ANEMOCYTE

Talent for Life

CELL AND GENE THERAPY BIOPROCESSING & COMMERCIALIZATION

EXPLORING MANUFACTURING PLATFORMS AND ANCILLARY MATERIALS TO ADDRESS CELL AND GENE THERAPY PRODUCT NEEDS

BOSTON 09/10/2019

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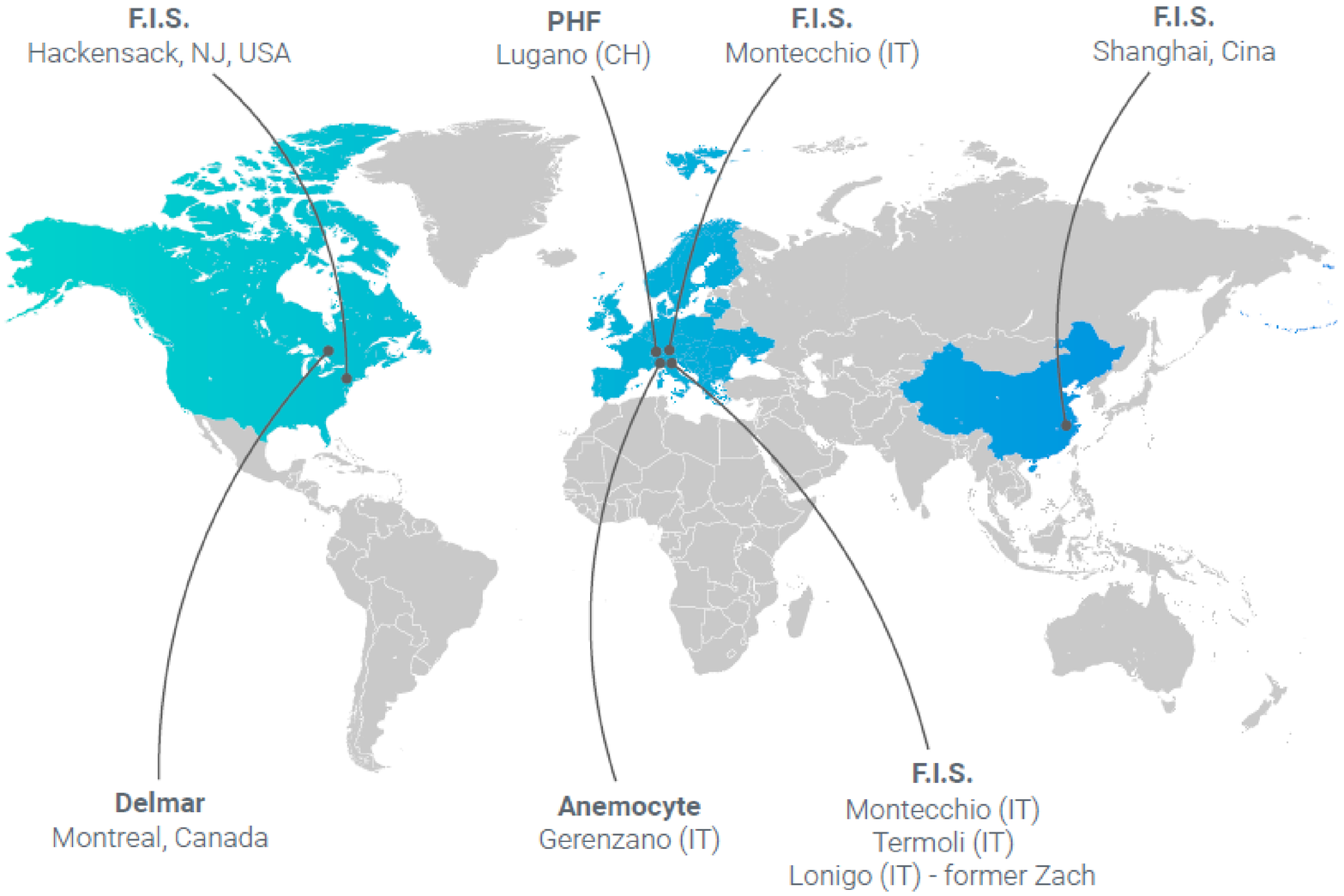
- Nine Trees Group – who we are and what we do
- Why BMO (Biotech Manufacturing Organization)
- CAR-T focused cases

i. Plasmid
ii. Non-viral
iii. Behind the Scene of I&D (Manufacturing and More)

- Non-Core Activities

i. Logistics
ii. Visual Inspection
iii. BIG DATA


F.I.S.
Hackensack, NJ, USA
PHF
Lugano (CH)
F.I.S.
Montecchio (IT)
F.I.S.
Shanghai, Cina
Delmar
Montreal, Canada
Anemocyte
Gerenzano (IT)
F.I.S.
Montecchio (IT)
Termoli (IT)
Lonigo (IT) - former Zach

nine trees GROUP

400 MIL €

Annual Revenue

1800

Employees (300 R&D)

60+ COUNTRIES

Active Business

Core Business: custom manufacturing of Drugs and API
Main Market: 70% of commercial products are sold in US and Canada

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Anemocyte is a biotech company active in the field of Cell and Gene Therapies that generates and implements innovation in all steps of product development and manufacturing

Manufacturing

Our Wealth Of Experience

MORE THAN
15
YEARS

of GMP manufacturing of CGTs
and biological drugs

MORE THAN
60
YEARS

of contract manufacturing within our
group (Nine Trees Group > 1800 employees
worldwide, 15% focused on R&D)

Existing site - 1000 m^2 at your service (10764 ft^2)

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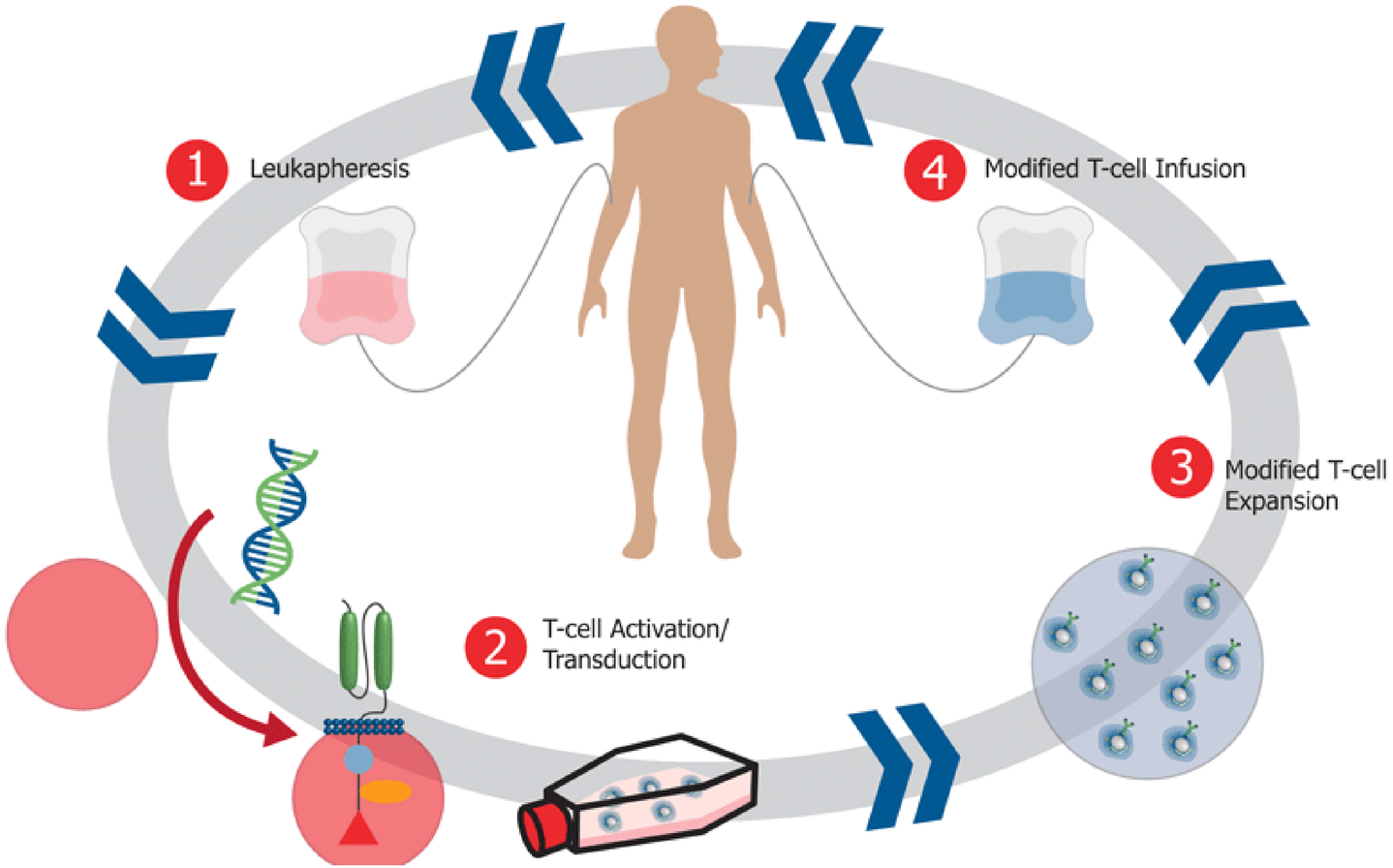
We are a **Biotech Manufacturing Organization** (BMO)

a biotech company that addresses Cell and Gene Therapy needs pro-actively offering one stop shop solutions and fostering exciting innovations

CAR-T manufacturing in a nutshell



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NEW INSIGHTS ON THE OPTIMIZATION OF THE CAR-T CELLS MANUFACTURING PROCESS

Marco Pupo, Serena Vella, Claudio Storini and Stefano Baila
Innovation and Development Department, Anemocyte Srl, Gerenzano, Italy.

INTRODUCTION

Over the last decades, **Advanced Therapy Medicinal Products (ATMPs)** have led a real revolution in medicine and healthcare, offering cures for many unmet medical needs. The translation of the research achievements from academia into clinical products requires several steps of development addressing **"manufacturability"** and **"sustainability"** of the product, balancing: *critical quality attributes, cost of goods, needle-to-needle logistic and scale.*

As a **Biotech Manufacturing Organization (BMO)**, we have been exposed to multiple ideas in need of process development and a multi-parameter approach was often required. Among the different parameters we have been evaluating, ***culture media and manufacturing platforms are often the most critical ones.***

Achieving Manufacturability and Managing COGs

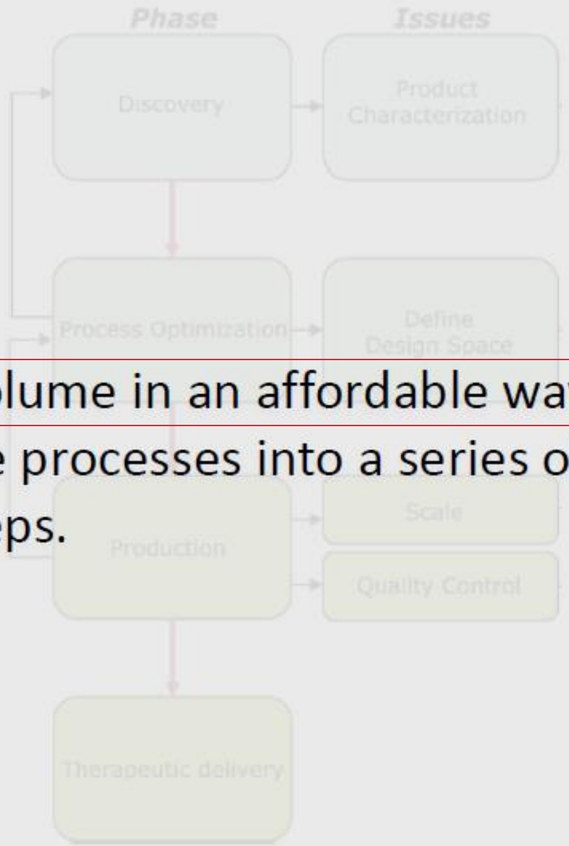
Understand your process

Eliminate skill-based processing steps

Integrate data flows

Manage cost of goods: critical quality attributes (CQAs) – they are not only release parameters, but also process control parameters that must be controlled throughout the manufacturing process

Transform the Manufacturing Process: Your process should be scalable and consist of closed-unit process operations in the lowest class clean space possible, selecting the appropriate technologies, processes, and equipment for manufacturing.



The vision is to ultimately treat patients at high volume in an affordable way, methods need to be identified to transform these processes into a series of repeatable, automated steps.

