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

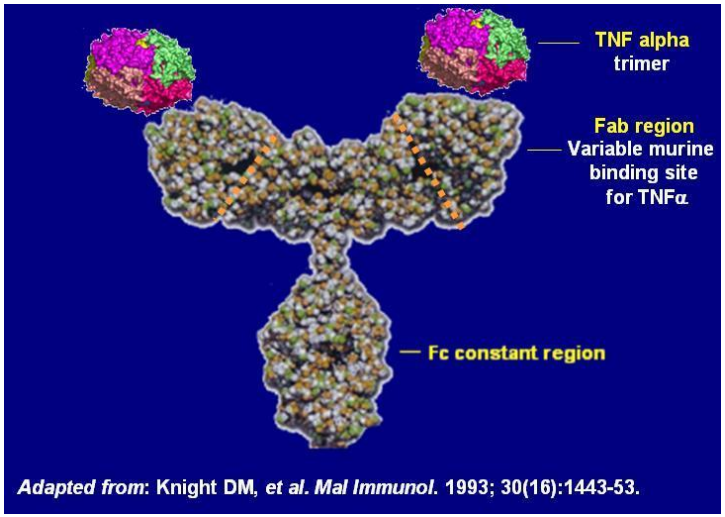
- Delivery: e.g. homing and uptake in cell (binding)
- For enzyme-replacement products uptake in the cells is also an pivotal aspect for their efficacy.
- Potency testing is often based on testing MoA (e.g. enzyme activity) only.



Modes of Action: Infliximab

Primary MoA

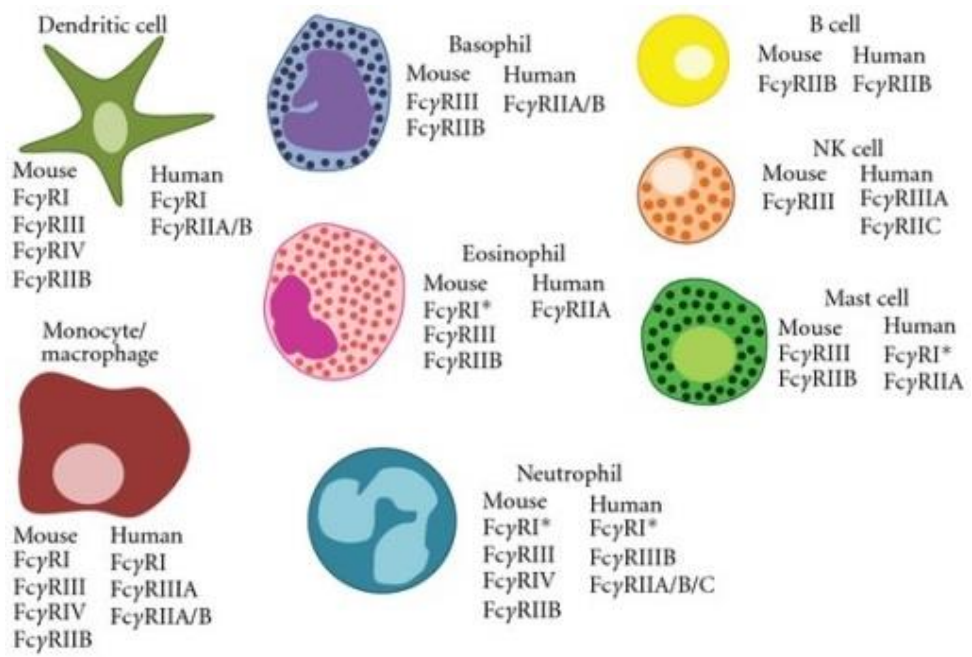
- Infliximab binds to both soluble and transmembrane TNFα and TNFα receptor activation is prevented
- Potency test: ability to block TNF-induced inhibition of (WEHI-164) cell proliferation (Ph.Eur. Draft monograph)

