Biological activity testing

Regulatory considerations

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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

• EU Regulatory expectations establishing biological activity
• Not only Primary Mode of Action
• Potency assays for Cell-based products
• Inherent variation biological assays
• VAC2VAC project
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**.

ICH Topic Q 6 B
Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products
ICH Q6B 2.1.2 Biological activity

- A valid biological assay to measure the biological activity should be provided by the manufacturer.
- Examples of procedures used to measure biological activity include:
  - Animal-based biological assays, which measure an organism's biological response to the product;
  - Cell culture-based biological assays, which measure biochemical or physiological response at the cellular level;
  - Biochemical assays, which measure biological activities such as enzymatic reaction rates or biological responses induced by immunological interactions
- Other procedures such as ligand and receptor binding assays, may be acceptable.
“based on the intended biological effect”: MoA

- Often exact Mode of Action (MoA) not fully known
- Developing representative *in vitro* assay not always straightforward
- If not practically feasible: sometimes surrogate assays as potency tests for release.
- Often >1 MoA: Difficult to capture in a single potency assay
How about other pivotal functional aspects

- Delivery: e.g. homing and uptake in cell (binding)
- For enzyme-replacement products uptake in the cells is also an pivotal aspect for their efficacy.
- Potency testing is often based on testing MoA (e.g. enzyme activity) only.
Modes of Action: Infliximab

Primary MoA

- Infliximab binds to both soluble and transmembrane TNFα and TNFα receptor activation is prevented
- Potency test: ability to block TNF-induced inhibition of (WEHI-164) cell proliferation (Ph.Eur. Draft monograph)

Secondary Mode of Action Infliximab

- Binding Fc receptors (FcγRI, FcγRIIa, FcγRIIb & FcRn) on effector cells
- ADCC (antibody-dependent cellular cytotoxicity)
- Binding Cq1
- CDC (Complement-dependent cytotoxicity)
- All affected by glycosylation

From: https://openi.nlm.nih.gov
Biosimilarity Inflectra vs Remicade

- Comparable binding activity to both monomeric and trimeric TNFα
- Comparable result in TNFα neutralisation assay
- Relative binding affinities to Fcγ receptors (FcγRI, FcγRIIa, FcγRIIb and FcRn) were comparable
- **Differences** in relative *in vitro* binding affinity of FcγRIIIa and FcγRIIIb
- **Differences** *Ex vivo* binding assay with isolated neutrophils and NK cells for Crohn’s Disease patients
- Genotype dependent **difference** in NK binding
- In presence of diluted CD patient serum all differences in binding were abrogated
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.
Cell-based medicinal products: the new biologicals

• Often exact MoA unknown (consequence: e.g. no surrogate markers available)
• Sometimes *in vitro* assay does not correlate with *in vivo* situation
  – Assay conditions are insufficient (e.g. presence of immune suppressiva *in vivo*)
  – Surrogate markers etc. are not appropriate read-out for biological activity
• Assay qualitative instead of quantitative
• Reference standard difficult to obtain
• Not up-to-date with most recent scientific knowledge (fast evolving field)
Mesenchymal Stem Cells

- Tissue homeostasis and regeneration capacities
- Immunomodulatory abilities with potential therapeutic applications
Mesenchymal Stem Cells

- Immunomodulatory abilities with 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
MSC modes of immunomodulation

From Mesenchymal Stem Cells: Immunology and Therapeutic Benefits. NE Haddad
MSC modes of immunomodulation

- Expression of receptors & adhesion molecules
- Paracrine effects via soluble mediators (IDO, PGE₂, TGF-β, 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
MSC Bioactivities

• 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

• Most studies only determined effect on cytokines produced
• Results impacted by culture method, tissue origin & assay conditions
MSC Potency test considerations

