Regulatory aspects of manufacturing and control of CAR-T cells, game-changing autologous immunotherapy products

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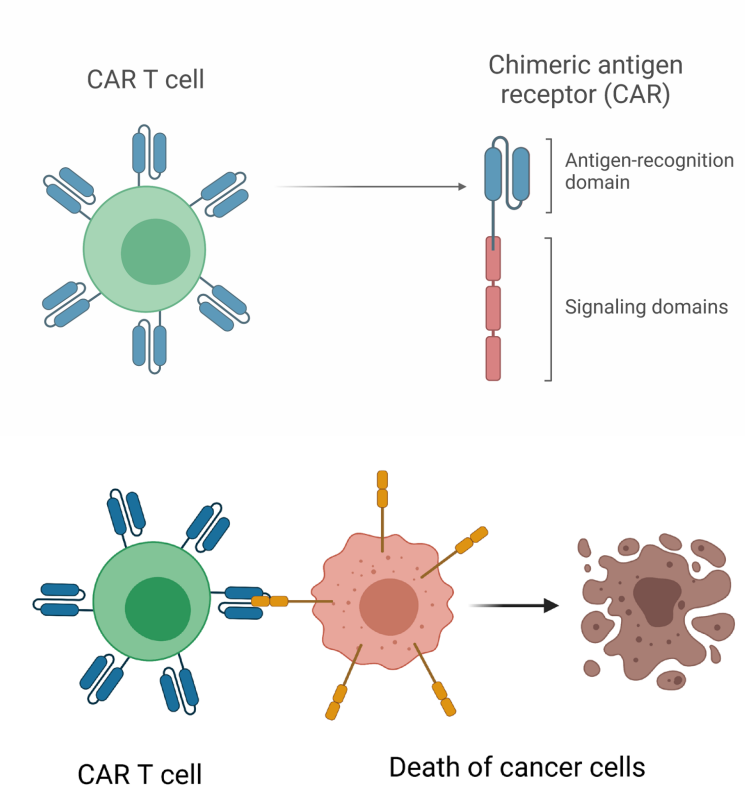
DISCLAIMER: Personal views only, meant to initiate further discussion; may not necessarily reflect views/opinions of MEB, EMA or EDQM.

Outline

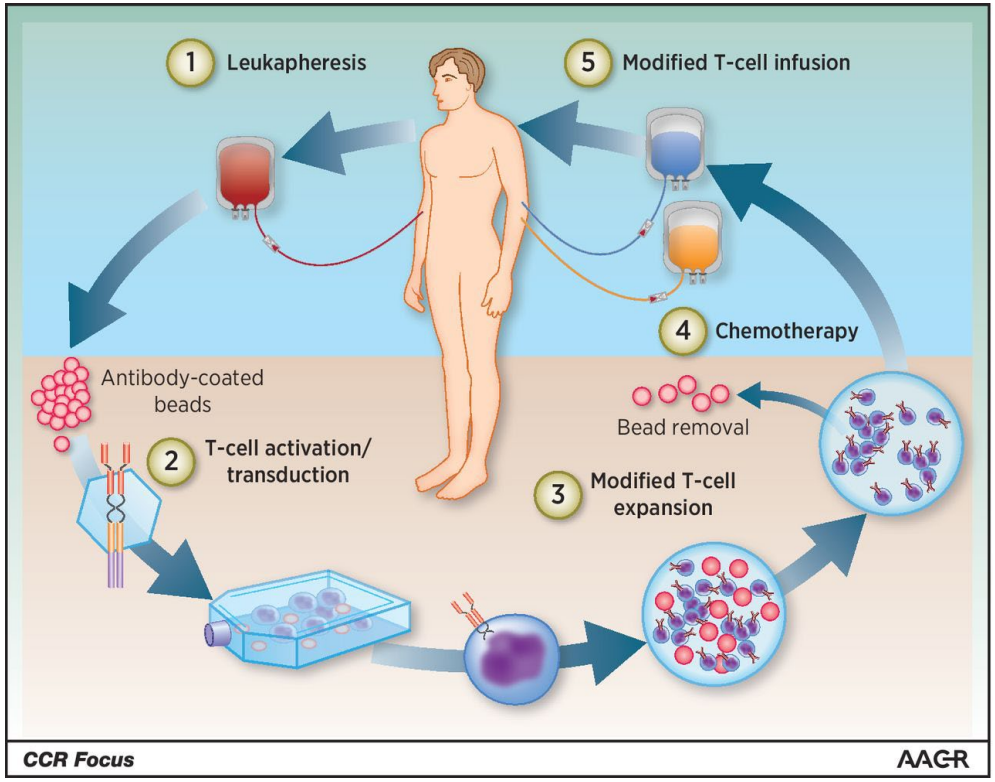
- Introduction of CAR-T cells
- Mode of Action & Clinical Aspects
- Manufacturing process and controls
  - Starting materials
  - Autologous cells
  - Potency testing
- CAR-T specific considerations
- Current developments

CAR T-cells

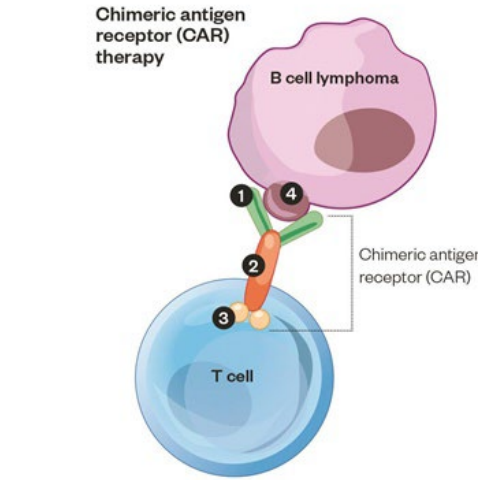
- Engineered (autologous) T-cell immunotherapy product
- Gene therapy (ATMP)
- Aimed to recognise specific target on patient/ recipient cells
- (Potential) Game Changer in treatment of (soluble) tumours

