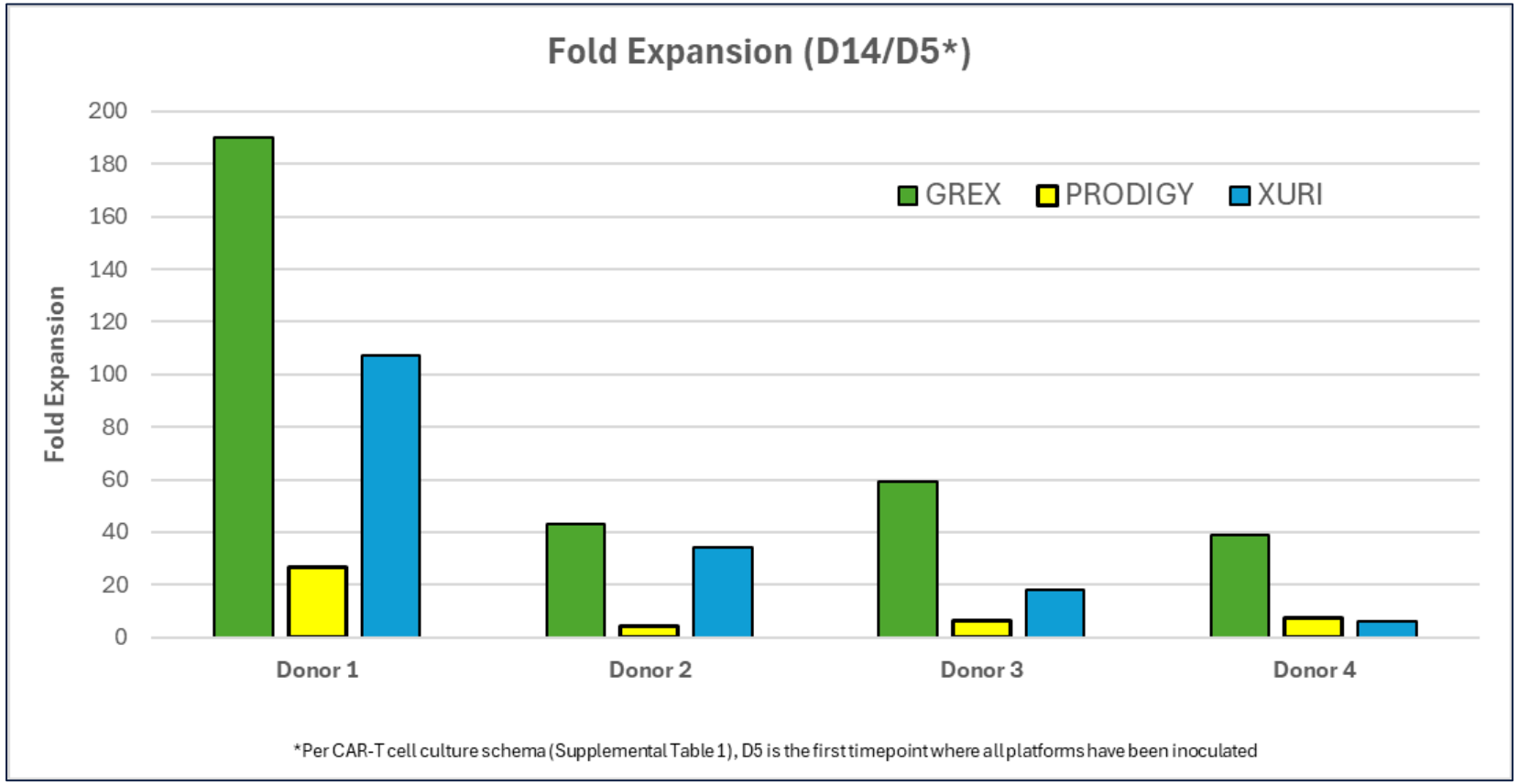
	Process			Output			
Patients	Batch Length (hr)	Batch Length (d)	Total Time (hr)	Batch / Hr	Batch / Day	Batch / Week	Batch / Year
1	170	7.08	170	0.0059	0.141	0.988	51
2	170	7.08	340	0.0059	0.141	0.988	51
5	170	7.08	850	0.0059	0.141	0.988	51
10	170	7.08	1700	0.0059	0.141	0.988	51
100	170	7.08	17000	0.0059	0.141	0.988	51
1,000	170	7.08	170000	0.0059	0.141	0.988	51
10,000	170	7.08	1700000	0.0059	0.141	0.988	51
100,000	170	7.08	17000000	0.0059	0.141	0.988	51

GREX

	Process			Output			
Patients	Batch Length (hr)	Batch Length (d)	Total Time (hr)	Batch / Hour	Batch / Day	Batch / Week	Batch / Year
1	170	7.08	170	0.0059	0.14	0.99	51
2	170	7.08	172	0.0116	0.28	1.95	102
5	170	7.08	178	0.028	0.67	4.72	245
10	170	7.08	188	0.053	1.28	8.94	465
100	170	7.08	368	0.272	6.52	45.65	2,374
1,000	170	7.08	2168	0.461	11.07	77.49	4,030
10,000	170	7.08	20168	0.496	11.90	83.30	4,332
100,000	170	7.08	200168	0.500	11.99	83.93	4,364

G-Rex® v. Prodigy v. Xuri

✓ **G-Rex®** produces **greater fold expansion** in 4/4 donors when compared with Prodigy/Xuri



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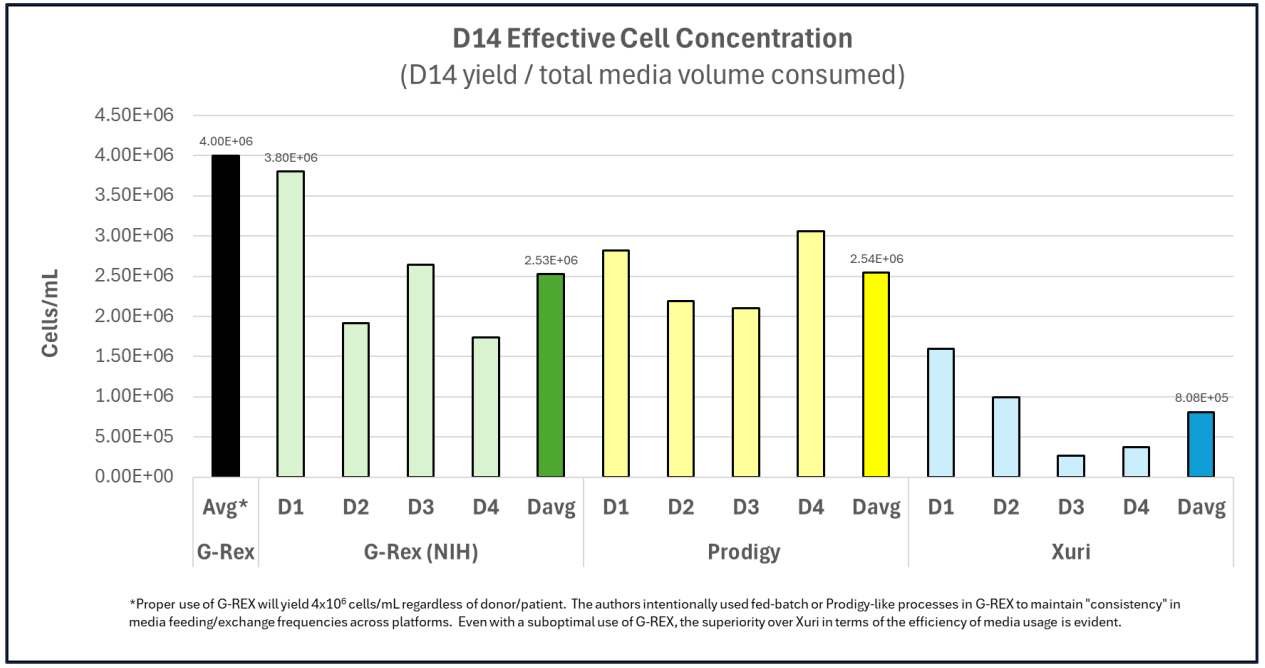
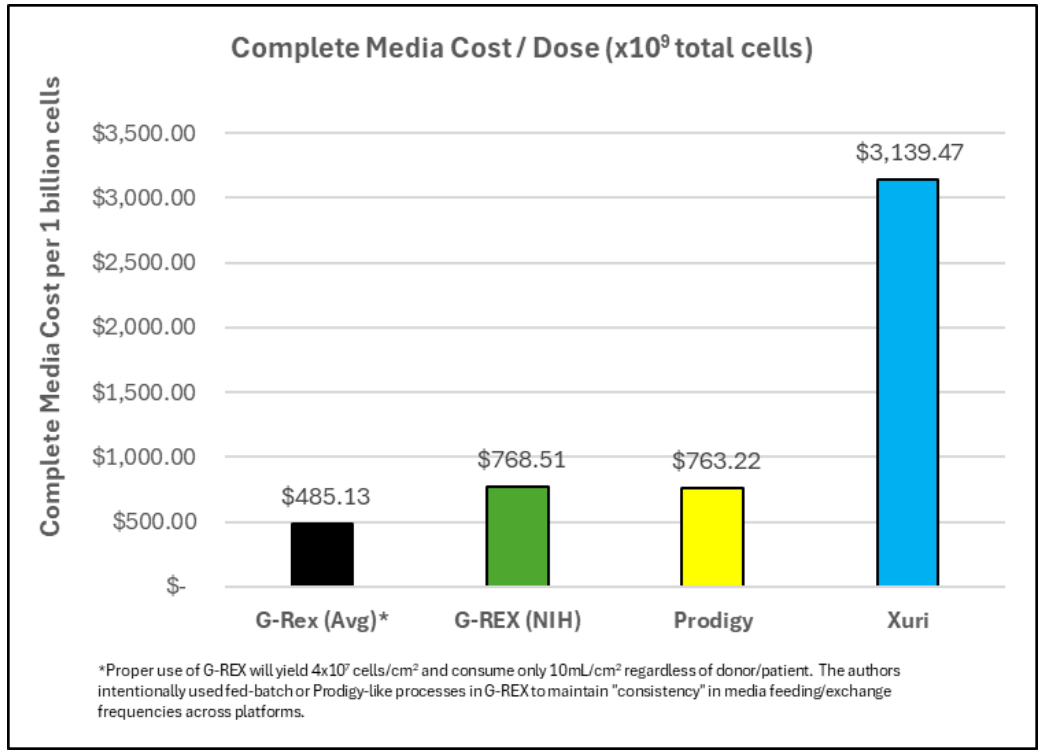
G-Rex® vs. Prodigy vs. Xuri

