CELL AND GENE THERAPY: TECHNOLOGIES, MANUFACTURING EQUIPMENT AND DESIGN CONSIDERATIONS

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Cell and Gene Therapies

01/10/2023

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SAFETY TOPIC

- Risks observed during clinical trials of cell therapies:
  - Neurotoxicity from CAR-T has been observed
  - CAR-T lot-specific differences in Interferon-Gamma results in mild-to-severe toxicity
  - Tumor growth due to stem cell progenitor spill-over in transplanted graft tissue
  - Potential carcinogenic mutations in CAR-T

CAR-T: Chimeric Antigen Receptor

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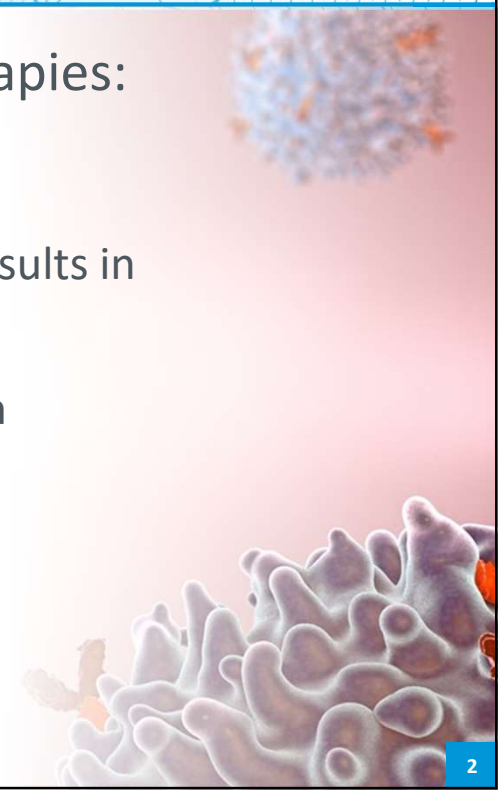

SAFETY TOPIC

- Account for and mitigate risks prior to suspension of pipeline by regulatory authorities:
  - Incorporate new assays to detect toxicity and profile critical genetic signatures during clinical phases
  - Expect lot-specific differences: Design-Of-Experiment for early dose escalation, prevent high efficacy doses whose toxicity can potentially put a permanent stop on the pipeline
  - Creative technologies for detecting and depleting tumorigenic progenitors from cell culture

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AGENDA
Cell-Gene Technology & Commercial Manufacturing
US and Global Cell & Gene Therapy Market
Cell & Gene Biotherapeutic Approaches
Examples of Emerging Therapies
Commercial Manufacturing Processes
Design Considerations
Cell Therapy Facility Components
Estimation, Design & Consultation
Case study for Autologous CAR-T
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DEFINITIONS

- *In Vivo*: In the Body
- *In Vitro*: In the Lab
- Carcinogenic: causing cancer
- iPSCs: induced pluripotent stem cells
- Progenitor: originating cell-type
- Autologous: cell therapy derived from the patient's own cells
- Allogeneic: cell therapy derived from a donor's cells

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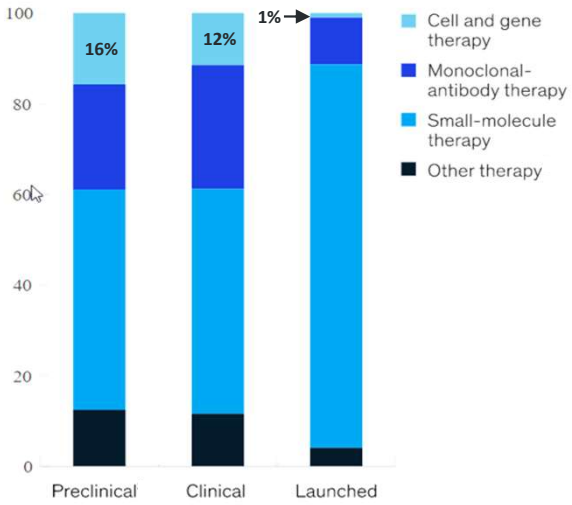
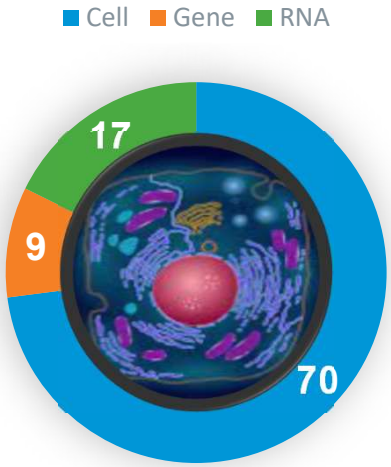
CELL-GENE THERAPY (CGT) MARKET OVERVIEW

