Understanding Mechanisms of Action and Identifying Relevant Biological Activities: Why are they so Important?

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Overview

- MoA and characterisation
  - Product (focus)
  - Using characterisation outputs
  - Process (brief)
- MoA and relevant biological function as part of characterisation
  - What is a potency assay?
  - Why is potency so important?

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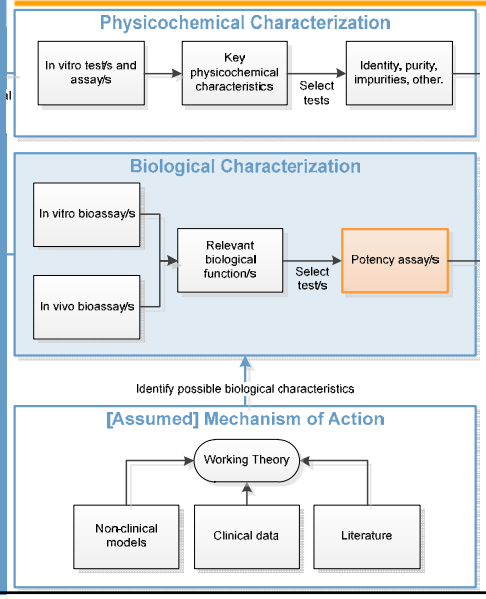
1.Mechanism/s of Action (MoA)

- What are the possible MoA?
- ‘We don’t know how it works’
  - The therapeutic idea must have come from somewhere, so there must be a preliminary theory as to why it might work.
  - How much the MoA is understood is a matter of degree:

Knowledge	**Analogy (to car)**
• Enough to pursue the idea (therapeutic rationale).	e.g. to operate/use a car (arguably transplantation is merely this).
• Enough to control product quality.	e.g. able to check the car is operational.
• Enough to make manufacturing changes.	e.g. Repair the car, performance/safety enhancements.
• Enough to redesign the product to make it better.	e.g. Design a new car from scratch.

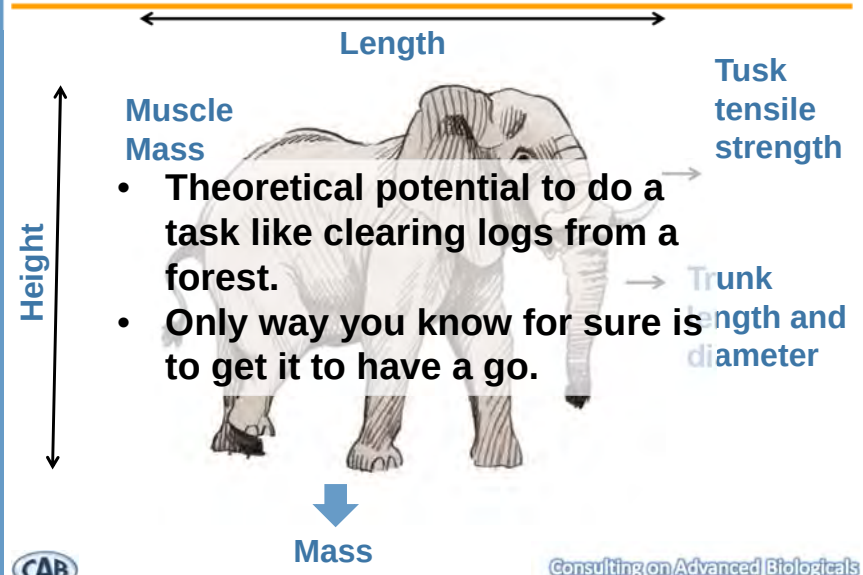
Characterisation Strategy



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.

The Elephant in the Room



- Theoretical potential to do a task like clearing logs from a forest.
- Only way you know for sure is to get it to have a go.