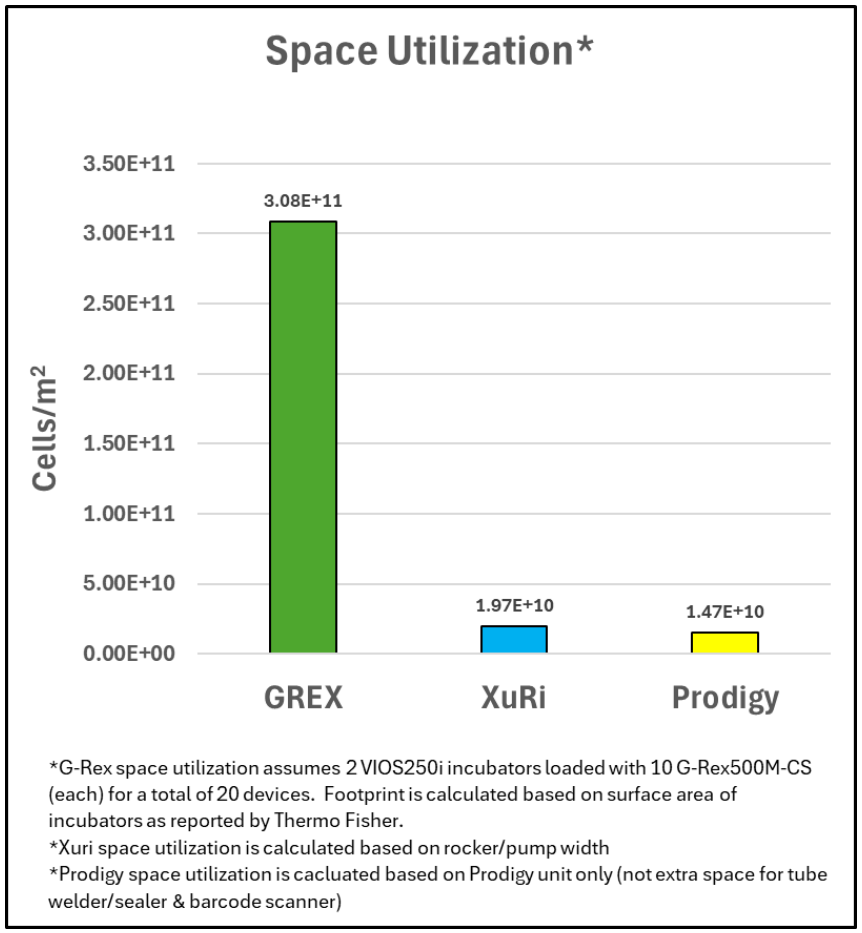
- ✓ **G-Rex®** achieves consistently **higher cell concentrations** than Prodigy or Xuri
  - ✓ **G-Rex®** has **greater efficiency** with media/cytokines consumption
- ✓ **G-Rex® lowers the cost of goods** sold by reducing inputs and maximizing outputs





Hannah W. Song, Michaela Prochazkova, Lipei Shao,Roshini Traynor, Sarah Underwood, Mary Black, Vicki Fellowes, Rongye Shi, Marie Pouzolles, Hsien-Chao Chou, Adam T. Cheuk, Naomi Taylor, Ping Jin, Robert P. Somerville, David F. Stroncek, Javed Khan, Steven L. Highfill, CAR-T cell expansion platforms yield distinct T cell differentiation states, *Cytotherapy* (2024), doi; https://doi.org/10.1016/j.jcyt.2024.03.003

G-Rex® vs. Prodigy vs. Xuri



*G-Rex space utilization assumes 2 VIOS250i incubators loaded with 10 G-Rex500M-CS (each) for a total of 20 devices. Footprint is calculated based on surface area of incubators as reported by Thermo Fisher.
*Xuri space utilization is calculated based on rocker/pump width
*Prodigy space utilization is cacluated based on Prodigy unit only (not extra space for tube welder/sealer & barcode scanner)

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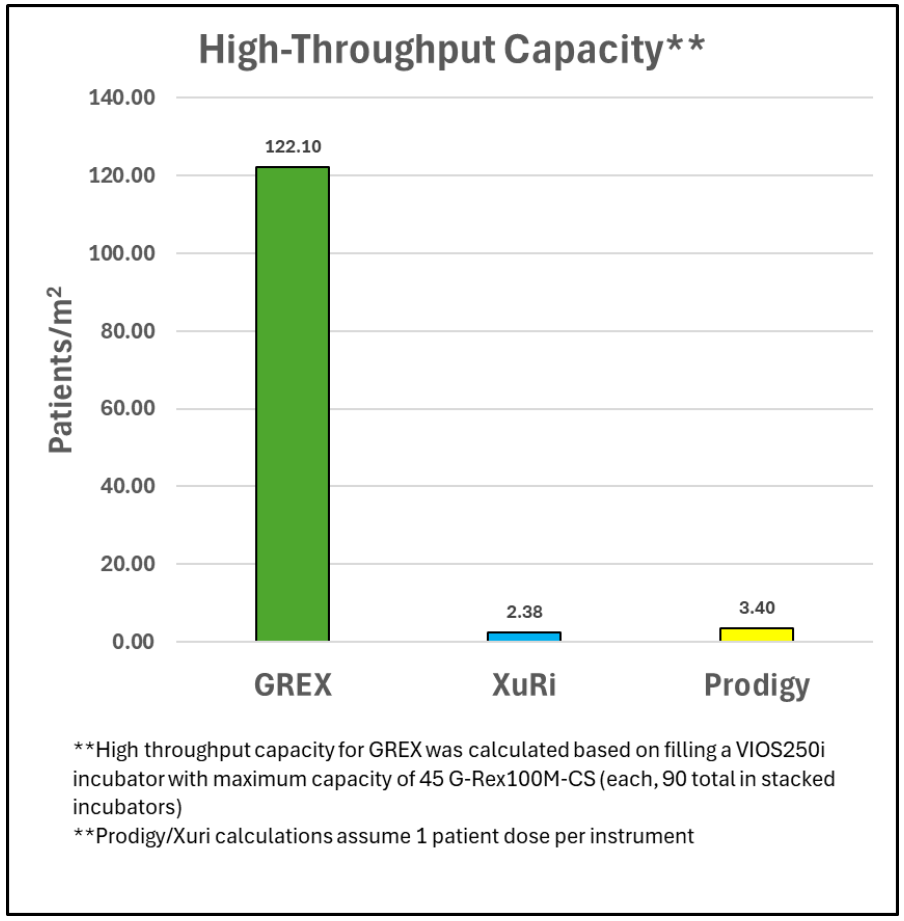
G-Rex® vs. Prodigy vs. Xuri



**High throughput capacity for GREX was calculated based on filling a VIOS250i incubator with maximum capacity of 45 G-Rex100M-CS (each, 90 total in stacked incubators)
**Prodigy/Xuri calculations assume 1 patient dose per instrument

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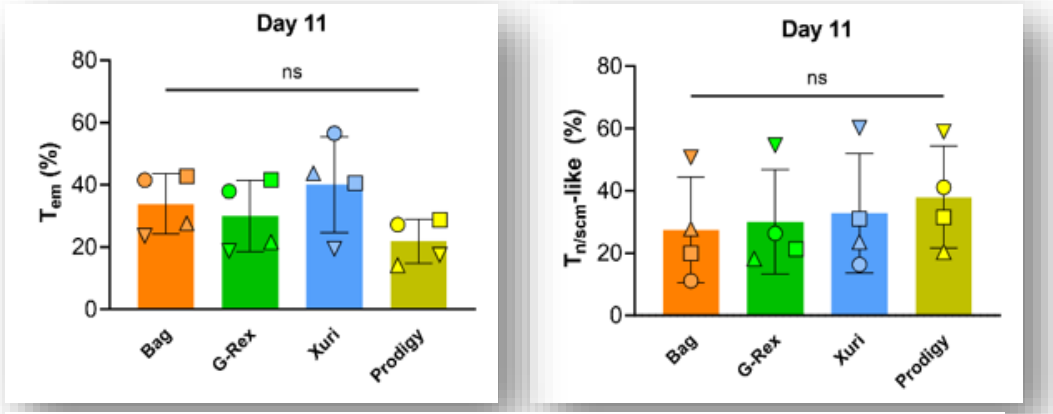
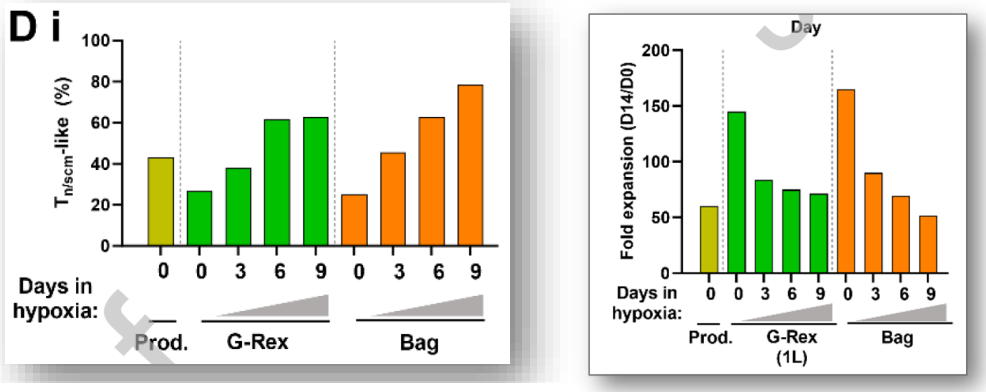
G-Rex® vs. Prodigy vs. Xuri



Supplementary Figure S5: Naïve/stem central memory-like and effector memory phenotype over time.

