EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

CBG MEB

Potency & Stability Testing for ATMP

SME Workshop EMA

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Outline

- Rationale of Potency & Stability testing
- Examples
  - Autologous DC
  - Tissue Engineering Product
  - Gene Therapy product
- Additional Recommendations
- Guidelines & Further reading

Rationale

ICH 6QB, **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

Guideline Cell-Based Medicinal Products (EMEA/CHMP/410869/2006)

Major cellular functions (viability, self renewal, death and differentiation) are pivotal to the quality, function and sustainability

Monitor these as IPC / at release using surrogate markers and appropriate technology (e.g. gene expression profiles by microarrays, flow cytometric immuno-fluorescent analysis, cell cloning, PCR and many others)

1) *in vitro* assays using cell systems

2) *in vivo* assays using animal models.

In vivo assays for potency may also be useful especially when experimental animal models are available

Characterisation or Release;

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**.

Carefully consider potency testing for characterization / comparability and release. Preferably, the potency assay should reflect the clinical Mechanism of Action.

Stability testing

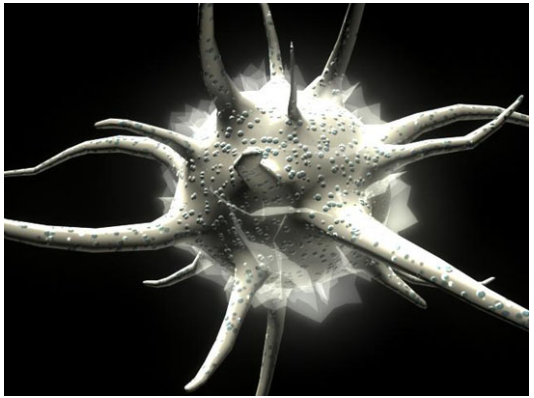
- A shelf life shall be determined for
  - i) Intermediates subject to storage
  - ii) Components of combined CBMP
  - iii) Active substance (Drug Substance)
  - iv) Finished product (Drug Product)
- Specified storage conditions
- Valid **in-use** shelf life (after opening from transport container) including temperature range
- Transportation & storage conditions supported by experimental data

Stability testing

- Document methods for freezing and thawing
- Combination products: stability testing for cellular / non-cellular components stored separately and in combination, where possible
- Determine impurities and degradation products originating from the structural component (matrix, scaffold, device)
- If limiting cell numbers (autologous cell products): test shelf life of structural components with (relevant) different cells (Justify!)

Casus 1: autologous DCs for immunotherapy

Dendritic cells pulsed with antigens *(e.g. tumour cell lysate)*



- DC = autologous
- tumour cell lysate = allogeneic
- cryopreserved in DMSO

Mode of action

1. presentation of tumour-associated antigen to lymphocytes
2. activation and induction of proliferation of $CD8^+$ and $CD4^+$ T cells
3. potent and specific anti-tumour response

Surrogate markers of DC maturation and potency.

Parameter	Method	Acceptance criteria
Viability	Trypan blue exclusion	> 80%
Phenotype	Flow cytometry	$CD11c^+$/$MHC\text{-}II^+$ > 95% $CD80^+$ > 60%
Phenotype additional markers	Flow cytometry	e.g. CD54, CD69, CD83, CD86