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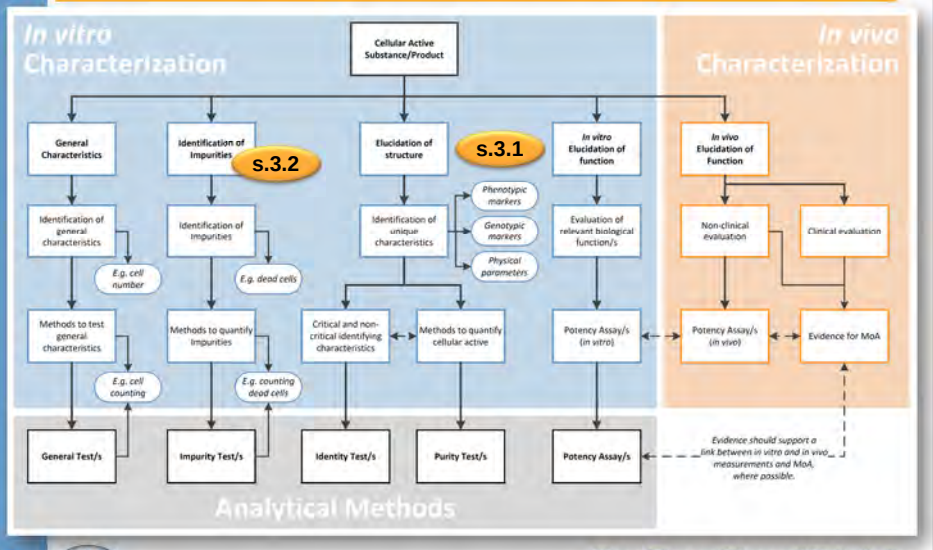
Embarking on Characterisation



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Objectives of product characterisation

- To understand the structure and function/s of the cellular active substance (product)
- Understand the interplay between the structure of the active substance and MoA
  - Identify critical quality attributes
    - Specifications (intermediates, release, stability), comparability
  - Identify stability indicating parameters
    - Specifications (shelf-life, in-use stability, shipping, stored intermediates)
  - Identify other useful parameters
    - pH, osmolality, content,

Objectives of process characterisation

To understand the process and how this achieves a consistent product

- Understand the role of each step
  - Operating parameters **and ranges**
  - Setting operating parameters (e.g. temp, CO_2, pH, reagent concentration, etc)
    - Overlap with specifications (intermediates), comparability (intermediates)
- Set in-process controls
    - Confirming critical steps are successful (fail batch early, trouble shooting).

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’

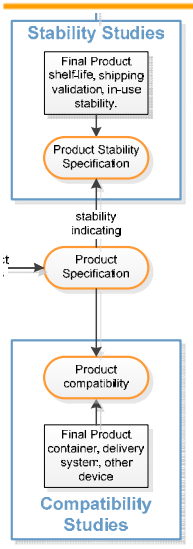
Selecting a potency assay?

- 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.

The Importance of Potency



- You cannot confirm stability without a potency assay
  - Biological function may be lost before viability
- Viability alone doesn't confirm the product is effective, you need a potency assay.
- Compatibility of the product with devices (e.g. syringes, containers, etc) or other biomaterials (or potentially drugs) cannot be confirmed without a potency assay.
  - Although generally minor issue

