Stability

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Overview

- Issues I see with Stability Studies (cell therapy)
- How its expected to be done (ICH principles)
  - Stress testing
  - Accelerated stability
  - Real-time stability
  - In-use stability
  - Other related studies (not discussed)

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CBMP – Do these Stability Data Look Suitable?

...eal-time s... 4 °C; Batch #1234.

Callout notes:
- Necessary but don't evaluate stability?
- Overall WEAK specification
- Too few time-points
- How can this be useful?
- Is this assay sensitive to change?
- Is this taking up too much of the DP?
- What does this really tell you?

Attribute	Test method	Specification	Time-point 0	Time-point 1	Time-point 2
Appearance	Visual inspection	red/orange turbid solution	complies	complies	complies
Identity	Phenotypic marker (flow cytometry)	complies	complies	complies	complies
Viability	Trypan blue (manual count)	≥ 70%	95	94	92
Potency	Bioassay	≥ 4 U/10^6 cells	12.1	9.8	6.8
Sterility	EP/USP	No growth	No growth	NT	No growth

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CBMP – Better?

Callout notes:
- Now viability can be interpreted
- Viability fairly stable
- Nearly 50% of cells lost

Attribute	Test method	Specification	Time-point 0	Time-point 1	Time-point 2
Appearance	Visual inspection	red/orange turbid solution	complies	complies	complies
Identity	Phenotypic marker (flow cytometry)	complies	complies	complies	complies
Viability	Trypan blue (manual count)	≥ 70%	95	94	92
Content	Manual cell count	≥ 10^6 cells/mL	2.1×10^6	1.8×10^6	1.1×10^6
Potency	Bioassay	≥ 4 U/10^6 cells	12.1	9.8	6.8
Sterility	EP/USP	No growth	No growth	NT	No growth

- Presenting viability as a percentage without total cells is meaningless.
  - *Yet I see this often?*

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CBMP – Different ways to present data

Attribute	Test method	Specification	Time-point 0	Time-point 1	Time-point 2
Appearance	Visual inspection	red/orange turbid solution	complies	complies	complies
~~Identity~~ Purity	Phenotypic marker (flow cytometry)	~~complies~~ >80 %	~~complies~~ 95%	~~complies~~ 93%	~~complies~~ 94%
~~Viability~~	~~Trypan blue (manual count)~~	~~≥ 70%~~	~~95~~	~~94~~	~~92~~
Non-viable cells	Trypan blue (manual count)	≤ 0.3 × 10^6 non-viable cells/mL	0.1 × 10^6	0.1 × 10^6	0.1 × 10^6
~~Content~~	~~Manual cell count~~	~~≥ 10^6 cells/mL~~	~~2.1 × 10^6~~	~~1.8 × 10^6~~	~~1.1 × 10^6~~
Content	Manual cell count	≥ 10^6 viable cells/mL	2.0 × 10^6	1.7 × 10^6	1.0 × 10^6
Potency	Bioassay	≥ 4 U/10^6 cells	12.1	9.8	6.8
Sterility	EP/USP	No growth	No growth	NT	No growth

Product-related Impurity

A measure of purity unlike identity is quantitative and likely to be stability-indicating.

Total cell content could be total cells or total viable cells.

- **Note: this is not a recommendation, merely to make the point that you need to consider the best way to present data.**

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Take-Home Message
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

See discussion in these

12 *Focus – Advanced therapies*

Measurement reliability over the
cellular therapeutic product lifecycle

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Cytotherapy, 2014; 0: 1–10

Cytotherapy 16(9): 1187-1196.

Reference materials for cellular therapeutics

CHRISTOPHER A. BRAVERY[1] & ANNA FRENCH[2]

[1]*Consulting on Advanced Biologicals Ltd. Advanced Biologicals Ltd, London, United Kingdom, and [2]The Oxford–UCL Centre for the Advancement of Sustainable Medical Innovation (CASMI), The University of Oxford, Oxford, United Kingdom*

Abstract
The development of cellular therapeutics (CTP) takes place over many years, and, where successful, the developer will anticipate the product to be in clinical use for decades. Successful demonstration of manufacturing and quality consistency is

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ICH - General Principles
Specifics = case-by-case

