Regulatory aspects of CAR-T cell therapy

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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

- Specifics of CAR-T cells
- Release tests CAR-T products
- Potency of ATMP and MoA
- Proposed assays
- Considerations for CAR-T potency tests
CAR-T cell therapy

1. Leukapheresis
2. T-cell activation/transduction
3. Modified T-cell expansion
4. Chemotherapy
5. Modified T-cell infusion

Chimeric antigen receptor (CAR) therapy

- B cell lymphoma
- Cancer cell

1st generation receptors

- CD3ζ
- Chimeric antigen receptor (CAR)
- Chimeric costimulatory receptor (CCR)

2nd generation receptors

- CD28

3rd generation receptors

- 4-1BB or OX40

Single-signaling domain

- Dual-antigen recognition and complementary signaling

Single-signaling domain

- Single-antigen recognition and complementary signaling
Two FDA approved CAR-T products also filed at EMA

Kite Files the Industry’s First CAR-T Marketing Authorization Application in Europe for Axicabtagene Ciloleucel

- Submission Based on Primary Analysis of ZUMA-1 in Patients with Aggressive NHL
- Accelerated Assessment Granted Through Priority Medicines (PRIME) Regulatory Initiative

SANTA MONICA, Calif.--(BUSINESS WIRE)-- Kite Pharma, Inc. (Nasdaq:KITE), a leading cell therapy company, today announced that it has submitted a Marketing Authorization Application (MAA) to the European Medicines Agency (EMA) for axicabtagene ciloleucel as a treatment for patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) transformed follicular lymphoma (TFL), and primary mediastinal B-cell lymphoma (PMBL). Approval is expected for autologous

Novartis receives another regulatory milestone for product CTL019 (tisagenlecleucel) with submission of its MAA* to EMA for children, young adults with r/r B-cell ALL and adult patients with r/r DLBCL

Kite’s Yescarta™ (Axicabtagene Ciloleucel) Becomes First CAR T Therapy Approved by the FDA for the Treatment of Adult Patients With Relapsed or Refractory Large B-Cell Lymphoma After Two or More Lines of Systemic Therapy

Novartis receives first ever FDA approval for a CAR-T cell therapy, Kymriah(TM) (CTL019), for children and young adults with B-cell ALL that is refractory or has relapsed at least twice
### Examples of CAR-T cell release tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Release testing for CAR-T introduced by retroviral and lentiviral vector</th>
<th>Release testing for CAR-T introduced by transposon/transposase</th>
<th>Release testing for CAR-T introduced by mRNA electroporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td>• Gram stain/sterility</td>
<td>• Gram stain/sterility</td>
<td>• Gram stain/sterility</td>
</tr>
<tr>
<td></td>
<td>• Mycoplasma</td>
<td>• Mycoplasma</td>
<td>• Mycoplasma</td>
</tr>
<tr>
<td></td>
<td>• Endotoxin level</td>
<td>• Endotoxin level</td>
<td>• Endotoxin level</td>
</tr>
<tr>
<td></td>
<td>• Copies of transgene insertion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• RCR/RCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Replication Competent virus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purity</td>
<td>• % CD3+ T cells</td>
<td>• % CD3+ T cells</td>
<td>• % CD3+ T cells</td>
</tr>
<tr>
<td></td>
<td>• %CAR-T cells</td>
<td>• %CAR-T cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Residual tumor burden</td>
<td>• Residual AAPCs Artifical Antigen Presenting cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Residual beads</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identity</td>
<td>• % CAR T cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potency</td>
<td>In vitro CTL or IFN-γ secretion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From Wang and Riviere, Mol Ther Oncolytics 2016*
ICH 6QB Definition Potency

- **potency** is the **quantitative measure of biological activity** based on the **attribute** of the product, which is linked to the **relevant biological properties**.
- The **assay** demonstrating the biological activity should be **based on the intended biological effect** which should **ideally** be **related to** the **clinical response**.

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**ICH Topic Q 6 B**
**Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products**
Cell-based medicinal products: the new biologicals

Potency is a key parameter for complex products which are difficult to characterise.