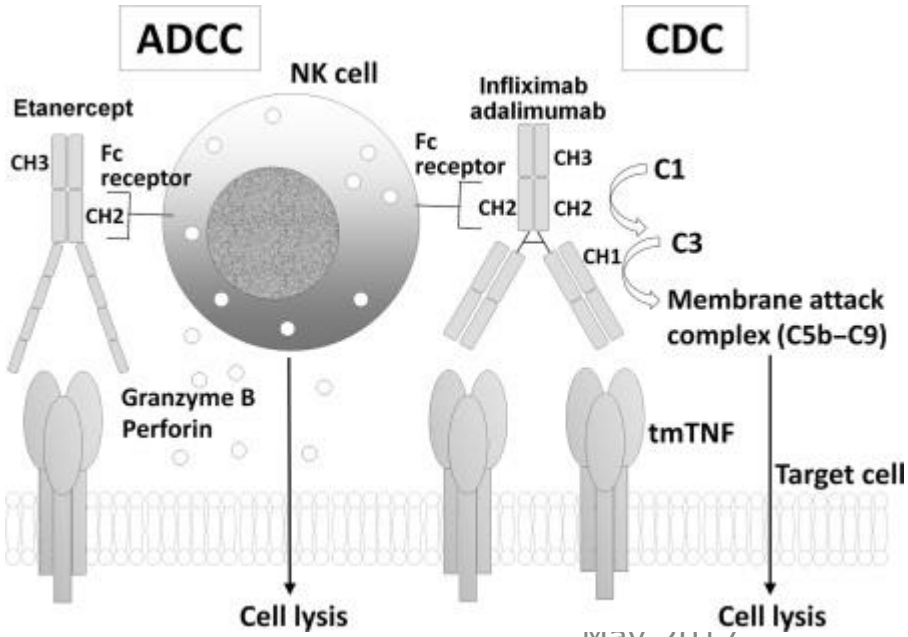


Secondary Mode of Action Infliximab

- 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

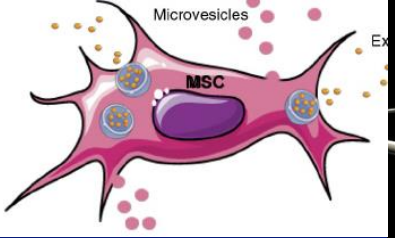
From: https://openi.nlm.nih.gov

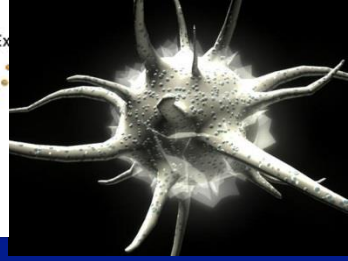




Biosimilarity Inflectra vs Remicade

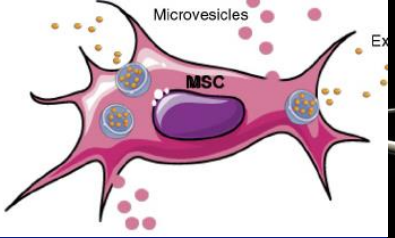
- Comparable binding activity to both monomeric and trimeric TNFα
- Comparable result in TNFα neutralisation assay
- Relative binding affinities to Fcγ receptors (FcγRI, FcγRIIa, FcγRIIb and FcRn) were comparable
- **Differences** in relative *in vitro* binding affinity of FcγRIIIa and FcγRIIIb
- **Differences** *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

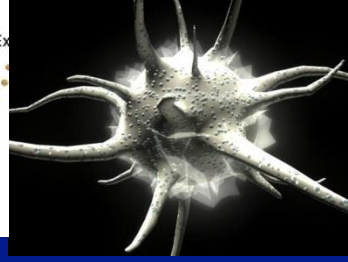




Cell-based medicinal products: the new biologicals

- 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.