Planning for Commercial Scale of Cell Therapy and Regenerative Medicine Products, Part 1. Bioprocess international April 2015

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- Why BMO (Biotech Manufacturing Organization)
- BMO in action

I. Plasmid

I. Non-viral

II. Behind the Scene of I&D (Manufacturing and More)

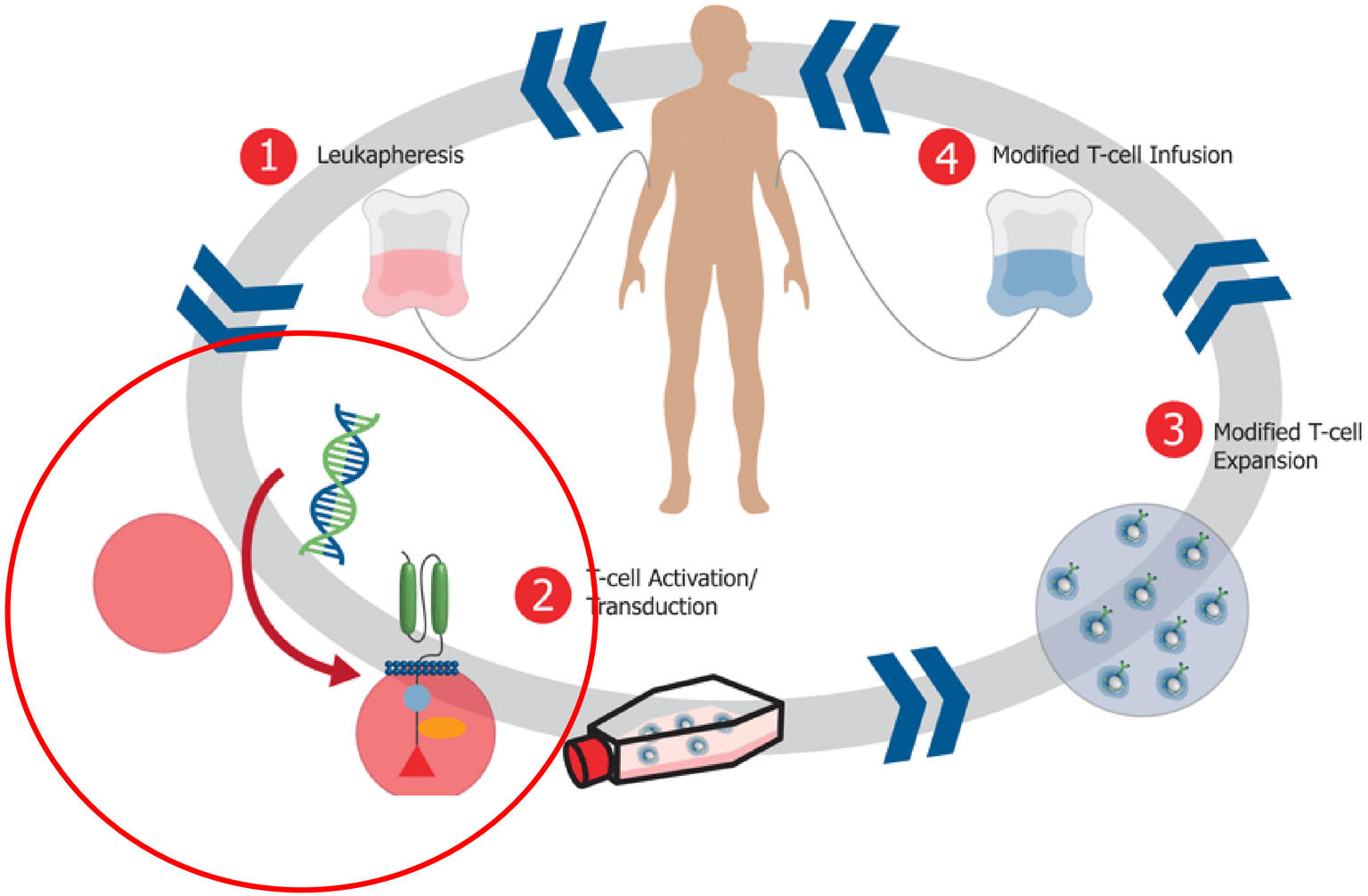
- Non-Core Activities

i. Logistics

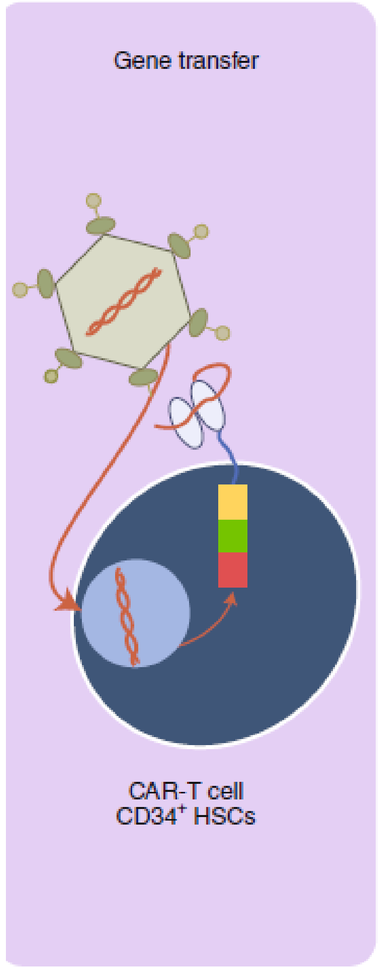
ii. Visual Inspection

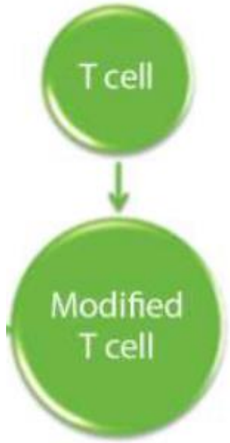
iii. BIG DATA

CAR-T manufacturing in a nutshell – Editing

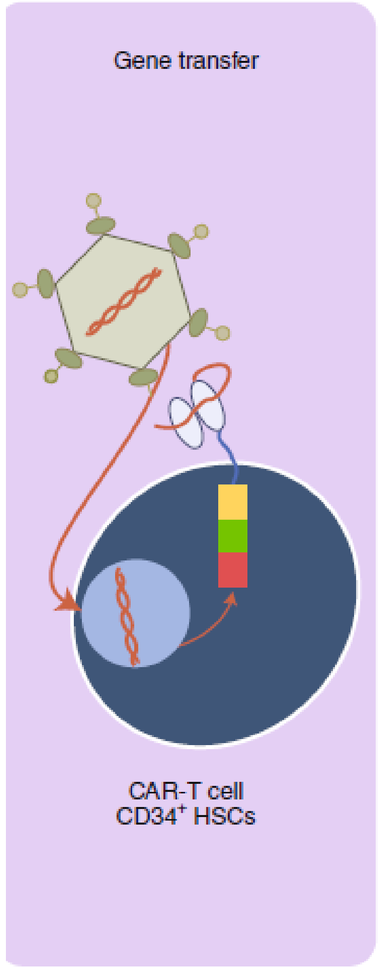


Most Common Manufacturing Solution





Plasmids - Building Blocks for Viral Vectors

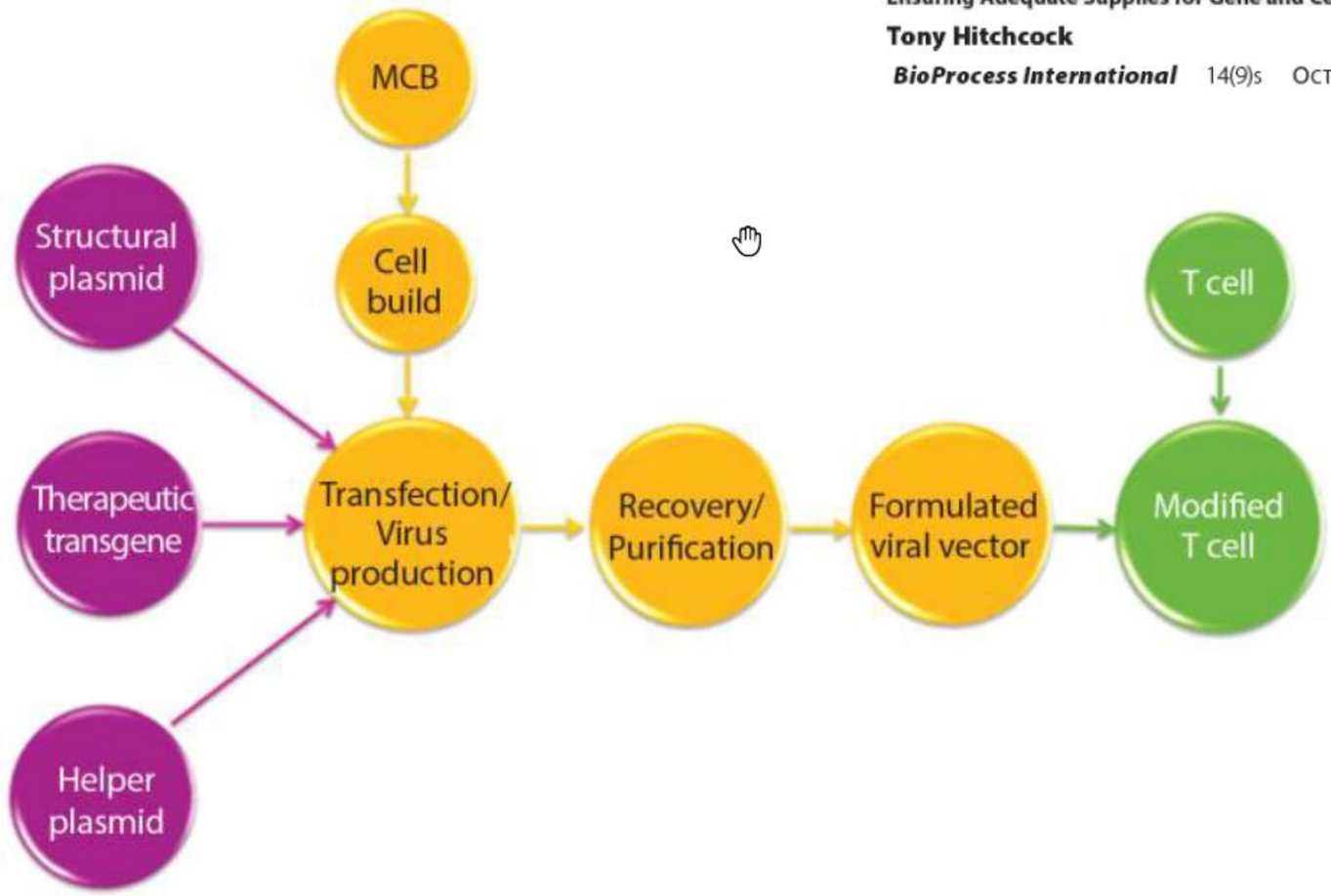


Manufacturing Plasmid DNA

Ensuring Adequate Supplies for Gene and Cell Therapies

Tony Hitchcock

BioProcess International 14(9)s OCTOBER 2016



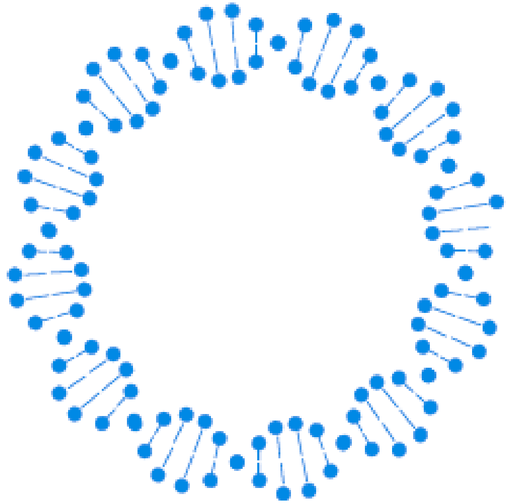
Plasmids – High Demand

Manufacturing Plasmid DNA

Ensuring Adequate Supplies for Gene and Cell Therapies

Tony Hitchcock *BioProcess International* 14(9)s October 2016

I do not doubt that the need to manufacture greater amounts of plasmid DNA will increase. To meet rising demand, production platforms will need to be scaled up significantly. It may be possible to meet the demands for niche therapies of <10,000 patients with small-scale production platforms making <10g/batch. But a reasonable prediction is that 100–1,000 g/year will be required for each plasmid vector for a marketed product.