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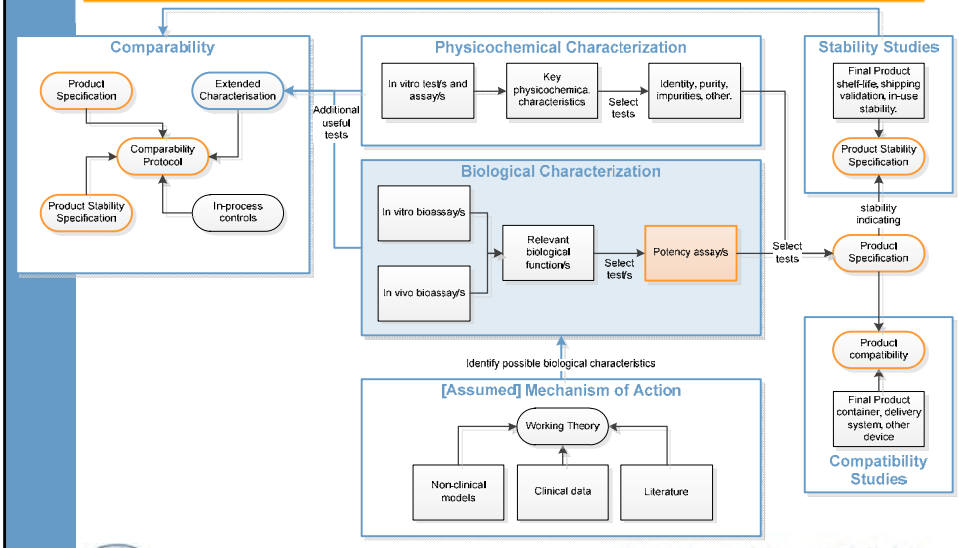
The Importance of Potency



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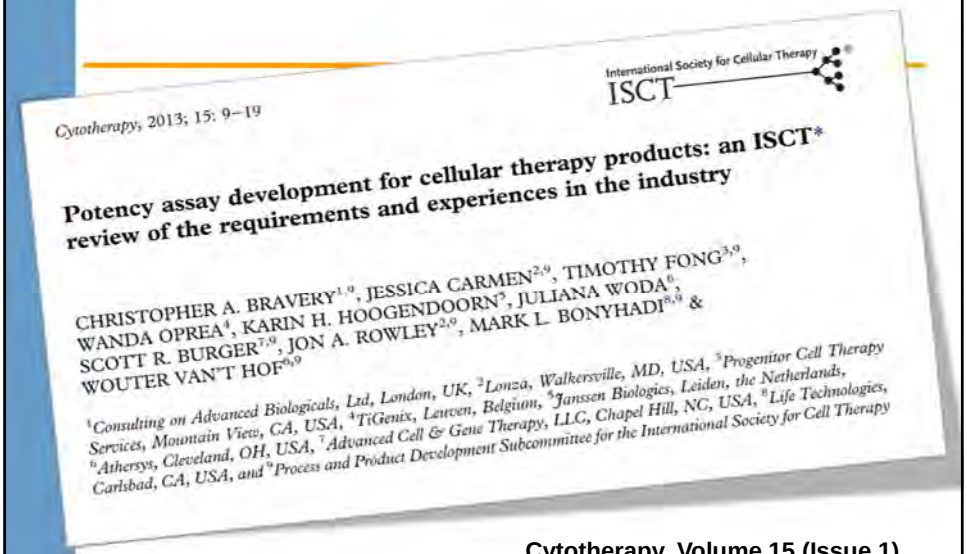
Conclusions

- 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
- Thorough biological characterisation should identify potential potency assay candidates
- Surrogate measurements of potency using physicochemical measurements can be acceptable but only when supported by true potency assays, *in vitro and/*or *in vivo*.



Cytotherapy, 2013; 15: 9–19

International Society for Cellular Therapy ISCT

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

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Cytotherapy, Volume 15 (Issue 1)
January 2013 (p 9-19)

Further Reading

- PAS-83 (BSI, 2012) Developing human cells for clinical applications in the European Union and the United States of America. Guide.
  - http://shop.bsigroup.com/en/forms/PASs/PAS-83/ (free download)
- PAS 93 (BSI, *2011*) Characterisation of cells and cell products
  - http://shop.bsigroup.com/en/forms/PASs/PAS-93/ (free download).
- PAS 84 (BSI, 2012) Cell therapy and Regenerative medicine glossary
  - http://www.futuremedicine.com/doi/pdfplus/10.2217/rme.12.38 (free download)