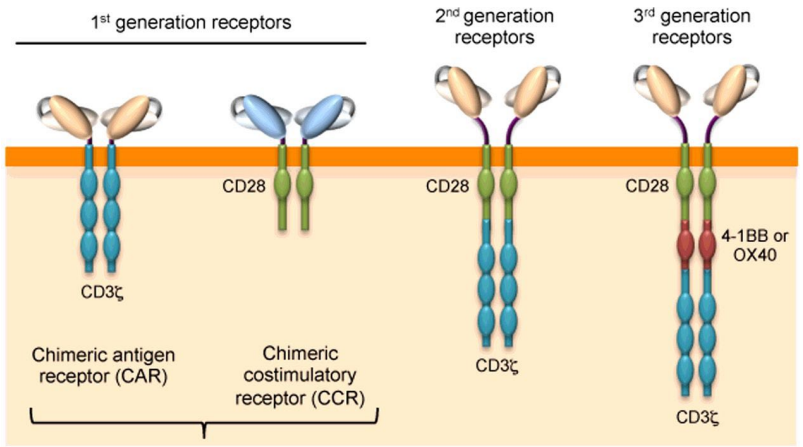


CAR-T cell therapy



McGuirk et al. 2017 Cytotherapy 19:1015-1024, DOI: 10.1016/j.jcyt.2017.06.001





Single-signaling domain	Single-signaling domain	Dual-signaling domain	Multiple-signaling domain
Dual-antigen recognition and complementary signaling		Single-antigen recognition and complementary signaling	

CAR-T treatment of B-Cell Lymphoma

DLBCL* and PMBCL* Patients:

- Clinically relevant efficacy
- Significant duration

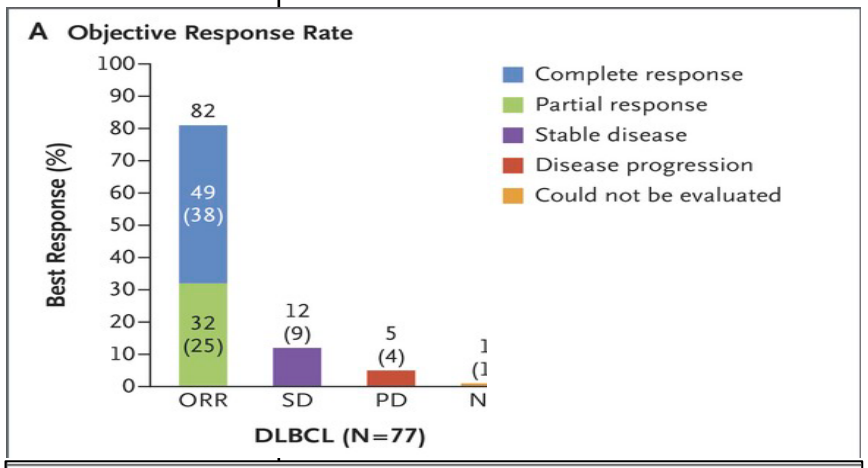
High incidence Adverse Drug Reactions

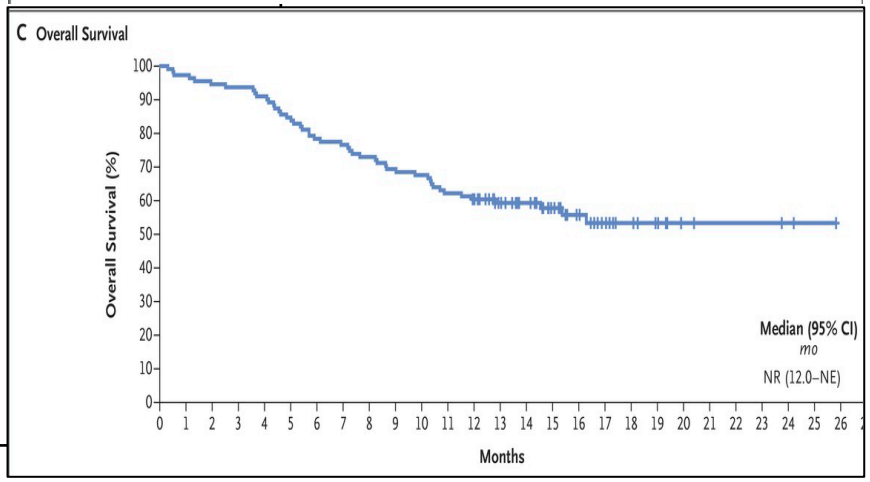
- Cytokine Release syndrome (CRS)
- Neurological adverse events
- Cytopenias (due to conditioning)

- CRS: Need to have Toculizumab (anti-IL6mAb) available

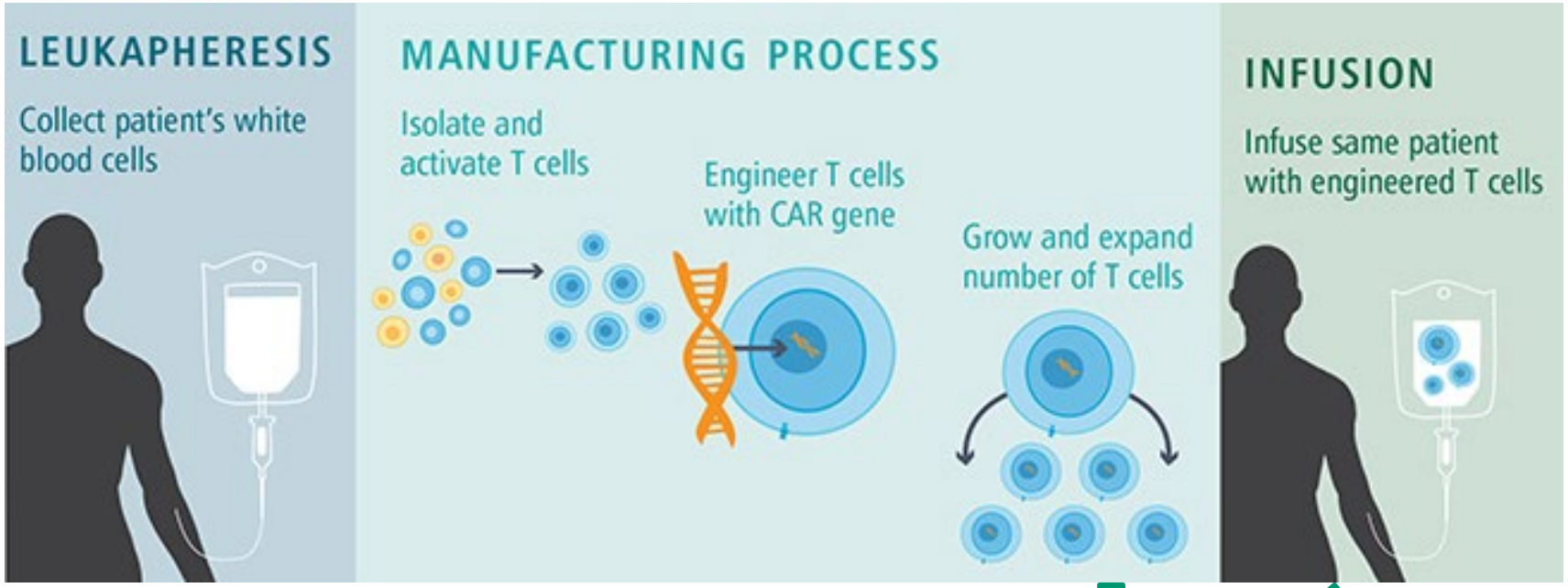
*DLBCL: diffuse large b cell lymphoma;

