Characterising your Gene Therapy Product and Ensuring Good Assay Development.

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Overview

- Overview of measurement concepts
- General concepts in characterisation
- MoA and relevant biological function as part of characterisation
  - What is a potency assay?
  - Why is potency so important?
- Measurement reference materials
- Conclusions

Quality – the foundation

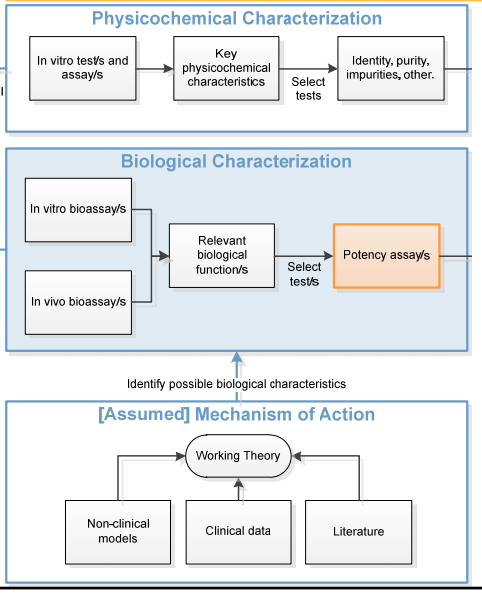
"*If you can't manufacture a consistent product, how can you expect a consistent clinical effect?*"

Christopher Bravery

The objectives of product quality are to identify critical quality attributes (and others that are useful)

- *Which are they? How do I know?*
- **How do I measure them (reliably over time)?**
- *How do I use them?*
  - *To set specifications?*
  - *To improve manufacturing?*

Characterisation Strategy



Physicochemical characterization
Refers to the use of methods that measure physical and chemical characteristics. Eg:
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. Eg:
Biological: *in vitro* and/or *in vivo* measurements of cytotoxicity, cell growth, de/differentiation, proliferation, migration, immunomodulation.

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, mRNA).
- **Measurand**: quantity to be measured

Simple Example: total protein in urine.

- **Analyte**: (total) protein.
- **Measurand**: Protein in urine.
- Is this useful?

- **Analyte**: (total) protein.
- **Measurand**: Protein in 24h urine.
- Is this more useful?

But measuring protein isn't simple

Table 2
Plasma protein concentration

Sample (plasma), $n=7$	Protein concentration (graph) (mg/ml)	Protein concentration formula (BSA standard[a]) (mg/ml)
Amido Black		
Median±S.D.	83.4±1.93	82.6±1.91
CV %	2.3	2.3
Lowry		
Median±S.D.	110.2±4.09	109.1±4.31
CV %	3.7	3.9
Bradford		
Median±S.D.	95.1±3.3	89.5±3.1
CV %	3.5	3.5
Biüret		
Median±S.D.	88.8±3.54	80.9±3.67
CV %	4	4.5
Ponceau-S/TCA		
Median±S.D.	92.3±5.71	94.7±5.86
CV %	6.2	6.2

[a] BSA concentration for Biüret assay 0.5 mg/ml; Lowry, Bradford, Amido Black and Ponceau-S/TCA assays were 0.1 mg/ml.
[b] HSA concentration for Biüret assay 0.5 mg/ml; Lowry, Bradford, Amido Black and Ponceau-S/TCA assays were 0.05 mg/ml.

RM=BSA

Different methods (different measurement principle) will give different results (same analyte) – this is why orthogonal methods are important as part of characterisation.

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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'.

GM Cells

- Less likely one single assay will capture all biological effects of the product (cell and genetic modification).
- 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 is may not be possible for GM cell therapy release testing, but could be (likely undesirable) for a gene vector.
- Where time or material (e.g.GM autologous cell) mean a true potency assay would not be possible (for release), a surrogate measure can be used, e.g. PCR, RT-PCR etc.

Warning!

- These surrogates for potency are only valid if correlated to other bioassays and/or *in vivo* effects relevant to the MoA. So you still need a proper potency assay.

Potency Example: Identifying RM

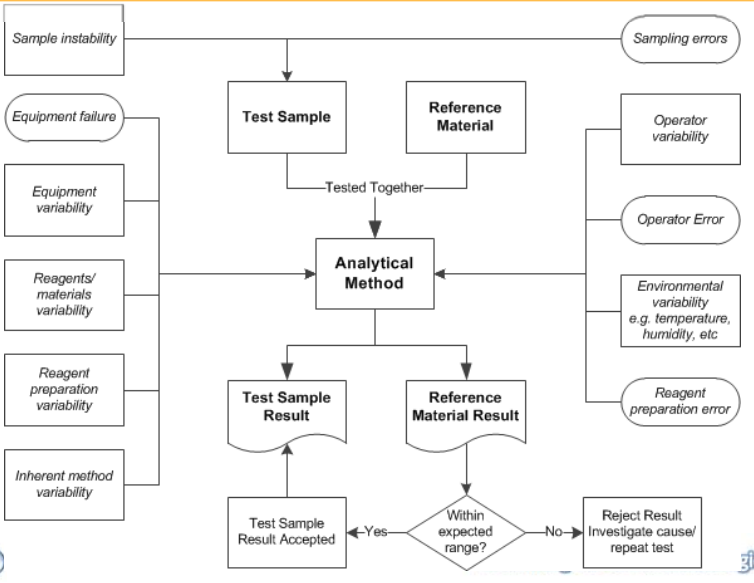
- **Assay:** product to transfect indicator cell line and measure gene product = protein X
- **Analyte**: soluble substance 'protein X'.
- Measurement system: ELISA
- Certified reference material (standard) for substance X from NIBSC/WHO/EP or in-house.
  - ! Check purpose of RM, e.g. is it just for activity or is it for protein content?
  - Metrological traceability (of RM)
- The RM here is used to calibrate (as a calibrant) the assay.

ENOUGH?

Potency Example: Identifying RM

- **Measurand**: Mass (concentration) of 'X' secreted over 24 hours by 10^5 cells/mL 48 hours after transfection with product (GT vector) in DMEM+10% FCS.
- Many things might affect this;

Why we use reference materials



Potency Example: Identifying RM

- ***Measurand**: Mass (concentration) of 'X' secreted over 24 hours by 10^5 cells/mL 48 hours after transfection with product (GT vector) in DMEM+10% FCS.*

- You need a GT vector RM (e.g. product RM) and a standard cell line
- Ideally the transfect efficiency will be consistent but the resulting secretion of factor X can be normalised to the RM, e.g. mass secreted X per transfected cell (per 24h)

Conclusions

- A thorough characterisation program will yield all the tools necessary to control the quality of the CTP.
- Surrogate measurements of potency using physicochemical measurements can be acceptable but only when supported by true potency assays, *in vitro and/*or *in vivo*.
- Reference materials help to demonstrate measurement reliability over time
- Reference materials need to be **sufficiently similar to the material to me measured** to be reliable
- Thinking carefully about what is being measured (analyte and measurand) is important

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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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Reference materials for cellular therapeutics

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