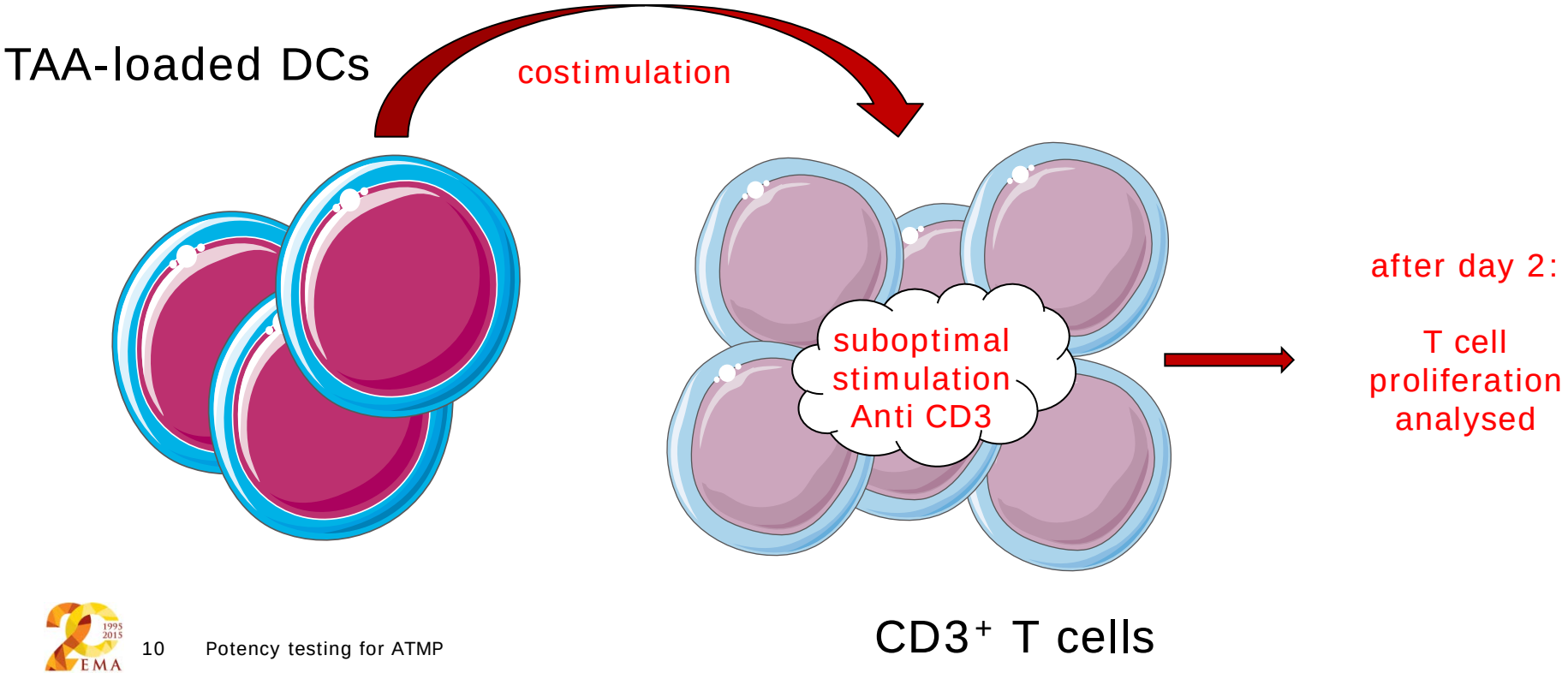
Insufficient: no functional assay

Additional potency assays

COSTIM bioassay: Proliferation of T-cells, after DC stimulation

Cytolysis assay: Using patient serum (T-cells) and a tumour cell line

COSTIM assay



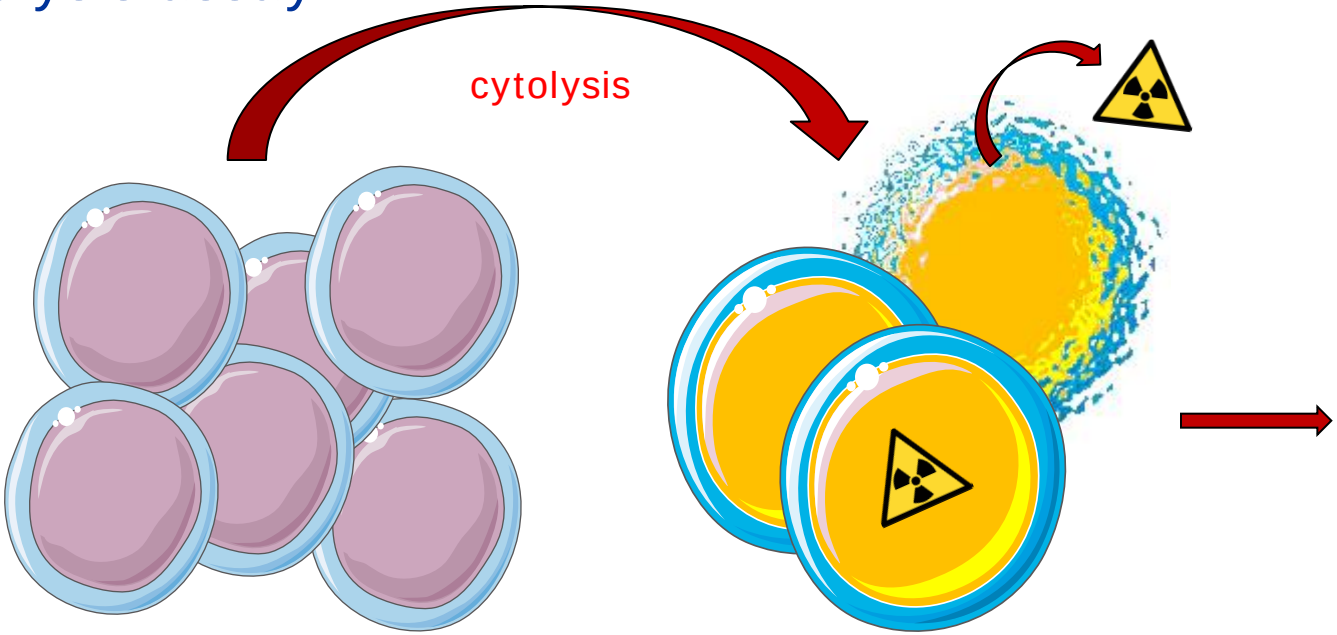
Relevance of COSTIM assay

COSTIM: useful assay to test the co-stimulation capacity of TAA-presenting DCs. Justify Biological relevance: T-cell proliferation not directly correlated with specific antigens presented.

Using one common T cell batch to monitor proliferative response is considered a MLR, mainly depending on mismatch between T cells and DCs. Compare to co-culture with autologous T cells.

Functional assays during clinical trials should use autologous T cells (or PBMCs). Only autologous cells will give correct information on patient-specific potency of product.

Cytolysis assay



Cytolysis assay

- Co-culture of patient's T-cells (before / after treatment) with tumour cell lines can show that treatment leads to T cells able to attack tumour cells.
- No release test.
- *In vitro* prior to immunisation not feasible
- Ideal = simulation of proposed MoA and biological effect

DC Potency Summary

- Validated functional assay required (e.g. COSTIM assay)
- DC viability and phenotype not sufficient
- Justification for chosen markers and controls required (include monitoring these markers prior to stimulation)