*PBCL: Polymorphic b cell lymphoma





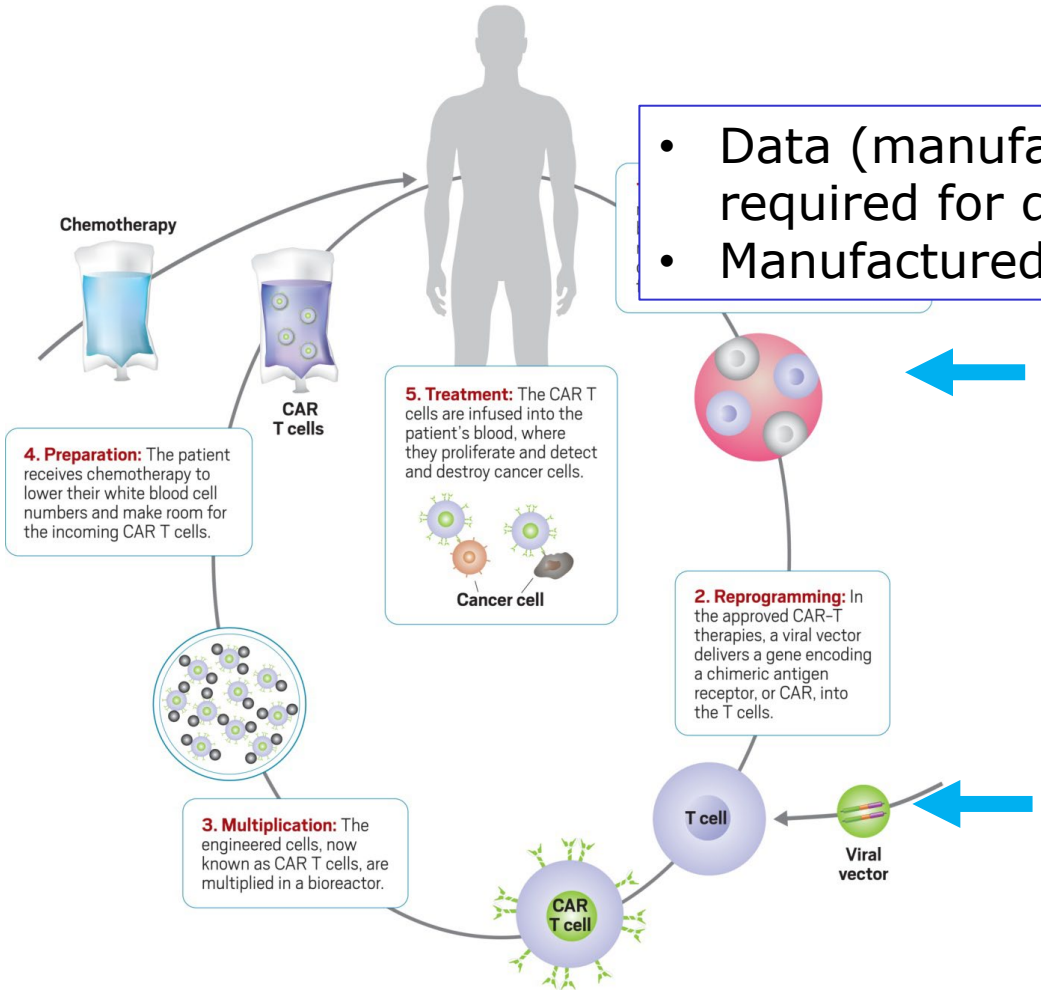
Overview CAR-T manufacturing



Transport

Transport

Overview CAR-T manufacturing



Starting materials:

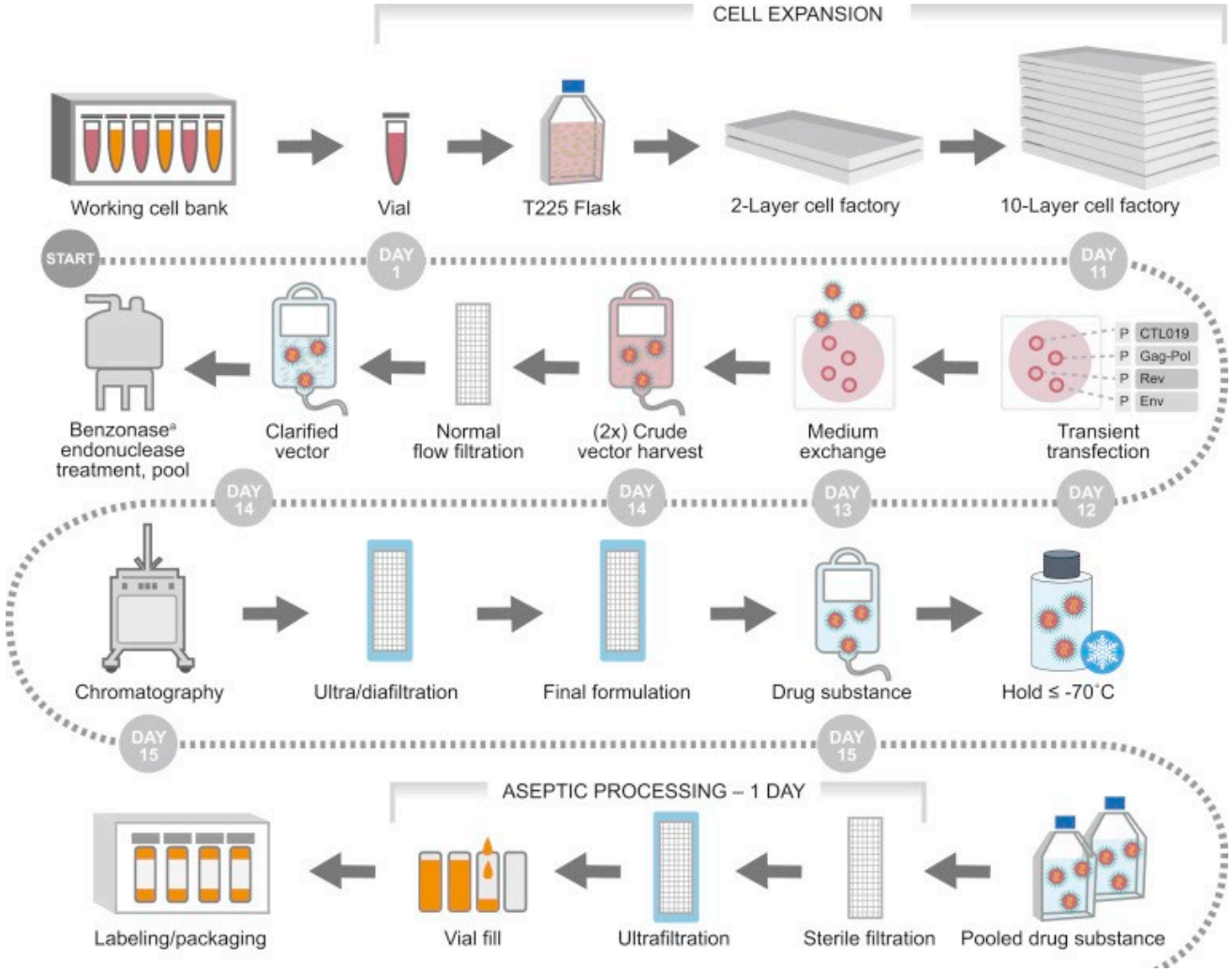
- Data (manufacturing & control) same as required for drug substance
- Manufactured according to (principles of) GMP

Apheresis: PBMCs
(Peripheral blood mononuclear cells)

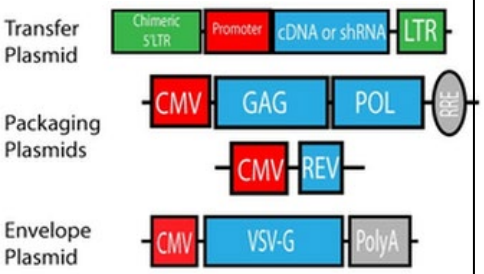
Viral Vector

Satyanarayana, Chem. Engin. News. October 1, 2018

Viral Vector manufacturing



Third generation Lentiviral vector 4 plasmids (starting material; principles of GMP)



www.addgene.org/guides/lentivirus

Levine et al. 2017. Global Manufacturing of CAR T Cell TherapyDOI:https://doi.org/10.1016/j.omtm.2016.12.006

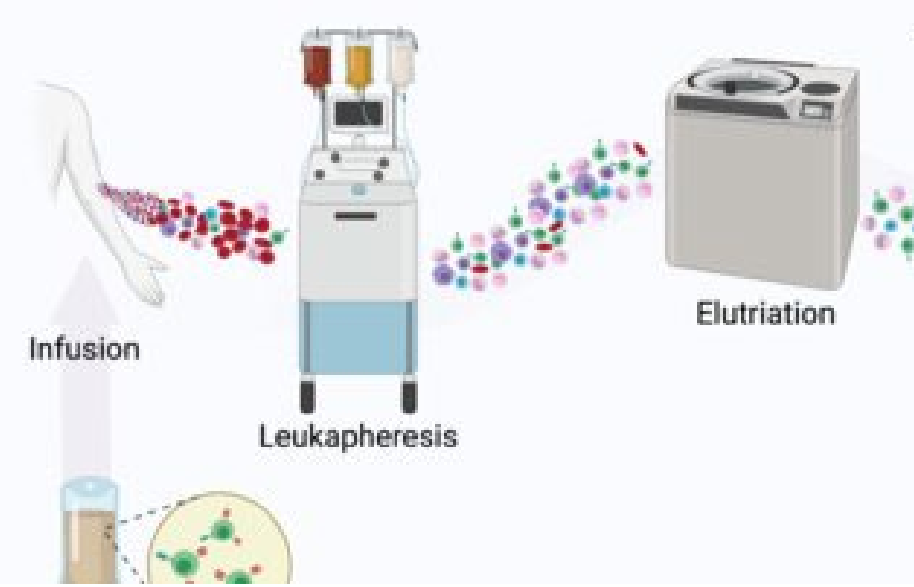
Viral Vector release specifications

Test	Method
Appearance	Visual Inspection
Identity	e.g. RT-PCR
Titer	Number Viral particles/volume
Infectivity	Infectivity (Suitable cells)
Potency	CAR-expression
Replication Competent Virus (RCV)	Virus replication in suitable cells
In vitro viral testing	14d in vitro assay for viral contaminants
Microbial safety	Sterility and Mycoplasma
Endotoxin	