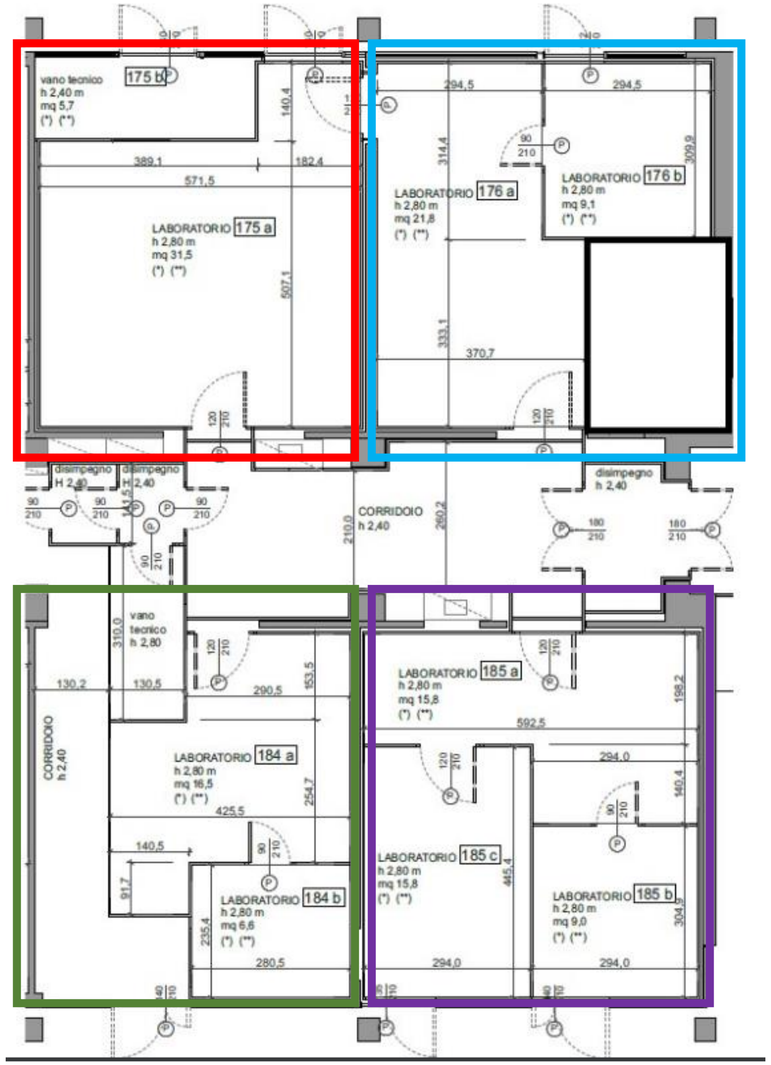
Plasmid Manufacturing - Classified Plant

Upstream

Up to 8 batches/month

5-200L (higher volume optional)

Final Filling & Buffers



Downstream

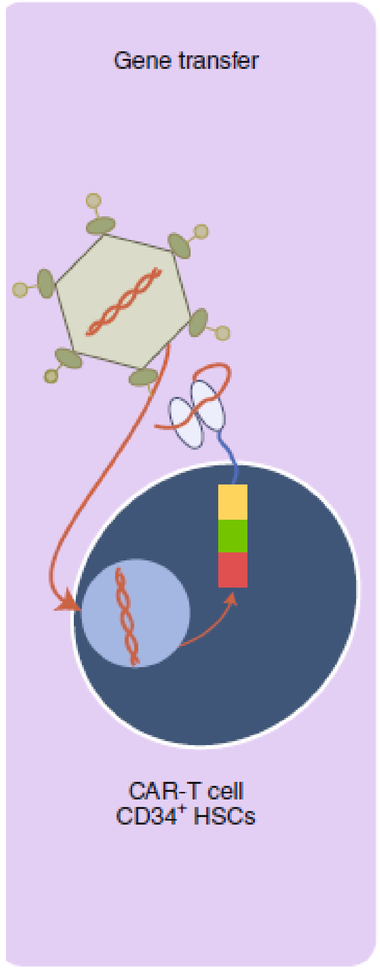
Two segregated areas

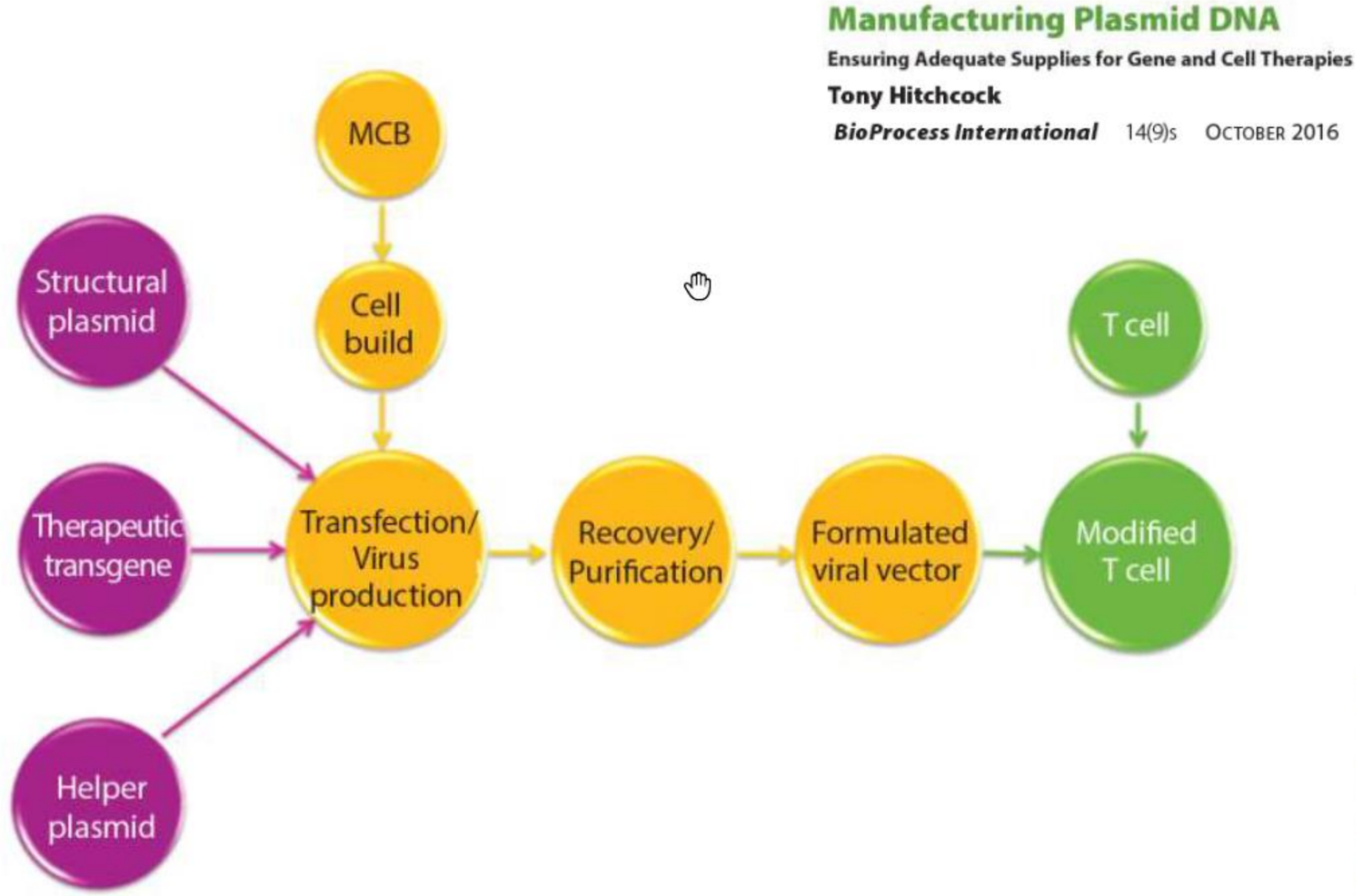
- Alkaline lysis
- RNA removal by the addition of $CaCl_2$
- Capture and Polishing by monolithic columns
- Final formulation by TFF in TE buffer (or customer choice)

Quality Control

(more details upon request)

Viral Vectors





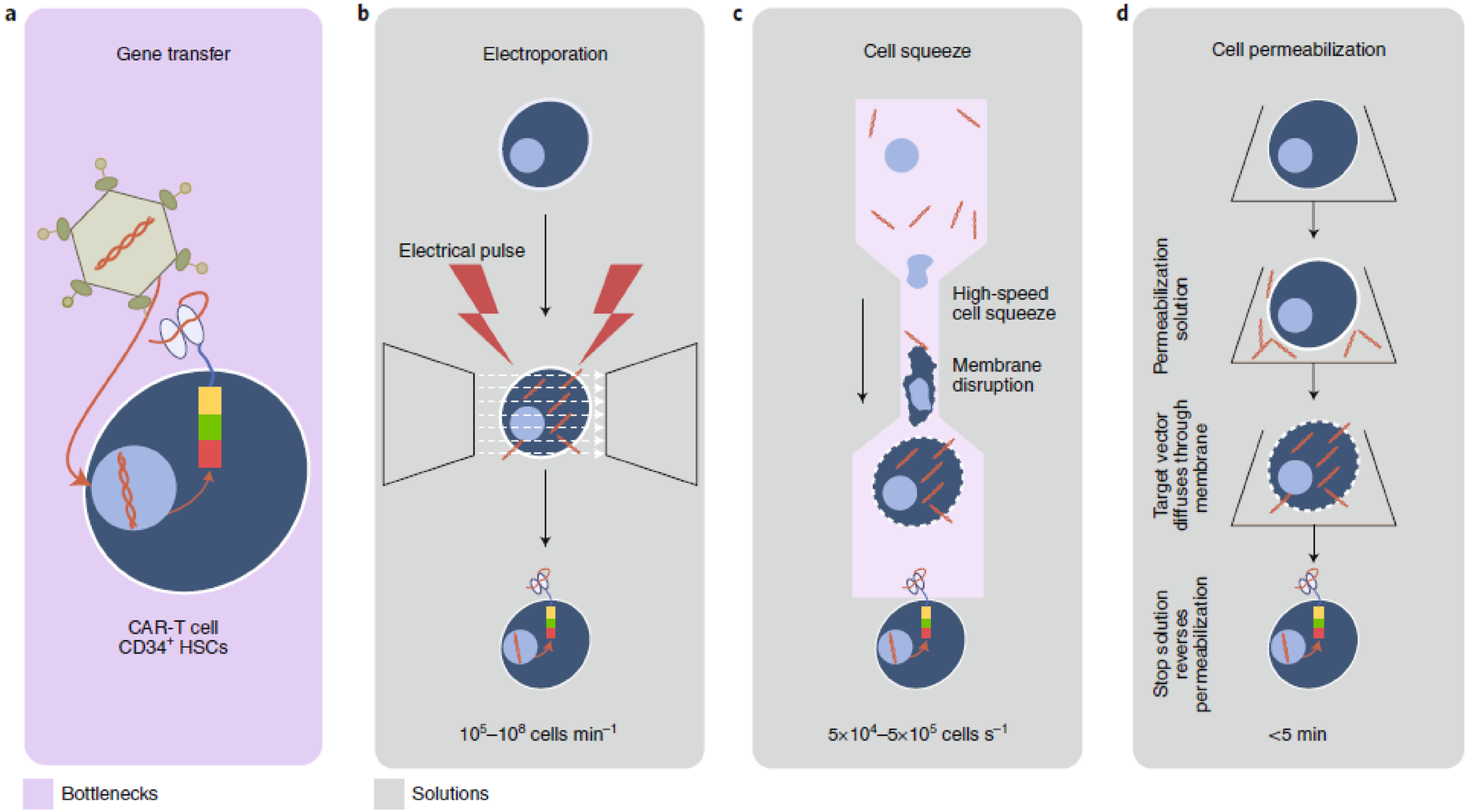
The New York Times

Gene Therapy Hits a Peculiar Roadblock: A Virus Shortage

By GINA KOLATA NOV. 27, 2017

Other gene therapy companies are not always able to afford the manufacturing costs or find a manufacturer. Some have taken to buying slots in virus production queues years in advance — like buying a nonrefundable airline ticket long before your vacation and hoping you can get away when the time comes.

Genetic Modification for Cell Therapy



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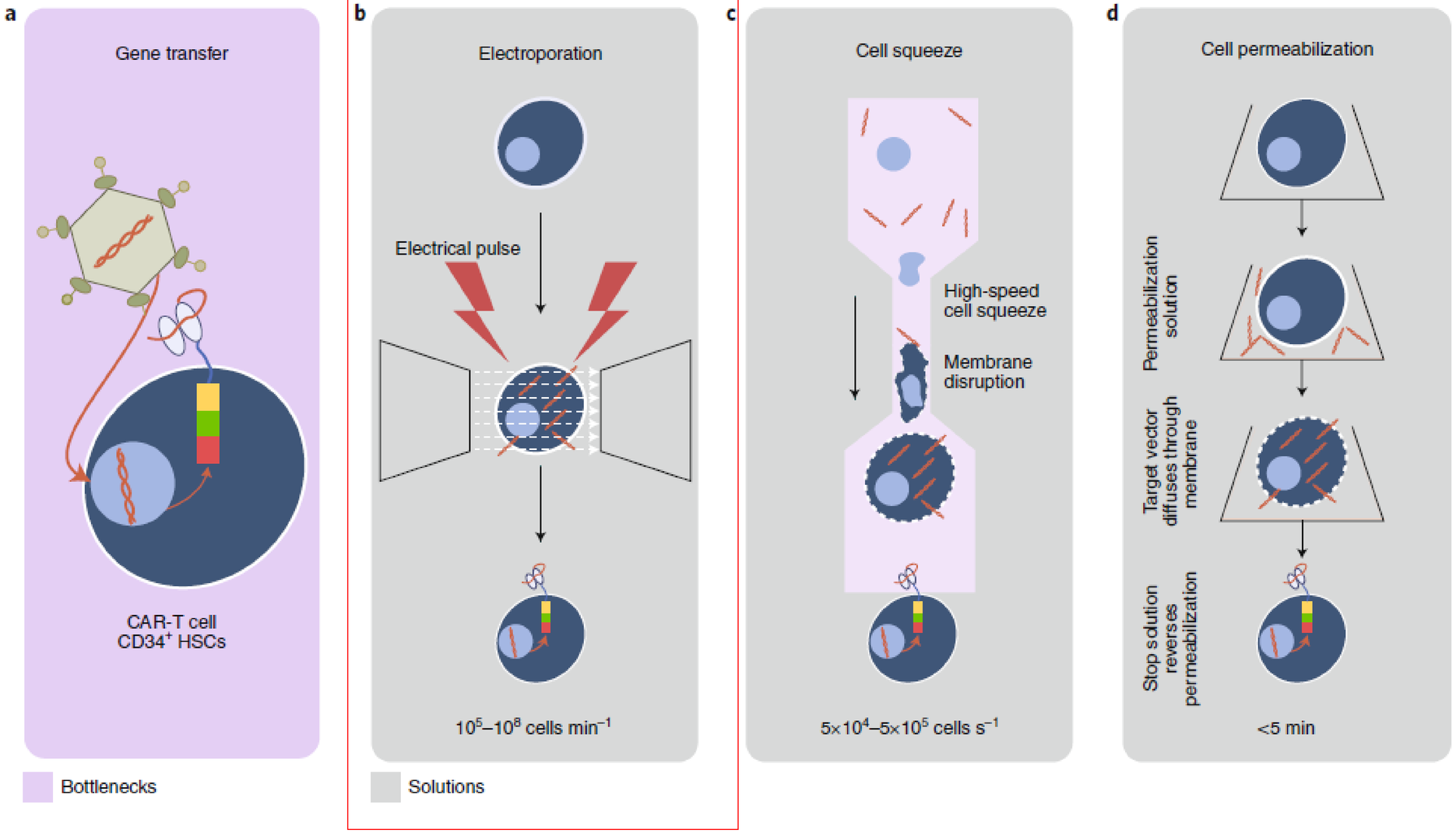
Genetic Modification for Cell Therapy



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Manufacturing Highlight - MaxCyte®

First center of excellence for the **MaxCyte Technology** applied to non -viral gene modification.

