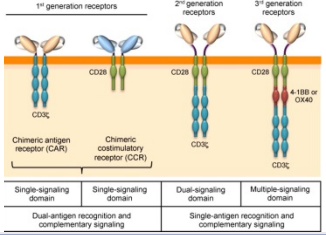
Regulatory perspective on Potency Assays for cell-based products

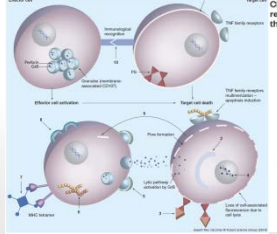
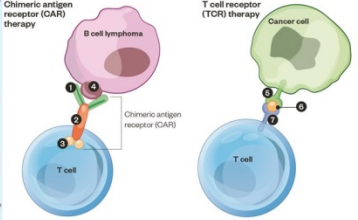
Marcel Hoefnagel
Medicines Evaluation Board, The Netherlands

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

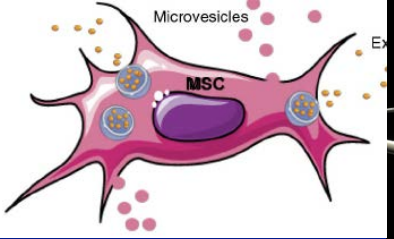



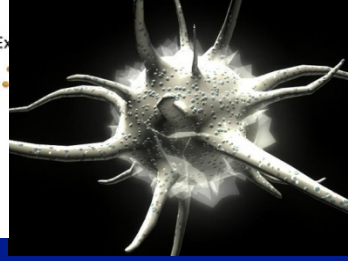



ICH 6QB Definition Potency

- **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**.

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



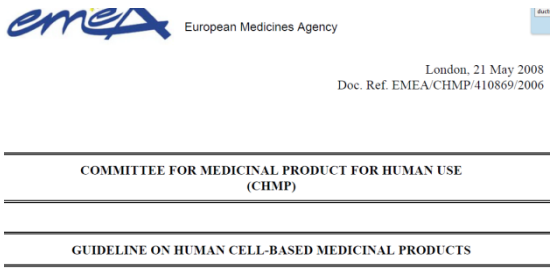


Cell-based medicinal products: the new biologicals

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**.

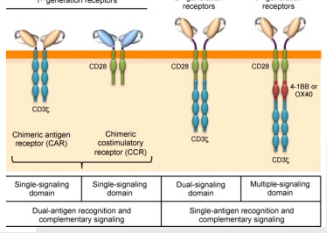
Preferably, the potency assay should reflect the clinical Mechanism of Action.

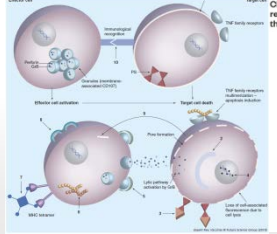

European Medicines Agency

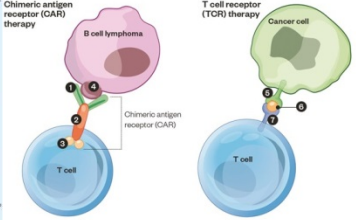
London, 21 May 2008
Doc. Ref. EMEA/CHMP/410869/2006

COMMITTEE FOR MEDICINAL PRODUCT FOR HUMAN USE
(CHMP)

GUIDELINE ON HUMAN CELL-BASED MEDICINAL PRODUCTS







Guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer (EMA/CHMP/BWP/271475/2006)

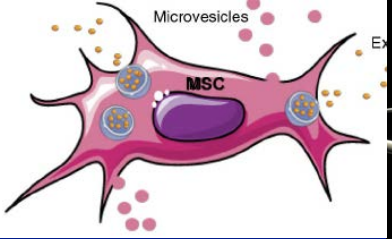
Potency assays for immunotherapy products will be based on complex immune mechanisms which are often **poorly or incompletely understood** and which may be complicated by multi-antigen formulations and **inherent variability of the starting material**.