Note: Viral vector is a starting material

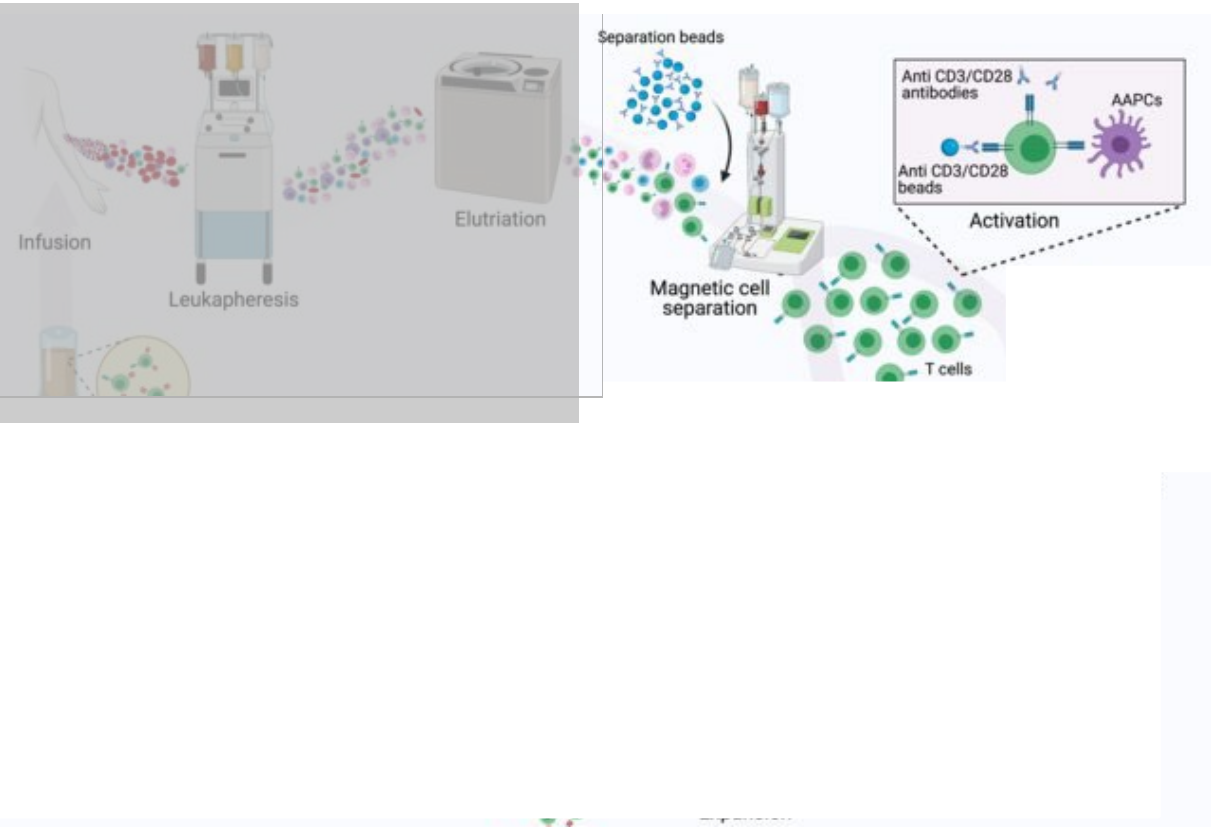
Starting material PBMCs

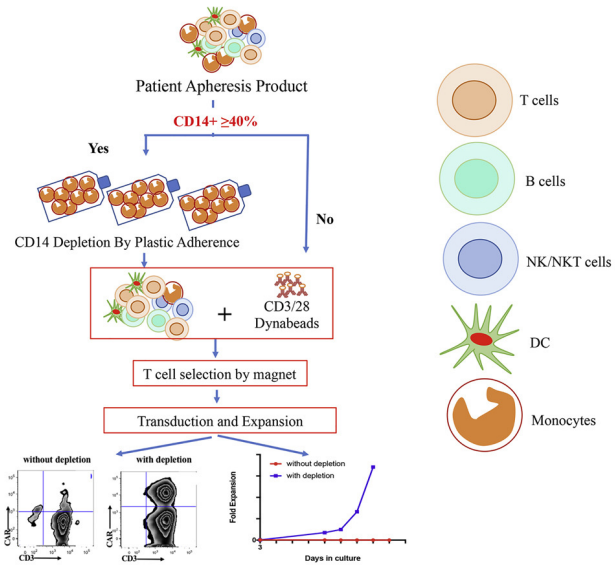
- (Enriched) PBMCs
- Apheresis centre: JACIE approved
- Compliance with Directives 2002/98/EC, 2004/23/EC
- Standard apheresis equipment
- If production starts at Apheresis centre: GMP required
- Sometimes Several apheresis sites for one product



From:Scalable Manufacturing of CAR T Cells for Cancer Immunotherapy August 2021 Mohamed Abou-el-Enein, Magdi Elsallab, Steven A. Feldman Barbara Savoldo DOI: 10.1158/2643-3230.BCD-21-0084