CGT Share in Pharma



Global CGT Launches



>750 Trials of **CGTs**

3,633 in development

> 30,000 patients enrolled

- Forecasted growth from 2021 to 2031:
  - 11-fold higher global sales to $55 million annually

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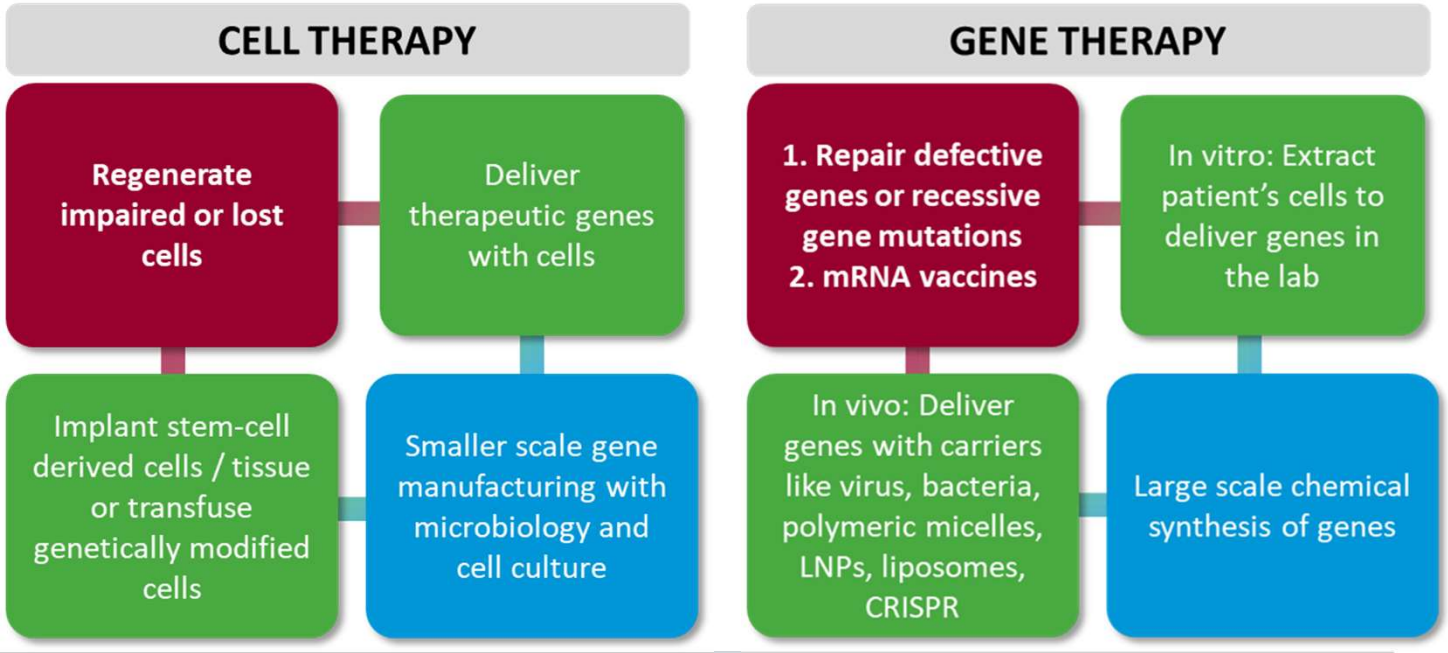
CELL & GENE BIOTHERAPEUTIC APPROACHES