- During characterisation / clinical studies use assay to demonstrate functionality
- Effect on other immune cells (as part of characterisation)

Further considerations / recommendations

- Preferably Quantitative assays
- Evaluate relation potency-efficacy
- Consider if Reference Standard (TAA-Loaded DCs) is feasible
- Does testing before cryopreservation ensure potency after thawing & washing?
- Does storage impact on other aspects: FACS analysis, viability, T-cell stimulation, etc. ?
- Include cells from patients in assay validation; disease may impact on e.g. patient's T-cell population.

Specific potency assay comments: autologous cells

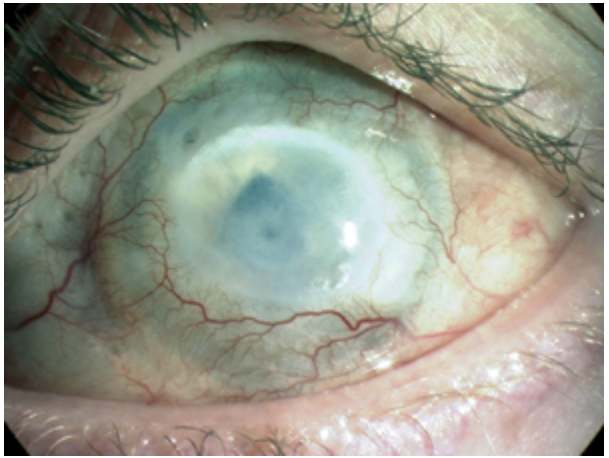
- Few cells available for potency assay (requires usually more cells than other release tests, especially bioassay)
- Aspecific stimulation (e.g. proliferation assay)
  - No determination of antigen-specific cell number / function
  - HLA type differences can hamper bioassay development
  - MLR used as bioassay (proliferative capacity based on HLA differences, only possible when used to analyse an effect with correct control: e.g. before/after vaccination, DCs without / with stimulation, etc.)
- Difficulties with correct reference standard due to e.g. donor variability
- Wide range for specifications due to e.g. donor variability