Working with **Anemocyte** allows therapy developers to perform a licence free evaluation and process optimization.

Manufacturing Highlight - MaxCyte®

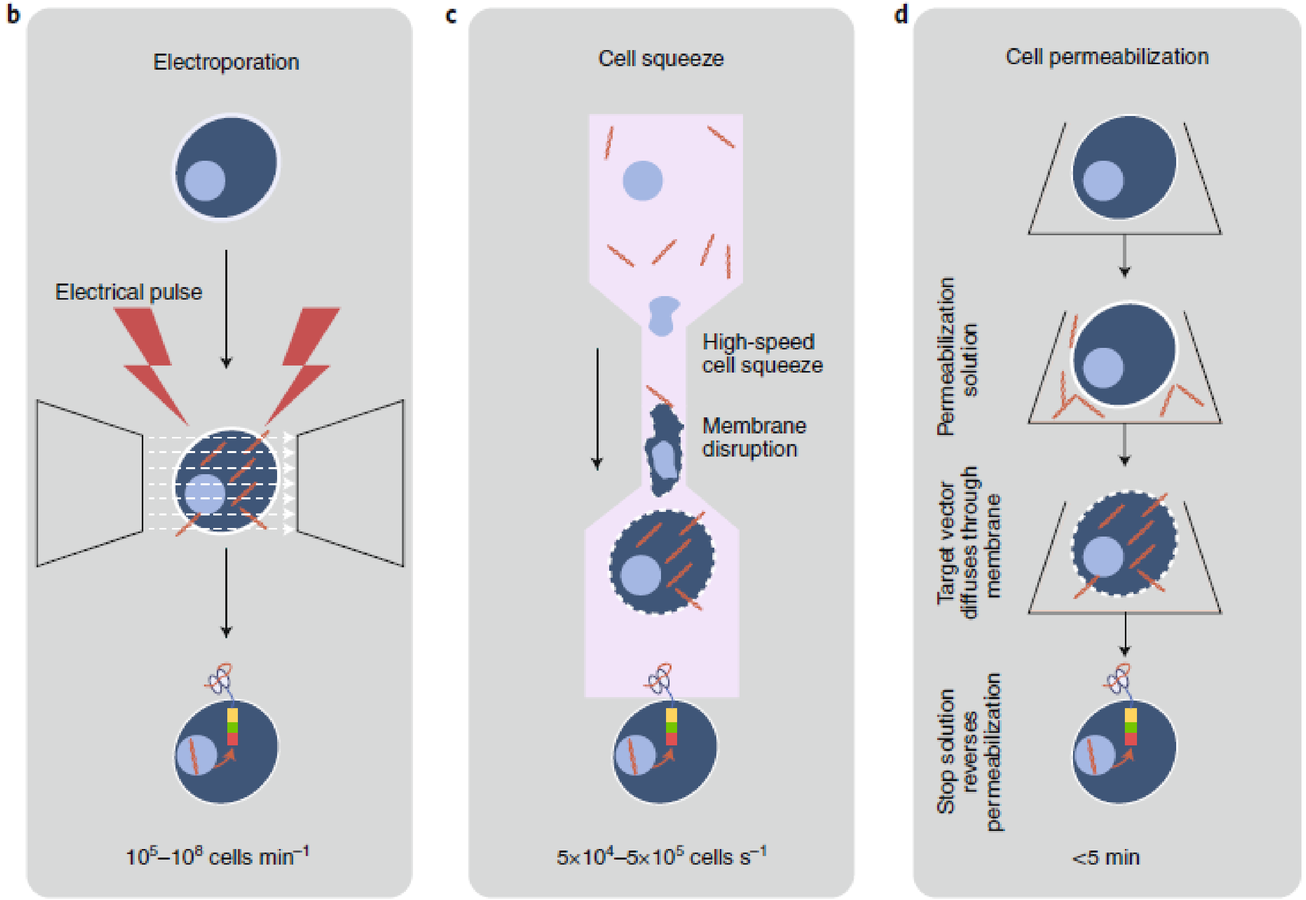
Gene Editing

Plasmids

Transposons

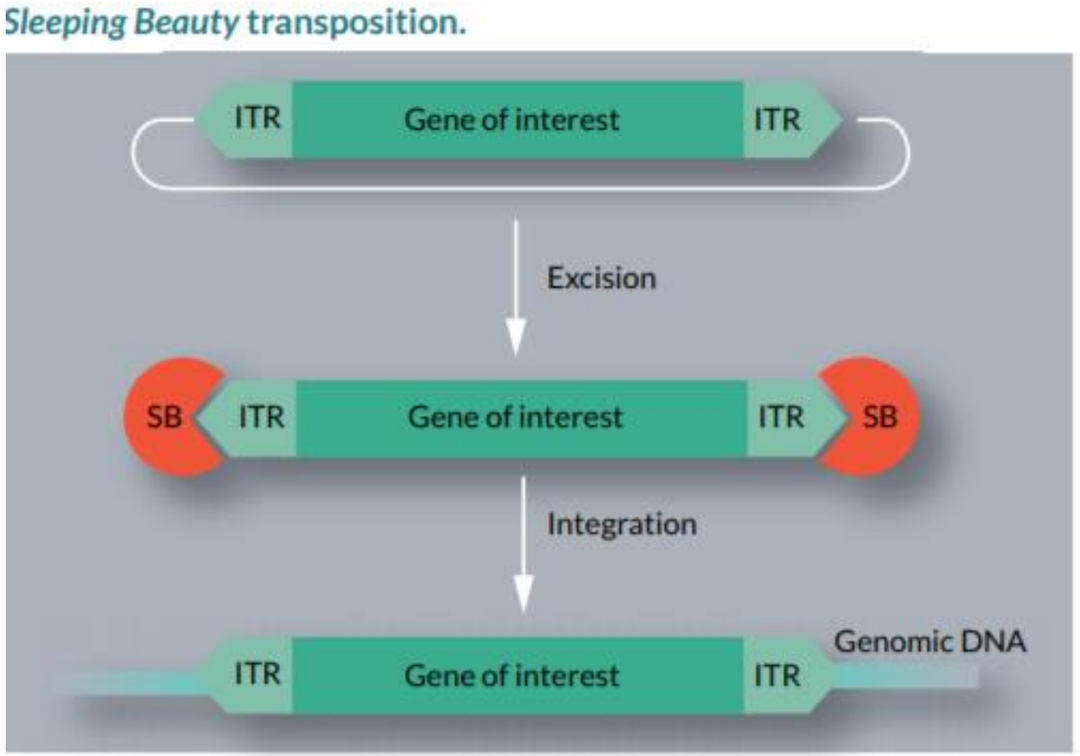
RNA

Non-Viral Gene Transfer for Cell Therapy



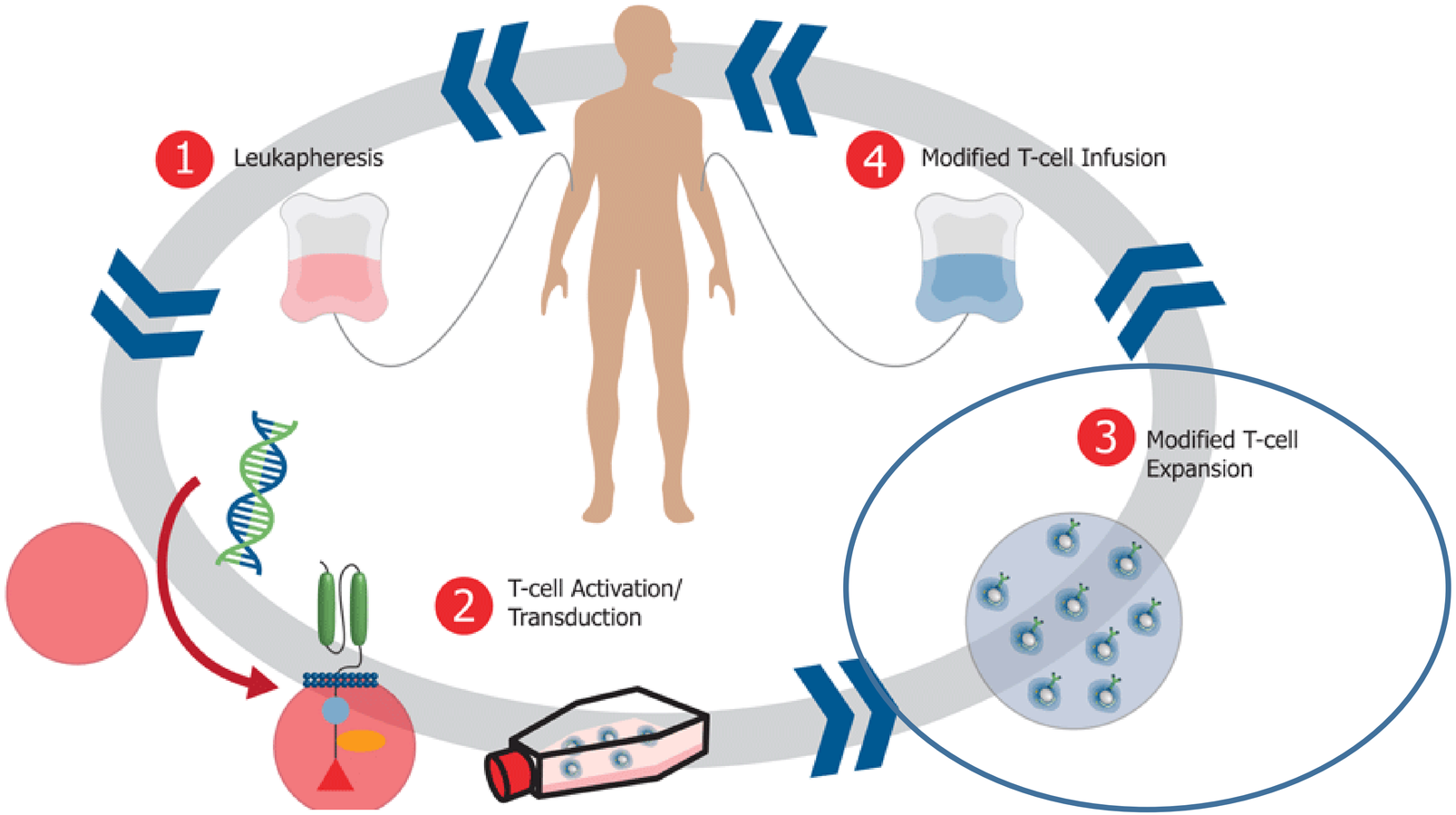
Non-viral do not lead to DNA integration of the gene of interest into the host genome.
This may compromise long-term gene expression due to degradation of the non-integrated episomes and/or dilution upon cell proliferation.

DNA Transposon



DNA transposons translocate via a **"non-replicative mechanism,"** whereby two **Terminal Inverted Repeats** (TIRs) are recognized and cleaved by a **transposase enzyme**, releasing the cognate DNA transposons with free DNA ends. The excised DNA transposons then integrate into a new genomic region where target sites are recognized and cut by the same transposase. This cut-and-paste mechanism usually duplicates DNA target sites upon insertion, leaving target site duplications (TSDs).