Color Legend: Diseases & Mechanism | Therapy Technology | Process & Scale

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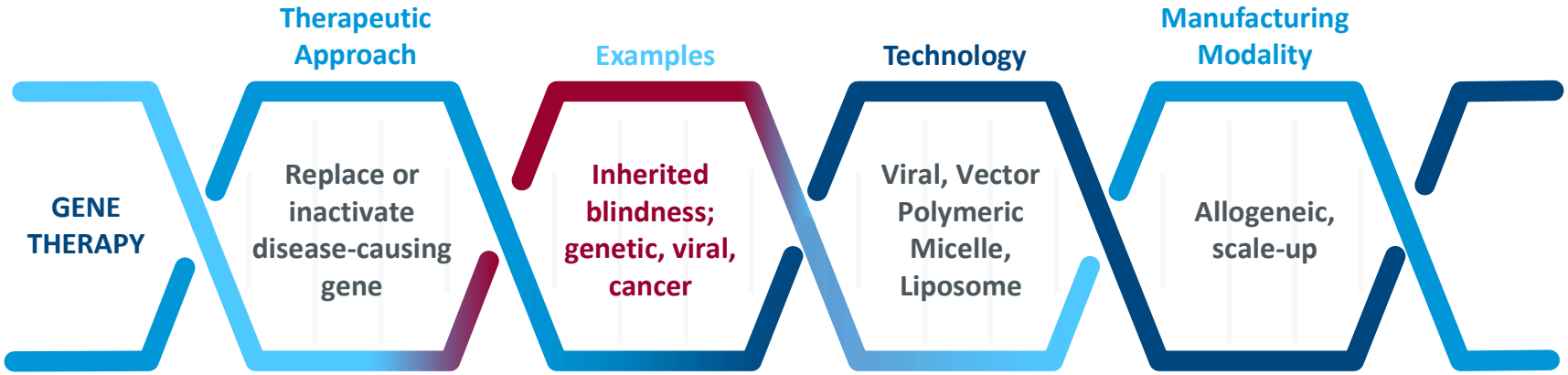
EXAMPLES OF EMERGING THERAPIES, THEIR DELIVERY TECHNOLOGY AND MANUFACTURING FORMAT

DEFECTIVE GENE



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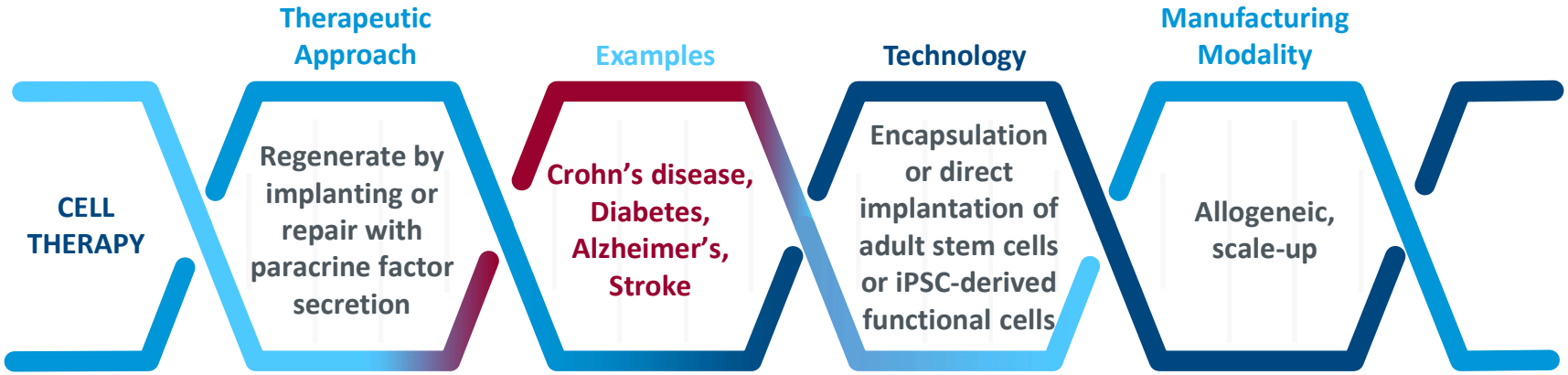
EXAMPLES OF EMERGING THERAPIES, THEIR DELIVERY TECHNOLOGY AND MANUFACTURING FORMAT