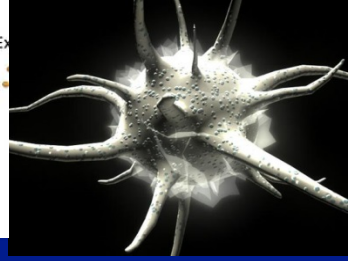
To **assure a consistent functional activity** of the medicinal product in the recipient, the potency of the product **within justified limits** should be demonstrated by a bioassay based on a defined biological effect as close as possible to the **mechanism(s) of action/clinical response**

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 July 2016
EMA/CHMP/BWP/271475/2006 rev.1
Committee for medicinal products for human use (CHMP)

Guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer



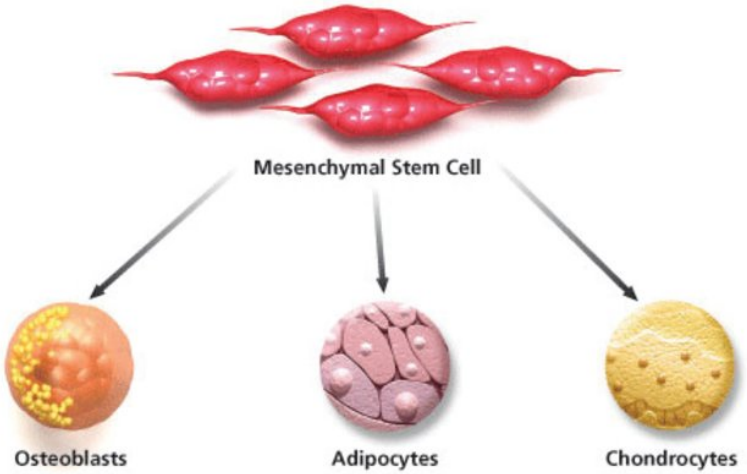


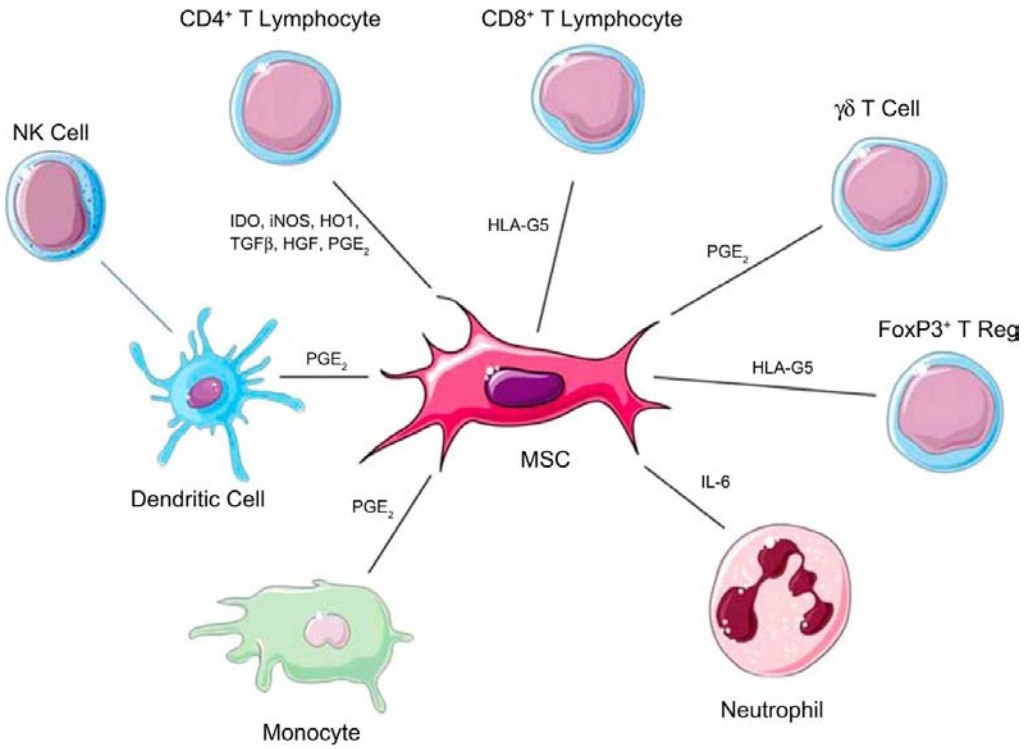
Challenges for Potency tests of Cell Based Products

