Analytical Considerations for Potency Testing

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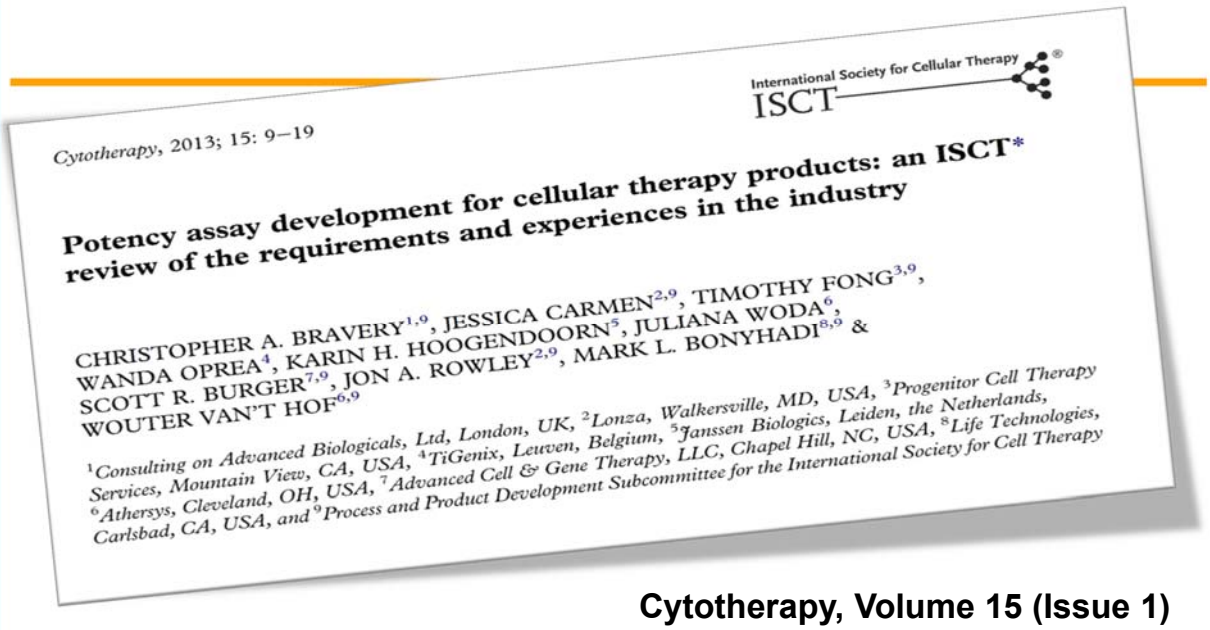

International Society for Cellular Therapy
ISCT

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Potency assay development for cellular therapy products: an ISCT* review of the requirements and experiences in the industry

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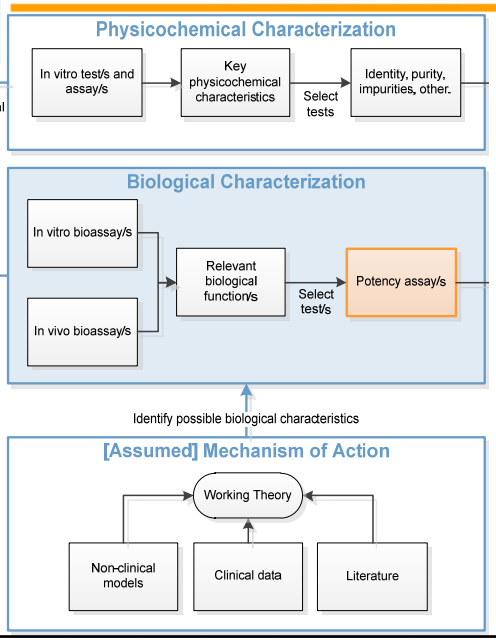
Outline

- Characterization
- Why is potency so important?
- Analytical considerations
  - validation

Characterisation Strategy



Physicochemical characterization
Refers to the use of methods that measure physical and chemical characteristics. E.g:
Physical: size, morphology, light scattering properties, tensile strength, cell number, confluence.
Chemical: identification of phenotypic markers and secreted substances, genotype, gene expression profile.

Biological characterization
Refers to the use of methods that measure biological function, i.e. how the physicochemical characteristics influence biological systems. E.g:
Biological: *in vitro* and/or *in vivo* measurements of cytotoxicity, cell growth, de/differentiation, proliferation, migration, immunomodulation.

What is a potency assay?

- Biological 'activity' implies a change over time; so single measurements are not biological assays.
- Any assay used for biological characterisation could be a potency assay if it gives a meaningful indication the product will be 'potent'.
- It is unlikely one single assay will capture all biological effects.
- One or more biological assays may be needed together to define potency.
- Biological characterisation will allow you to identify which assays are candidate 'potency assays'

Selecting a potency assay?

- Practicalities will necessarily limit those that could be used for product release, e.g. An in vivo assay (e.g. Botox) is unlikely to be possible for cell therapy release testing.
- Where time or material mean a true potency assay would not be possible, a surrogate measure can be used, e.g.
  - ChondroCelect – expression of gene markers
  - Provenge – Expression of CD54

Warning!

- These surrogates for potency are only valid if correlated to other bioassays and/or *in vivo* effects relevant to the MoA.

What Are You Trying to Measure?