IMPAIRED CELLULAR FUNCTION IN ORGAN



iPSC: Induced Pluripotent Stem Cells

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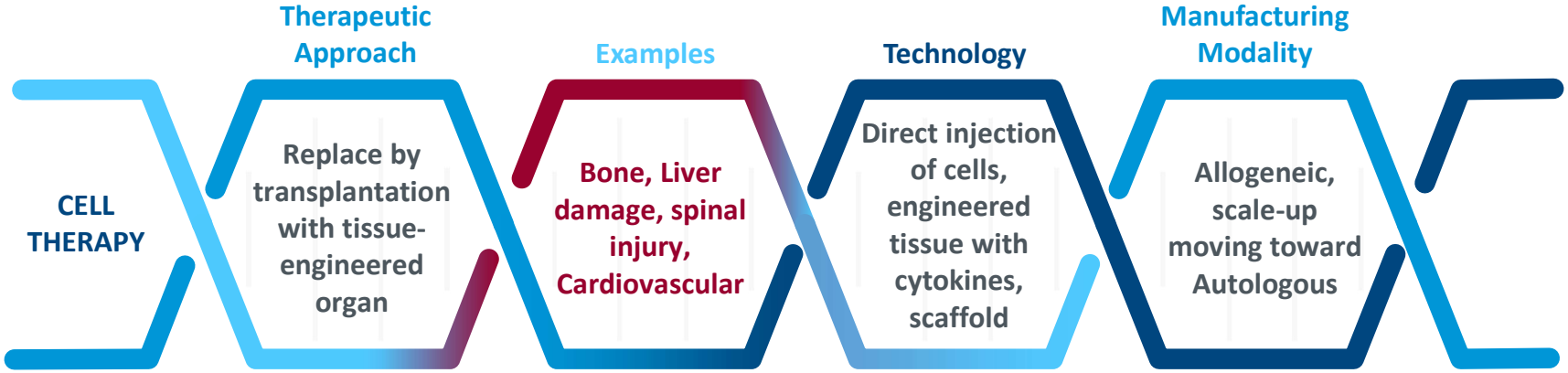
EXAMPLES OF EMERGING THERAPIES, THEIR DELIVERY TECHNOLOGY AND MANUFACTURING FORMAT

LOSS OR DAMAGE TO TISSUE



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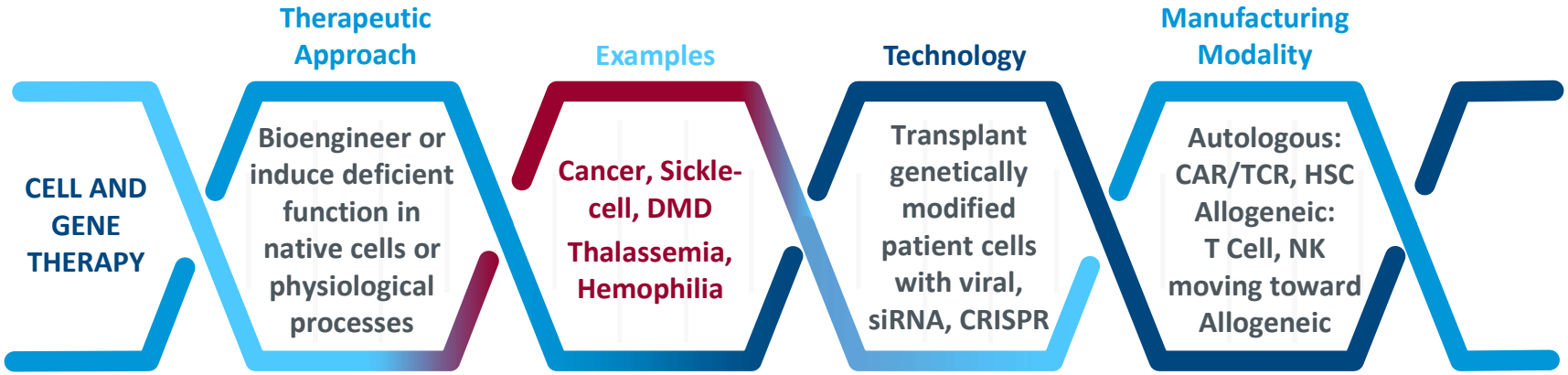
EXAMPLES OF EMERGING THERAPIES, THEIR DELIVERY TECHNOLOGY AND MANUFACTURING FORMAT