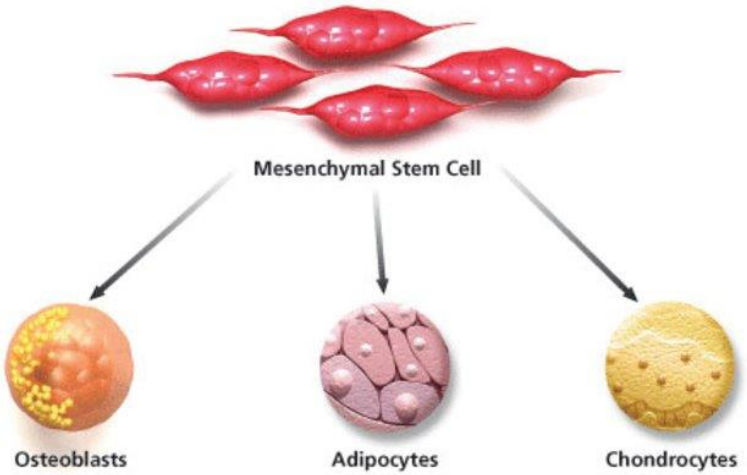


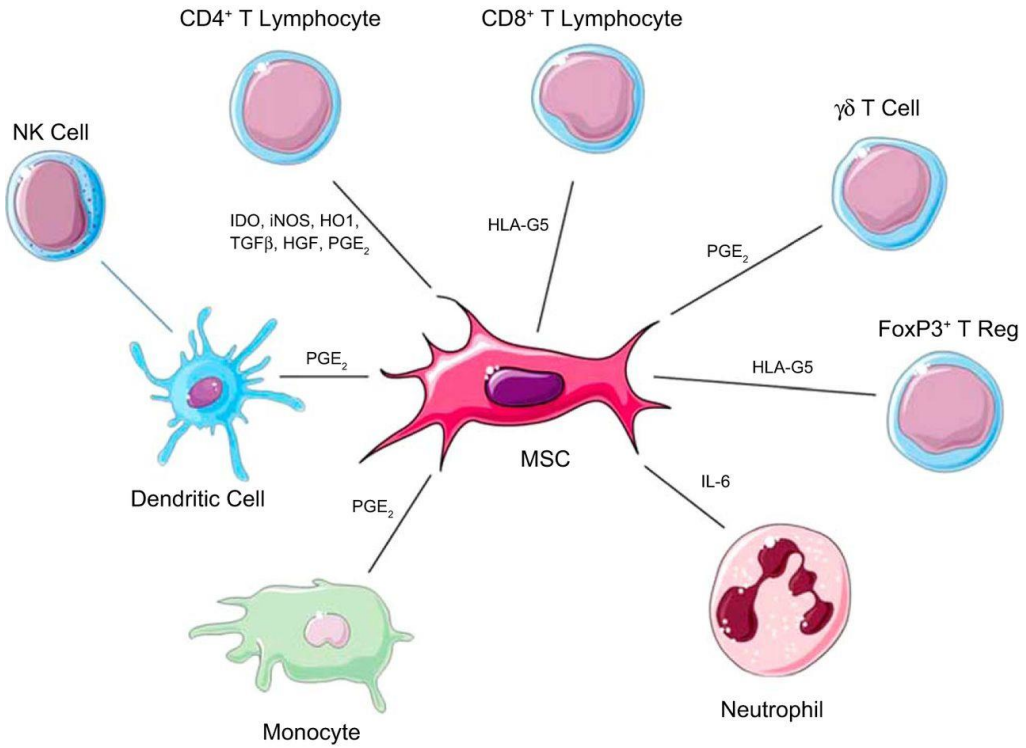
Cell-based medicinal products: the new biologicals

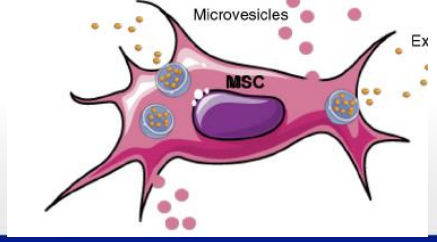
- Often exact MoA unknown (consequence: e.g. no surrogate markers available)
- Sometimes *in vitro* assay does not correlate with *in vivo* situation
  - Assay conditions are insufficient (e.g. presence of immune suppressiva *in vivo*)
  - Surrogate markers etc. are not appropriate read-out for biological 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