Manufacturing process Drug Substance
T-Cell selection and specific activation





Wang et al 2021
https://doi.org/10.1016/j.omtm.2021.06.014

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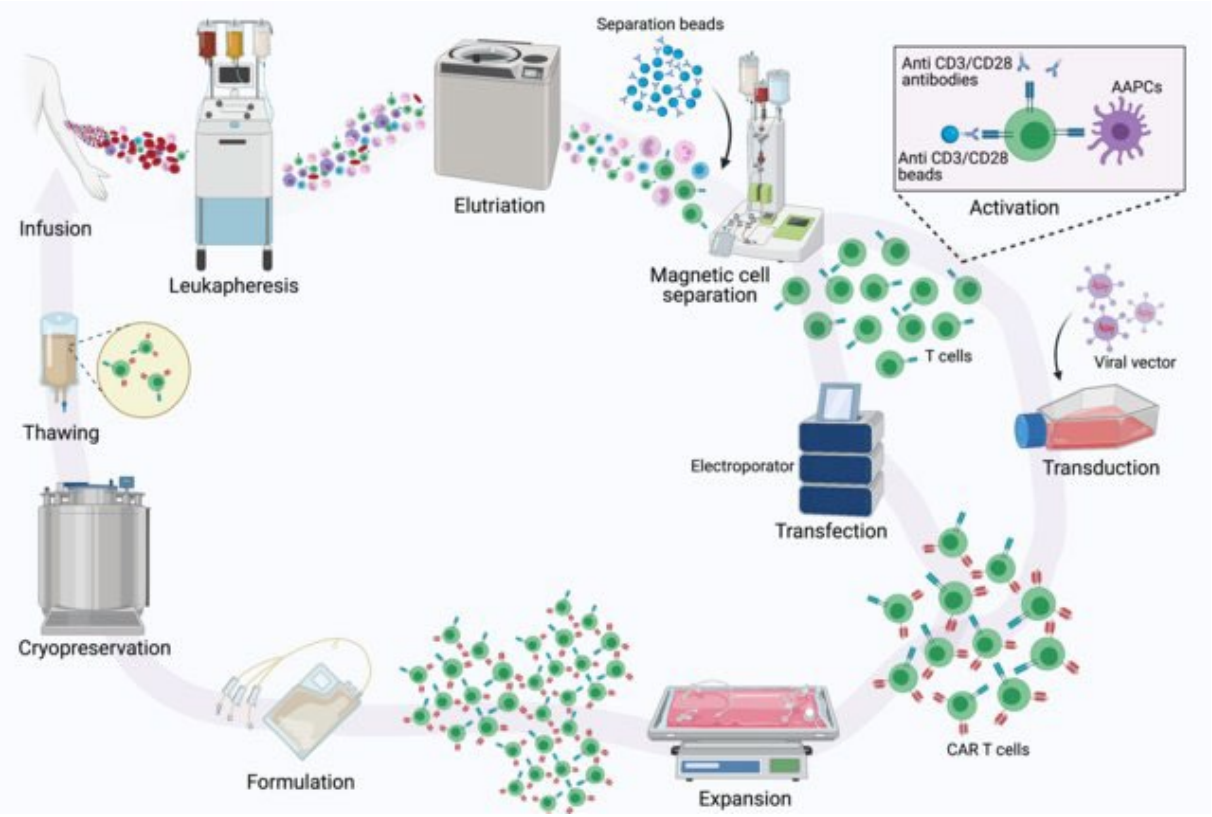


- Transduction efficiency: autologous cell batches varying in size/quality
- MoI (multiplicity of Infection = Virus:cells ratio)
- How to set control ranges during manufacturing?
- Patient material to validate process and stability?
- Control critical reagents
- Raw materials (Ph.Eur. 5.2.12)
- Viral safety reagents (HSA, AB-serum)
- GMP for ATMP

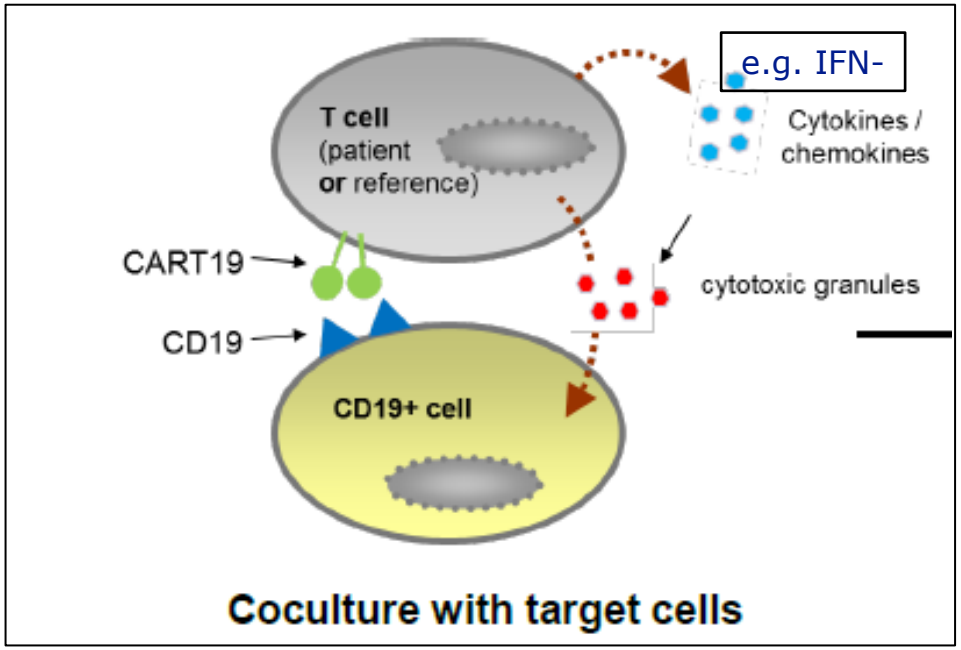
From:Scalable Manufacturing of CAR T Cells for Cancer Immunotherapy
Abou-el-Enein et al. 2021. DOI: 10.1158/2643-3230.BCD-21-0084

Drug Product release specifications

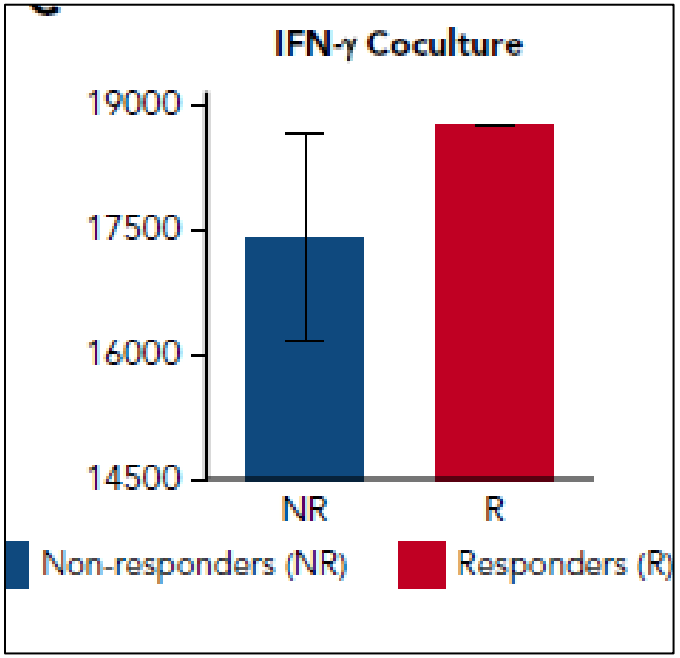
Test	Method
Appearance	Visual Inspection
Dose (Viable; CAR^+ cells)	e.g. FACS
Viability	e.g. Cell staining
Potency	e.g. IFN-gamma production
Anti-Target CAR expression	% CAR^+ cells (FACS)
Vector Copy Number	e.g. RT-PCR
Purity	% $CD3^+$ cells (FACS)
(Specific composition; subpopulations; Characterisation)	(Ratio $CD4^+$:$CD8^+$ e.g. Effector or Memory T-cells)
Process-related impurities	
Endotoxin	