LIMITED PHYSIOLOGICAL CAPABILITIES



siRNA: Small-Interference RNA
NK: Natural Killer cells

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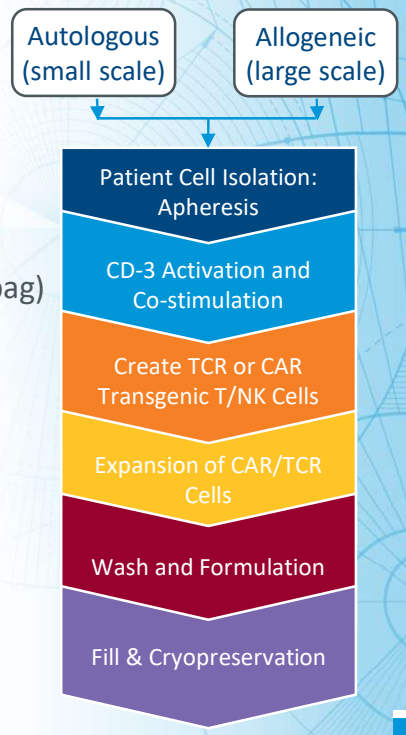
CANCER CELL THERAPY BIOPROCESSES

PBMCs and T cells are enriched from patient's WBCs and genetic material with native or engineered T cell receptors are delivered to enable them to identify and fight cancer cells.

- Starting Material: 10 – 30 × 10^6 patient WBCs
- Maximum Densities: 0.8-1.2 × 10^6 cells/mL
- CAR-T Dose Size: 0.04 – 0.400 × 10^9 cells/dose (small scale: 1-2 × 10^9 cells: static bag)
- CAR-T Autologous Scale: 1-3L per patient-specific dose
- CAR-T Allogeneic Scale: 200-2000L/100 doses (large scale; stirred-suspension)
- TCR Dose Size: 5 – 100×10^9 cells/dose (medium scale: 100×10^9 cells: wave bag)
- TIL Dose Size: 0.3 – 30×10^9 cells/dose (medium scale 1-50×10^9 cells: wave bag)



CAR-T: Chimeric Antigen Receptor
TCR: T Cell Receptor
TIL: Tumor Infiltrating Lymphocytes

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REGENERATIVE THERAPIES BIOPROCESSES

Induced Pluripotent Stem Cells (iPSCs) are derived from patient fibroblasts, PBMCs, or organ-specific cells. Advanced and optimized cell culture direct iPSCs to desired tissues.

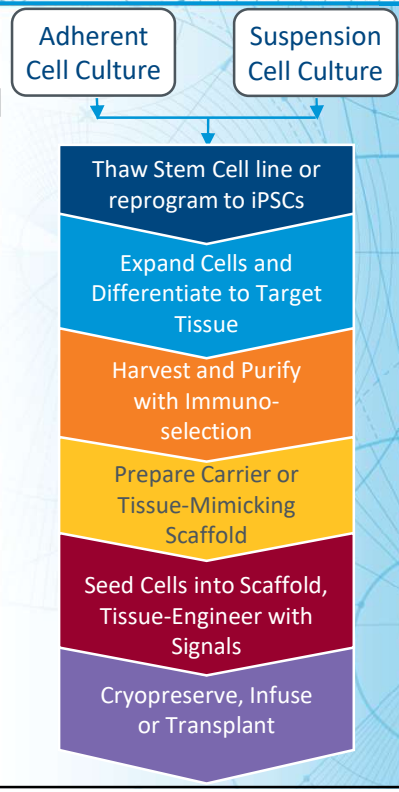
Pancreatic β Cells for Type 1 Diabetes:

- Dose: 340 – 750 × 10^6 cells for 150lb patient
- Concentration: 0.6 × 10^6 cells/mL
- Scale: 500mL – 1L

Ischemic Cardiomyopathy:

- iPSC-derived Heart Cell Dose: 1.5 – 2.9 × 10^6 cells
- iPSC-derived Scale: 1.2L
- hMSC Dose: 100-150 × 10^6 cells from 25mL bone marrow
- hMSC Scale: 3 weeks small scale culture; or Allogeneic: 200L large scale

hMSC: Human Mesenchymal Stem Cells



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GENE THERAPY PROCESSES

Chemical synthesis of small genes or E.Coli fermentation of larger genes. Viral Vectors, siRNA or CRISPR/CAS9 for *in vitro* gene therapy.

Viral Vector produced from HEK-293T Cells:

- iCELLis Fixed Bed Yield: 70L -> 5×1012 TU RVV; 3×1016 VP AAV; 2×1014 TU LVV
- Allegro Stirred Tank: Scale up to 2000L

Patient hMSCs or HSCs derived from iPSCs:

- Isolate MSCs: 25mL (100-150 × 106 cells)
- iPSC Culture & Differentiated: Up to 500L for allogeneic gene therapy

Expand Cells With Inserted Genes:

- Corning CellStack: 1.5-2L adherent culture
- Eppendorf CelliGen Blu: 3-5L suspension culture

HSC: Hematopoietic Stem Cells
RVV: Retro Viral Vector
LVV: Lenti Viral Vector
AAV: Adeno Viral Vector
iPSCs: Induced Pluripotent Stem Cells

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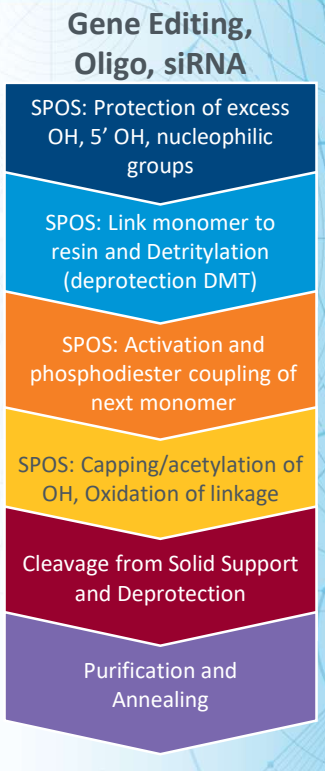

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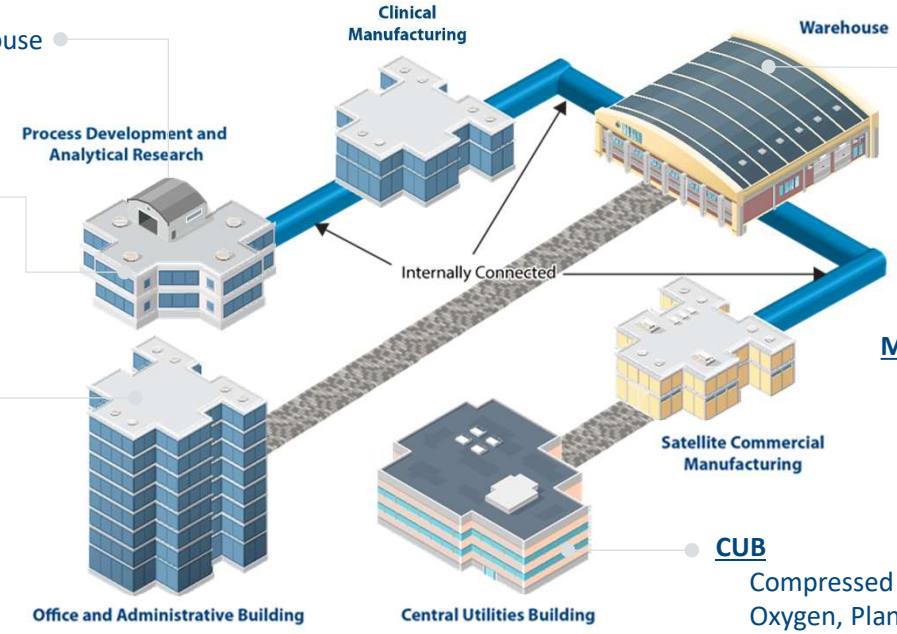
COMMERCIAL CELL THERAPY FACILITY COMPONENTS

WH Local Warehouse

Labs
RnD, Process Dev, Pilot Plant, Analytical, Microbiology, Cell Culture, Virology, etc.