- ✓ **G-Rex®** produces CAR-T product with equivalent % $T_{n/scm}$ in normoxia conditions



- ✓ **G-Rex®** produces CAR-T product with **greater % $T_{n/scm}$** in hypoxia conditions and **greater fold expansion**

Figure 5: Hypoxia culture yields a more naïve/stem central memory ($T_{n/scm}$)-like and less activated phenotype, with longer time in culture corresponding to greater proportion of $T_{n/scm}$-like cells. (A) Dissolved oxygen (DO) concentration in Prodigy,

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G-Rex® Grant Program

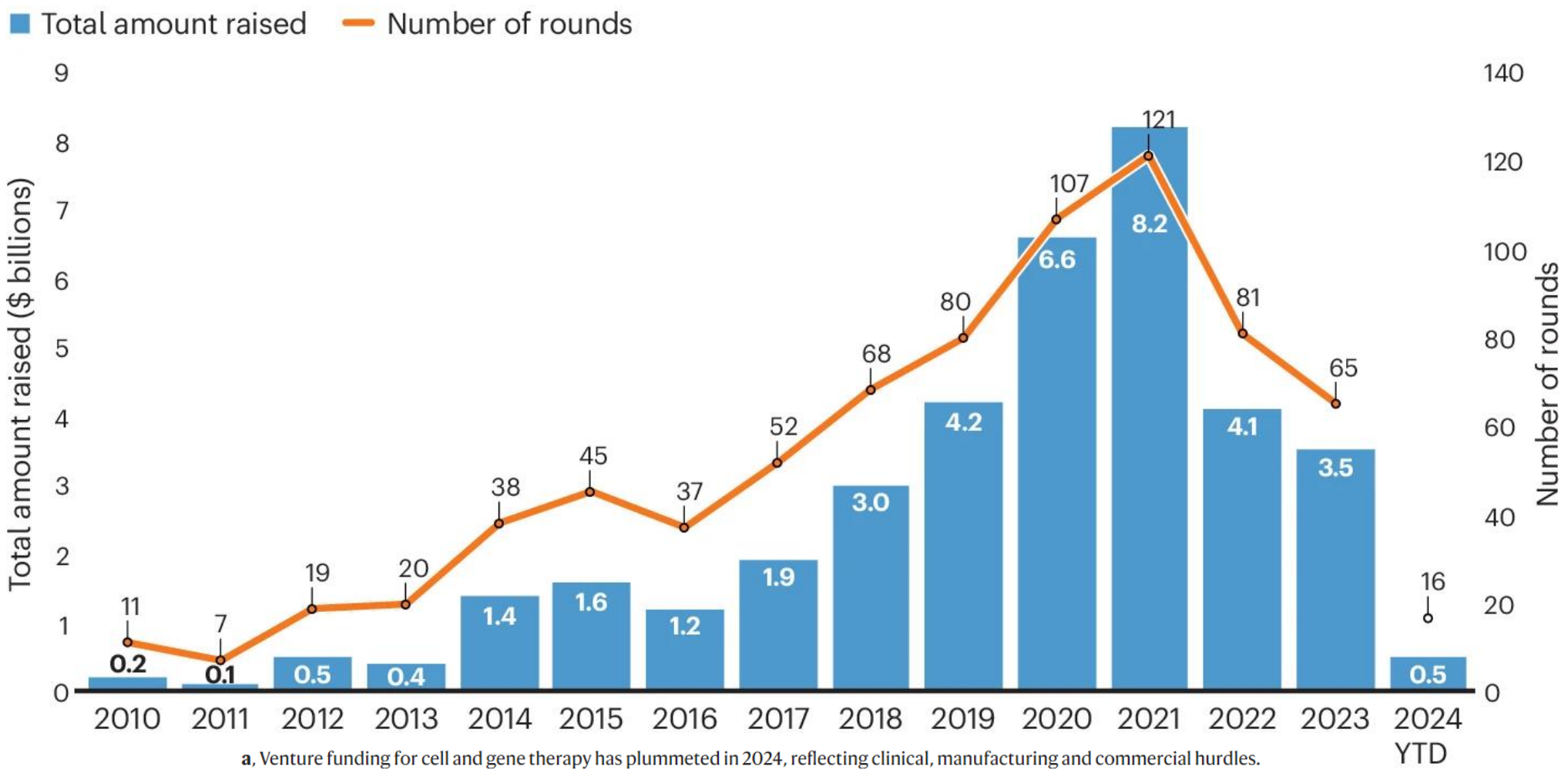
for the advancement of CGT development and manufacturing

CGT Investment Overview

CGT Investment Overview

nature biotechnology

Biotech financing: darkest before the dawn

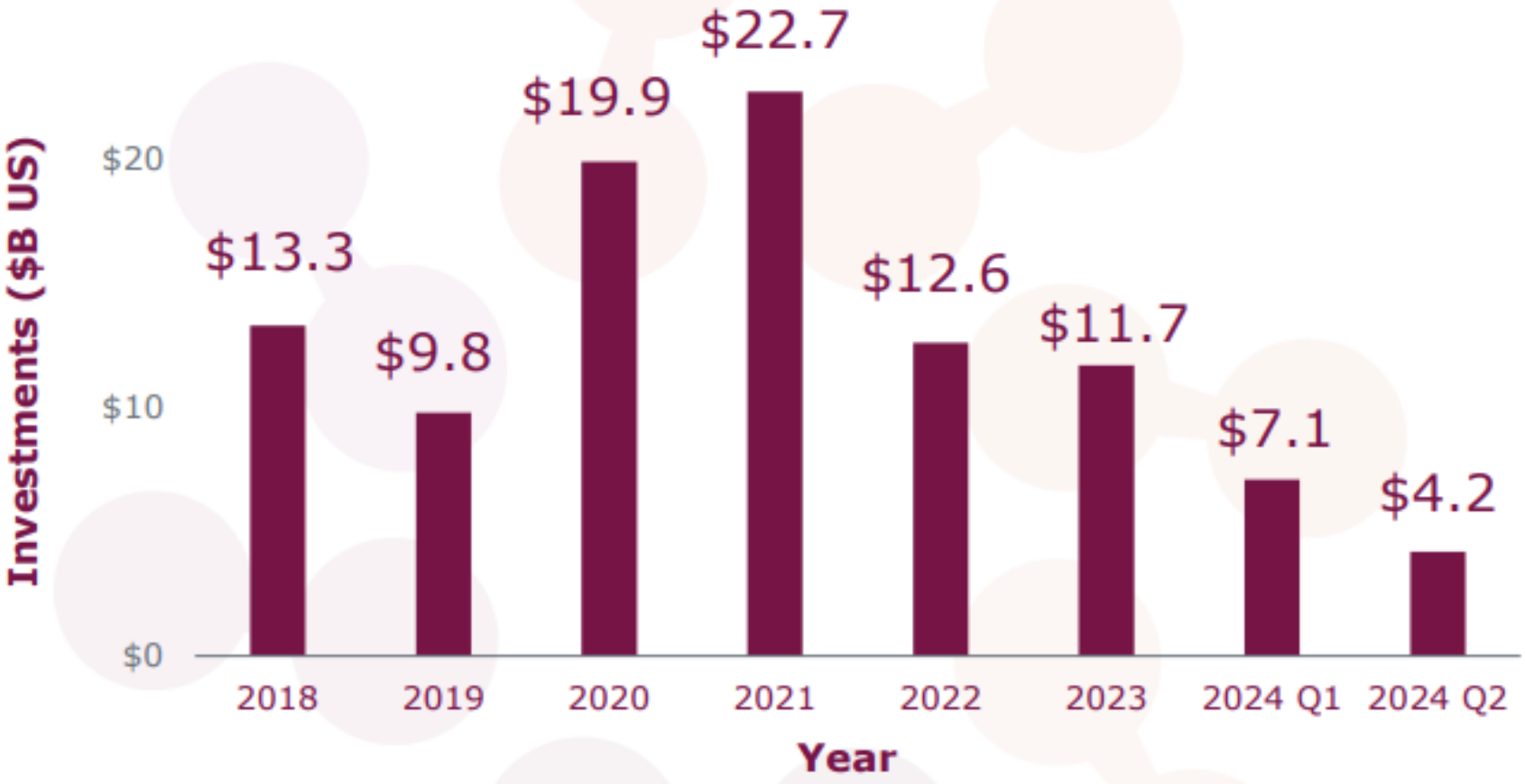


a, Venture funding for cell and gene therapy has plummeted in 2024, reflecting clinical, manufacturing and commercial hurdles.