CAR-T manufacturing in a nutshell



T cells manufacturing platforms

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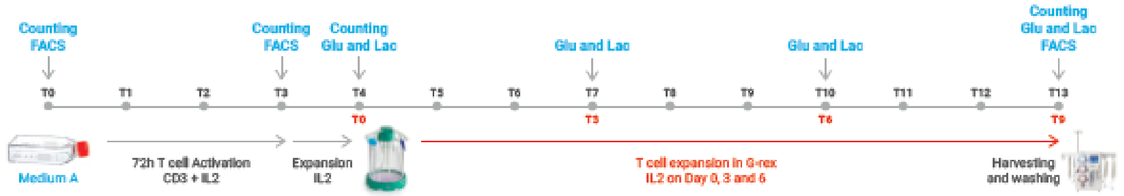
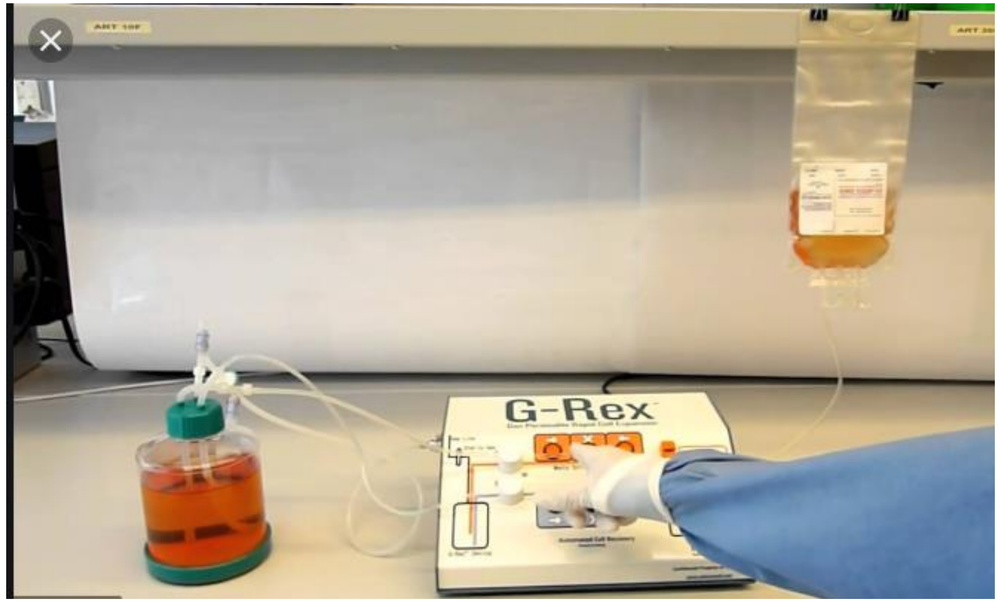
SCALING-UP AND AUTOMATING THE MANUFACTURING PROCESS



T CELL ACTIVATION AND EXPANSION



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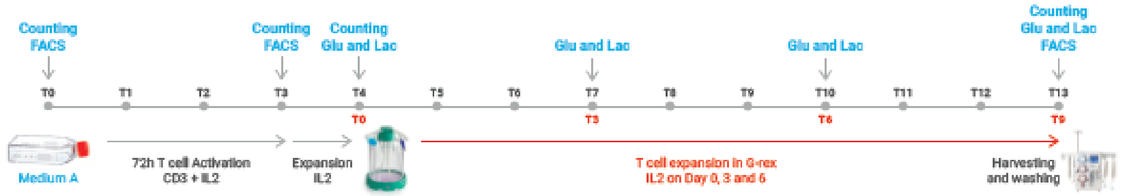
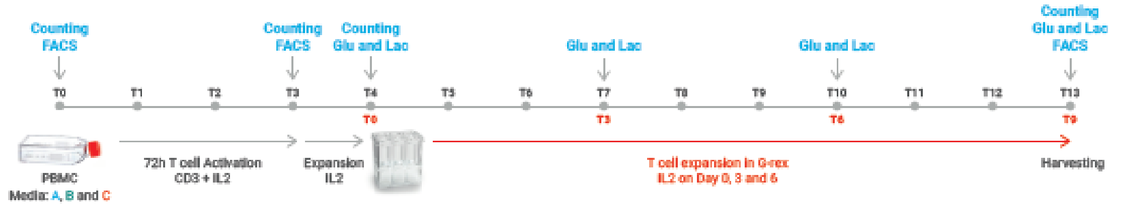
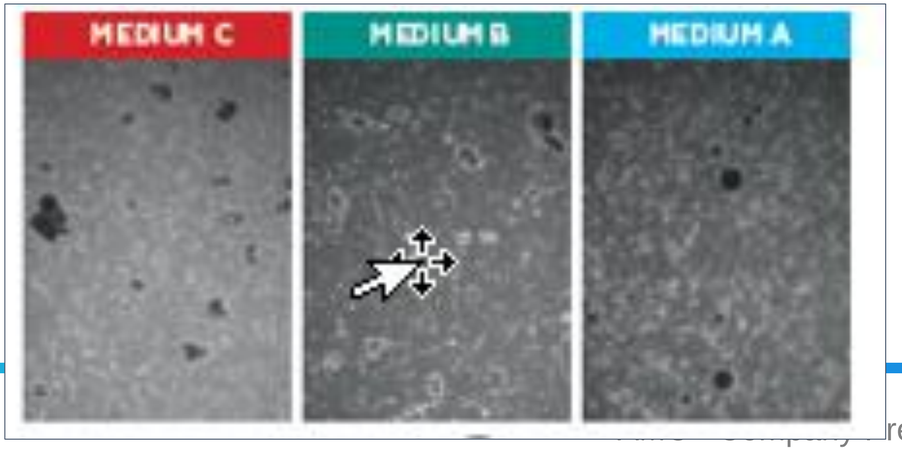


T CELL ACTIVATION AND EXPANSION

CHOOSING THE RIGHT CULTURE MEDIUM



T CELL ACTIVATION AND EXPANSION IN DIFFERENT GMP-GRADE MEDIA



Choosing the right media

Comparing Media

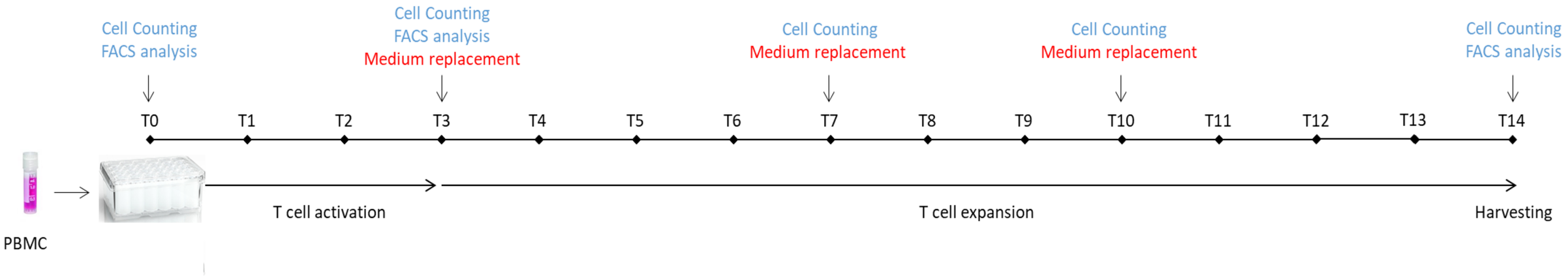
Xeno- and Serum free Medium

PRIME-XV T CELL CDM

GREX – Process Development

G-REX - 24 well plate
Individual well specification 2 cm2 gas permeable membrane
8 ml media capacity
Recommended seeding 1M / well

Comparing Media – Protocol



PBMC were obtained from buffy-coats of three healthy donors through density gradient separation (Ficoll)

Culture Condition

- **Media A - Xeno and Serum free media**

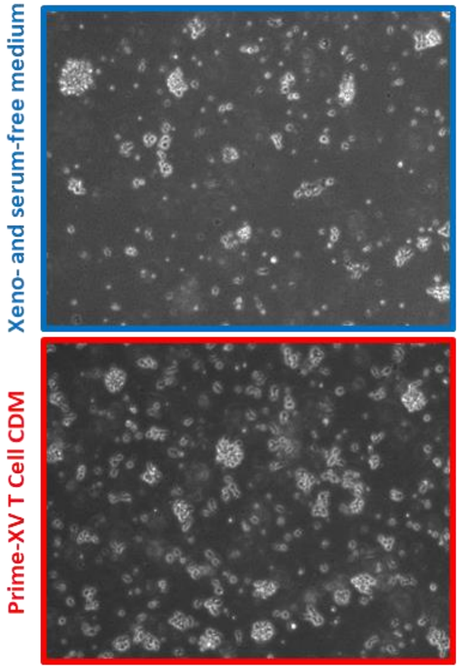
Or

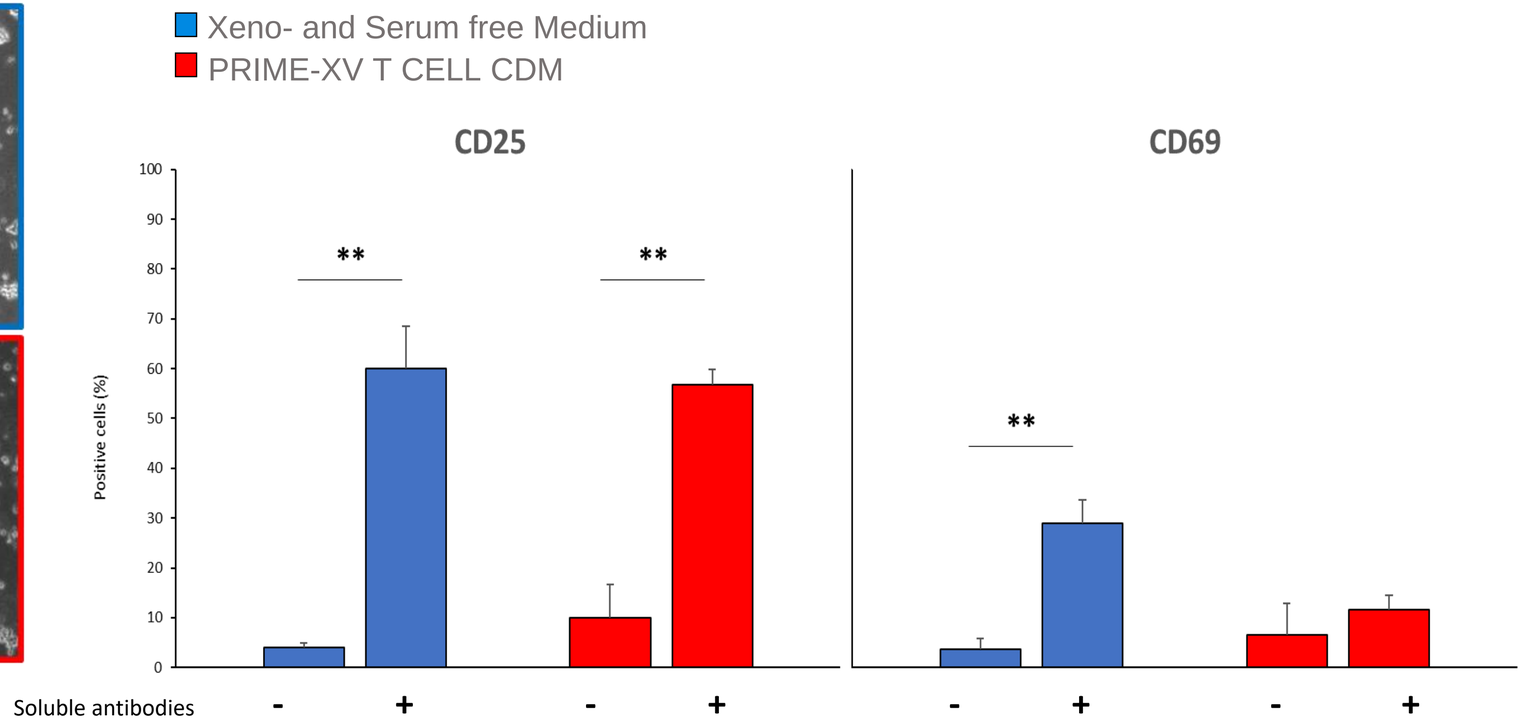
- **PRIME-XV T cell CDM (Xeno/human free + Chemical Defined)**

IL2: 300UI/ml

Cell seeding: 1 x10^6 cells (density: 0.5x10^6 cells/cm^2)

Results: T cell Activation (T3)