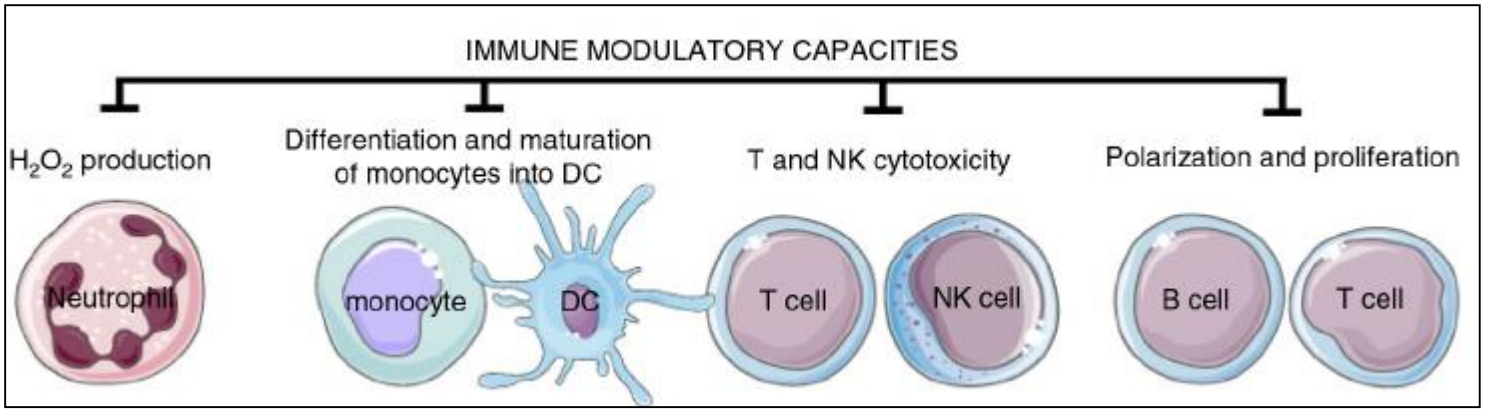




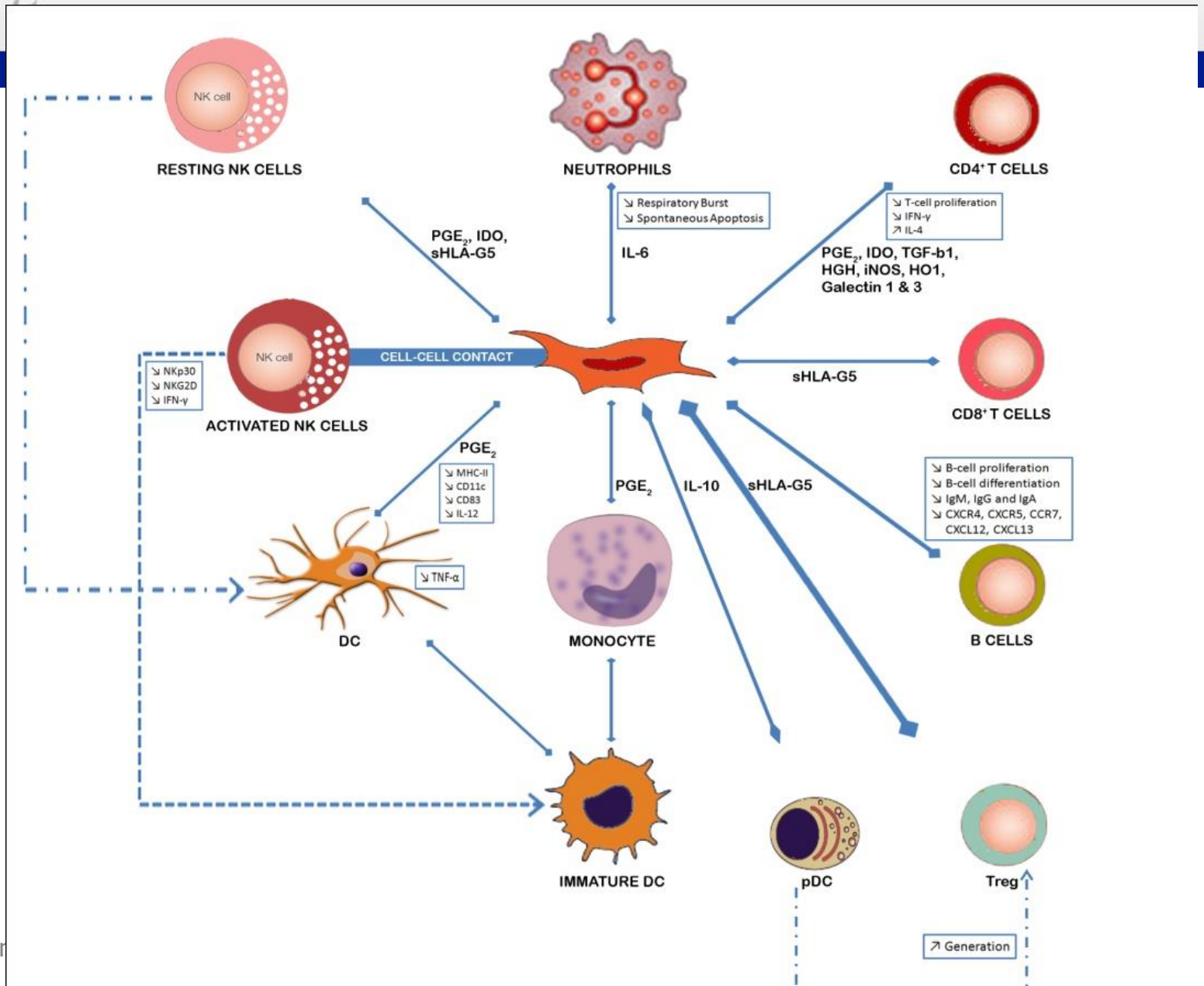


Mesenchymal Stem Cells

- 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



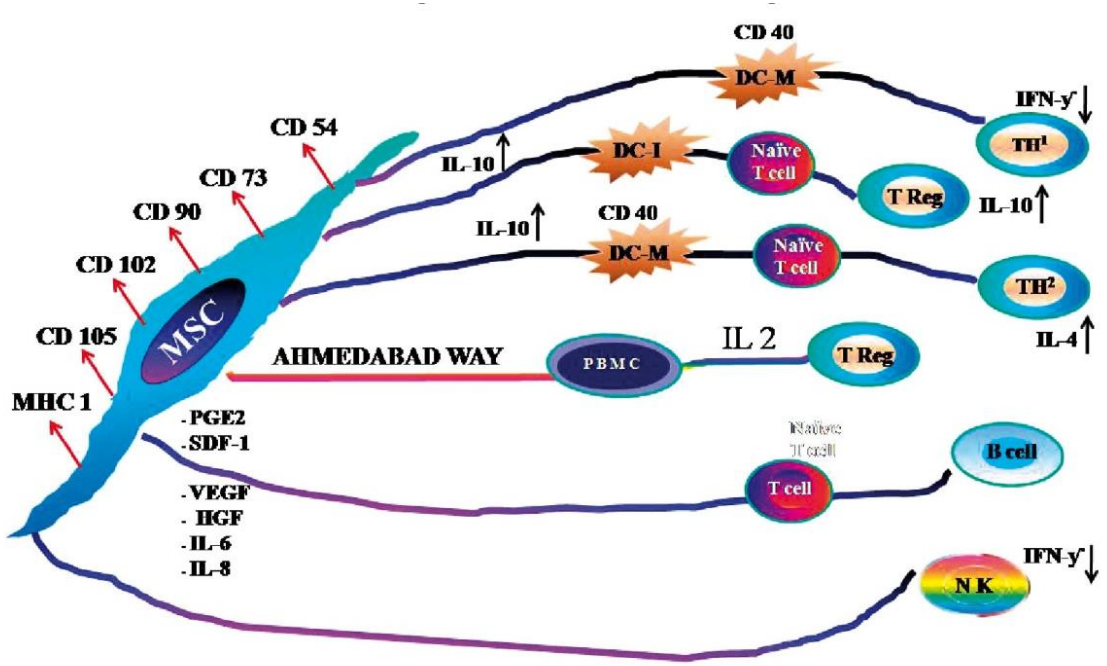
MSC modes of immunomodulation



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, 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 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