Admin
Site Management, Procurement, Supply Chain, Quality Assurance, Engineering, Security, IT, etc.



WH Shipping/Receiving, Ambient/Cold/Freezer Storage, Hazardous Materials Storage, Solid Waste Handling.

Manufacturing
Upstream & Downstream Processes, Formulation & Fill, QC, Inspection, Packaging, Clean Utilities, etc.

CUB
Compressed Air, CO_2, Nitrogen, Oxygen, Plant Steam, Chilled Water, Water Pre-Treatment, Waste Treatment, etc.

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PHASE-APPROPRIATE, PIPELINE-DEPENDENT ESTIMATIONS

Cell Therapy Site Design Parameters	Lab/RnD	Phase 1/2	Phase 3	PPQ/Commercial
Space, Utility, & Processing Capabilities				
cGMP Materials and Qualification	Not Typical	Space & Storage for Fully Qualified Materials		
Space Classification	ISO 7 – Grade C	ISO 7 – Grade B (closed), ISO 5 – Grade A (semi-open)		
Process Scale	Lab Scale	Varying scale/dose escalation		Medium for autologous
Equipment				
cGMP Compliance	Automation; Rest is less essential during discovery phase	From cGMP certified to full compliance		
Automation and Closed Capabilities		Increases from Phase 1-3		Automation
Precision, Control, Reproducibility, QA		Gradual increase with equipment change/process		

Legend: Design Parameter Rating/Level Required at Each Stage

N/A — Low — Medium — High

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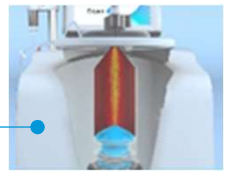
CASE STUDY FOR AUTOLOGOUS CAR-T: PHASE-APPROPRIATE EQUIPMENT AND MANUFACTURING CAPABILITIES

PROCESS	Lab/RnD	Phase 1/2 (cGMP)	Phase 3 (cGMP Larger Scale)	PPQ/Manufacturing (Automation/Closed)
Cell Thaw/Isolation	AutoMACS Pro/Centrifugation	CliniMACS Plus/Sepax/Plasmatherm	CliniMACS Plus (30-60 x10^9 cells)/ThawStar Biolife 2m vial, 1L bag	CliniMACS Prodigy/Cytiva Sefia (end-to-end)

AutoMACs Pro:
High-throughput sorting for RnD

Plasmatherm:
Timed thawing without water contact



SEPAX:
Automated Closed System Density Centrifugation

CliniMACS Plus
Magnetic Separation

Thawstar®
Automated, Algorithm
1.8-2.0ml (top photo)
or 25ml-1L (bottom photo)

CliniMACS Prodigy

Sefia

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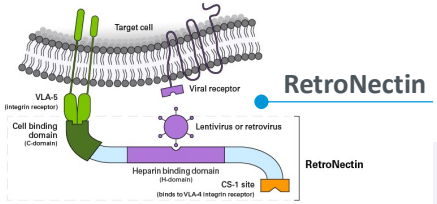
CASE STUDY FOR AUTOLOGOUS CAR-T: PHASE-APPROPRIATE EQUIPMENT AND MANUFACTURING CAPABILITIES

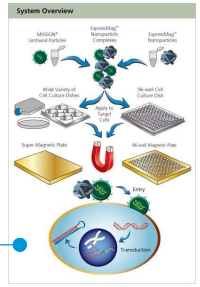
PROCESS	Lab/RnD	Phase 1/2 (cGMP)	Phase 3 (cGMP Larger Scale)	PPQ/Manufacturing (Automation/Closed)
Gene Insertion	Transfection (Lipofectamine/MACSfectin)	Static Transduction (Retronectin; Beads like ExpressMag and MACSductin)/Electroporation (Lonza Nucleofector)		

