Design Considerations
Stability Testing for Biologics

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Topics to Cover

1. Biologic Regulation (FDA/EMA)
2. Agency Perspectives on Bio/Chem Differences
3. Strategy for Expiry Dating
What are biologics?

- Proteins
- Monoclonal antibodies
- Conjugated protein systems
- Some polypeptides

- Drug Substance (DS) is macromolecule
  - Developed biologically e.g. CC expression, recombinant, harvesting.

- Related classification is Biologicals
  - Blood plasma products, vaccines, immunoglobulins and allergenic extracts.
Key Aspects

- Biologics are more labile than small molecules
- Require low-temp storage
- Costly to manufacture
- Time consuming to manufacture
- Often produced in small batches

- Inventory preservation is very important and particular
- Cold-chain issues and distribution are encountered
  - Excursion studies are of paramount importance
  - Shipping methods should be qualified prior to launch
Common Issues with Protein Stability

- Light, heat, air, trace-metal sensitivity
- Stress factors large/small disrupt protein folding
- Multiple degradation pathways possible
- Multiple physical degrading pathways
- Non-Arrhenius behavior
- Collaborative degradation
Strategy for Expiry Dating

- Molecular and structural alterations
  - Primary amino acid sequence
  - Higher order structures

- Alterations post MFG
  - Deamidation
  - Oxidation
  - Glycosylation
  - Aggregation

- Stability Goal: determine what specific changes occur in the biologic molecule that could lead to its instability.
Denaturation

- Disruption of secondary/tertiary structure
- Assumed irreversible
- Most likely due to heat
  - Acids/Bases
  - Organic Compounds
  - Heavy metal ions
  - Agitation
- Exposes protected protein to degradation
- Both covalent and noncovalent forces dictate conformation and bioactivity.
Aggregation

- Formation of macromolecular complexes
- Covalent/Hydrophobic association
- Affect binding
- Affect activity
- Irreversible
- Immunogenic

- Control and detection of aggregates is important.
- What are the main factors that contribute to aggregation?
Oxidation

► Occurs on side chains.
  ► Air
  ► Peroxide
  ► Light

► Most prone
  ► Met e.g. CH$_3$S(O)CH$_2$CH$_2$CH(NH$_2$)CO$_2$H
  ► Cys
  ► Tyr
  ► Trp

► What manufacturing infrastructure can contribute to oxidation?
Deamidation

- Occurs with Asn and Gln
  - to Asp and Glu
- In proteins and monoclonals
- iso residues can form
  - Can decrease activity
- Case by case study of effects is warranted.
Excipients

- Residual peroxides
  - Chain oxidation
- Saccharides and Polyols
  - Glycosylation
    - Site, content, chain structure
    - Case by Case: range from immunogenic to no clinical impact
Overall Control Strategy

- QC testing is interconnected to characterization testing
- Control Strategy for manufacturing has many parts

Control Strategy: a planned set of controls, derived from current product and process understanding, that assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components.

ICH Q10

Control Strategy Processes assure the identity, quality, purity, safety and potency of the drug. It also enriches understanding of required Stability.

What are some process controls for biologic manufacturing?
Required Testing of CQAs

- Critical Quality Attributes generally require QC release and Stability testing

A CQA is “a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.”

ICH Q8 (R2)

“The identification of CQAs for complex products can be challenging. Biotechnological/biological products, for example, typically possess such a high number of quality attributes that it might not be possible to fully evaluate on impact, safety and efficacy of each one.”

ICH Q11

- Stability testing panels for biologics are often more populated.
Similarities to Chemical Drugs

- Stability Indicating Methods
- Long-Term and Accelerated Storage Conditions
- Frequency of Testing
- Stress Conditions
Stability Program Methods

- Stability Indicating Methods

“A stability indicating assay is a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product [...] Assay analytical procedures for stability studies should be stability-indicating unless scientifically justified.”

-FDA (Draft) Guidance for Industry Analytical Procedures and Methods Validation (August 2000)
Stability Indicating Methods

- Consult ICH Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological Products
- Compare to ICH Q1A (R2) Stability Testing of New Drug Substances and Products

“On the whole, there is no single stability-indicating assay or parameter that profiles the stability characteristics of a biotechnological product. Consequently, the manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity and potency of the product will be detected.”

-ICH Q5C

Additional mandatory monitoring for Final Container:
- Appearance, Sterility, Excipients
  - What are they and when should they be performed?
- CCI - preferred post-marketing commitment.

“The ability of a container closure system to maintain the integrity of its microbial barrier and hence the sterility of a drug product throughout its shelf-life should be demonstrated. Submit a summary report and data in a CBE-0 supplement.”