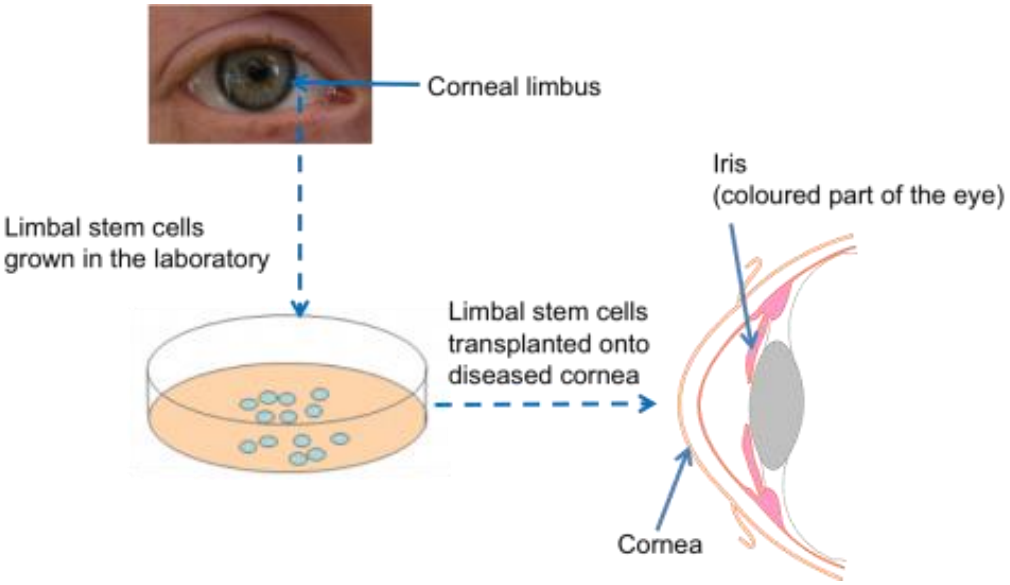
Most proposed MSC potency assay

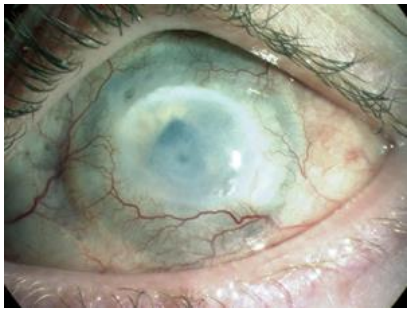
- Inhibition of T cell activation/proliferation in co-culture with MSCs
- Induction of T cells:
  - with memory antigens
  - mitogens (e.g. PHA, PMA or ConA)
  - T cell receptor cross-linking and co-stimulation (aCD3/aCD28)
  - Allogeneity (e.g. allogeneic PBMCs or DCs in a mixed lymphocyte reaction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

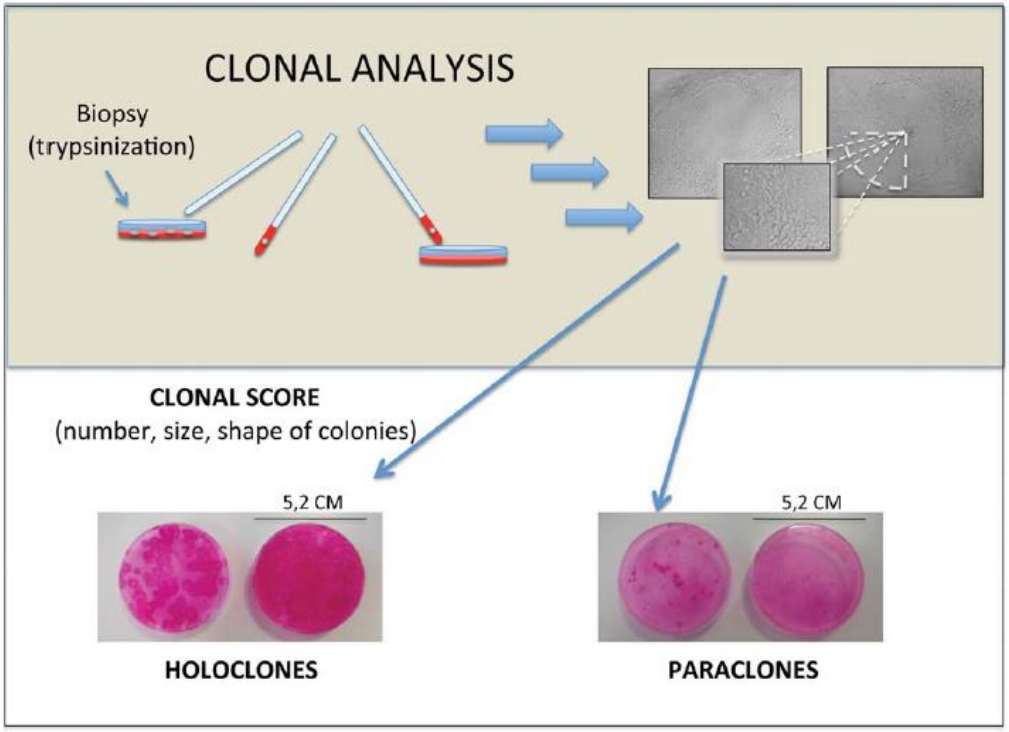




Based on Pellegrini *et al*., Stem Cells (2014)

Potency assay limbal stem cells

- **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

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%

Not accepted!

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

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