- **Measurement**: process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity.
- **Analyte**: specific substance to be measured (e.g. IL-2, haemoglobin).
- **Measurand**: quantity to be measured

For detailed description of terms see: International vocabulary on metrology (VIM)

Example: Viability (e.g. trypan blue)

Identifying the analyte

- **Analyte**: non-viable cells (those that stain).
- **Measurand**: % cells in a sample that are viable
  - *Or, the inverse; % of non-viable cells in sample.*
- Is the measurand correct (given the analyte)?

Analyte 1 isn't sufficient to achieve the desired measurand

- **Analyte 1**: non-viable cells (those that stain).
- **Analyte 2**: total cells (stained or not)

Viability (e.g. trypan blue)

Getting the measurand right

- **Analyte 1**: non-viable cells (counted, those that stain).
- **Analyte 2**: total cells (counted)
- **Measurand 1**: % of total cells that are viable
  - analyte (2-1)/2 *100.
- Is this measurand useful?

Viability (e.g. trypan blue)

Getting the measurand right

- **Analyte 1**: non-viable cells (counted, those that stain).
- **Analyte 2**: total cells (counted)
- If we include a second measurand (using same data from analyte 2)
- **Measurand 2**: Total cell count (cell content) [analyte 2]
- Is this more useful in conjunction with measurand A?

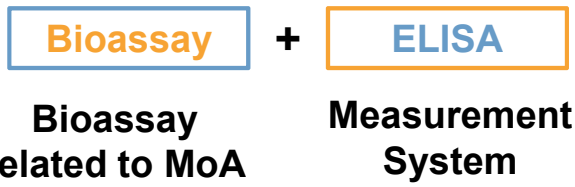
Or

- **Measurand 3**: Total viable cells [analyte 2 – analyte 1]

Hypothetical Potency Assay

Assay Overview:

- Cells (e.g. product) plus 'stimulator (e.g. another cell)'
- Coculture over time (hours/days)
- Response = release of a cytokine (cytokine 'X')
- Cytokine is measured in supernatant by ELISA

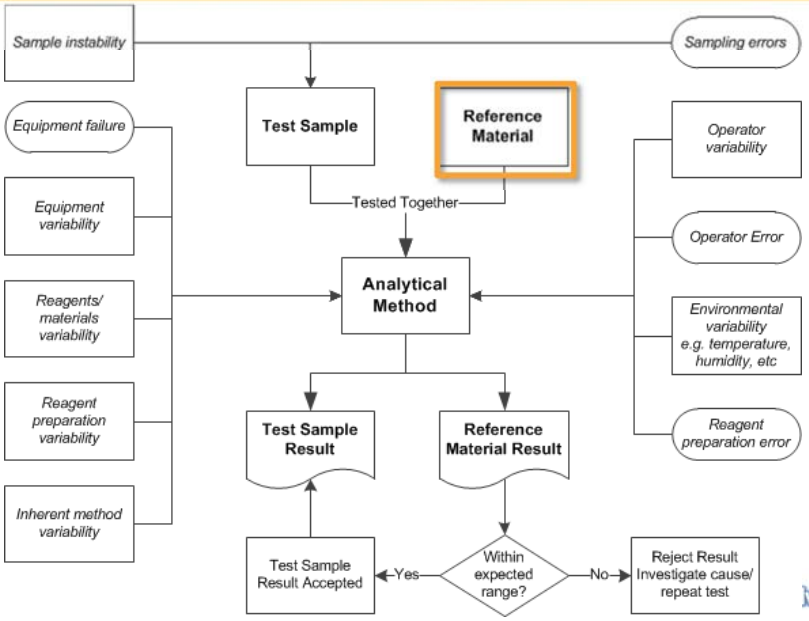


Analyte/Measurand

- **Analyte:** cytokine X
- **Measurand**: Mass (concentration) of cytokine 'X' secreted in response to a stimulus over 48 hours by 10^5 cells/mL in DMEM+10% FCS.

- Many things might affect this;

Sources of assay variability



Qualification, Validation and Verification

Qualification

Qualification (also validation and other terms)

- This could be described as *characterising the method*
  - Overlaps with method development
- Qualification aims to understand the critical parameters of the method and explores its capabilities
  - Implication – most or all of validation criteria should be explored
  - Might be less rigorous
  - **Note: You CANNOT fail qualification**
    - Its exploratory.

Qualification, Validation and Verification

Validation

Validation (Validation)

- This is confirming the method behaves as expected.
- You need to include pre-defined acceptance criteria for the validation parameters
  - How can you do this without qualification?
    - You get estimates from development/qualification
  - **Note: You CAN fail validation**
    - Its confirmatory
    - get the acceptance criteria wrong e.g. missing qualification and/or incomplete method development = no data to set validation acceptance criteria.

Qualification, Validation and Verification

Verification

Verification (re-validation)

- This is reconfirming the validation after small changes that do not affect all performance aspects
  - New lab, New sample matrix, small refinements to the method, New reagent, New equipment
  - Routine QA (lifecycle management)
    - E.g. might be a concern whether its behaving correctly.
- Appropriate parameters re-evaluated as required.

A few things to consider

- Method validation guidance tends to focus on the analyte
- Bioassay + ELISA (measurement system)
  - Don't forget both elements in validation
  - e.g linearity of ELISA for analyte **and** linearity of bioassay – i.e. is there a linear relationship between number of product cells and quantity of cytokine released?

Conclusions

- A thorough characterisation program will yield all the tools necessary to control the quality of the CTP.
- Biological characterisation should focus on all the possible MoA
- Analytical guidance focusses on the analyte; but consider also the **measurand** (quantity to be measured).
- Considering the measurand can help avoid mistakes in qualification and later validation.