A combination of **multiple methods** may be needed to adequately define the potency of these products **during the development**. Certain assays may be needed to **control process changes**, whereas others are more suitable for **release testing**.

Preferably, the potency assay should reflect the clinical Mechanism of Action.
Challenges for Potency tests of Cell Based Products

• Functionality should be demonstrated
• Viability and cell markers not sufficient
• Often exact MoA not fully known
• Often ≥ 1 suspected/suggested MoA
• Sometimes *in vitro* assay does not correlate with *in vivo* situation
• (Semi-)Quantitative
T cell cytolytic activity

Zaritskaya et al. 
Expert Rev 
Vaccines 2010
# (CAR) T cell potency tests

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Responder cells</th>
<th>Stimuli</th>
<th>Read out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotyping assay</td>
<td>T cells</td>
<td></td>
<td>CD markers, antigen (e.g. CD19) specific receptor</td>
</tr>
<tr>
<td>Avidity assay</td>
<td>T cells</td>
<td>MHC multimers loaded with antigens</td>
<td>Amount/duration of TCR-MHC-peptide binding ($EC_{50}$)</td>
</tr>
<tr>
<td>Proliferation assay</td>
<td>Effector T cells</td>
<td>Irradiated (peptide-pulsed) tumour cells</td>
<td>Proliferation (via CFSE dilution)</td>
</tr>
<tr>
<td>Cytokine production assay</td>
<td>Effector T cells</td>
<td>Irradiated (peptide-pulsed) tumour cells</td>
<td>Intracellular cytokines (e.g. IFN-$\gamma$ and IL-2)</td>
</tr>
</tbody>
</table>
## (CAR) T cell potency tests (2)

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Responder cells</th>
<th>Stimuli</th>
<th>Read out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effector molecule release assay</td>
<td>Effector T cells</td>
<td>Irradiated (peptide-pulsed) tumour cells/peptides</td>
<td>- Secretion of cytokines (e.g. IFN-γ, TNF-α, IL-2)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Secretion of cytotoxic factors (e.g. granzyme B)</td>
</tr>
<tr>
<td>Degranulation assay</td>
<td>Effector T cells</td>
<td>Irradiated (peptide-pulsed) tumour cells</td>
<td>Surface expression CD107a/b</td>
</tr>
<tr>
<td>Growth inhibition assay</td>
<td>Effector T cells</td>
<td>(Patient specific) Tumour cells</td>
<td>Tumor cell proliferation (³H incorporation)</td>
</tr>
</tbody>
</table>
# (CAR) T cell potency tests (3)

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Responder cells</th>
<th>Stimuli</th>
<th>Read out</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxicity assay (various</td>
<td>(Patient specific) Tumour cells (GFP transduced)</td>
<td>Effector T cells</td>
<td>- Release intracellular proteins (e.g. LDH, β-gal or luciferase), or</td>
</tr>
<tr>
<td>types of release assays)</td>
<td></td>
<td></td>
<td>radioactive $^{51}$Cr</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Intracellular $^3$H thymidine, GFP</td>
</tr>
</tbody>
</table>

De Wolf et al Cytotherapy 2018
In vitro: Cytotoxicity ($^{51}$Cr release)  In vivo: Survival Mice with lymphoma

From Hollyman et al. J. Immunother. 2009
Considerations for (CAR-)T cell potency tests

- Anticipated MoA involves tumour recognition & cell death
- Potency assays based on cytotoxic potential of antigen-specific T cells are most evident
- Release assay based on surrogates due to practical limitations (time, sample size)
- Biological relevance & correlation with in vivo functionality need to be substantiated with sufficient product-specific (non-)clinical data.
- Based on characterisation, (non)clinical studies & literature
- Animal results not necessarily representative for human
Considerations for (CAR-)T cell potency tests

- Establish correlation with clinical response based on potency characterisation studies of **clinical batches**
- Autologous product: inherent variability between batches
- How to set specifications?
- Avoid rejection of good batches
- Can detect clinically relevant defects & sub-potent batches
- Generally not 100% clinical success
- Link clinical data/outcome and potency test
- Post-approval: Evaluation Specifications/ Appropriateness test