-FDA Letter to BLA Application for Yervoy (Ipilimumab) (2011)
Long-Term and Accelerated Storage Conditions

- Long-Term storage condition is the one on the label
- Accelerated storage is performed at higher temperatures and humidities

“Studies under accelerated conditions may provide useful support data for establishing the expiration date, provide product stability information for future product development, assist in validation of analytical methods for the stability program, or generate information which may help elucidate the degradation profile of the drug substance or drug product.”

-ICH Q5C

<table>
<thead>
<tr>
<th>Long-Term Storage Temp</th>
<th>Accelerated Storage Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20°C ± 5°C</td>
<td>5°C ± 3°C or 25°C ± 2°C</td>
</tr>
<tr>
<td>5°C ± 3°C</td>
<td>25°C ± 2°C/60% RH ± 5% RH</td>
</tr>
<tr>
<td>25°C ± 2°C/60% RH ± 5% RH</td>
<td>40°C ± 2°C/75% RH ± 5% RH</td>
</tr>
</tbody>
</table>

-ICH Q1A (R2)
**Frequency of Testing**

- ICH Q5C provides minimum testing frequency

<table>
<thead>
<tr>
<th>Desired Shelf-Life</th>
<th>Testing Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1 year</td>
<td>0, 1, 2, 3, 6, 9, 12 months</td>
</tr>
<tr>
<td>&gt; 1 year</td>
<td>0, 3, 6, 9, 12, 18, 24, 36 months</td>
</tr>
</tbody>
</table>

**Importance of Stress Conditions**

“Studies under stress conditions may be useful in determining whether accidental exposures to conditions other than those proposed (e.g. during transportation) are deleterious to the product and also for evaluating which specific test parameters may be the best indicators of product stability.”

-ICH Q5C

What are some stress conditions for biologics?

Freeze-thaw for frozen liquids is important.
Differences to Chemical Drugs

- Minimum requirement for stability data at time of market application submission.
- Definition of “significant change”
- Extrapolation of shelf-life
- Regression analysis to set shelf-life
Stability Data Requirement

- 6 month minimum requirement.
- 3 representative batches
  - DS & DP

“Where bulk material is to be stored after manufacture but prior to formulation and final manufacturing, stability data should be provided on at least three batches for which manufacture and storage are representative of the manufacturing scale production. A minimum of 6 months stability data at the time of submission should be submitted in cases where storage periods are greater than 6 months.”

ICH Q5C

What does ICH Q1A (R2) state about the required minimums of chemical drugs?
What does representative batch mean according to ICH Q5C?
Definition of “Significant Change”

Case-by-case basis.

“Recommendations for maximum acceptable losses of activity, limits for physicochemical changes, or degradation during the proposed shelf-life have not been developed for individual types or groups of biotechnological/biological products but are considered on a case-by-case basis.”

ICH Q5C

“Significant change” in ICH Q1A (R2) for chemical products

1. Drug substance failure of specification
2. Drug product 5% change in assay from t initial
3. Degradation products exceeding acceptance criteria
Extrapolation for Shelf-Life

- Is extrapolation of biologic shelf-life permitted/encouraged?

“Primary data to support requested storage period for either drug substance or drug product should be based on long-term, real-time, real-condition stability studies. Thus, the development of a proper long-term stability program becomes critical to the successful development of a commercial product.”

ICH Q5C

- ICH Q1A (R2) permits extrapolation beyond long-term data.
- ICH Q1E permits double shelf-life with no more than 12-months.
“Regression analysis is considered an appropriate approach to evaluating the stability data for a quantitative attribute and establishing a retest period or shelf-life. [...] An appropriate approach to retest period or shelf-life estimation is to analyze a quantitative attribute (e.g. assay degradation products) by determining the earliest time at which the 95% confidence limit for the mean intersects the proposed acceptance criterion.”

ICH Q5C

- Make sure that the acceptance criterion is known prior to regression.
- Arrhenius can often underestimate degradation in biologics.

“ICH Q1E is a guideline for evaluation of stability for setting shelf-life (when the acceptance criterion is known), not for determining stability specifications. Careful consideration must be given when using linear regression for biotechonology products, because many times it is not appropriate; these products often do not behave in the same manner as small molecules.”

FDA Letter to Zaltrap BLA (2012)
Clinical Phase-Appropriate

- Agencies have an approach for setting expiry dates using stability data.
- Extrapolation using preliminary data during clinical development is permitted.
  - Full, formal stability assessment is required to assign expiry - without extrapolation.

Early-Stage Clinical Development

- Agencies require it regardless of how limited it is.
- Stability protocol outlining desired storage period of API is provided.
  - Specifications, analytical methods and testing panel are included.
- Batches should be “representative batches” in desired condition/containers.
- Methods should be stability indicating.
Other Details in Expiry Dating

- FDA and EMA differ in expiry dating.
  - FDA does not require expiry dating during clinical development.
    
    \[(g)\] *New Drug Products for investigational uses are exempt from the requirements of this section, provided that they meet appropriate standards or specifications as demonstrated by stability studies during their use in clinical investigations.\]*

    
    *CFR 21 Part 211.137*

  - EMA requires expiry dating during early stage development.
    