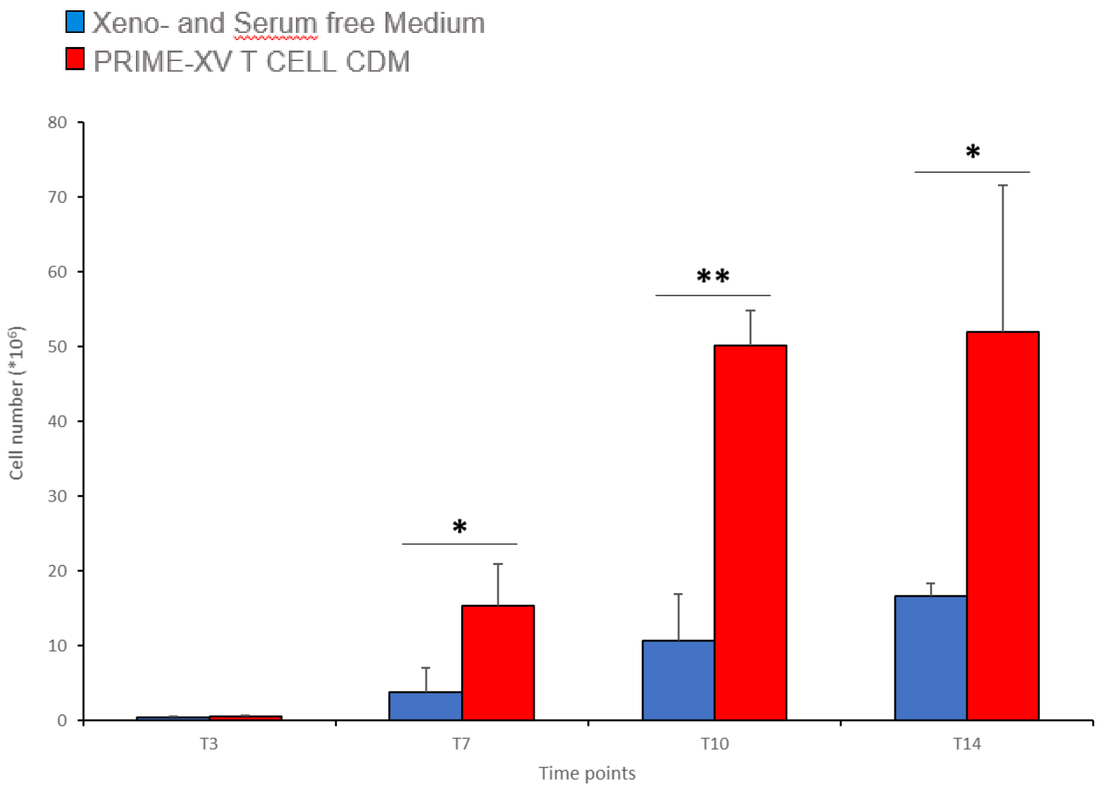


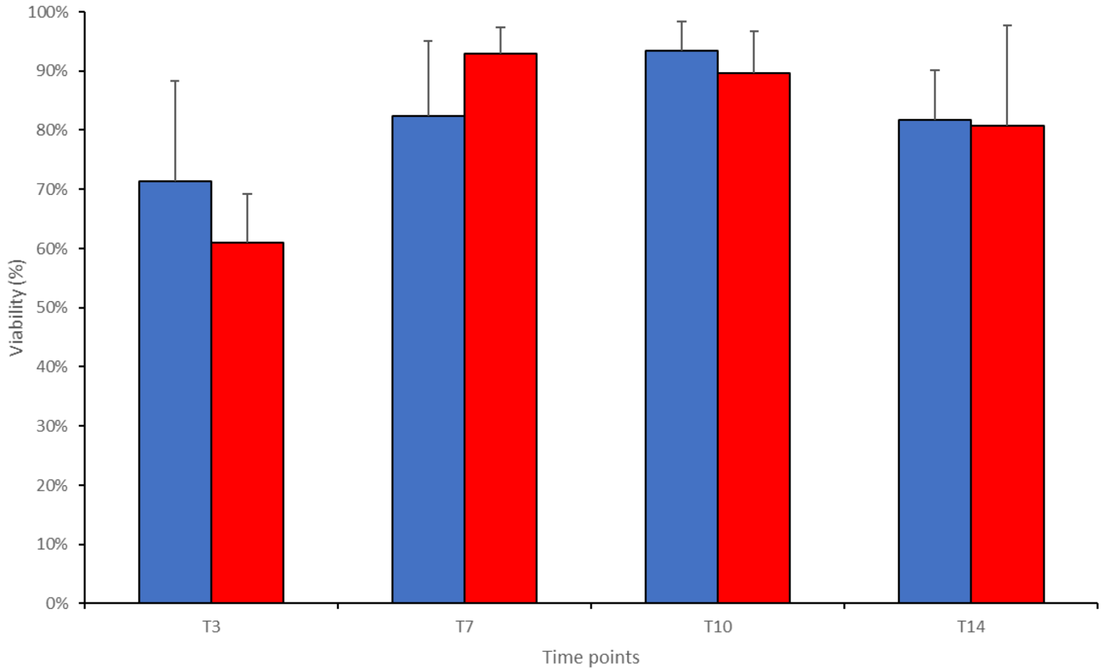
** p value ≤ 0.01

Results – successful activation with both media
(PRIME media seems a little more efficient)

Results: Cell number and viability

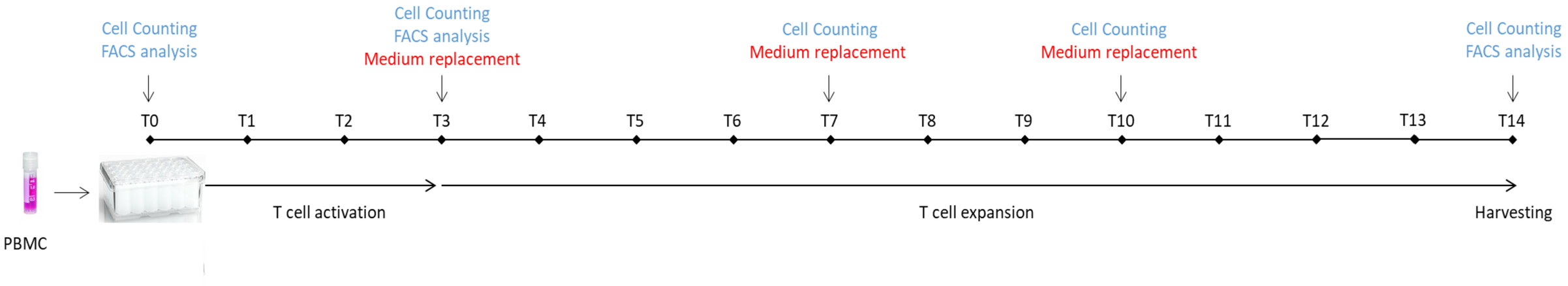
* p value ≤ 0.05
** p value ≤ 0.01





Results – Prime sustains higher expansion rate (2 to 5 folds) than control media while keeping comparable viability

Protocol: Exploring IL2 and Seeding



PBMC were obtained from buffy-coats of three healthy donors through density gradient separation (Ficoll)

Culture Condition

- Media A - Xeno and Serum free media

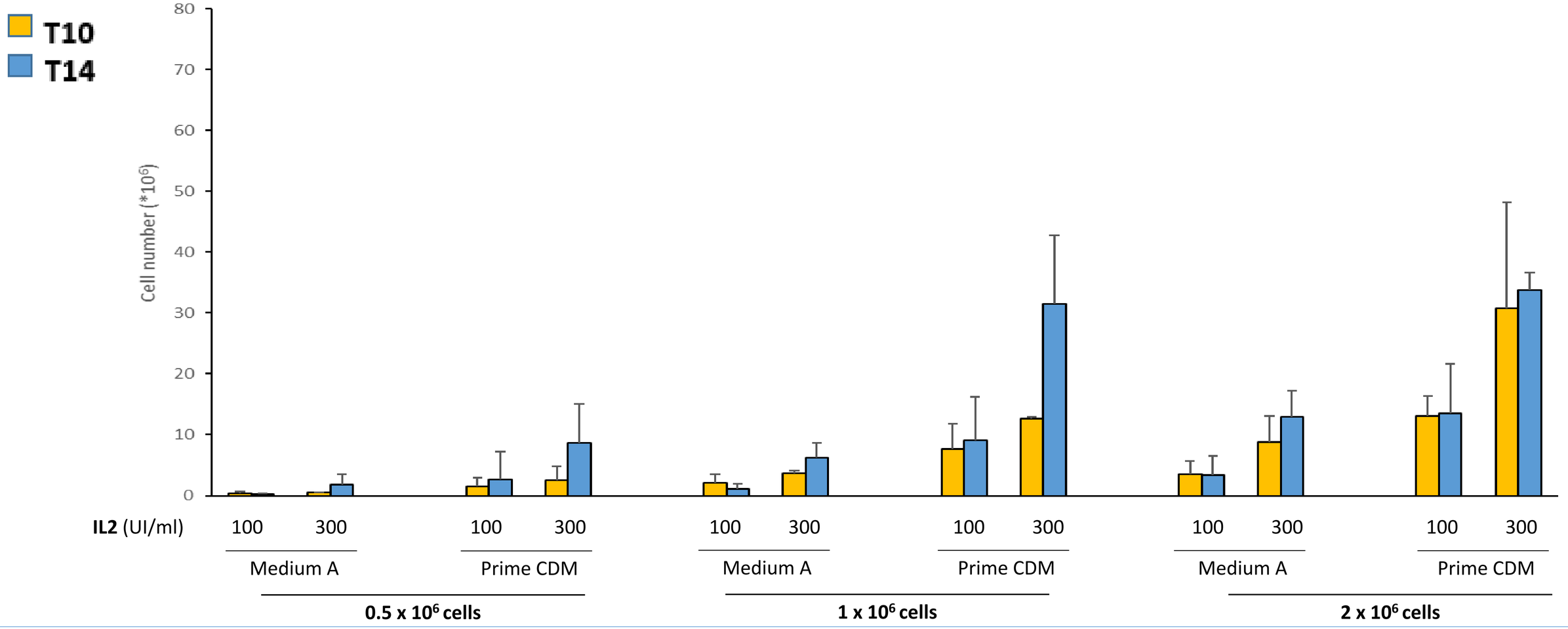
Or

- PRIME-XV T cell CDM (Xeno/human free + Chemical Defined)

IL2: 100UI/ml or 300UI/ml

Cell seeding: 0,5 – 1- 2 x10^6 cells (density: 0,25 - 0.5 - 1x10^6 cells/cm^2)