- 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

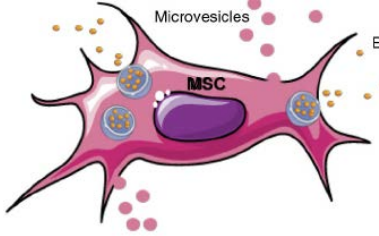
Mesenchymal Stromal Cells

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

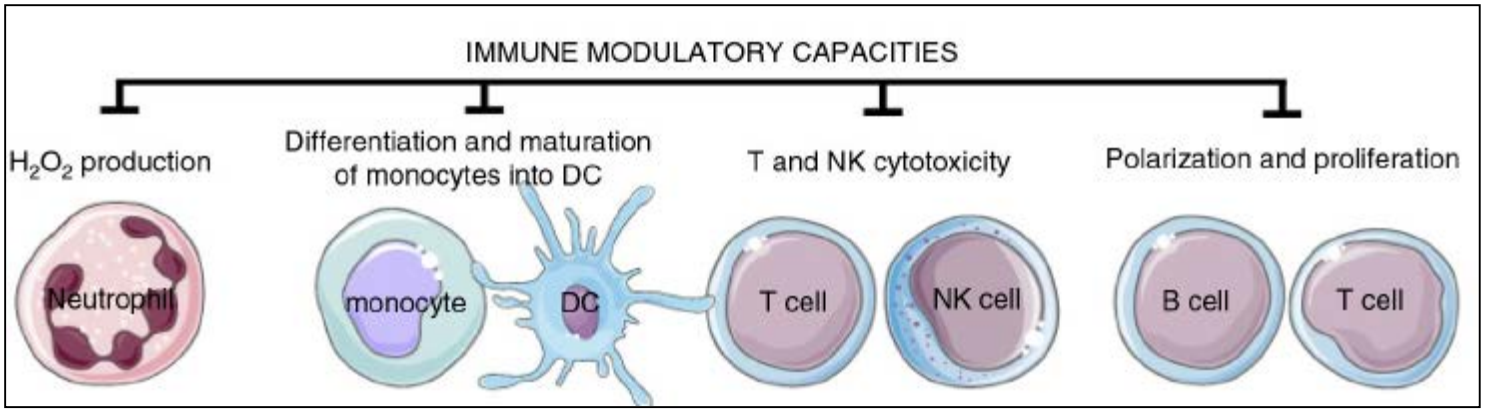




Immunomodulatory abilities

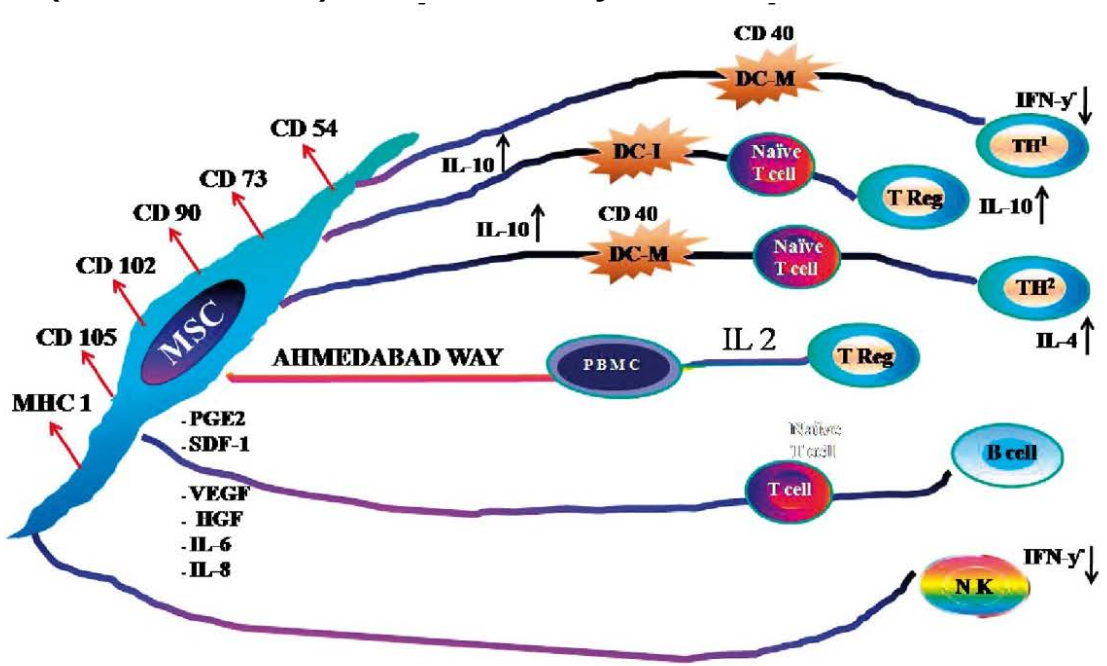


- Low immunogenicity (allowing allogeneic use; cross species barriers)
- Potential therapeutic applications
  - Graft-versus-host disease (GvHD)
  - Transplant rejection
  - Autoimmunity
- **Direct**: Suppression of activation, proliferation and effector functions of pro-inflammatory cells
- **Indirect**: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



