The Stability of Monoclonal Antibodies (mAbs)

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What are Abs 1

- Antibodies are proteins produced by the B lymphocytes of the immune system in response to foreign proteins, called antigens.

- Structurally antibodies are proteins consisting of four polypeptide chains. These four chains form a quaternary structure somewhat resembling a Y shape.
What are mAbs 2

- Monoclonal antibodies are antibodies which have been artificially produced against a specific antigen.

- They are extremely specific and bind to their target antigens.

- Monoclonal antibodies (mAb or moAb) are monospecific antibodies that are the same because they are made by identical immune cells that are all clones of a unique parent cell.

- Sources of MABs – mouse and human (chimeric)
Source subthemes: naming

Human parts are shown in red, non-human parts are blue.

Mouse

-\textit{O}\textit{-}

Chimeric

-\textit{Xi}\textit{-}

Humanized

-\textit{Zu}\textit{-}

Chimeric/humanized

-\textit{XiZu}\textit{-}

Human

-\textit{U}\textit{-}
**Mode of Action**

- Bind to specific target antigens
- May - inhibit antigens biological actions
- May - cause death of target cells

*Specificity of action*

Used to treat many diseases
Protein Structure

- **Primary Structure** – the sequence of a chain of amino acid

- **Secondary Structure** – linking of sequences of amino acids by hydrogen bonding (formation of regular sub-structures such as pleated sheets or alpha helices)

- **Tertiary Structure** – occurs when there are certain attractions between pleated sheets and alpha helices (3-d structures)

- **Quaternary Structures** – protein consisting of more than one amino acid chain (complex of protein molecules)
Protein Structure 2

- Primary structure: amino acid sequence
- Secondary structure: regular sub-structures
- Tertiary structure: three-dimensional structure
- Quaternary structure: complex of protein molecules

- Alpha helix
- Beta sheet
- Tertiary structure
- Quaternary structure
Methods of mAbs Degradation

- Chemical degradation of amino acids
- Fragmentation
- Denaturation of tertiary structure – disruption of bonds essential for native configuration
- Aggregation to form dimers, tetramers or larger aggregates
- Adherence - extremely interactive with surfaces of all types. They can potentially bind or interact with containers, filers and tubing
Potential MAB instability

- **Physical**
  - denaturation of tertiary structure – disruption of bonds essential for native configuration
  - self-association into dimers, tetramers or larger aggregates

- **Chemical**
  - disulphide formation and exchange
  - deamidation
  - isomerisation
  - oxidation
  - crosslinking – non-reducible
  - formation of acidic and basic species - fragmentation
Factors affecting degradation

- Temperature
- Freezing
- pH extremes
- Surfactants
- Pressure
- Shaking \ Shearing
- Light (uv)
- Metals
- Oxygen
- Presence of water
- Absence of water
- Denaturants
- Excipients
- Interfaces

Factors influence development, production, preparation, storage & handling
Source Guidance 1

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS

Q1A(R2)

Current Step 4 version
dated 6 February 2003

Generally applies to small medicine molecules
QUALITY OF BIOTECHNOLOGICAL PRODUCTS:

STABILITY TESTING OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Q5C

Current Step 4 version

dated 30 November 1995

RQC Yellow Cover document - Stability
Scope of Q5C

Q5c - characterised proteins and polypeptides
- their derivatives and products of which they are components
- and which are isolated from tissues, body fluids, cell cultures, or produced using rDNA technology
  - cytokines (interferons, interleukins, colony-stimulating factors, tumour necrosis factors)
  - erythropoietins
  - plasminogen activators
  - blood plasma factors
  - growth hormones and growth factors
  - insulins
  - monoclonal antibodies
  - vaccines consisting of well-characterised proteins or polypeptides

Q5C - does not cover antibiotics, allergenic extracts, heparins, vitamins, whole blood, or cellular blood components.
Stability Approach

- Primary data to support a requested storage period long-term, real-time, real-condition stability studies

- Preferable to not use accelerated stressed stability testing
Biologicals specifically mAbs

- active components are typically proteins and/or polypeptides

Therefore

- maintenance of molecular conformation and, hence of biological activity, is dependent on non-covalent as well as covalent forces.

- particularly sensitive to environmental factors such as temperature changes, oxidation, light, ionic content, and shear.

In order to ensure maintenance of biological activity and to avoid degradation, **stringent conditions for their storage are usually necessary.**
Potential MAB instability

- Chemical instability
- Physical Instability
- Loss of Biological activity
Evaluation of mAbs stability

Potency

• assays for biological activity, where applicable, should be part of the pivotal stability studies

Purity and quality

• appropriate physicochemical, biochemical and immunochemical methods for the analysis of
  - the molecular entity
  - the quantitative detection of degradation products
Stability-indicating Profile

- no single stability-indicating assay or parameter - profiles the stability characteristics of a biotechnological/biological product

- the stability-indicating profile should provide assurance that changes in the
  - Identity
  - Potency
  - Purity
  - Other characteristics

of the product will be detected

- the determination of which tests should be included will be product-specific
Potency

**Potency** - potency is the specific ability or capacity of a product to achieve its intended effect.

When the intended use of a product is linked to a **definable and measurable biological activity**, testing for potency should be part of the stability studies.