Stability Testing of New Drug Substances and Products — **ICH Q1A**

Photostability Testing of New Drug Substances and Products — **ICH Q1B**

Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Drug Products — **ICH Q1D**

Evaluation of Stability Data — **ICH Q1E**

Validation of Analytical Procedures: Text and Methodology — **ICH Q2**

Impurities in New Drug Substances — **ICH Q3A**

Impurities in New Drug Products — **ICH Q3B**

Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin — **ICH Q5A**

Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products — **ICH Q5B**

Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products — **ICH Q5C**

Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products — **ICH Q5D**

Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process — **ICH Q5E**

Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products — **ICH Q6B**

Quality

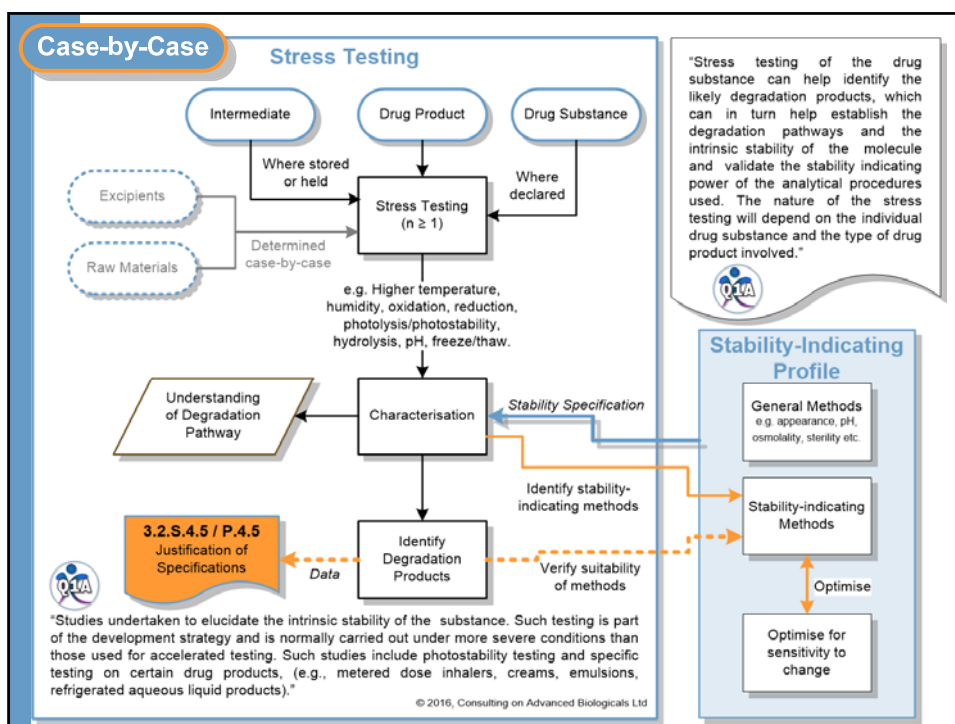7 · CAB · http://advbiols.com/documents/ICH.swf · Consulting on Advanced Biologicals

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Before undertaking any stability studies, you need to determine:

✓ **what the *Stability-Indicating* parameters are**
✓ **the *most appropriate* analytical methods to evaluate these.**

✓ The obvious way to do this is to '*degrade*' the product (intermediate, DS etc).

Identifying Stability-Indicating Parameters
Considerations-1

- Unlike (inert) proteins, cells are not in equilibrium with their environment (i.e. viable/alive/living).
  - Resistant to oxidation/reduction etc
    - protective mechanisms, repair mechanisms etc
  - How to identify changes leading up to (sudden?) loss of cell integrity/viability?
- Common viability methods (e.g. trypan blue) give only yes/no result for viability of each cell
  - explore earlier changes preceding loss of viability
    - e.g. apoptosis, autophagy, necrosis
    - Classification of cell death: Cell Death Differ. 2009 January ; 16(1): 3–11. doi:10.1038/cdd.2008.150.
    - Molecular definitions: Cell Death and Differentiation (2012) 19, 107–120

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Identifying Stability-Indicating Parameters
Considerations-2

- Measures of cell stress, e.g.
  - Biochemical
  - Physical (membrane integrity)
  - Secreted factors, e.g. heat-shock proteins etc.
- Receptor/ligand modulation
  - Surface receptor/ligands involved in the MoA may be induced, up/down-regulated, internalised/shed.
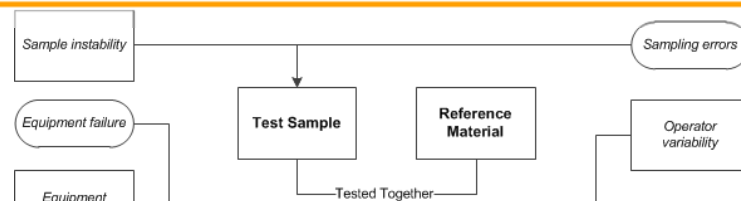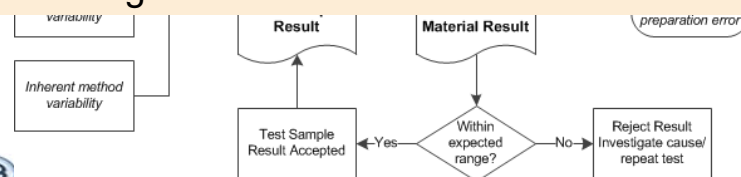   - mRNA changes normally precede protein changes by hours to days.

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Sources of Analytical Error

Bravery & French; Cytotherapy 16(9): 1187-1196.

- Method needs to be 'sensitive to change'
- Sufficiently precise/accurate, robust etc.
- Such that can detect small changes in stability-indicating attributes.