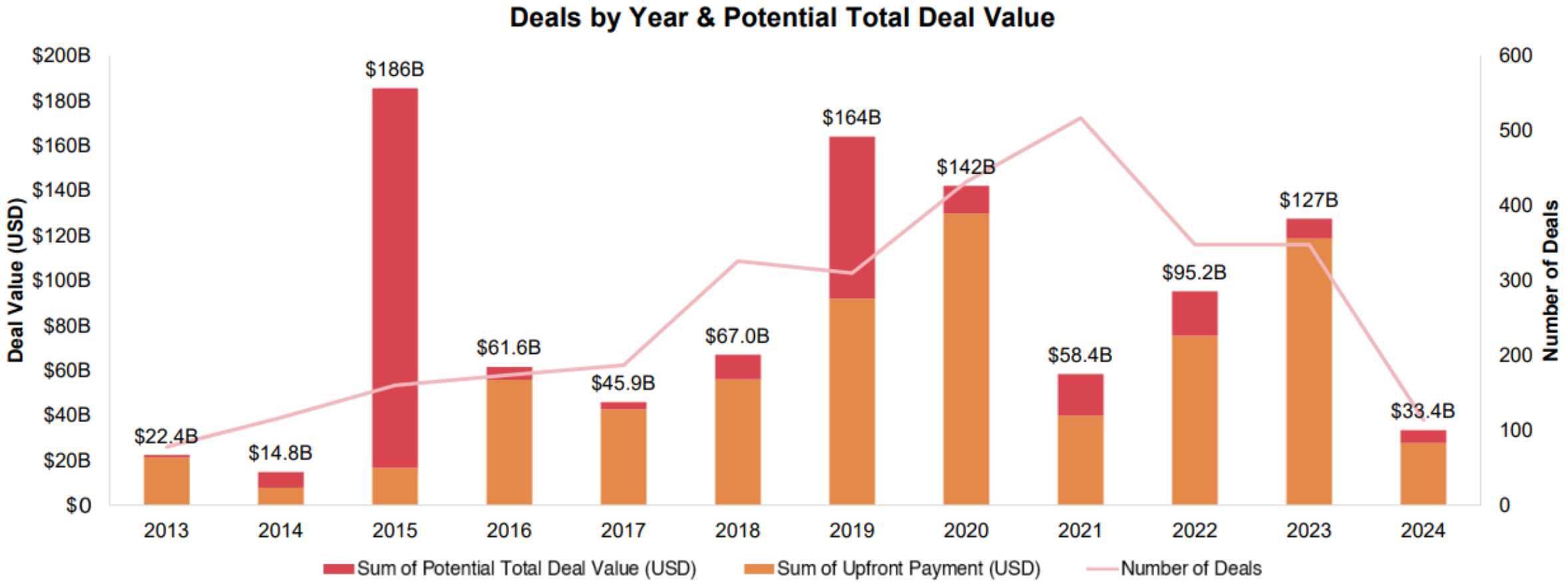
ScaleReady

Senior, M. (2024). Biotech financing: darkest before the dawn. *Nature Biotechnology*. https://doi.org/10.1038/s41587-024-02357-2

Investments by Year



Deal and Company Landscape



What do investors want to see?

What do investors want to see?

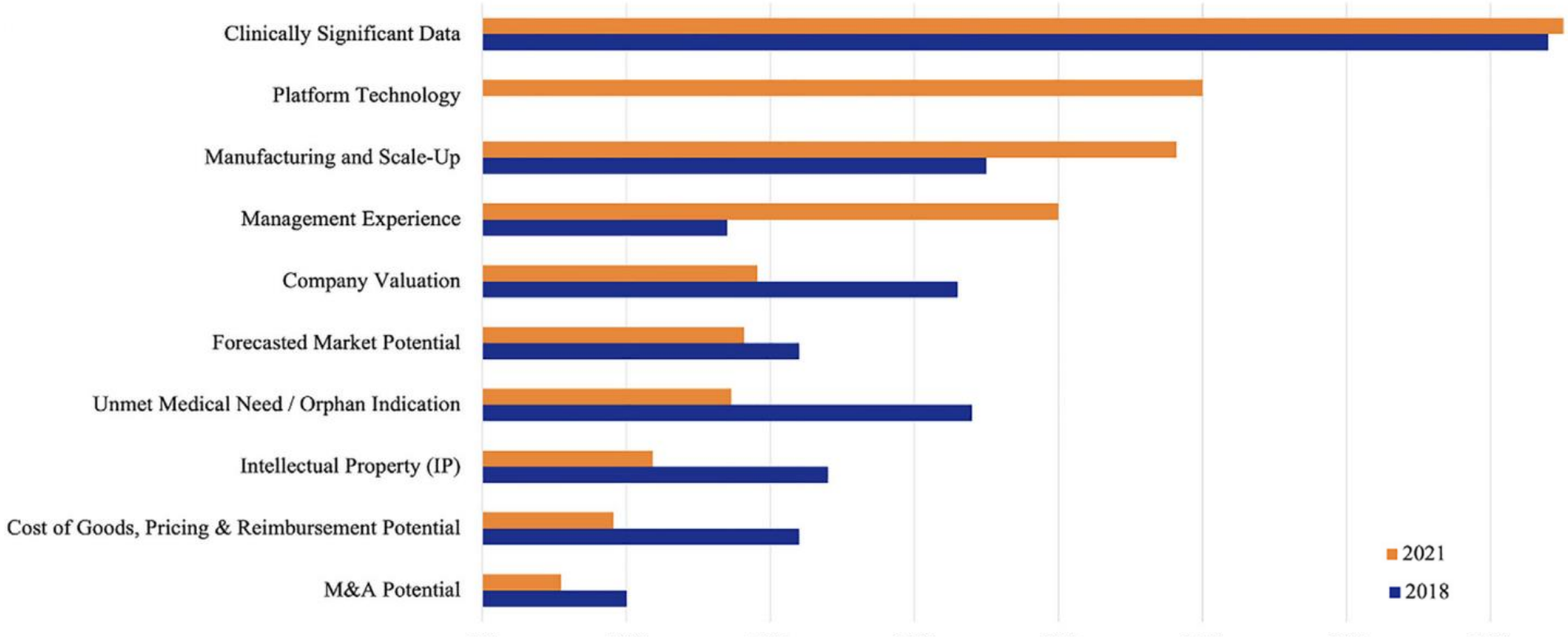


Figure 11. (A) Rank of factors selected as first-place for most influential in decision-making to invest in a CGT product development opportunity. (B) Rank of factors selected in the top three for most influential in decision-making to invest in a CGT product development opportunity. M&A, mergers and acquisitions. (Color version of figure is available online.)

Kunze-Küllmer, M., Goonewardene, A., Kili, S., Theoharis, S., & Rivers, P. (2024). Cell and Gene Therapy Investment: Evolution and Future Outlook on Investor Perspectives. Cytotherapy. https://doi.org/10.1016/j.jcyt.2024.02.017

What do investors want to see?

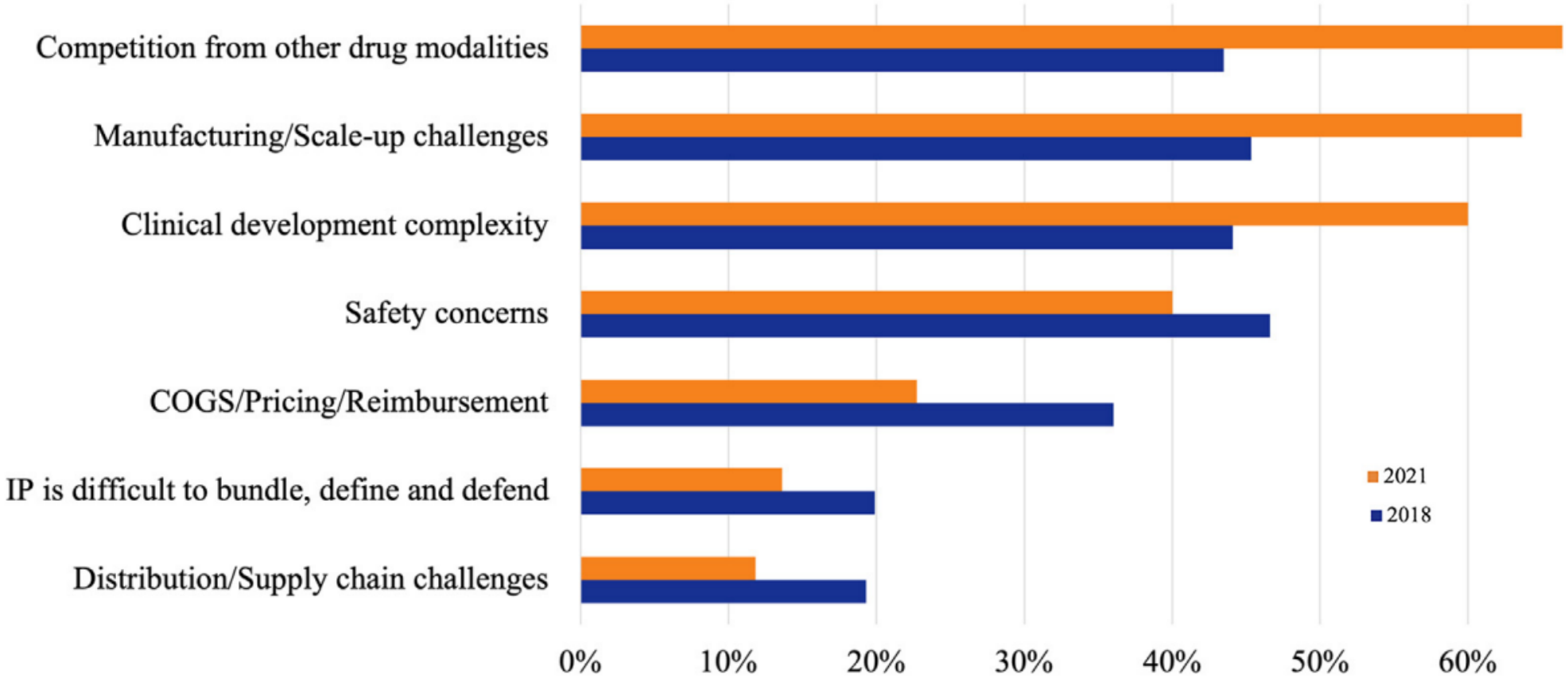


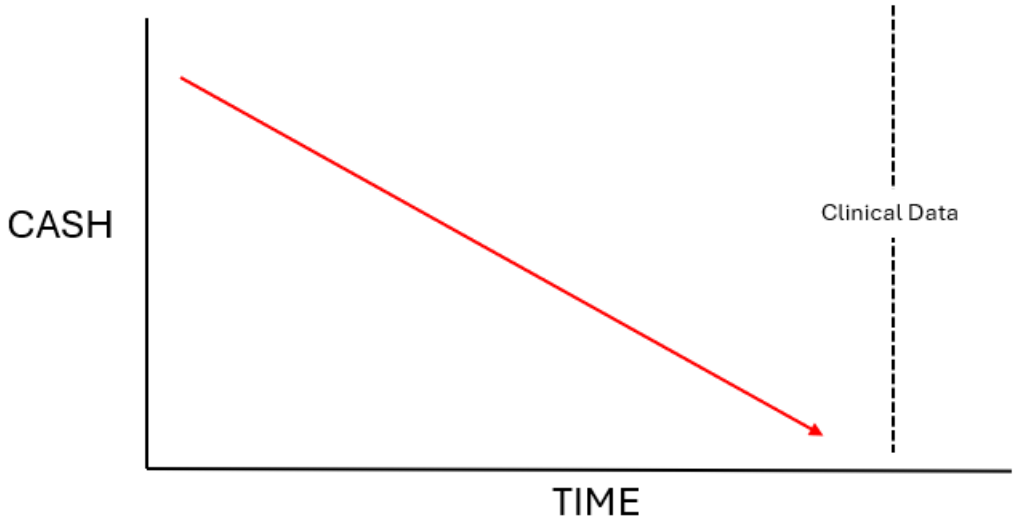
Figure 12. Rank of factors selected in the top three for greatest barriers in decision-making to invest in a CGT product development opportunity.