MSC modes of immunomodulation

- Expression of receptors & adhesion molecules
- Paracrine effects via soluble mediators (IDO, PGE_2, 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



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

Assay type	Responder cells	Stimuli	Read out
MSC activation	MSC	IFN-γ, TNF-α or IL-1β	e.g. CD200, cytokine receptors, Soluble mediators
Immune cell Inhibition	PBMC or T-cells	Memory antigen, Mitogen or aCD3/aCD28	Cytokine production, Surface markers T cell proliferation
Inhibition of MLR (mixed lymphocyte Reaction	PBMC or T-cells	Allogeneic PBMCs or DCs	Cytokine production, Surface markers T cell proliferation
Suppressor cell induction assay	Immune cells (e.g. T cells)	MSC	Treg induction surface markers, cytokine production, suppressive function
Immune cell Migration assay	T cells (after MSC contact)	Chemokines (e.g., CXCL10)	T cell chemotaxis

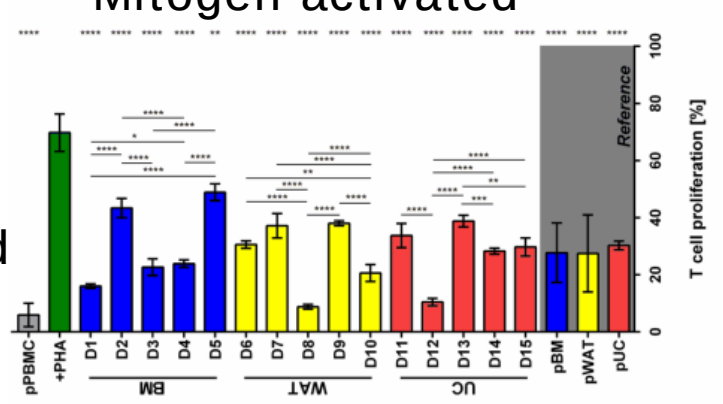