    “*The claimed shelf-life of the active substance under the proposed storage conditions should be stated and accompanied by an evaluation of the available data. Any observed trends should be discussed.[...] extension of the shelf life beyond the period covered by real-time stability data may be acceptable...”*

    
    *EMA/CHMP/BWP247713/2012*

- Cannot exceed 2x or 12 months of real data.
- Can you extend beyond the intended duration of long term stability studies?
Analysis and Extrapolation

<table>
<thead>
<tr>
<th>Time Point (x)</th>
<th>Result (y)</th>
<th>X•Y</th>
<th>X•X</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>104</td>
<td>312</td>
<td>9</td>
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<tr>
<td>6</td>
<td>90</td>
<td>540</td>
<td>36</td>
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<td>9</td>
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<td>12</td>
<td>97</td>
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<td>18</td>
<td>100</td>
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<tr>
<td>36</td>
<td>97</td>
<td>3492</td>
<td>1296</td>
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</tbody>
</table>

- expected result = intercept + (slope × time)
- To find control limits
  - Calculate the expected result ± (k × s)
  - k = multiplier of normal quantiles for protection
  - s = square-root of the mean square error of regression
\[ \sum X = 108 , \sum Y = 782 , \sum X \cdot Y = 10542 , \sum X^2 = 2466 \]

\[
a = \frac{\sum Y \cdot \sum X^2 - \sum X \cdot \sum XY}{n \cdot \sum X^2 - (\sum X)^2} = \frac{782 \cdot 2466 - 108 \cdot 10542}{8 \cdot 2466 - 108^2} \approx 97.951
\]

\[
b = \frac{n \cdot \sum XY - \sum X \cdot \sum Y}{n \cdot \sum X^2 - (\sum X)^2} = \frac{8 \cdot 10542 - 108 \cdot 782}{8 \cdot 2466 - (108)^2} \approx -0.015
\]

\[ y = a + b \cdot x \]

\[ y = 97.951 - 0.015 \cdot x \]
95% confidence extrapolation to lower/upper specification by mean response

Let \( \hat{y} \) be the predicted mean for \( x \), i.e.

\[
\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x
\]

It so follows that:

\[
E(\hat{y}) = \beta_0 + \beta_1 x
\]

\[
\text{var}(\hat{y}) = \sigma^2 \left( \frac{1}{n} + \frac{(x - \bar{x})^2}{SXX} \right)
\]

\[
\hat{y} \pm t_{(1-\frac{\alpha}{2}),n-2} \times \hat{\sigma} \times \sqrt{\frac{1}{n} + \frac{(x - \bar{x})^2}{SXX}}
\]
Arrhenius Modeling

\[ k = Ae^{\left(-\frac{E_a}{RT}\right)} \]

Rewrite to aid graphically:

\[ \ln k = \ln A e^{\left(-\frac{E_a}{RT}\right)} \]

\[ = \ln A + \ln e^{\left(-\frac{E_a}{RT}\right)} \]

\[ = -\frac{E_a}{RT} + \ln A \]

Familiar format? Hint: \( y = mx + b \)
Arrhenius Isoconversion (possible extrapolation)

<table>
<thead>
<tr>
<th>Time</th>
<th>5°C</th>
<th>25°C</th>
<th>40°C</th>
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<tbody>
<tr>
<td>0M</td>
<td>850</td>
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<tr>
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<td>9M</td>
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<td>550</td>
<td>520</td>
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<tr>
<td>12M</td>
<td>700</td>
<td>500</td>
<td>450</td>
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</tbody>
</table>

\( \ln(k) \) vs. 1/T is negatively sloped

\( \ln(\tau_{fail}) \) vs. 1/T is positively sloped

\( \tau_{fail} \times \text{rate} \) is a constant for each reaction at all temps
Stability Protocol

- Same general requirements as small molecule
- Duration of at least intended shelf-life.
- Accelerated performance and photostability.
- Stability indicating tests will be different than small molecule.
- Cold storage conditions
  - Frequent testing due to relative instability
    - Time units of days/weeks in addition to months
    - Consider the development data (e.g. if ultra low temp, then fine)
## Time/Temperature Schedule for DP

<table>
<thead>
<tr>
<th>Test/Time</th>
<th>T=0</th>
<th>1M (25 ºC)</th>
<th>3M (5 ºC)</th>
<th>6M (5 ºC)</th>
<th>9M (5 ºC)</th>
<th>12M (5 ºC)</th>
<th>18M (5 ºC)</th>
<th>24M (5 ºC)</th>
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<tbody>
<tr>
<td>App</td>
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<td></td>
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