Casus 2: Tissue engineered Product: Autologous Limbal Epithelial stem cells

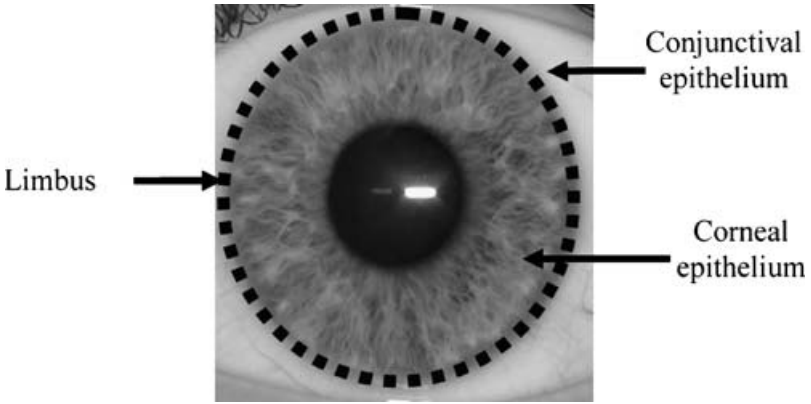
Loss of corneal stem cells (injury/disease)

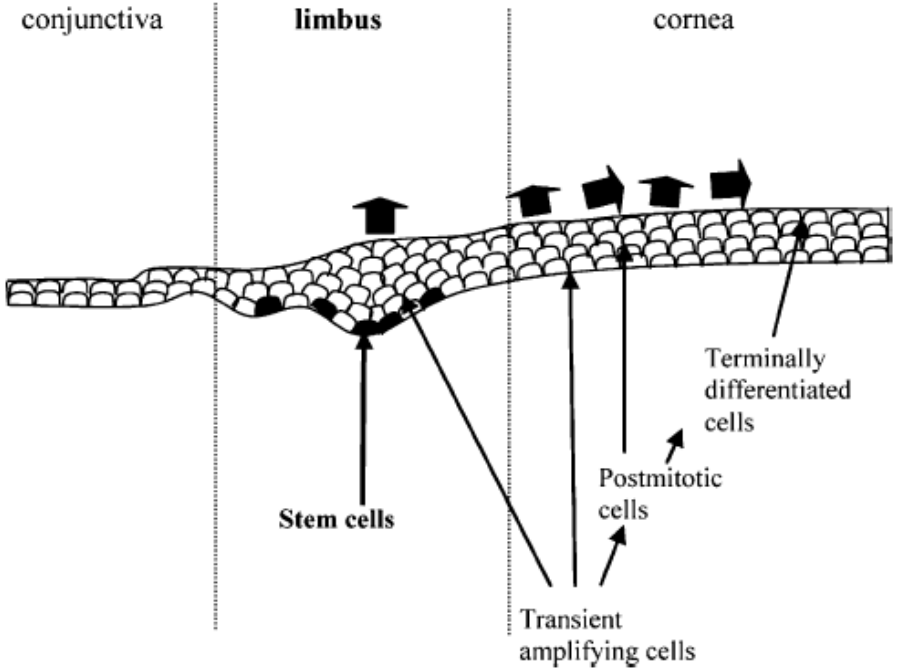
→ no cornea repair and overgrowing of conjunctival epithelium