Elements in MSC Potency Assay Design

- **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

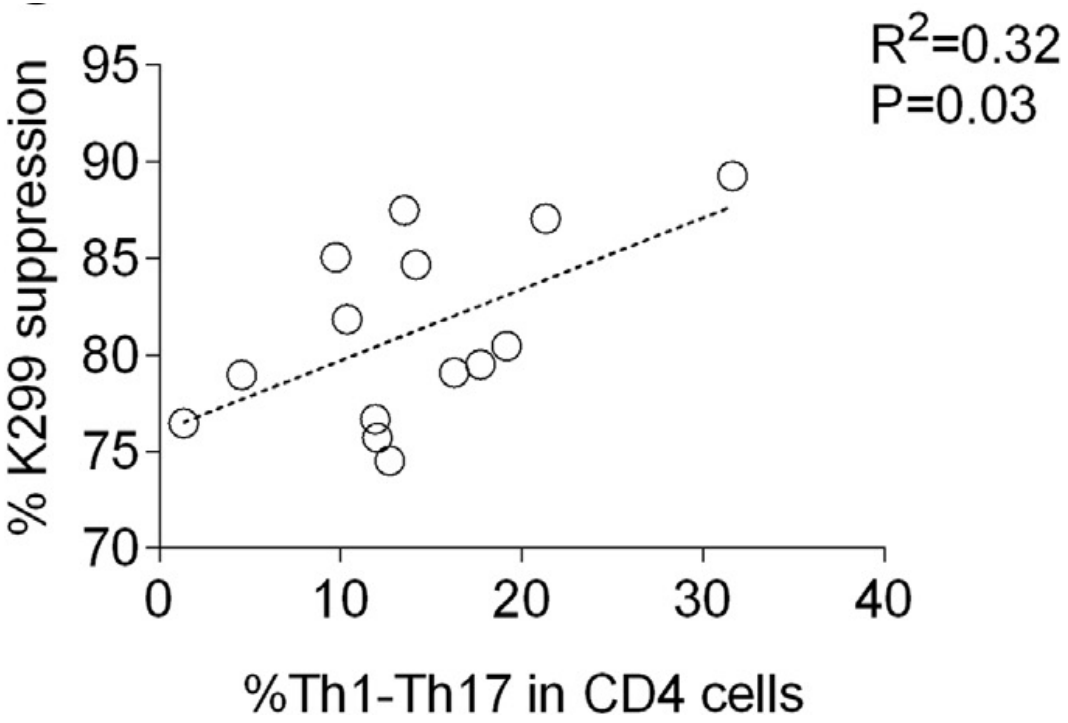
Mitogen activated

Freshly Thawed



From: Ketterl et al. Stem Cell Res Ther 2015

Impact of Response Cells composition

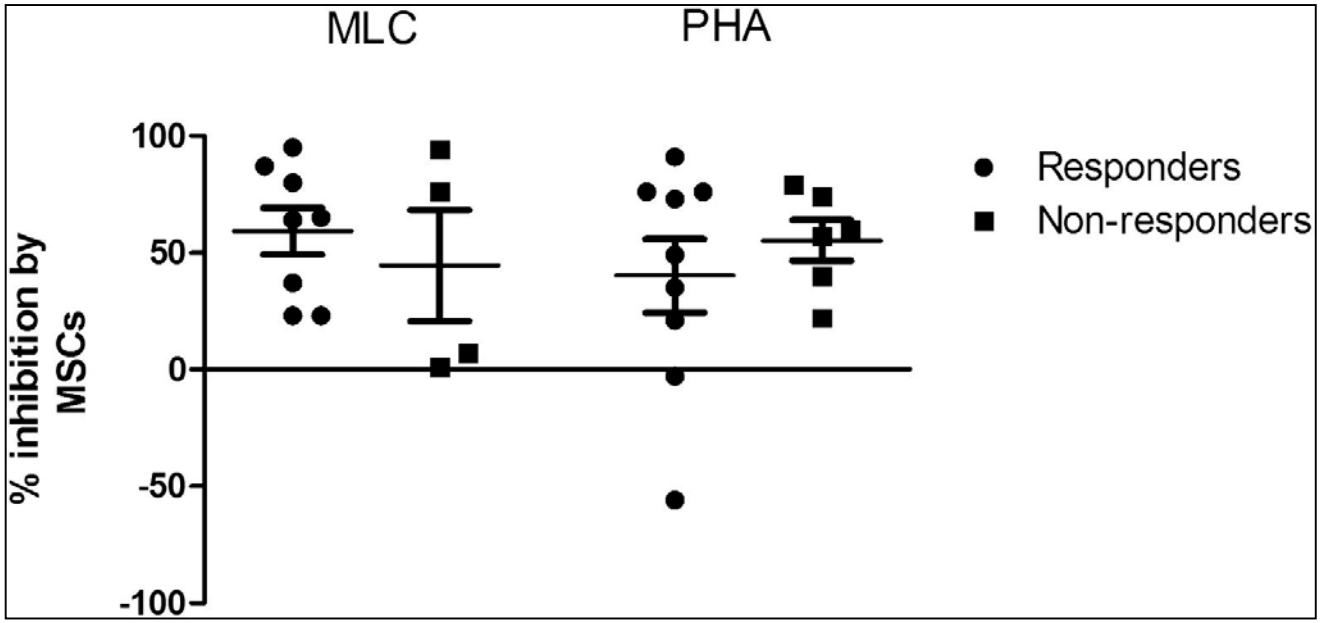


From: Salem et al
Cytotherapy, 2015; 17

In vitro potency *vs* clinical *response*

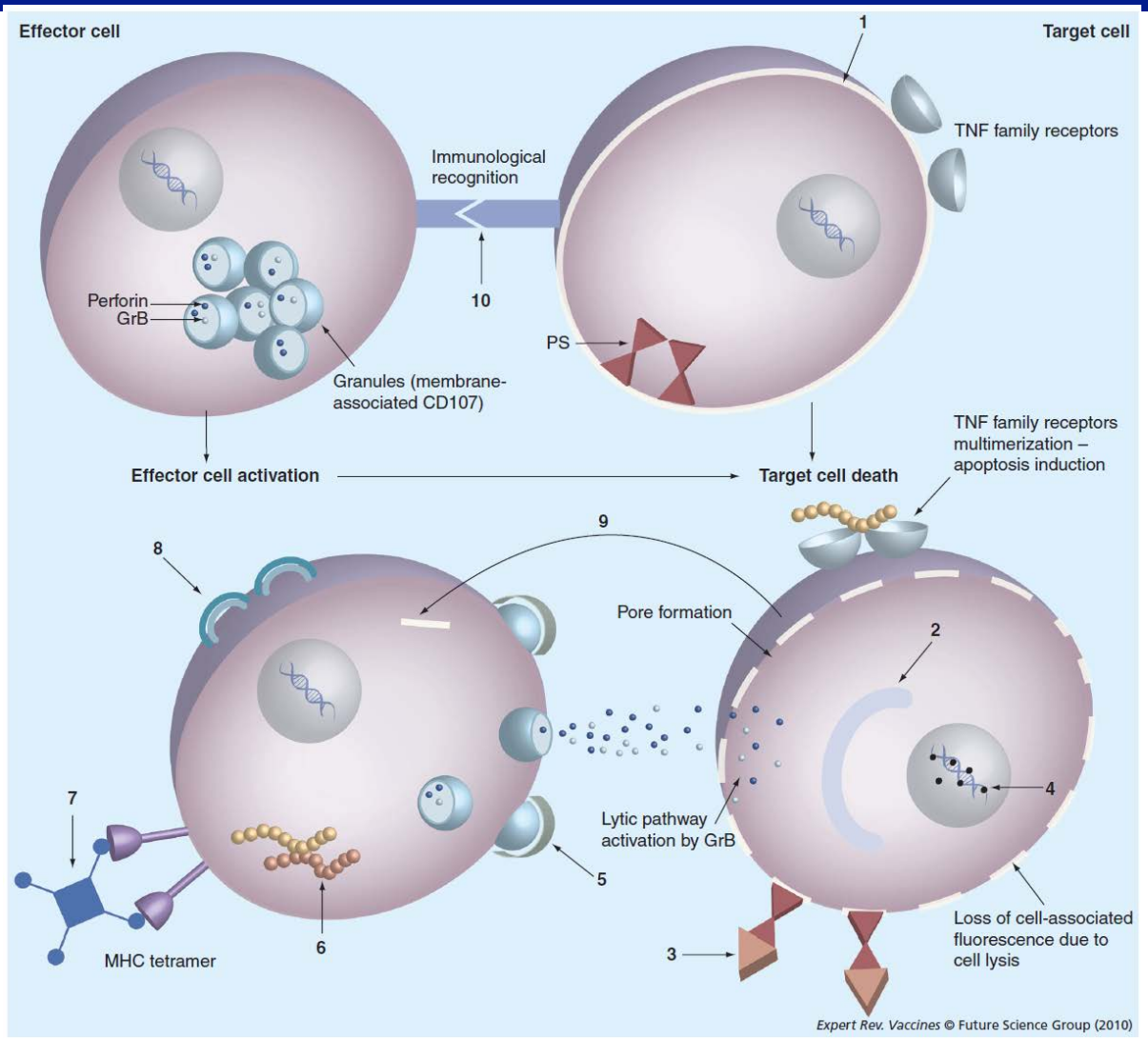
MSC treatment in GvHD and hemorrhagic cystitis patients