In general, potencies of biotechnological/biological products tested by different laboratories - **expressed in relation to an appropriate reference material** (linked to national or international reference standard).

Biological characteristics
- immunoreactivity and crossreactivity
- the determination of relevant functional characteristics
- binding studies to determine affinity

How do we measure? What is acceptable?
Potency 2

- Receptor Studies
- Cell Line Studies
- Clinical Effect
Purity and Molecular Characterisation

- Purity is a relative term
  the absolute purity of biologicals is extremely difficult to determine

- The purity of a biotechnological/biological product should be typically assessed by
  - more than one method
  - purity value derived is method-dependent
  - tests for purity should focus on methods for determination of degradation products

- The degree of purity, as well as individual and total amounts of degradation products of the biological product
  - limits of acceptable degradation should be derived from the analytical profiles of batches of the drug substance and drug product used in the preclinical and clinical studies.
Purity and Molecular Characterisation 2

- The use of relevant **physicochemical, biochemical and immunochemical** analytical methodologies should permit a comprehensive characterisation

- drug substance and/or drug product (e.g., molecular size, charge, hydrophobicity)

- accurate detection of degradation changes during storage
## Purity and Molecular Characterisation

<table>
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<tr>
<th>Method</th>
<th>Information</th>
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<tbody>
<tr>
<td>Visual</td>
<td>Appearance of solution – particulate material</td>
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<tr>
<td>pH</td>
<td>Optimised conditions for stability</td>
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<tr>
<td>Particle Counting / Microflow Imaging</td>
<td>Presence of aggregates\ absence of silicone oil droplets</td>
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<tr>
<td>Size Exclusion Chromatography (SEC HPLC)</td>
<td>Size distribution, degradation products (high molecular weight), dimers and aggregates, quantification (mg/ml) Identification, higher structure (2° and 3° structure), adsorption, physical changes</td>
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<tr>
<td>Capillary electrophoresis (SDS-PAGE)</td>
<td>Size distribution, degradation products (small molecular weight). Information on identification and chemical changes</td>
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<tr>
<td>Peptide Mapping</td>
<td>AA sequence – chemical changes</td>
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<tr>
<td>Total Protein Assay (BCA)</td>
<td>Loss of protein - adsorption</td>
</tr>
<tr>
<td>High-resolution chromatography (e.g., RP, gel filtration, ion exchange, affinity)</td>
<td>Identification and quantification of biological and degradation products</td>
</tr>
</tbody>
</table>
The following product characteristics should be monitored and reported for the drug product in its final container:

- **Visual appearance of the product**
  - colour and opacity for solutions/suspensions
  - colour, texture and dissolution time for powders
- **visible particulates**
  - Aggregates
  - Silicone oil droplets
- **pH**
- **moisture level** of powders and lyophilised products
Extended stability

Maximise

- dose banding
- production throughput - batching
- vial sharing
- quality evaluation and assurance
  - chemical
  - microbiological
- patient experience
Examples of extended stability

Rituximab

- Genetech – RTX – no change in activity when stored at 5°C during 154 days

Trastuzumab

- US direction leaflet – a reconstituted vial with Bacteriostatic Water for Injection – stable for 28 days after reconstitution when stored refrigerated at 2-8°C
Examples of extended stability

Case 1: Baxter 1

- Baxter
  - Rituximab (MabThera) – 24 hrs 2-8°C - (12 hrs RT)
  - Trastuzumab (Herceptin) – 24hrs <30°C

- Baxter – undertaken following
  - visual inspection (appearance and particles)
  - pH (optimal stability)
  - size excision chromatography (SEC HPLC) – size distribution, degradation products (↑), aggregates, quantification
    - ID, 2° 3° structure, adsorption, physical changes
  - Capillary electrophoresis (SDS PAGE) – size distribution, degradation products (↓)
    - ID, 2° 3° structure, chemical changes
  - BCA assay – quantification of total protein content
Examples of extended stability
Case 1: Baxter 2

- Viaflo, Viaflex and Intermate SV
- 7, 14, 21, 28, 35 days 0-8°C + 48hrs at RT
- Essentially – mAbs stable for the parameters described
- Genetech – RTX – no change in activity when stored at 5°C
Examples of extended stability
Case 1: Baxter 3

*In vitro* Bioassays?

