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
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 Tocilizumab (anti-IL6mAb) available

*DLBCL: diffuse large b cell lymphoma;
*PBCL: Polymorphic b cell lymphoma
Overview CAR-T manufacturing

LEUKAPHERESIS
Collect patient’s white blood cells

MANUFACTURING PROCESS
Isolate and activate T cells
Engineer T cells with CAR gene
Grow and expand number of T cells

INFUSION
Infuse same patient with engineered T cells

Transport

Hoefnagel, CASSS-NL
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)

Transfer Plasmid
Packaging Plasmids
Envelope Plasmid

www.addgene.org/guides/lentivirus

Hoefnagel, CASSS-NL
March 2022
# Viral Vector release specifications

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

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

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

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</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Visual Inspection</td>
</tr>
<tr>
<td>Dose (Viable; CAR(^+) cells)</td>
<td>e.g. FACS</td>
</tr>
<tr>
<td>Viability</td>
<td>e.g. Cell staining</td>
</tr>
<tr>
<td>Potency</td>
<td>e.g. IFN-gamma production</td>
</tr>
<tr>
<td>Anti-Target CAR expression</td>
<td>% CAR(^+) cells (FACS)</td>
</tr>
<tr>
<td>Vector Copy Number</td>
<td>e.g. RT-PCR</td>
</tr>
<tr>
<td>Purity</td>
<td>% CD3(^+) cells (FACS)</td>
</tr>
<tr>
<td>(Specific composition; subpopulations; Characterisation)</td>
<td>(Ratio CD4(^+):CD8(^+) e.g. Effector or Memory T-cells)</td>
</tr>
<tr>
<td>Process-related impurities</td>
<td></td>
</tr>
<tr>
<td>Endotoxin</td>
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*Note: Release testing prior to Cryopreservation*
Potency test and..... Relation Clinical response

CASS Bioassay 2015| Erik Rutjens

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

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

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?
Developments

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

Eradication CAR T-cells

Armored CAR T-cells

Overcome tumor microenvironment
(Source: Biorender.com)

Prevent allograft rejection

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