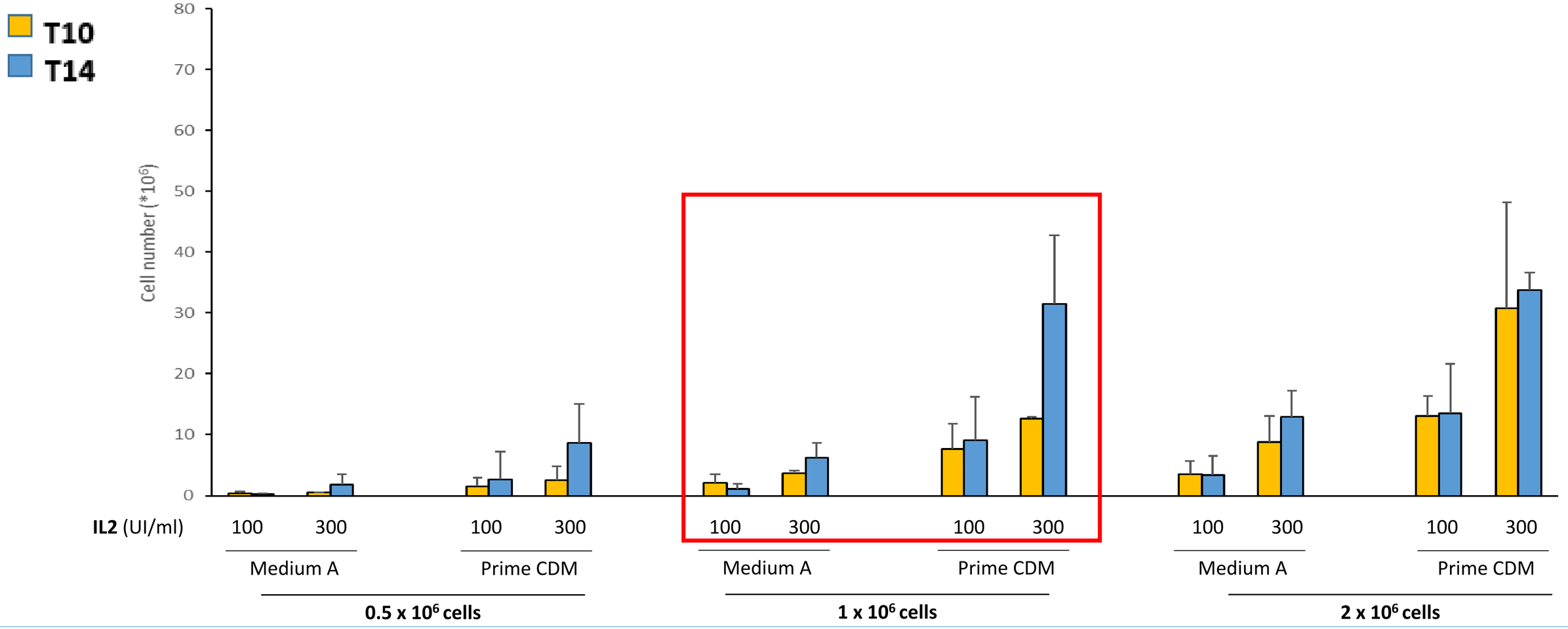
Exploring IL2 and Seeding



Results – IL2 at 100 IU limits expansion capabilities

PRIME CDM allows higher expansion potential at specific condition

Exploring IL2 and Seeding



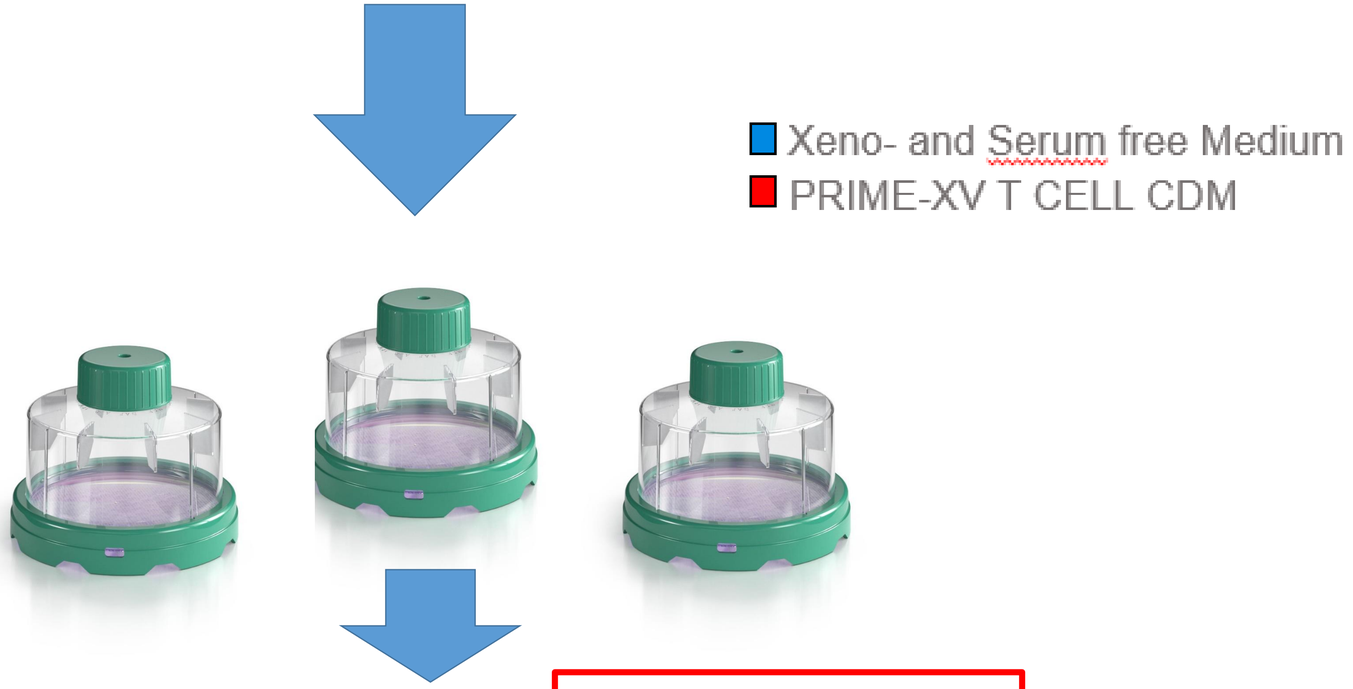
Results – IL2 at 100 IU limits expansion capabilities

PRIME CDM allows higher expansion potential at specific condition

Exploring Expansion potential

Buffy coat

600M (range 300-800) PBMCs in 50 ml

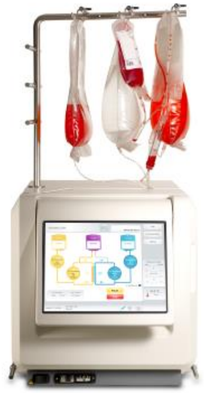


3X10^9 T-CELLS

9X10^9 T-CELLS
or
3X10^9 T-CELLS
saving 60%IL2

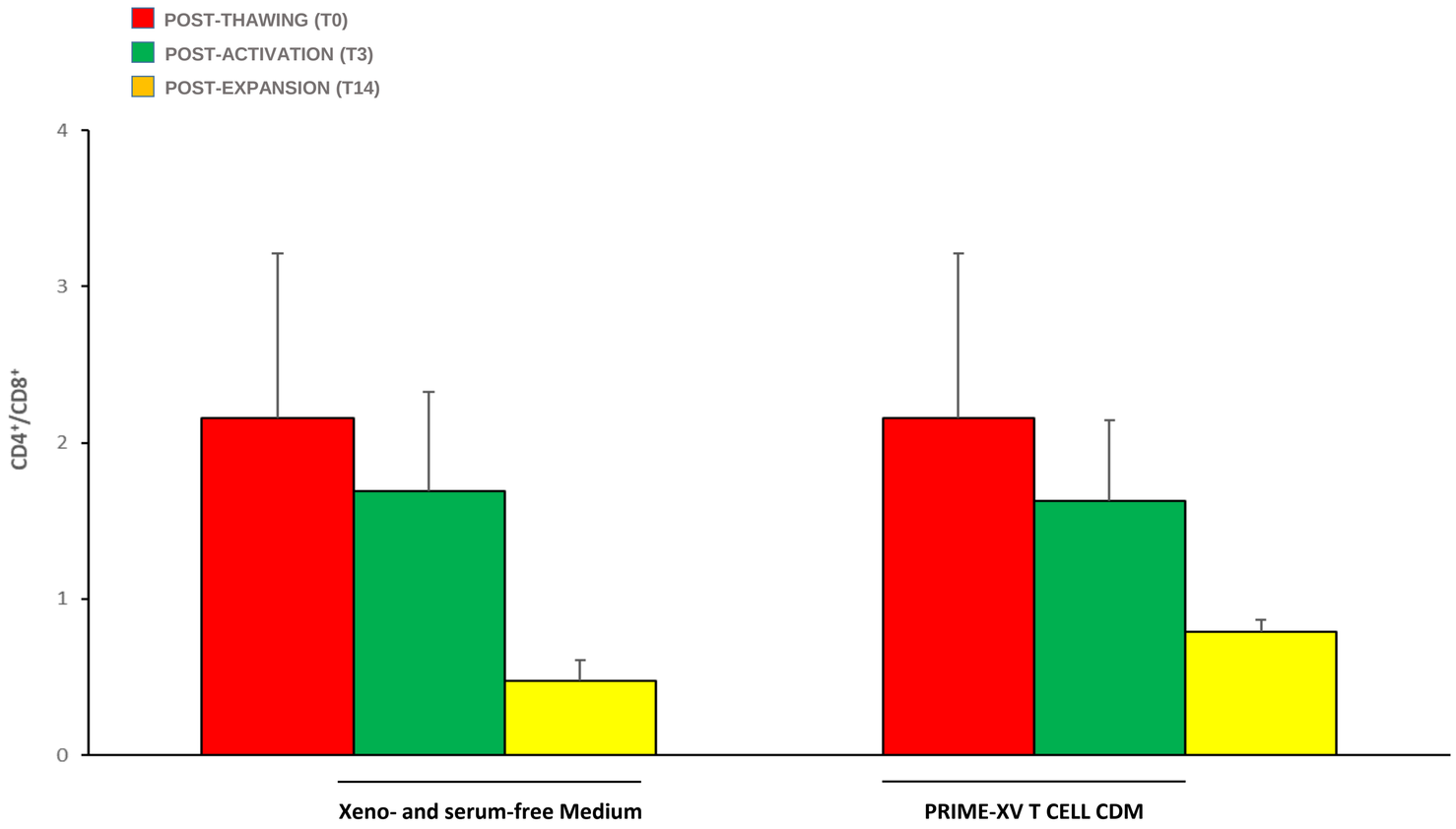
What is next

- Characterization of the obtained cells
- Scale Up

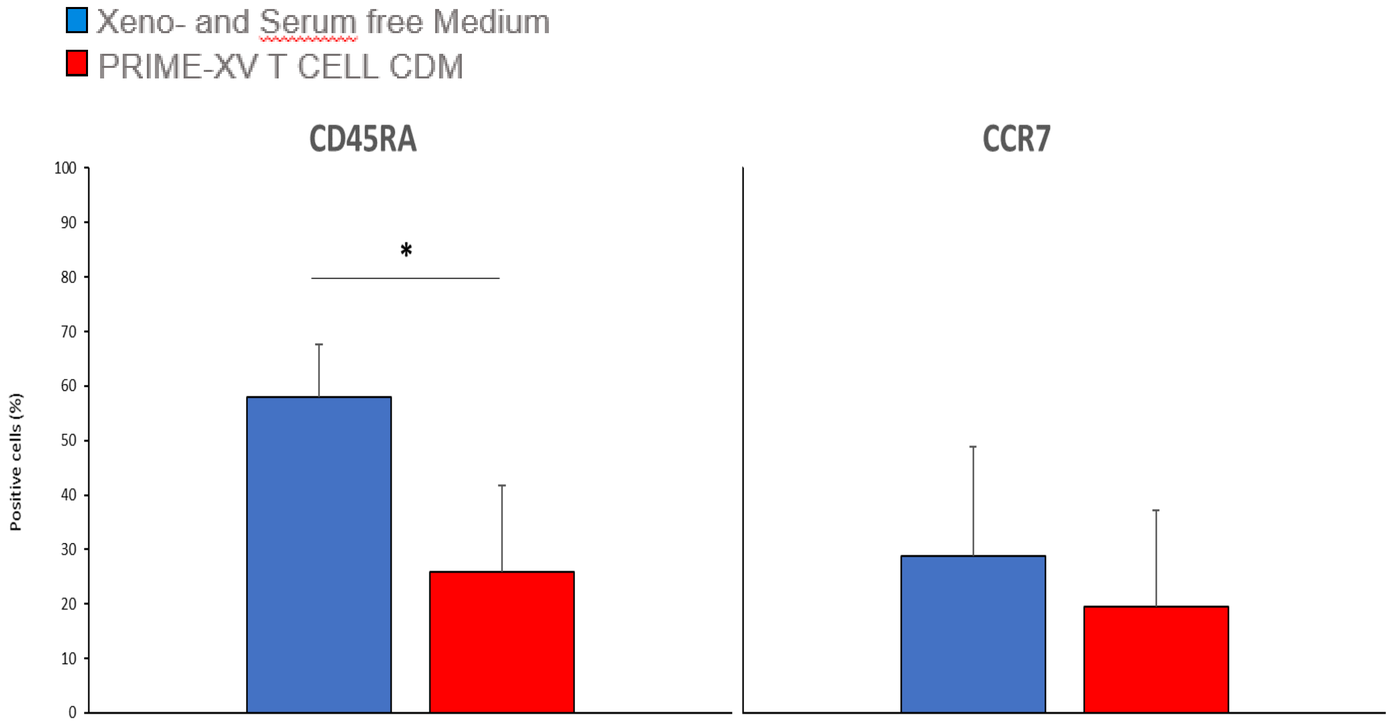


- A CAR-T process

Preliminary info: CD4/CD8 after expansion (T14)



Preliminary info : CD45RA and CCR7 after expansion (T14)



*** p value ≤ 0.05**

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- CAR-T focused cases

i. Plasmid
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iii. Behind the Scene of I&D (Manufacturing and More)

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Non-Core Collaborations

Advanced Logistic for cell and gene therapy
In collaboration with RPS Aerospace

brevetti cea
INSPECTION MACHINES

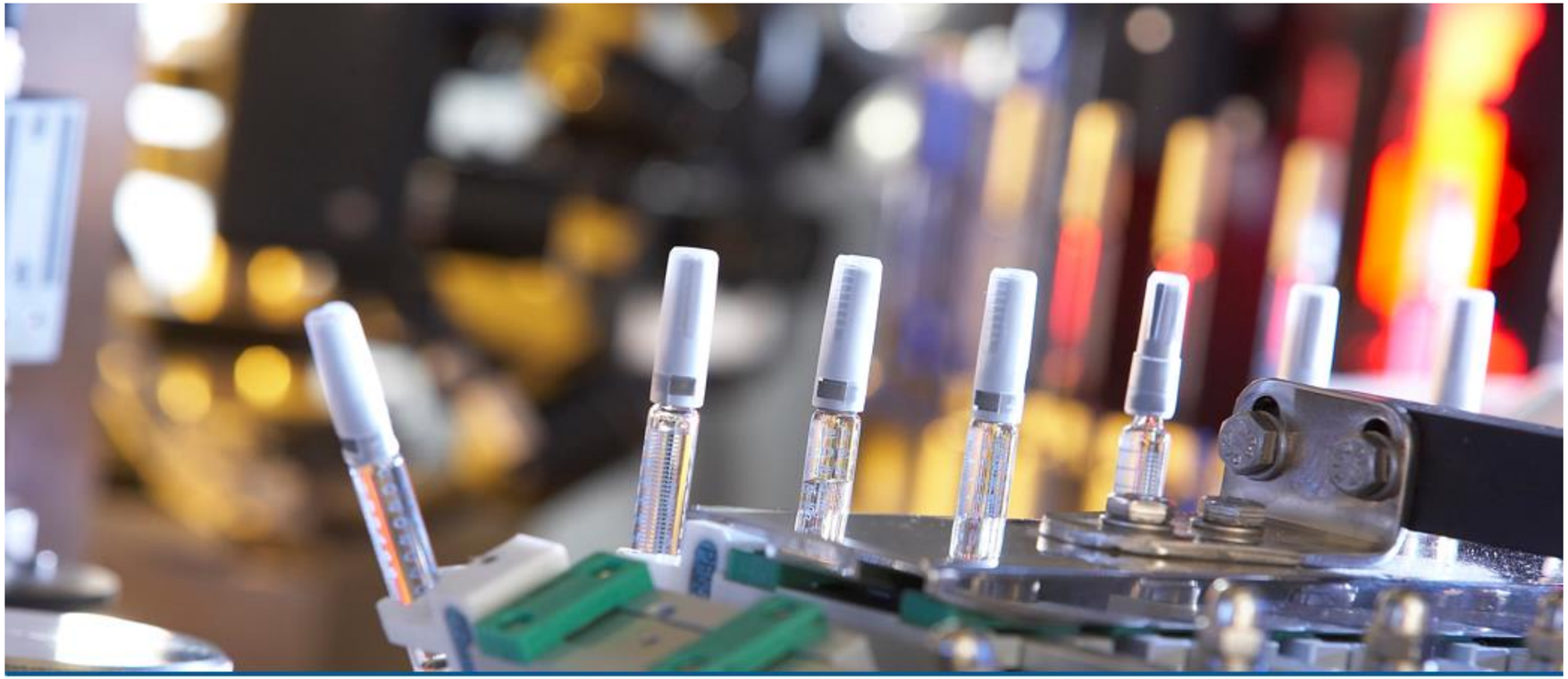
Visual Inspection applied to cell and gene therapy
In collaboration with brevetti cea