Kunze-Küllmer, M., Goonewardene, A., Kili, S., Theoharis, S., & Rivers, P. (2024). Cell and Gene Therapy Investment: Evolution and Future Outlook on Investor Perspectives. Cytotherapy. https://doi.org/10.1016/j.jcyt.2024.02.017

What do investors want to see?

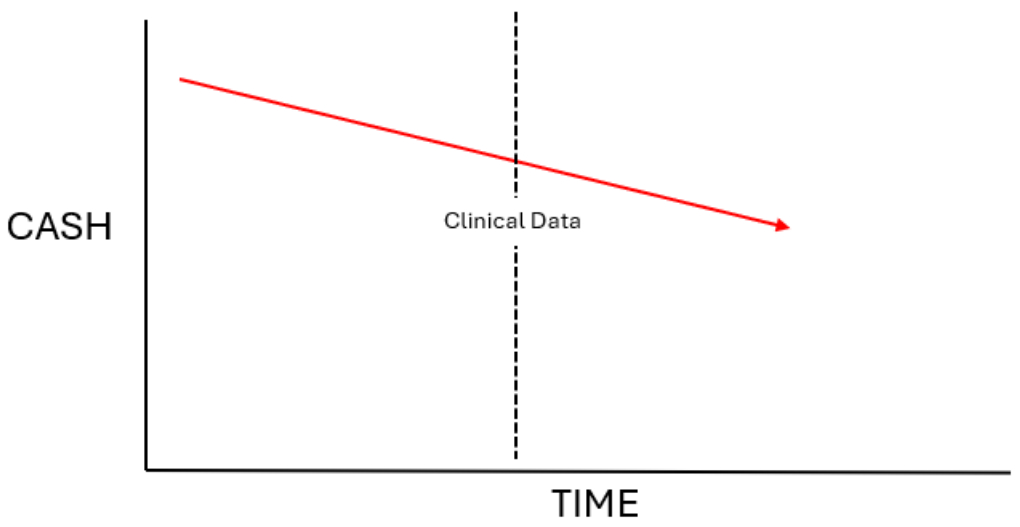
Company A

- ! Excessive cash burn rates
  - ! Imprudent decision making
  - ! Inefficient use of people, space, and materials
- ! **Slow to generate clinically significant data**
- ! Doesn't keep the main thing the main thing



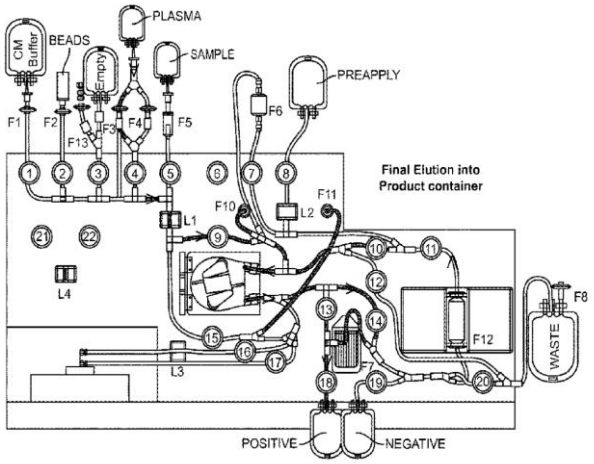
Company B

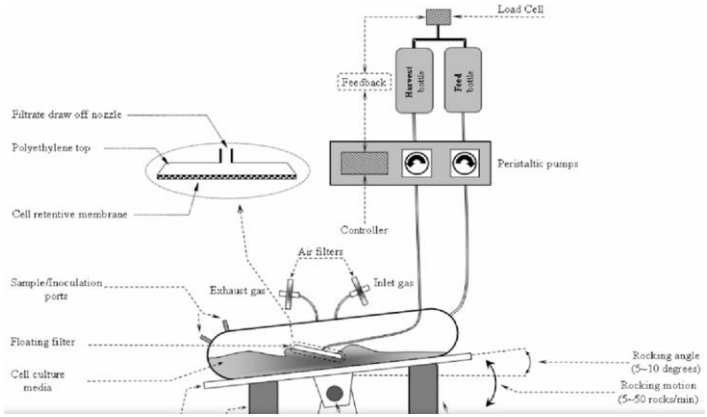
- ✓ Modest and reasonable cash burn rate
  - ✓ Prudent decision making
  - ✓ Highly efficient use of people, space, and materials
- ✓ **Quickly generates clinical significant data**
- ✓ Keeps the main thing the main thing!

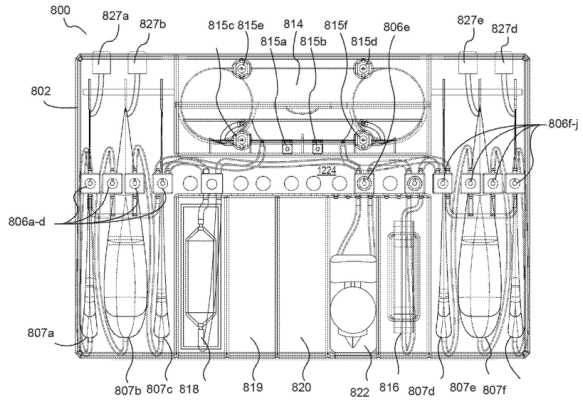


Where do we go from here?

CGT needs to transition away from “all-in-one” bioreactors







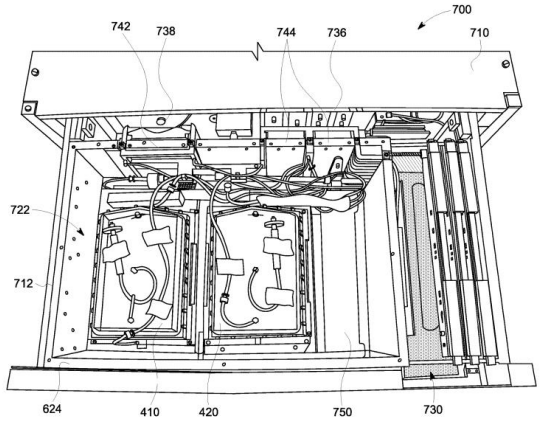


FIG. 34

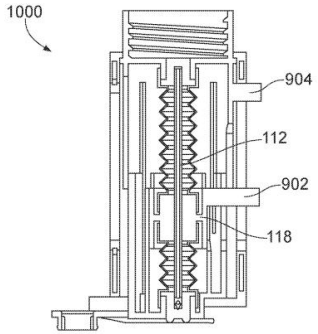
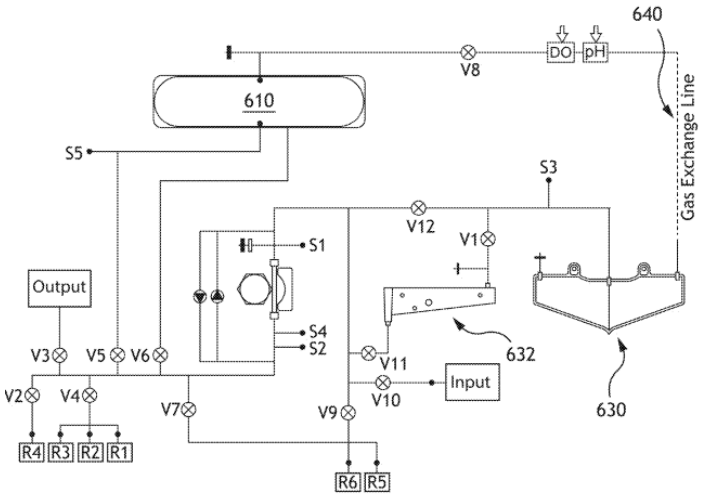


FIG. 37



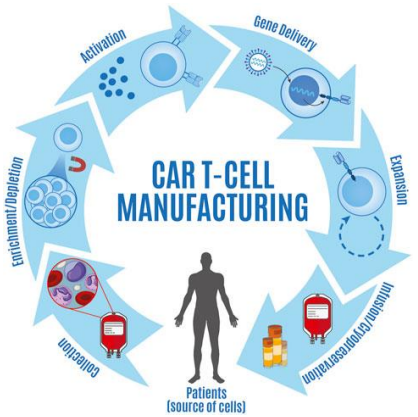
Where do we go from here?