→ vision loss



Background information limbal stem cells



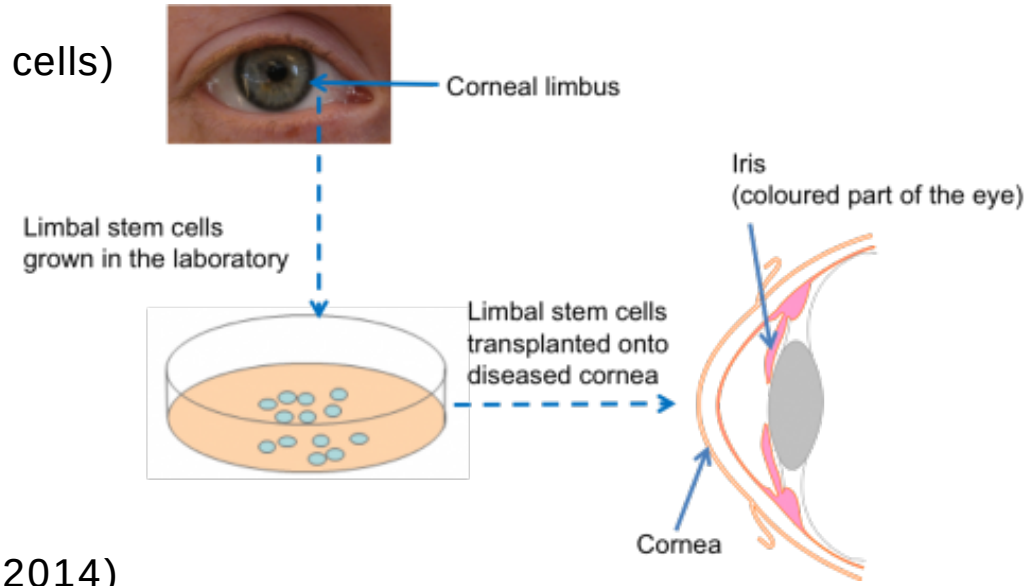


Notara *et al*., Cell Tissue Res, vol. 331, pp. 135-143

Background information

Autologous LESC (limbal epithelial stem cells) expanded on a cellular matrix to:

- Maintain stem cells undifferentiated
- Form an epithelial cell sheet for transplantation



- Based on Pellegrini *et al*., Stem Cells (2014)