ANEMOCYTE
Talent for Life
BIG DATA APPLIED TO HEALTHCARE

Big Data applied to cell and gene therapy
Internal project

FLY N’ICE

Advanced Logistic for cell and gene therapy
In collaboration with RPS Aerospace

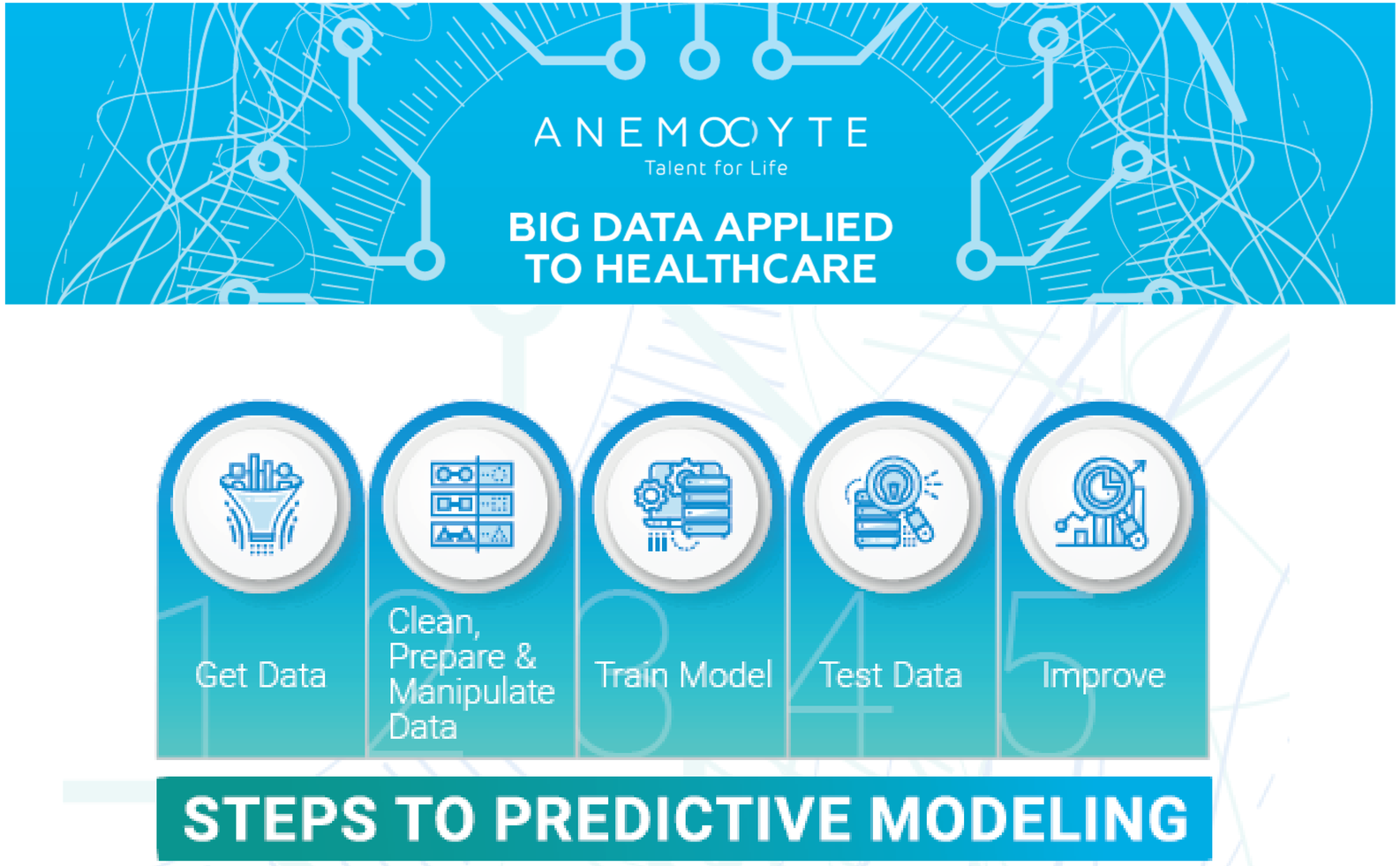


Mastering inspection technologies

brevetti cea
INSPECTION MACHINES

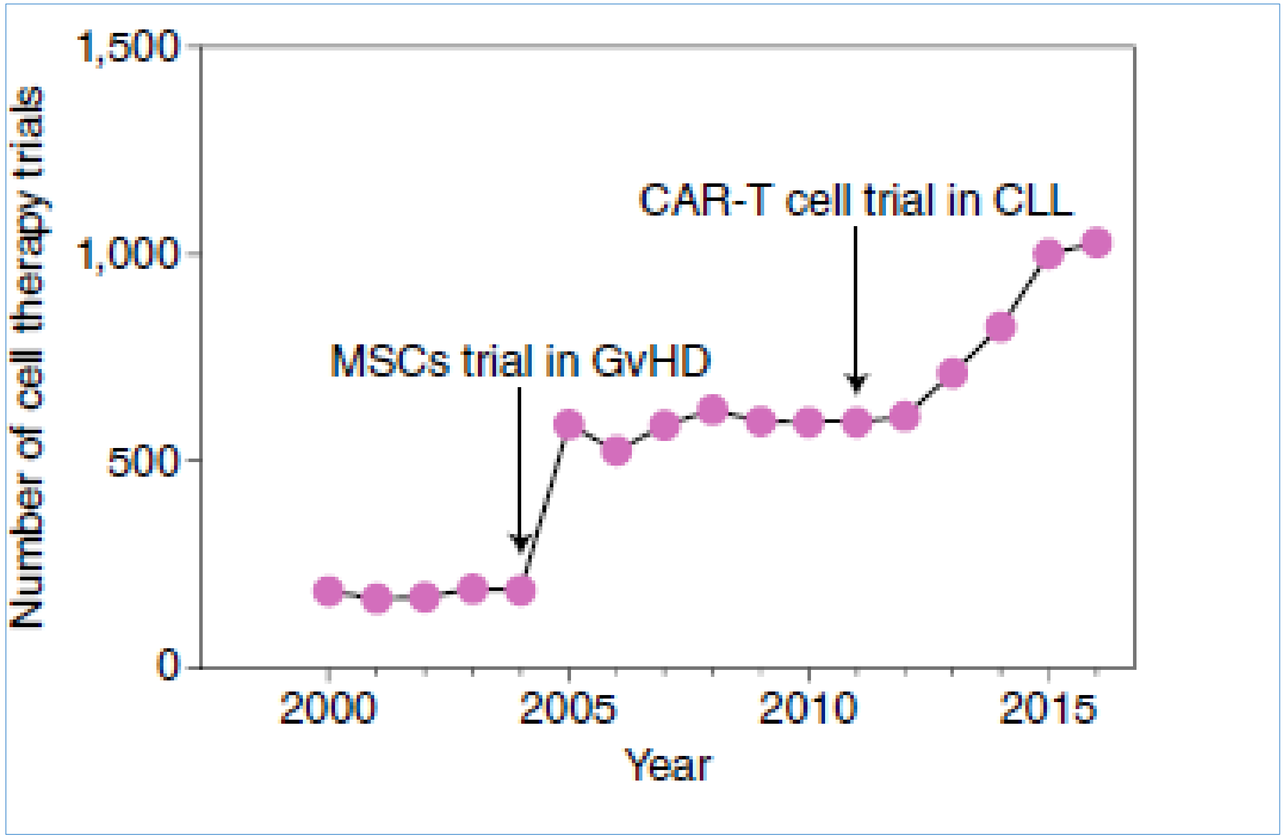
www.brevetti-cea.com

Big Data = Create a Predictive Modeling



Conclusions

ATMP trials overview



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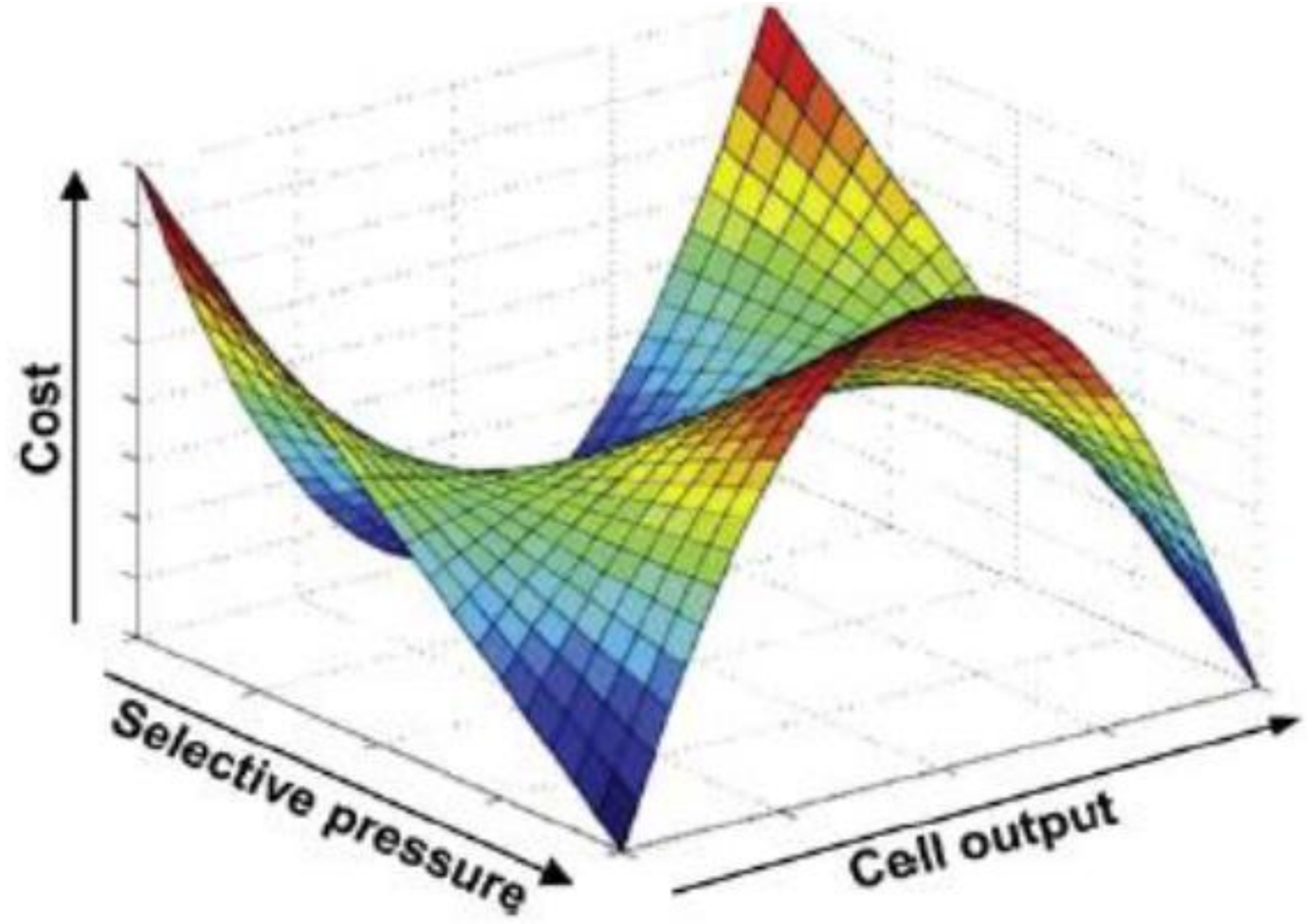
Find both process and product “sweet spot”



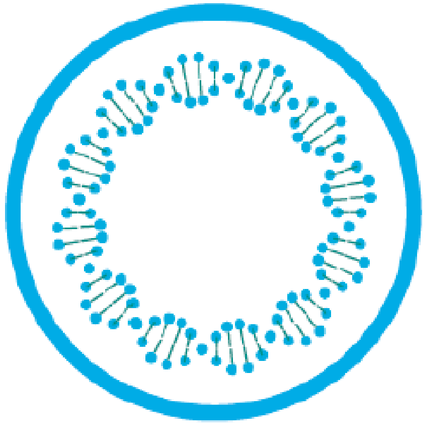
The Systematic Production of Cells for Cell Therapies - Cell Stem Cell 3, October 9, 2008

Our Core Business – Development and Manufacturing

Cell Therapy (Autologous and Allogenic)

Non-Viral gene modified Cells

Extracellular Vesicles



Plasmid DNA manufacturing
for viral vector production

Grade: GMP or High-Quality

excellula project

GMP

Custom GMP Spaces

Custom GMP spaces to address up to commercial needs.

Scale: up to Commercial

ANEMOCYTE

Talent for Life

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