Lipofectamine

MACSfectin



RetroNectin



ExpressMag

MACSductin

Nucleofector:
cGMP, Automated, Efficient, Electroporation

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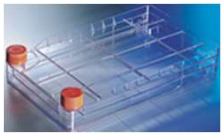
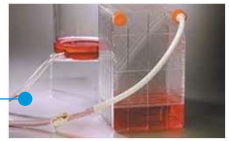
CASE STUDY FOR AUTOLOGOUS CAR-T: PHASE-APPROPRIATE EQUIPMENT AND MANUFACTURING CAPABILITIES

PROCESS	Lab/RnD	Phase 1/2 (cGMP)	Phase 3 (cGMP Larger Scale)	PPQ/Manufacturing (Automation/Closed)
Cell Culture	Open flasks/plates/cell stacks (0.5-2.5m^2)	Closed Bags (0.1-2L) (PermaLife/OriGen)	Pall iCELLis (500m2/70L) Rockers, controlled pH/gas (Wave/Xuri up to 25L); Suspension Mobius/Allegro (50-2000L) – encapsul./microcarrier	


Flasks


Multilayer Flasks




Cell Stacks


Closed Bags


Allegro™ Stirred Tank
10L to 2000L


Mobius
3L to 2000L


CelliGen® BLU
3-5L suspension culture


Xuri Rockers
25L

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CASE STUDY FOR AUTOLOGOUS CAR-T: PHASE-APPROPRIATE EQUIPMENT AND MANUFACTURING CAPABILITIES

PROCESS	Lab/RnD	Phase 1/2 (cGMP)	Phase 3 (cGMP Larger Scale)	PPQ/Manufacturing (Automation/Closed)
Harvest	Centrifugation	Sepax (0.1-1L)	TFF (Sartorius/Pall – 500-2000L or 15kg)	

Centrifugation

Automated, Closed-System Centrifugation

Cadence BioSMB
cGMP Compliant, 2000L, Continuous

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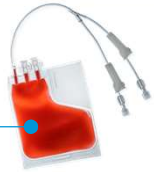
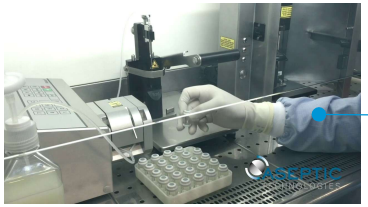
CASE STUDY FOR AUTOLOGOUS CAR-T: PHASE-APPROPRIATE EQUIPMENT AND MANUFACTURING CAPABILITIES

PROCESS	Lab/RnD	Phase 1/2 (cGMP)	Phase 3 (cGMP Larger Scale)	PPQ/Manufacturing (Automation/Closed)
Fill/Finish	Manual Vial/Bag	Crystal M1	Crystal M1 with L1 Robotic Line	Pure M1/Terumo FINIA

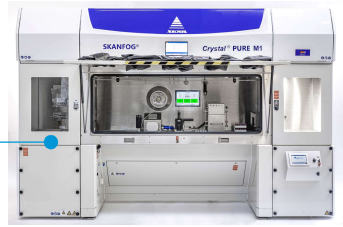
Cryo Vials



Cryo Bags



Crystal M1



Pure M1

Corning CoolCell

Controlled Rate Freezer (CRF)

L1 Robotic Filling Line

FINIA

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CASE STUDY FOR AUTOLOGOUS CAR-T: PHASE-APPROPRIATE EQUIPMENT AND MANUFACTURING CAPABILITIES

PROCESS	Lab/RnD	Phase 1/2 (cGMP)	Phase 3 (cGMP Larger Scale)	PPQ/Manufacturing (Automation/Closed)
Store/Ship	Dry ice/LN2	Vapor LN2 Cryopod (Brooks) and MVE Cryoshipper		

Dry Ice

Liquid Nitrogen Tank and Dewer

Cryopod:
Short distances (3hrs)

Cryoshipper:
Vapor Phase, Long distance (1 day)

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THANK YOU FOR YOUR ATTENTION

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