MLC= mixed lymphocyte; PHA = mitogen stimulation



From: Von Bahr et al. Biol Blood Marrow Transplant 18:2012

T cell cytolytic activity



Zaritskaya et al. Expert Rev Vaccines 2010

(CAR) T cell potency tests

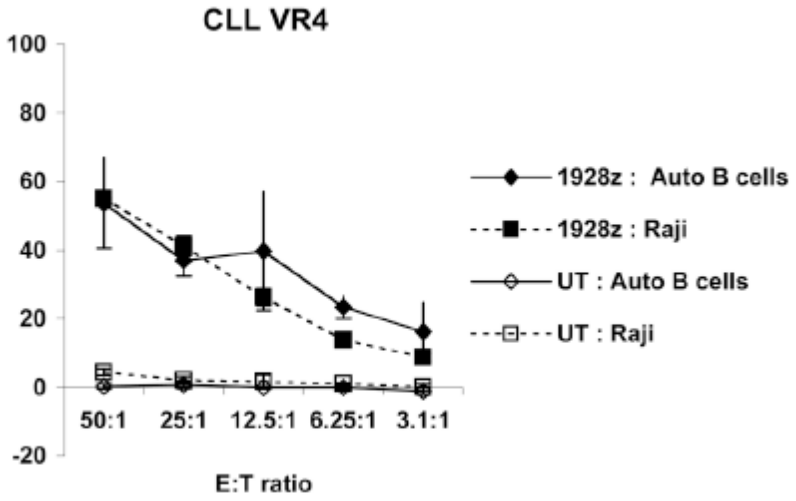
Assay type	Responder cells	Stimuli	Read out
Phenotyping assay	T cells		CD markers, antigen (e.g. CD19) specific receptor
Avidity assay	T cells	MHC multimers loaded with antigens	Amount/duration of TCR-MHC-peptide binding (EC_{50})
Proliferation assay	Effector T cells	Irradiated (peptide-pulsed) tumour cells	Proliferation (via CFSE dilution)
Cytokine production assay	Effector T cells	Irradiated (peptide-pulsed) tumour cells	Intracellular cytokines (e.g. IFN-γ and IL-2)

(CAR) T cell potency tests (2)

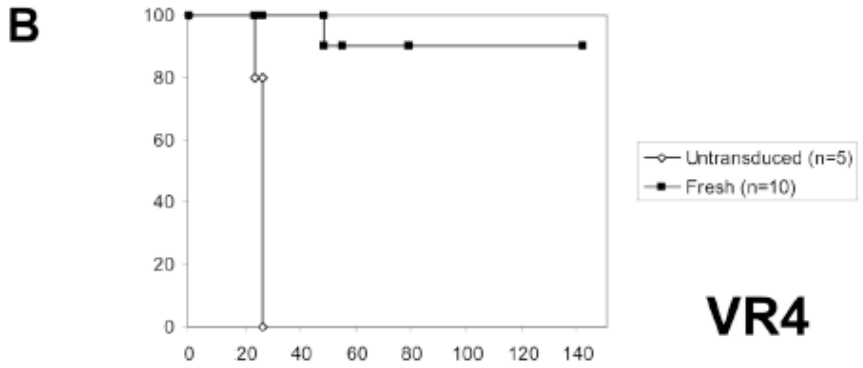
Assay type	Responder cells	Stimuli	Read out
Effector molecule release assay	Effector T cells	Irradiated (peptide-pulsed) tumour cells/ peptides	- Secretion of cytokines (e.g. IFN-γ, TNF-α, IL-2) - Secretion of cytotoxic factors (e.g. granzyme B)
Degranulation assay	Effector T cells	Irradiated (peptide-pulsed) 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/In vivo correlation

In vitro: Cytotoxicity (^{51}Cr release)



In vivo: Survival Mice with lymphoma

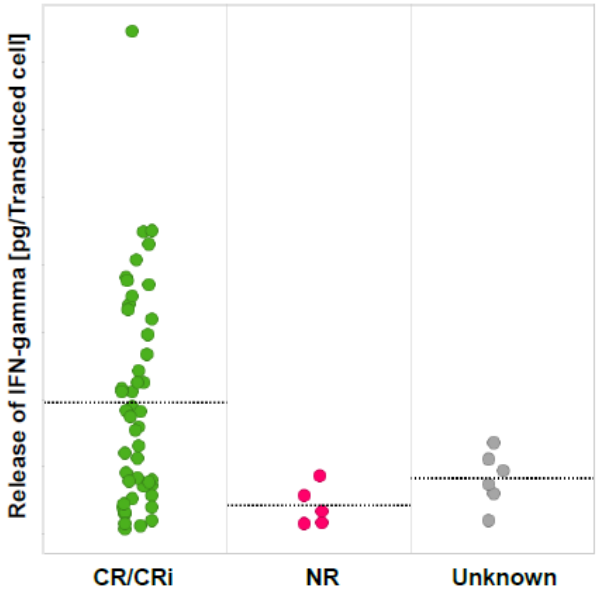


From Hollyman et al J. Immunother. 2009

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

In vitro/In vivo correlation
Kymriah CAR-T cells

Response vs product in vitro potency Study B2202

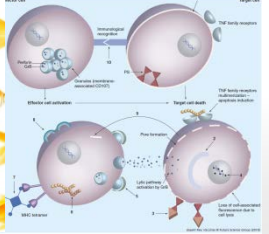


Courtesy Novartis

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].

https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM567385.pdf





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