Development

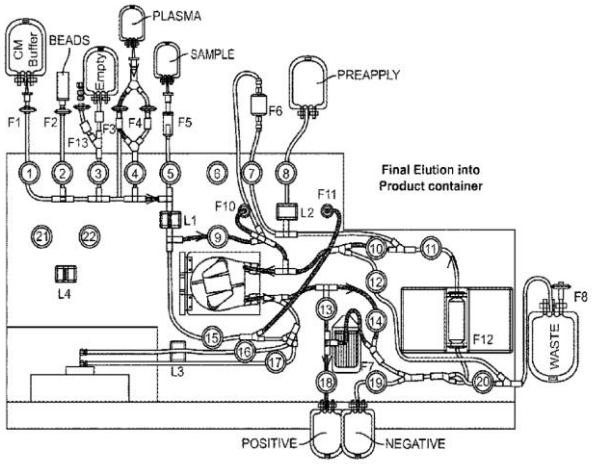
X No native small-scale model
! Unpredictable translation of small-scale data
! Excessive time and money spent attempting to create poorly predictive scale down models
! Excessive time and money spent optimizing CMC at full-scale

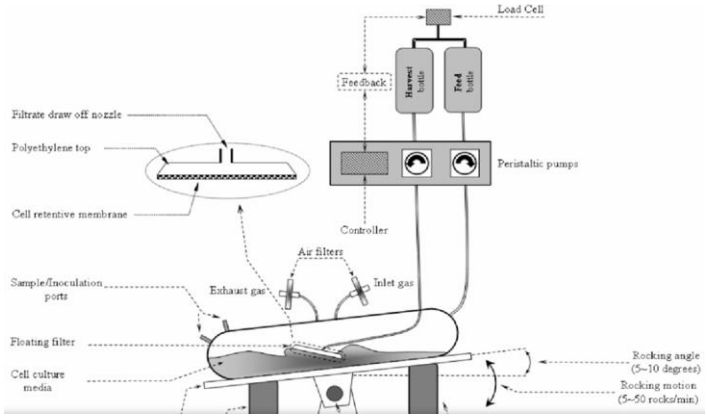
Manufacturing

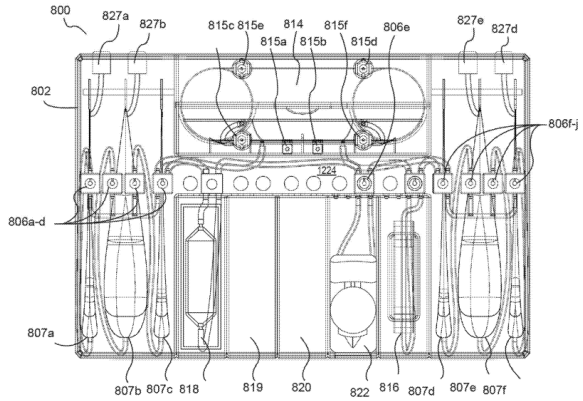
X Continuous fluidic connection of drug product
! Constraint is the manufacturing process itself
! Excessive investment in capital equipment for minimal throughput
! Limited potential for further innovation



CGT needs to transition away from “all-in-one” bioreactors







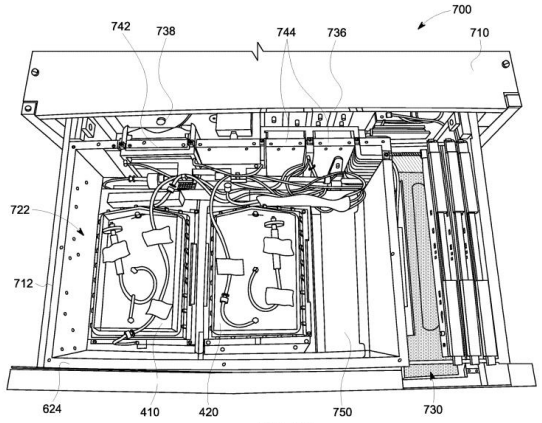


FIG. 34

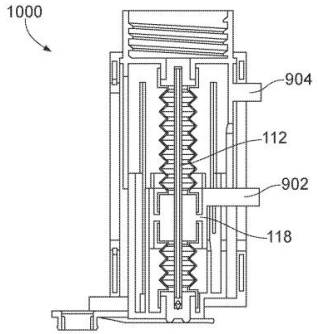
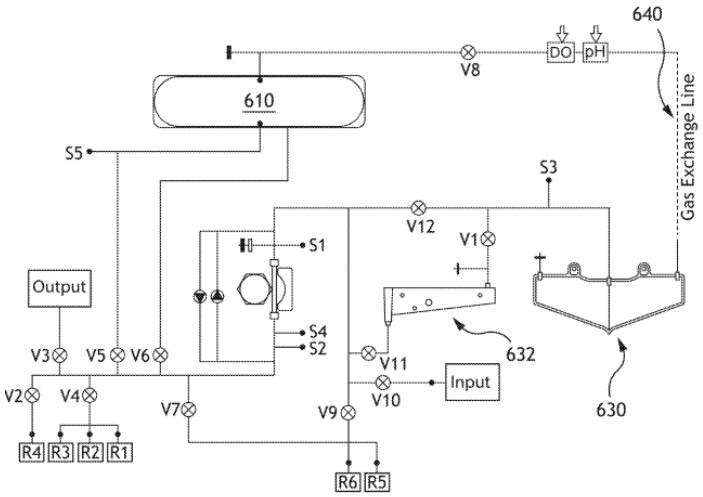
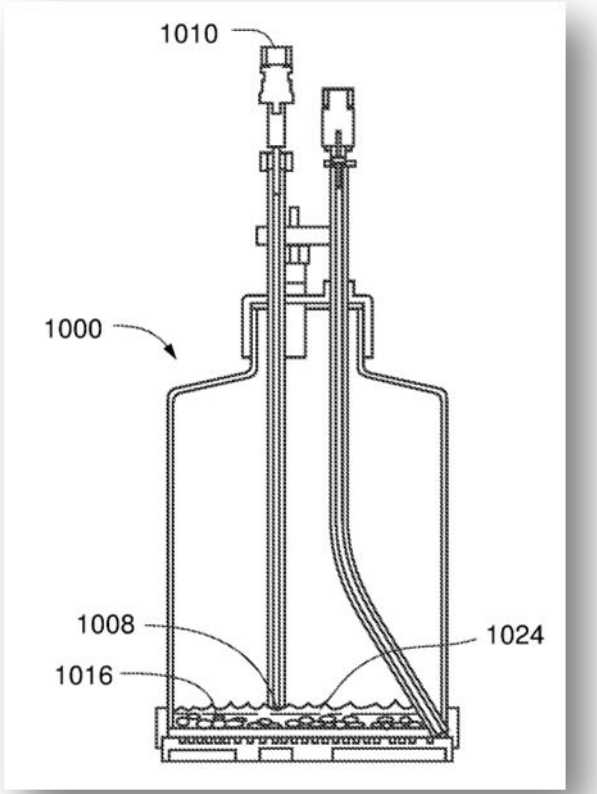


FIG. 37



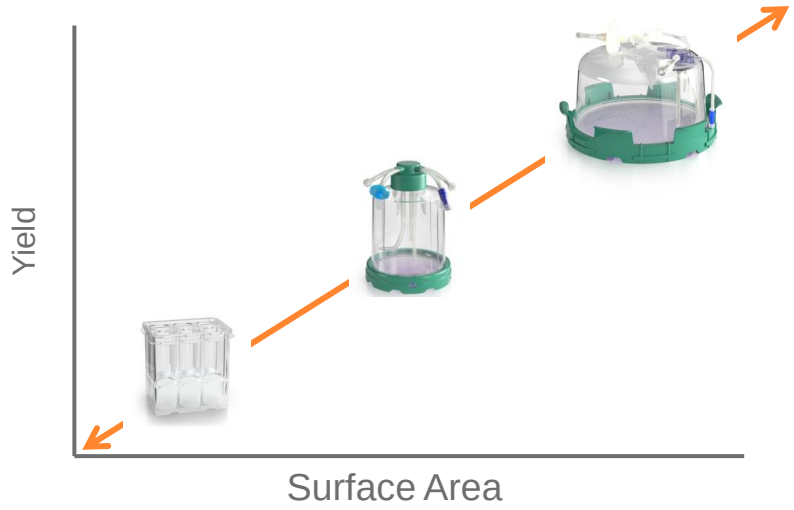
CGT needs to embrace **simplicity** and recognize it as the ultimate **sophistication**



Where do we go from here?

Development

- ✓ Native small-scale model
- ! Predictable translation
- ! Linear path of least resistance to IND
- ! Cost- and time-efficient CMC improvements



Manufacturing

- ✓ De-couple drug product from instruments
- ! Constraints are individual unit operations
- ! Modest investment for high throughput
- ! Future-proofed

G-Rex® Grant Program

for the advancement of CGT development and manufacturing

G-Rex® Grant Program

$20,000,000
in-kind contributions of G-Rex® & GMP reagents

$5k - $300k
per G-Rex® Grant Award

18 months
to utilize the G-Rex® Grant

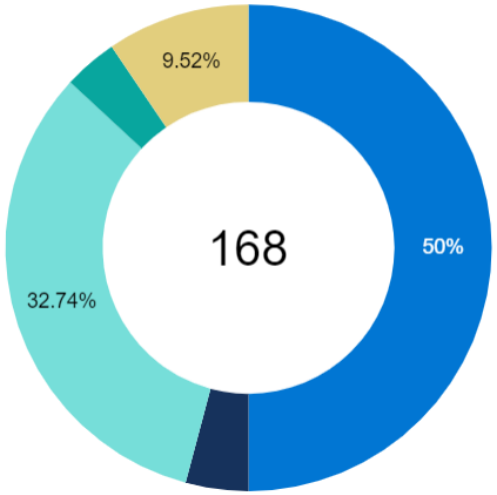
157 applicants
have applied since May

95% funding
rate of all applicants

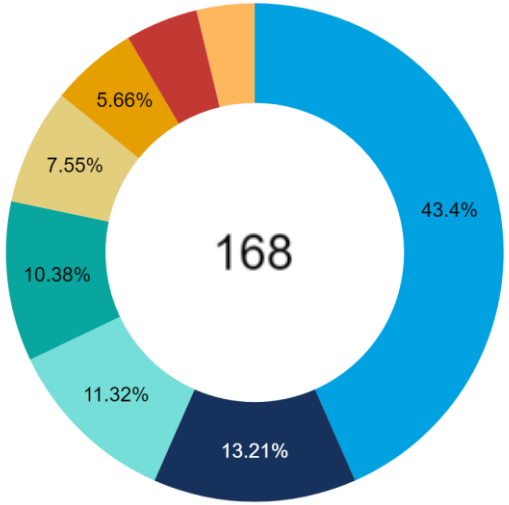
10 partners
offering exclusive savings to G-Rex Grant Recipients

G-Rex® Grant Program: Who? / What? / Where? / Why?

