Regulatory perspective on Potency Assays for cell-based 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

- Regulatory guidelines
- Considerations for MSC potency tests
- Considerations for T cells potency tests
- Recommendations
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.
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.
Guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer (EMA/CHMP/BWP/271475/2006)

**Potency assays for immunotherapy products** will be based on complex immune mechanisms which are often **poorly or incompletely understood** and which may be complicated by multi-antigen formulations and **inherent variability of the starting material**.

To **assure a consistent functional activity** of the medicinal product in the recipient, the potency of the product **within justified limits** should be demonstrated by a bioassay based on a defined biological effect as close as possible to the **mechanism(s) of action/clinical response**
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
Mesenchymal Stromal Cells

- Tissue homeostasis and regeneration capacities
- Homing to inflammation sites
- Immunomodulatory abilities with potential therapeutic applications
Immunomodulatory abilities

- Low immunogenicity (allowing allogeneic use; cross species barriers)
- Potential therapeutic applications
  - Graft-versus-host disease (GvHD)
  - Transplant rejection
  - Autoimmunity
- **Direct**: Suppression of activation, proliferation and effector functions of pro-inflammatory cells
- **Indirect**: Stimulation of anti-inflammatory cell types

![Diagram of immune modulatory capacities](image)
MSC modes of immunomodulation

- Expression of receptors & adhesion molecules
- Paracrine effects via soluble mediators (IDO, PGE$_2$, TGF-$\beta$, NO, several ILs) after cross-talk with activated immune cells
- Both on innate (i.a. NK, neutrophils, monocytes, DCs) and adaptive (T & B cells) immune system

From: Mesenchymal Stem Cells And Solid Organ Transplantation CellR4 2013
MSC Bioactivities

- MSC migration (response to TNF-α)
- MSC activation (CD200, or TNF-αR)
- Cytokine production
  - E.g. PGE2 or IDO production (tryptophane depletion)
- MSC effects on innate cells (DC, NK)
  - CD markers & cytokine secretion profiles
- Effects on CD4⁺ T cells
  - mainly inhibition of proliferation
  - alterations in Th subtype proportions
  - induction of regulatory T cells (Tregs)
- Effects on CD8⁺ T cells
  - MSCs suppress stimulation of antigen-specific cytotoxic T cells
# MSC 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>MSC activation</td>
<td>MSC</td>
<td>IFN-γ, TNF-α or IL-1β</td>
<td>e.g. CD200, cytokine receptors, Soluble mediators</td>
</tr>
<tr>
<td>Immune cell Inhibition</td>
<td>PBMC or T-cells</td>
<td>Memory antigen, Mitogen or aCD3/aCD28</td>
<td>Cytokine production, Surface markers T cell proliferation</td>
</tr>
<tr>
<td>Inhibition of MLR (mixed lymphocyte Reaction)</td>
<td>PBMC or T-cells</td>
<td>Allogeneic PBMCs or DCs</td>
<td>Cytokine production, Surface markers T cell proliferation</td>
</tr>
<tr>
<td>Suppressor cell induction assay</td>
<td>Immune cells (e.g. T cells)</td>
<td>MSC</td>
<td>Treg induction surface markers, cytokine production, suppressive function</td>
</tr>
<tr>
<td>Immune cell Migration assay</td>
<td>T cells (after MSC contact)</td>
<td>Chemokines (e.g., CXCL10)</td>
<td>T cell chemotaxis</td>
</tr>
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Elements in MSC Potency Assay Design

- **Type of assay**
  - Immune cell inhibition (e.g. GvHD)
  - Suppressor cell (Treg) induction (e.g. Autoimmune disease)
- **Responder cells**:
  - T-cells (CD4+, CD8+, Tregs), PBMC, B-cells
  - Origin (healthy-patient; heterologous-autologous)
  - Reproducibility (standard batch, fresh/frozen; consider pooling different donors, cell line)
- **Read-out parameters**: activation, cytokine production, proliferation, chemotaxis
- **Stimuli**: Mitogens, natural antigens, anti-CD3/anti-CD28, MLR
- **Reference control/batch**: Pooled MSC batch (random) or immunosuppressive cell line (Karpas 299 is more Treg like)
- Timing of response (IDO & PGE2 production time dependent)
T cell proliferation inhibition assay

Mitogen activated

Freshly Thawed

From: Ketterl et al. Stem Cell Res Ther 2015
Impact of Response Cells composition

From: Salem et al
Cytotherapy, 2015; 17

\[ R^2 = 0.32 \]
\[ P = 0.03 \]

From: Salem et al
Cytotherapy, 2015; 17
In vitro potency vs clinical response

MSC treatment in GvHD and hemorrhagic cystitis patients

MLC = mixed lymphocyte; PHA = mitogen stimulation


Hoefnagel, CASSS GCTP
July 2018
T cell cytolytic activity

Zaritskaya et al.  
*Expert Rev Vaccines* 2010
**CAR** T cell potency tests

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<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&lt;sub&gt;50&lt;/sub&gt;)</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</td>
<td>Effector T cells</td>
<td>Irradiated (peptide-pulsed) tumour cells</td>
<td>Intracellular cytokines (e.g. IFN-γ and IL-2)</td>
</tr>
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Hoefnagel, CASSS GCTP July 2018
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| Effector molecule release assay    | Effector T cells         | Irradiated (peptide-pulsed) tumour cells/peptides | - Secretion of cytokines (e.g. IFN-γ, TNF-α, IL-2)  
- Secretion of cytotoxic factors (e.g. granzyme B) |
| Degranulation assay                | Effector T cells         | Irradiated (peptide-pulsed) tumour cells | Surface expression CD107a/b                                                                         |
| Growth inhibition assay            | (Patient specific) Tumour cells | Effector T cells                     | Tumor cell proliferation ($^3$H incorporation)                                                       |
| Cytotoxicity assay (various release assays) | (Patient specific) Tumour cells (transduced) | Effector T cells | - Release intracellular proteins (e.g. LDH, β-gal or luciferase), or $^{51}$Cr  
- Intracellular $^3$H thymidine, GFP |
In vitro: Cytotoxicity (\(^{51}\)Cr release)  

In vivo: Survival Mice with lymphoma

- Künkele et al. (2015): discordance between in vitro potency and in vivo anti-tumour effects of CAR T cells. (Activation induced cell death)

From Hollyman et al J. Immunother. 2009
In vitro/In vivo correlation
Kymriah CAR-T cells

Response vs product in vitro potency
Study B2202

Pediatric ALL/B2202—63 patients (52-CR/CRI, 5-NR, 6-Unknown)
Best Overall Response within 3 months:
CR=complete remission; CRI=complete remission with incomplete blood count recovery; NR=nonresponder; Unknown [response].


Courtesy Novartis
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)
- Substantiate biological relevance & correlation with in vivo functionality using product-specific (non-)clinical data
- % Memory T cells (CD45/CD197) was also considered
- Based on characterisation, (non)clinical studies & literature
- Animal results not necessarily representative for human
Considerations for (CAR-)T cell potency tests (2)

- 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