- Potency test: differentiate potent and sub-potent batches
- (Semi-)quantitative assay is required
- Viability is not potency
- Activation status: phenotype CD markers not sufficient
- Promising Markers Contradictory results: CD200, TNF-αReceptor expression, IDO (time-dependent)
- Culture and Activation conditions of both MSCs and responder cells determine whether or not an immunomodulatory factor can be tested
Most proposed MSC potency assay

- Inhibition of T cell activation/proliferation in co-culture with MSCs
- Induction of T cells:
  - with memory antigens
  - mitogens (e.g. PHA, PMA or ConA)
  - T cell receptor cross-linking and co-stimulation (aCD3/aCD28)
  - Allogeneity (e.g. allogeneic PBMCs or DCs in a mixed lymphocyte reaction (MLR))
- Mitogen- or aCD3/aCD28-based assays: not specific nor natural; result in 3-4 days
- MLR mimics \textit{in vivo} response GvHD; result in 6-8 days
Considerations T-cell proliferation inhibition assay

- Assay does not reflect all relevant biological properties: i.e. no analysis of effect on other cell types
- PBMCs more representative of *in vivo* *(more variability!)*
- Assay susceptible to non-obvious differences
  - T cell proliferation dependent on mismatch (assay variability)
  - Allogeneic MSCs can cause alloreactivity
  - Age, gender & infection history MSC donor
  - Same responder cell preparation throughout products’ lifecycle
  - Acceptance criteria for % of subpopulations (CD4, CD8, Tregs)
Holoclar: Limbal Stem cells

Based on Pellegrini et al., Stem Cells (2014)
**Clinical data**: most important biological criterion for graft quality (likelihood successful outcome) is evaluation of number of stem cells detected as **p63 bright holoclones** in the culture.

**Release testing:**
- Viability
- Cell number
- Colony-forming efficiency
- % p63 bright cells
- % K3^+ cells

Pellegrini *et al.*, Stem Cells, vol. 32, pp. 26-34
Potency test for release of ATMP

- Surrogate markers could be acceptable
- Characterization studies should include bioactivity assay
- Evidence needed that surrogate marker is
  - linked to effect at cellular level (e.g. decreased T cell proliferation)
  - correlated with relevant clinical effects
  - detecting clinically relevant defects and sub-potent batches as these could occur in the specific manufacturing process
- Rejection of failing autologous / patient-specific batches (B/R)

- Example: Using ELISA to measure Cytokines it is not clear which cells produce these. Use of Flow Cytometry resolves this
Inherent variation of bioassays

- Example: Well-defined biological (rDNA protein)
- *In vivo* potency testing in rodents
- High variation: % RSD (intermediate precision) 59%
- Range **Clinical** batches 92-362%
- **Proposed** limits (95% confidence): 20-569%

- **Limits tightened**
- Risk of rejecting suitable batches
- *In vivo* assays generally high variability
- Bioactivity assays for well-defined products?

Not accepted!
VACCINE BATCH TO VACCINE BATCH COMPARISON BY CONSISTENCY TESTING

Proof of concept of consistency approach for batch release testing of established vaccines using sets of *in vitro* and analytical methods

- Develop, optimise & evaluate *non-animal methods* that cover key-parameters for demonstrating batch consistency, safety and efficacy

- *(Pre-)*validate methods and define with *regulators* guidance for regulatory approval and routine use
OVERVIEW

• 21 participants: 15 public partners, 6 EFPIA companies

• Total **budget**:
  - €7.85M EU funding in cash
  - €8.13M from EFPIA partners in kind

• Seven work packages
  - WP 1: **Physicochemical** methods
  - WP 2: **Immunochemical** methods
  - WP 3: **Cell-based** assays
  - WP 4: Multi-parametric assays and **bioinformatics**
  - WP 5: (Pre)**validation**
  - WP 6: Promotion of consistency testing to **regulatory acceptance**
  - WP 7: Consortium **management**
Thanks to Charlotte De Wolf

De Wolf et al. Cytotherapy  April 2017 (in press)
Regulatory perspective on in vitro potency assays for human mesenchymal stromal cells used in immunotherapy