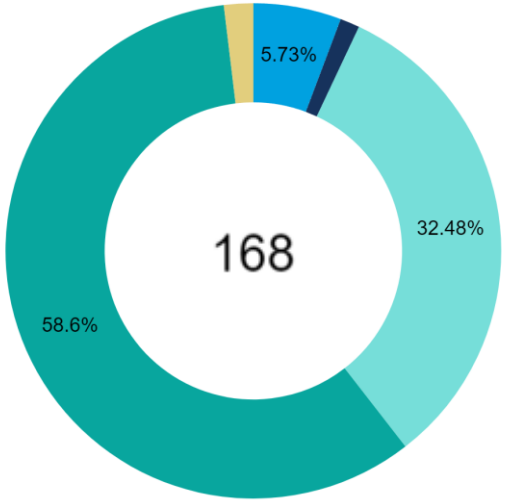
Who?



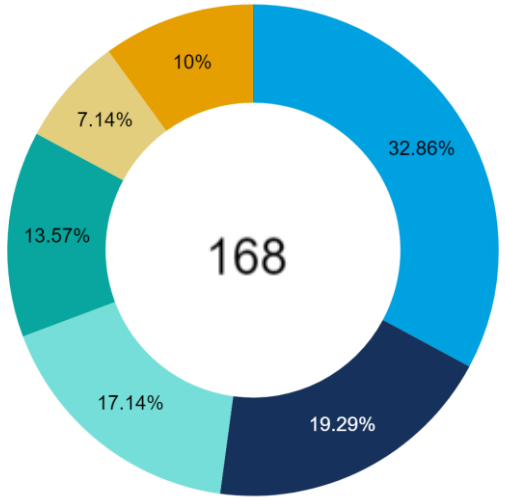
What?



Where?



Why?



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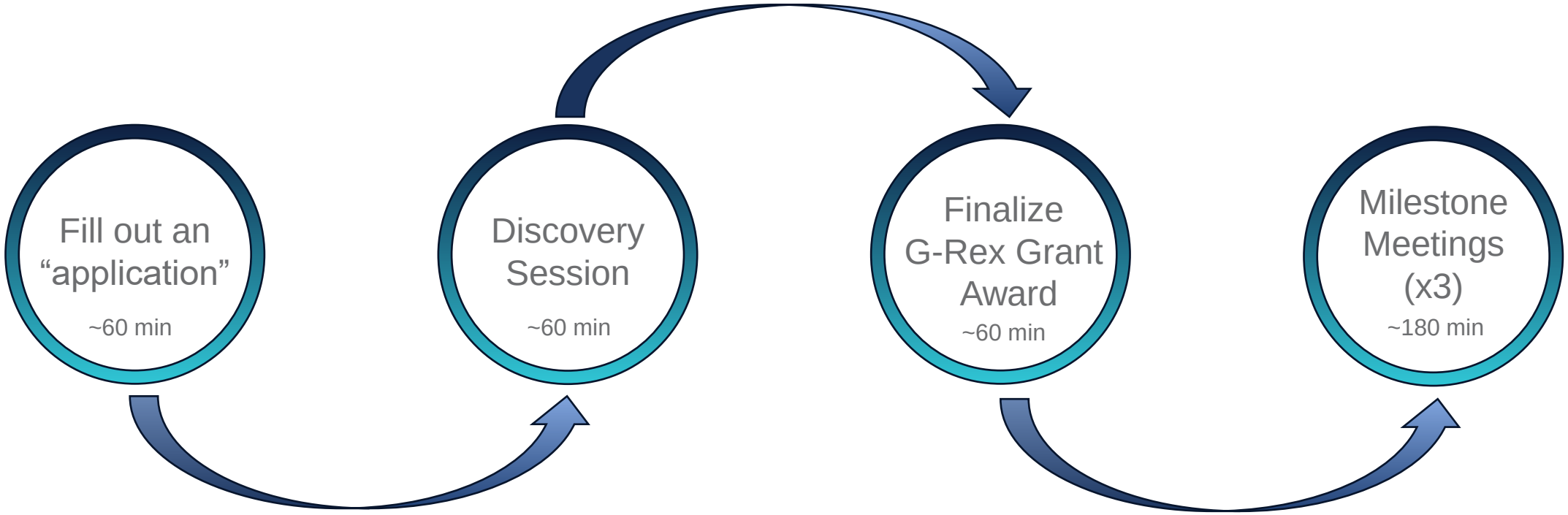
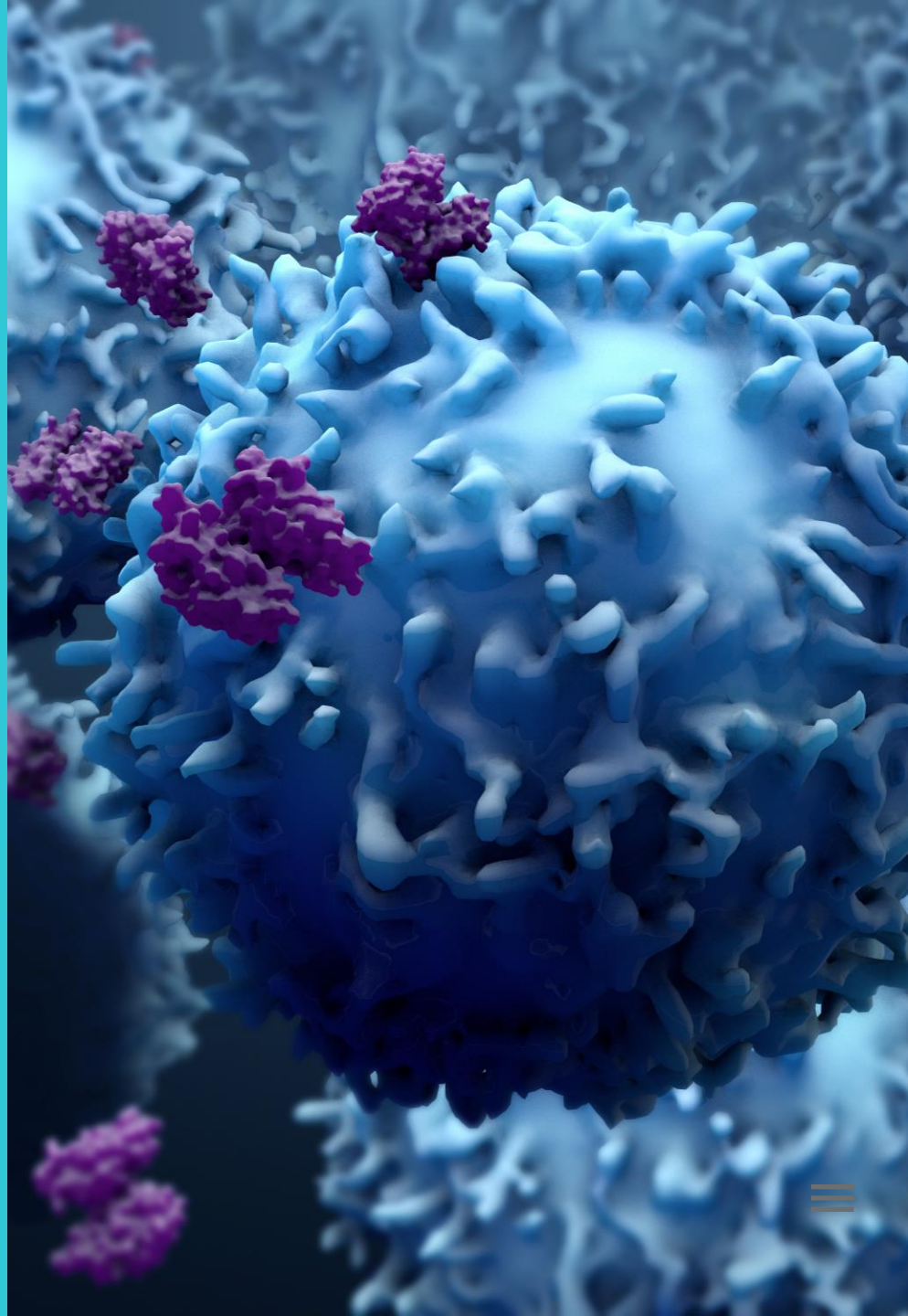
G-Rex® Grant Program: How?



Estimated Time Requirements *over 18 months* = **6 hours**

G-Rex® Grant Partners

G-Rex® is the most future-proofed manufacturing platform in the industry



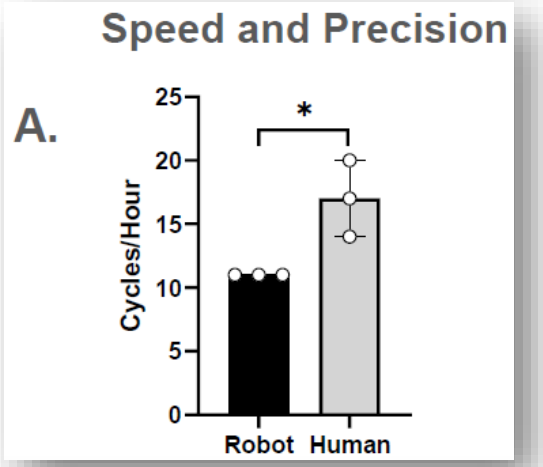
ScaleReady

G-Rex® with integrated robotic automation

Author Email: asmith@markertherapeutics.com

Robotic Automation of T Cell Generation for the Treatment of Acute Myeloid Leukemia (AML)

Anastasiya Smith[2], **John d'Aigle**[1], Tara Shahim[2], Sherket Peterson[1], Thorsten Demberg[2], Derian Salas[1], Sam Chang[2], Aditya Tandon[1], Jeannette Crisostomo[2], Alex Riley[1], Tsvetelina Hoang[2], Jaime Avalos[1], Juan Vera[2], Jose-Manuel Collados[1].
[1]Division of Healthcare, Consumer Segments & Service Robotics, ABB Inc, Houston, Texas 77021, USA; [2]Marker Therapeutics, Inc., Houston, Texas, 77027, USA.