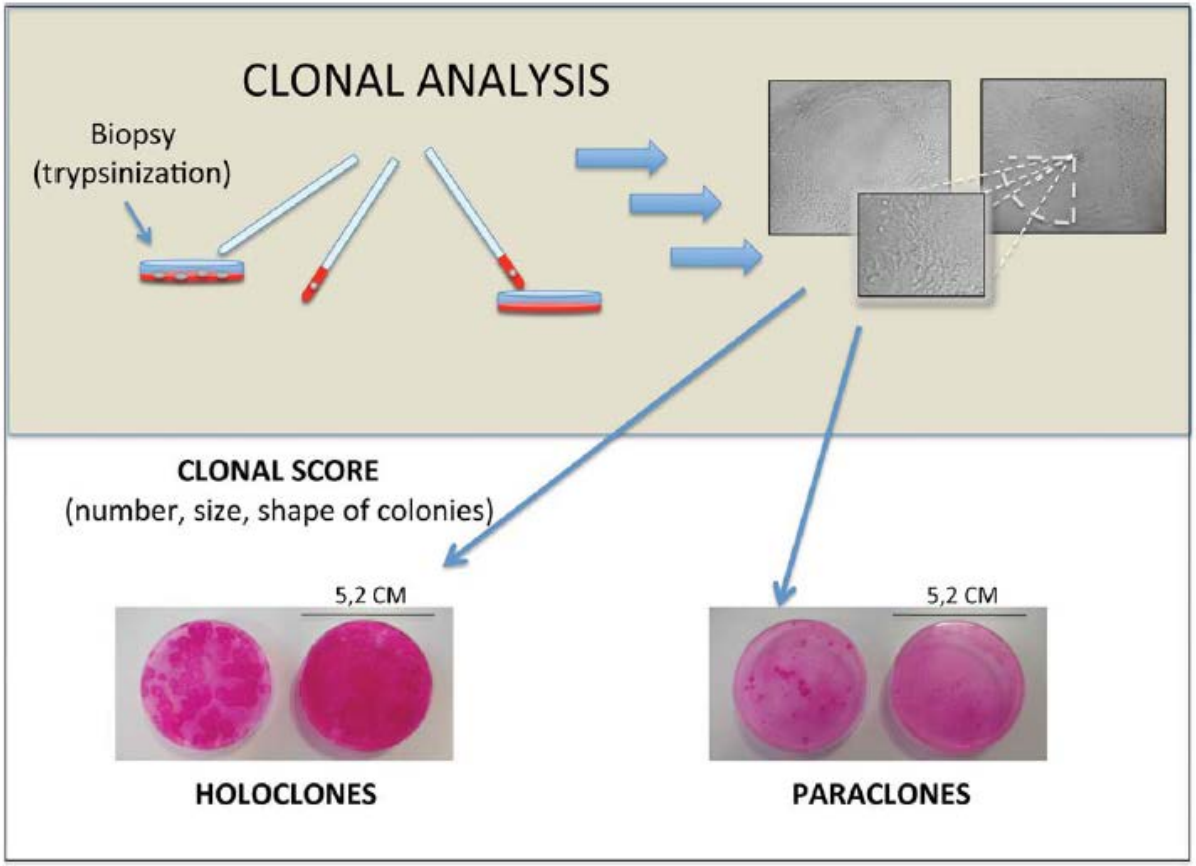
Characteristics

- Identity and purity
  - Small cuboidal cells with high nucleus-cytoplasm ratio
  - Undifferentiated stem cells after expansion (transient amplifying)
  - Phenotypic markers comparable to *in vivo* cells
- Potency (based on *in vivo* mechanism of action)
  - Potential of proliferation with self renewal and differentiation
  - 3 types of clonogenic keratinocytes: holoclones, meroclones, and paraclones
  - Stem cells: holoclone-forming cells

Potency Assay (1)

- Number of clonogenic cells, colony size & cell growth rate are conditions **necessary but**
- **not sufficient** to predict performance of the graft.

From Pelegrini *et al.*, Stem Cells (2014)



Pellegrini *et al.*, Stem Cells, vol. 32, pp. 26-34

Potency assay (2)

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

Rama *et al.*, N Engl J Med, vol. 363, pp. 147-155

Pellegrini *et al.*, Stem Cells, vol. 32, pp. 26-34

Casus 3: Gene therapy product: Eyelight

Lentiviral vector

hERP = human Eye Repair Protein gene

Mode of action

1. transfection of human retinal cells with LV
2. ERP gene transcription and translation → functional protein
3. protein deficiency solved to stop progressive eye disease

Eyelight Gene therapy: potency testing

- Infectious titre
- Transfection efficiency of target cells (or representative cells)
- ERP gene expression & functionality in target cells
- Functionality → can be difficult to model, but at least show that:
  - surrogate marker for function is linked to *in vivo* function (scientific rationale)
  - cell type in bioassay is representative for *in vivo* target cell
  - assay conditions represent *in vivo* transduction
- Include reference batch

Gene therapy: stability protocol

- Appearance
- pH
- Genomic titre
- Infectious titre
- *In vitro* potency (cellular infectivity, protein expression & functionality)
- SDS-PAGE (purity)

Further ATMP potency testing issues (1)

- Assay qualitative instead of quantitative
- 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
- Reference standard difficult to obtain
- Not up-to-date with most recent scientific knowledge

Further ATMP potency testing issues (2)

- Assay does not reflect all relevant biological properties (e.g. T cell suppression by MSCs → no analysis of effect on other cell types or analysis of self-renewing capacity of MSCs)
- Assay is not specific enough
  - Effect may also be caused by impurities
  - Not clear which cells produce the factor (e.g. ELISA versus flow cytometry)

Guidelines & Further Reading

- ICH Q6B Note For Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. (CPMP/ICH/365/96)
- EMEA/CHMP guideline on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer (CHMP/BWP/271475/06)
- EMEA/CHMP guideline on human cell-based medicinal products (EMEA/CHMP/410869/2006)
- ICH Considerations Oncolytic Viruses (EMEA/CHMP/ICH/607698/2008)
- Bravery *et al*. (2013) Cytotherapy 15, 9–19 (Gives examples!)
- Guthrie *et al*. (2013) Trends in Biotechnology, Vol. 31, 505-514