Rituximab

- CD20 protein
  - Cytotoxic mechanism (complement-dependent)
- Validation – specificity, linearity, robustness etc
- Proved stable

Trastuzumab

- HER2
- Validation – specificity, linearity, robustness etc
- Proved stable
Infliximab

Stabilité de l’infliximab en solution diluées (Guirao, S et al)

3 months in NaCl 09%; PE bags; 0.7-1.6mg/ml; 4-22°C

Methods

- Turbidity
- Dynamic light scattering
- SEC HPLC
- CEX HPLC

NHS studies underway
Rituximab

Stabilité d’un anticorps monoclonal d’intérêt thérapeutique après reconstitution: Le Rituximab (Jaccoulet et al)

3 months in NaCl 09%; 1 and 4 mg/ml; 4°C

Methods

- HPLC SE
- HPLC CEX
- Optical density
- Turbidity
- Dynamic light scattering
- Peptide mapping
Cetuximab

Stability of cetuximab and panitumumab in glass vials and PVC bags (Ikesue, H et al)

14 days in NaCl 09%; PVC bags; 2 mg/ml; 4°C

Methods
- Enzyme-linked immunosorbent assay (ELISHA)

Cf. Aggregation studies (Astier et al)
Case 2

Six-month stability of bevacizumab (Avastin) binding to vascular endothelial growth factor after withdrawal into a syringe and refrigeration or freezing

*Bakri SJ et al 2006 Retina 26:519*

- Primary reference source for Bevacizumab
- Biological effect - binding to vascular endothelial growth factor
- No physico-chemical analysis
- No visual examination
Six-month stability of bevacizumab (Avastin) binding to vascular endothelial growth factor after withdrawal into a syringe and refrigeration or freezing
Bakri S.J et al 2006 Retina 26:519
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Bakri S.J et al 2006 Retina 26:519
Bevacizumab 2

Sustained Elevation in Intraocular Pressure Associated With Intravitreal Bevacizumab Injections

Kahook et al 2009 Ophthalmic Surgery, Lasers & Imaging 40:3

- 6 cases of patients exhibiting increased Intraocular Pressure (IOP) after single or repeated Bevacizumab
- IOP lowering therapy was required
Bevacizumab 3.1

High–molecular-weight Aggregates In Repackaged Bevacizumab
887-892

Bevacizumab syringes (repackaged) obtained from 3 outside compounding pharmacies vs samples obtained directly from the original vial

Methodology

- enzyme-linked immunosorbent assay
- size exclusion chromatography
- polyacrylamide gel electrophoresis
- microflow imaging was used to examine particulate material within samples
Bevacizumab 3.2

- All syringes contained statistically similar amounts of protein, consisting of immunoglobulin (IgG) heavy and light chains (polyacrylamide gel electrophoresis).

- However, two of the three compounding pharmacies’ batches had significantly less functional IgG in the solution (enzyme-linked immunosorbent assay).

- Additionally, the compounding pharmacies with the lowest IgG (circa 50%) also contained 10-fold the number of micron-sized particulate matter (microflow imaging).

- Increase in micron-sized protein aggregates with the decrease in IgG concentration.

- Large particulate matter within some samples may lead to obstruction of aqueous outflow and subsequent elevation in intraocular pressure.
Bevacizumab 4.1

Silicone Oil Microdroplets and Protein Aggregates in Repackaged Bevacizumab and Ranibizumab: Effects of Long-term Storage and Product Mishandling


Bevacizumab syringes (repackaged) obtained from 4 outside compounding pharmacies vs samples obtained directly from the original vial

Controlled laboratory conditions

Plastic syringes – incubated at -20°C, 4°C and RT for 12 weeks

Subject to light, mechanical shock and freeze-thawing
Bevacizumab 4.2

Methodology

- Particle counting and size distribution
- SE-HPLC

Results

1. Particle Counts (>1um)
   1. Syringes 89,006 + 56,406 /mL to 602,062 + 18,349 /mL
   2. Glass Vial 63,839 + 349/mL

Intercompany and intra\inter batch variation

Large particles observed >111um

High proportion identified at silicone oil microdroplets
Bevacizumab 4.3

2. SE-HPLC

Dimers to tetramers – 2-4%

Octamers to decamers – 0.2-0.4%

Could not always correlate loss of monomers with increase in aggregates – loss on syringes or adsorption on silicone oil droplets

3. Freeze-thawing (single and repeated)

Increased particle levels - >1.2 x 10^6 particles per ml

Also due primarily to silicone oil droplets

Concern re. freezing during transportation
Bevacizumab 4.4

4. Mechanical Shock

Increase in silicone oil droplets

5. Light

Short term exposure caused clogging of the syringe needle

Ranibizumab

Comparable particle counts (5um syringe filter reduces)
Bevacizumab Summary

Studies have observed the
- formation of aggregates

**Physico-chemical considerations**
- decrease in biological activity (anti VEGF activity)

**Biological activity considerations**
- release of silicone oil droplets

**Physical considerations**
Conclusion

- Complex area of stability
- Heavy investment by pharmaceutical industry
- Stability indicators
  - Physical
  - Physico-chemical
  - Biological activity
- Multiple test approach required
- Care with preparation, transportation, procurement
- Ongoing review of science