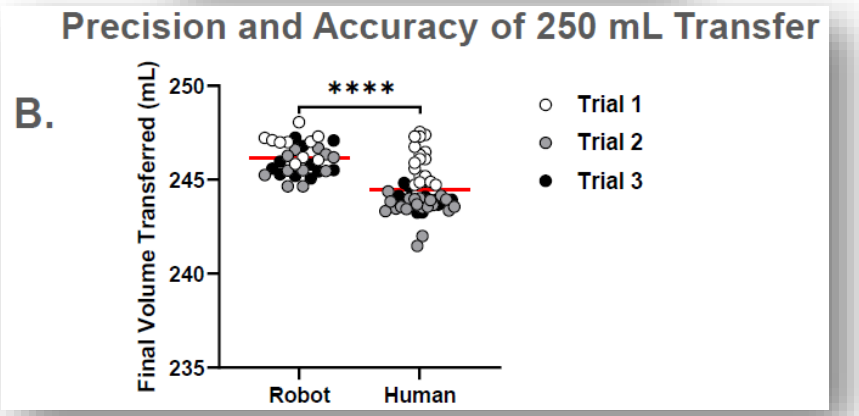


ABB Robotics for Healthcare

Robotic Automation of T-Cell Generation
for the treatment of Acute Myeloid Leukemia (AML)

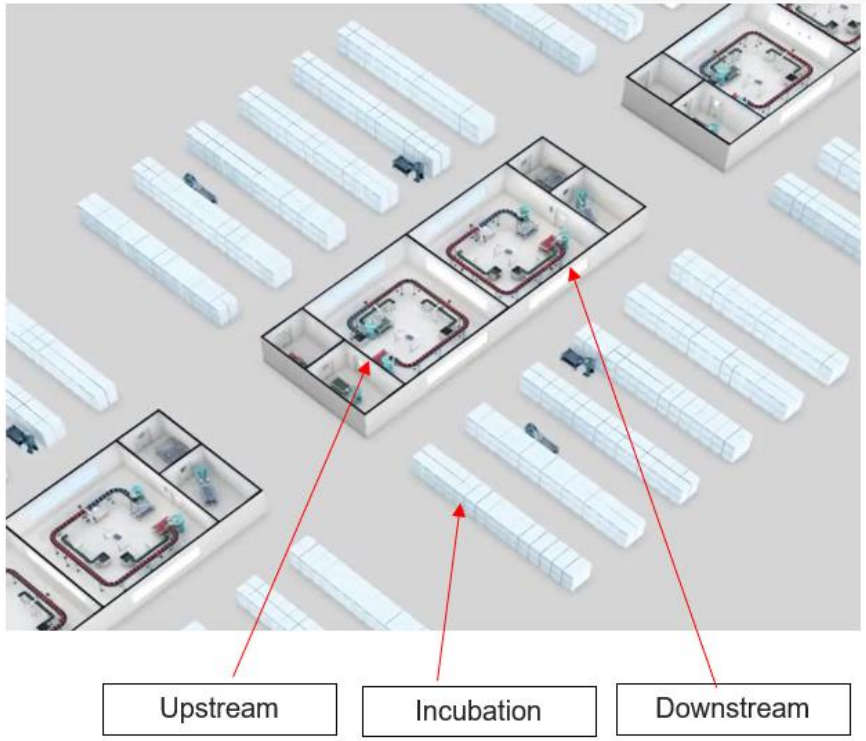
embedded video; does not work as PDF

G-Rex® & Robotic Automation

Cellular Origins Partners With ScaleReady to Simplify, Standardise, and Automate Cell Therapy Manufacturing

- *Partnership aims to integrate ScaleReady's CGT manufacturing workflow with Cellular Origins' robotic technology*
- *Initially focussed on automation of ScaleReady's G-Rex platform*

May 11, 2023 07:28 AM Eastern Daylight Time



Link to press release

G-Rex® & Robotic Automation

- ✓ **G-Rex®** is being automated in ways common to other mature industries
- ✓ Robots can be trained to maneuver and manipulate **G-Rex®** with consistency
- ✓ “Lights off” manufacturing with a **G-Rex®** centric approach
- ✓ **G-Rex®** based approaches are agonstic to centralized or de-centralized manufacturing

G-Rex® & Robotic Automation

MultiplyLabs

- ✓ **G-Rex®** is being automated in ways common to other mature industries
- ✓ Robots can be trained to maneuver and manipulate **G-Rex®** with consistency
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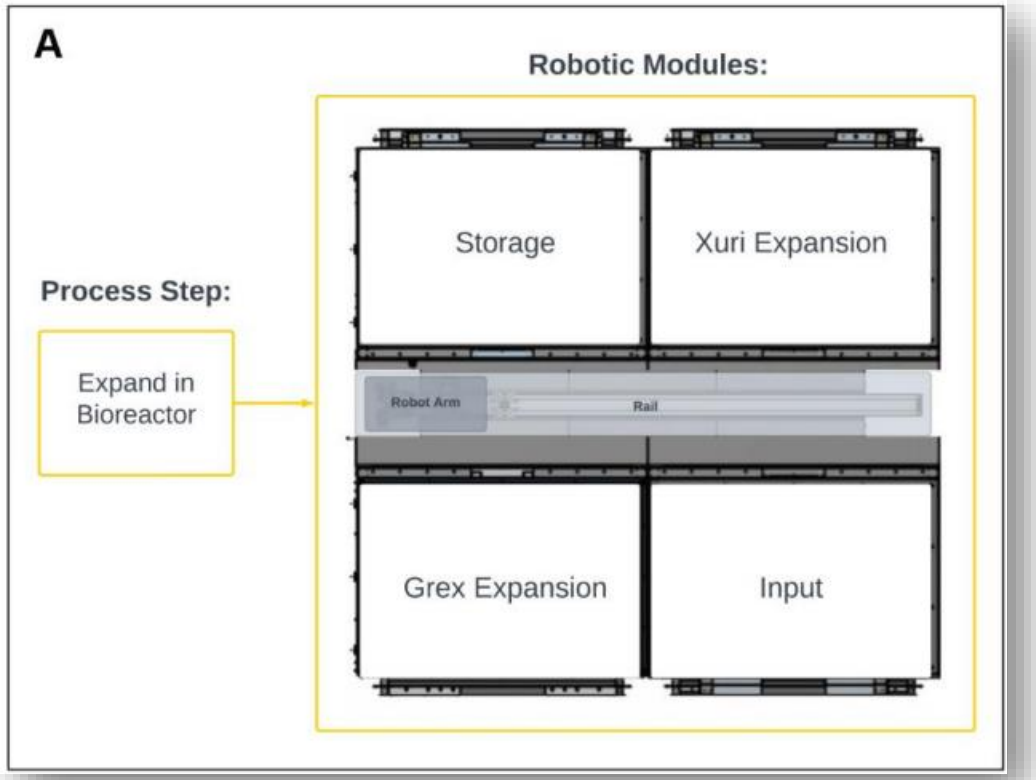
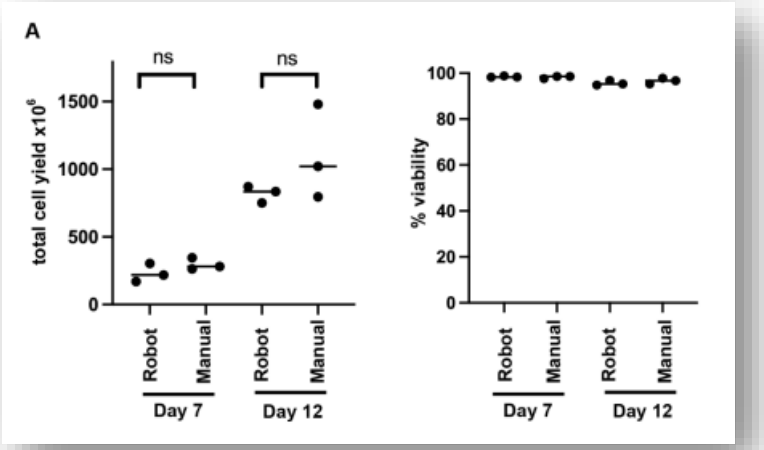
G-Rex® & Robotic Automation

MultiplyLabs



Here, we describe the performance of a new prototype robotic system designed to operate industry-standard cell therapy manufacturing equipment. We robotically automate the culture of CD8+ T cells using standard G-Rex 10M CS and Xuri Cell Expansion System W25 platforms, in combination with a modified Heracell Vios 250i cell culture incubator. We show that cell yields, phenotype, and viability are comparable between robotic and manual processes. The use of industry-standard equipment in a robotic process can greatly increase the pace of both process development and scale up for novel cell therapy products.



Melocchi, A., Schmittlein, B., Jones, A. L., Ainane, Y., Rizvi, A., Chan, D., Dickey, E., Pool, K., Harsono, K., Szymkiewicz, D., Scarfogliero, U., Bhatia, V., Sivanantham, A., Kreciglowa, N., Hunter, A., Gomez, M., Tanner, A., Uboldi, M., Batish, A., … Esensten, J. H. (2023). Development of a Robotic Cluster for Automated and Scalable Cell Therapy Manufacturing. https://doi.org/10.1101/2023.12.21.572854

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