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Formulation Development

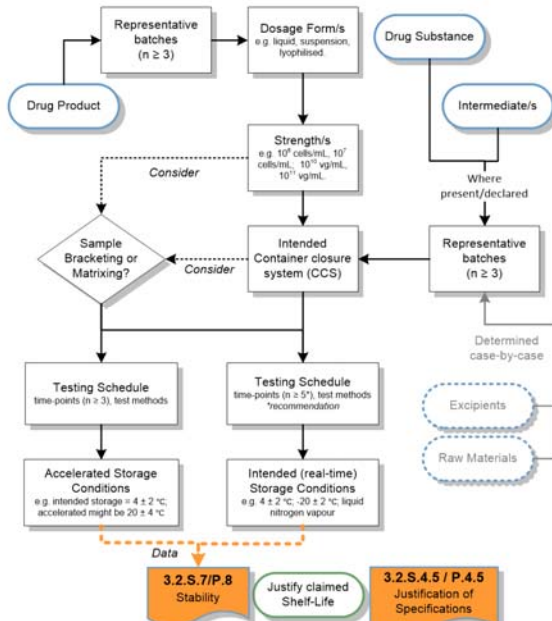**There should be studies to identify the optimal formulation**

- Issue – off-the-shelf media are not optimised for <u>your product</u> and <u>route of administration</u>

- Data support justification for excipient specifications 3.2.P.4.4.
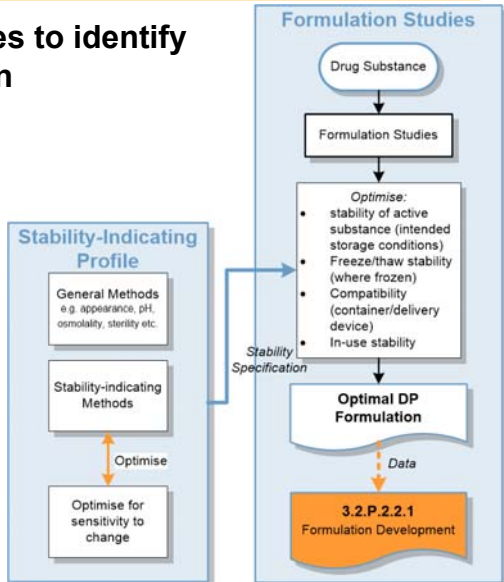
Note: The End of Shelf-life Specification should be in **3.2.S.4.1/P.5.1** (with Release Specification)

Accelerated Testing

"Studies designed to increase the rate of chemical degradation/ physical change of a substance by **using exaggerated storage conditions** as part of the formal stability studies. Data from these studies, in addition to long term stability studies, can be used to assess longer term chemical effects at non-accelerated conditions and to evaluate the effect of **short term excursions outside the label storage conditions** such as might occur during shipping. Results from accelerated testing studies are not always predictive of physical changes."

In-Use Stability (3.2.P.8)
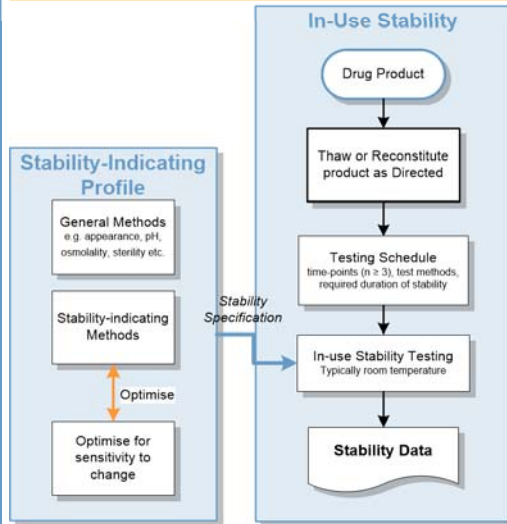
Confirm the product is stable during clinical handling
- ✓ Thawing
- ✓ Reconstitution
- ✓ How soon
- ✓ Leave on side/put in fridge?

Other Stability Studies
(Not discussed in this talk)

- Shipping
  - Shipping studies (real ± simulated) to evaluate impact on stability
- Raw materials
  - Where stability uncertain
  - Buffers, media, and other solutions prepared on-site
- QC samples
  - Duration of storage and handling before testing
  - Shipping to external tests labs

Other Uses for Stability-Indicating Methods

- Extended characterisation
- **Comparability Studies**
    - Can be very useful because they are *sensitive to change*
        - for *extended characterisation* (i.e. beyond routine testing)
        - as well as stability studies undertaken as part of comparability.

Conclusions 1/2

Before a stability specification and tests protocol can be devised;

- Need to understand the stability-indicating profile (including stability-indicating parameters)
    - General characterisation
    - Stress-testing
- Need to identify stability-indicating methods (to measure stability-indicating parameters)
    - Sensitive to change
    - Sufficiently precise, (accurate), robust etc.
- These data will be needed to set and justify your stability specification and acceptance criteria.

Conclusions 2/2

- Overall testing can be reduced in some instances by the use of Bracketing and/or Matrixing
  - Warning: use appropriately
- Release and Stability specifications and acceptance criteria can differ
  - Not all release tests are stability-indicating
    - e.g. process-related impurities unlikely to change
  - It may be necessary to accept some parameters change gradually over the shelf-life;
  - Stability (end of shelf-life) > Release
    - Needs justification, e.g. toxicity data (safe levels)
    - e.g. viability for fresh cell products.

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