Note: Release testing prior to Cryopreservation

Potency test and…..
Relation Clinical response

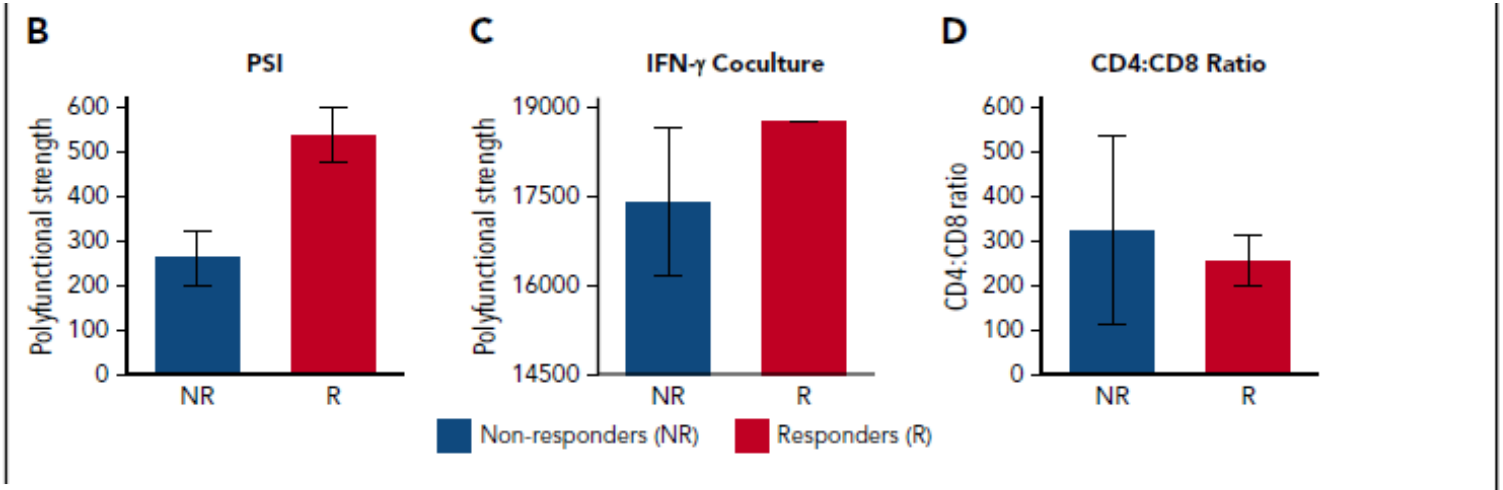


CASS Bioassay 2015| Erik Rutjens

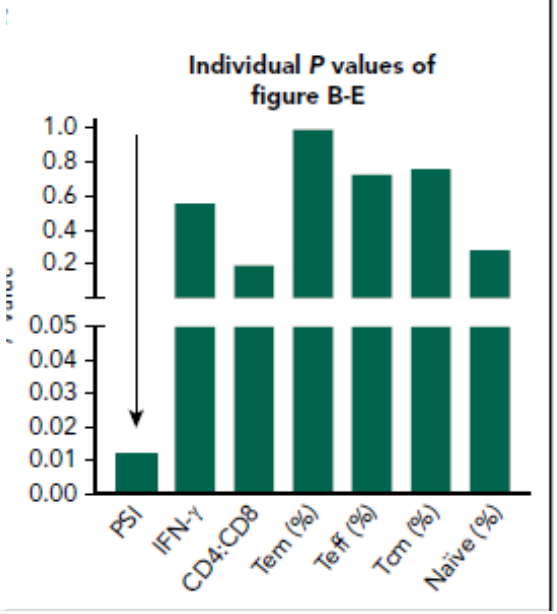


Rossi et al. Blood. 2018;132(8):804-814

Potency test and…..
Relation Clinical response (2)



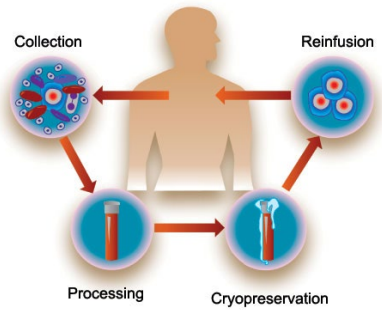
PSI=Polyfunctional Strength Index
(secretion of 2+ cytokines per cell)



Rossi et al. Blood. 2018;132(8):804-814

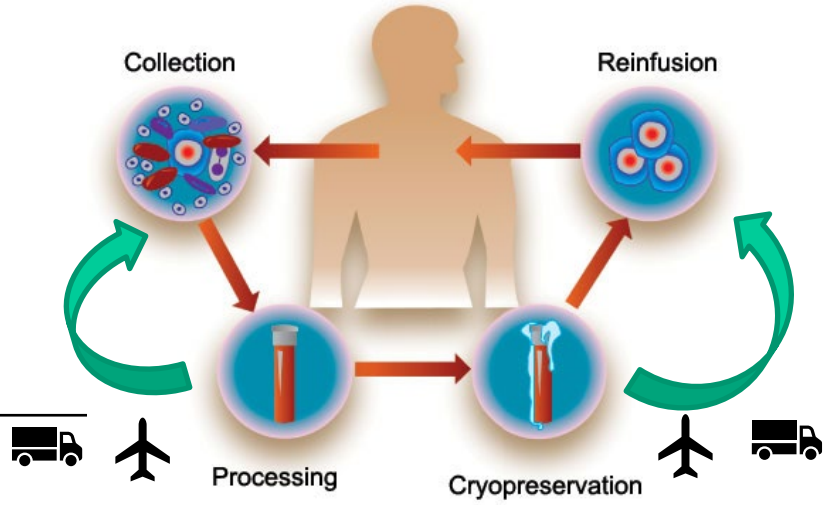
CAR-T specifics

- Autologous product
- Variability (e.g. cell numbers/populations/potency)
- Release of OOS batches (GMP for ATMP)
  - Q&A on use OOS batches of authorised ATMP (EMA/CAT/224381/2019)
  - Under exceptional circumstances (Sect. 11.5 GMP for ATMP) the administration of OOS cell/tissue-based ATMP may be considered to avoid immediate significant hazard to patient
  - Inform treating physician, patient, Competent authorities, EMA
  - Evaluate risk (No time to generate new batch; any effect of batch may be beneficial)
- Import testing into EU (incl. Potency testing)



CAR-T specifics (2)

- Limited material for testing
- Short in-use shelf life
- Cryopreservation
- Reconstitution
- (Bed-side) preparation (sterility)
- Needle-to-Needle time
- Transport
- Decentralised manufacturing



Automated systems

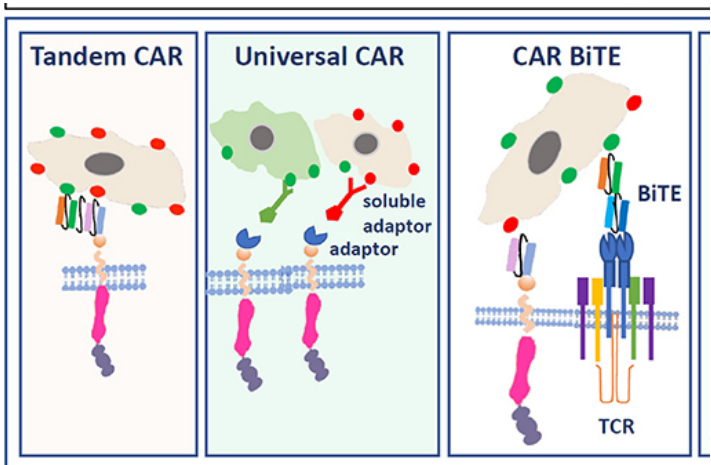
- Allows standardised decentralised production?
- Hospital manufacturing?
- No cryopreservation needed (better yield)
- No transport
- Shorter Needle-to Needle time
- Standardisation different sites & Central QP?

Clinimacs Prodigy®
semi-automated platform

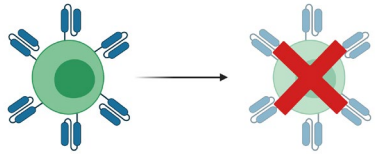
Cocoon®
automated platform

Developments

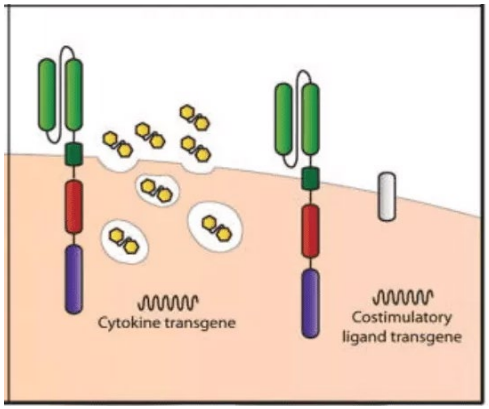
- Enhance specificity
- Overcome limitations (e.g. off the shelf)
- Control activity
- Target solid tumors
- Indications other than cancer



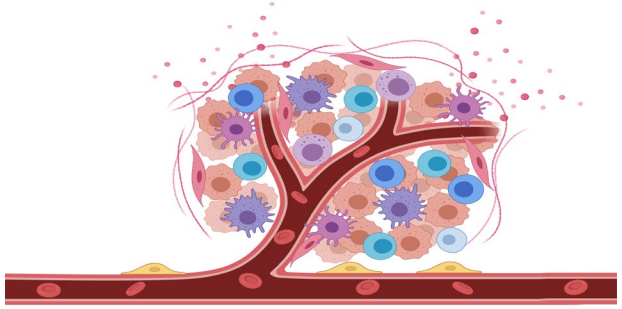
https://doi.org/10.3389/fimmu.2020.565631



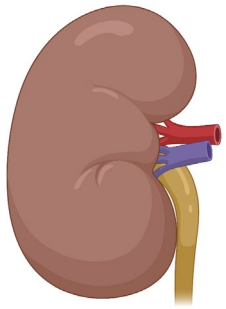
Eradication CAR T-cells



Armored CAR T-cells



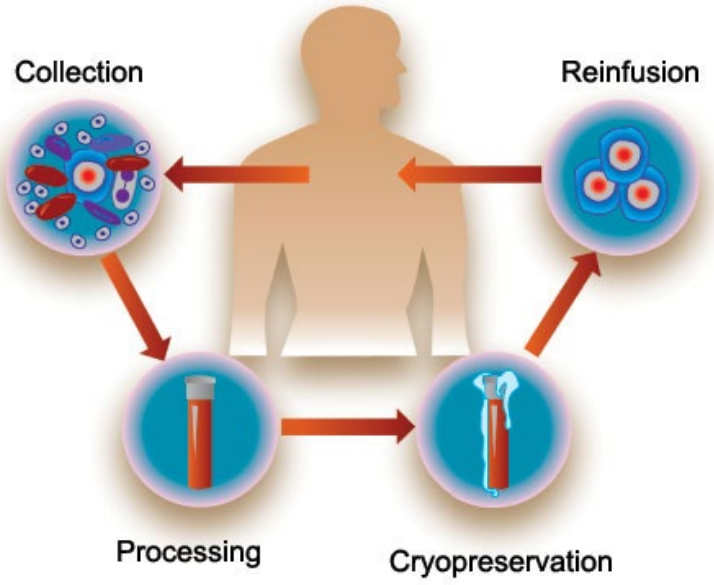
Overcome tumor microenvironment
(Source: Biorender.com)



Prevent allograft rejection

CAR-T Summary

- High clinical benefit
- Manufacturing:
  - Vector (principles of GMP)
  - PBMC Apheresis (CTD; part of manufacturing?)
  - Transduced cells
- Autologous, Variability
- Release of OOS batches
- Needle-to-Needle time
- Developing field



Thanks to:

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