Using Structure/Function Relationships to Identify CQAs and Develop Analytical Specifications

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ICH Q6B: “A specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described.”

Options for setting Acceptance Criteria (AC):
- Published guidelines (sterility, endotoxins, etc)
- Manufacturing capability / clinical experience
- Link AC to product safety and efficacy

Use structure / function data to set “clinically relevant” AC based on impact to biological functions
Multi-point, forced degradation studies with structural modeling and full analytical characterization are the foundation for PTM control strategy. Use these data to:

- Clearly identify PTM CQAs (deamidation & oxidation)
- Set “clinically relevant” AC based on S/F correlations rather than statistical analysis of mfg batches
Forced Deg Workflow

- Evaluate heat, peroxides, light, pH and glucose stress
- Prep 4-6 samples under each stress condition
- Fully characterize all samples using the assays below to identify CQAs and establish S/F correlations

<table>
<thead>
<tr>
<th>Quality Attributes</th>
<th>Analytical Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Structure</td>
<td>Peptide Map</td>
</tr>
<tr>
<td>Glycosylation and Glycation</td>
<td>Oligo Map and Intact Mass</td>
</tr>
<tr>
<td>Charge Heterogeneity</td>
<td>cIEF</td>
</tr>
<tr>
<td>Size Heterogeneity</td>
<td>SE-HPLC and cSDS</td>
</tr>
<tr>
<td>Higher Order Structure</td>
<td>CD, AUC and DSC</td>
</tr>
<tr>
<td>Biological Functions (depending on MOA)</td>
<td>Bioassay and Ag Binding</td>
</tr>
<tr>
<td></td>
<td>CDC, ADCC, Fc(\gamma)R and FcRn Binding</td>
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</tbody>
</table>
Heat Stress Impacts Biological Function
6M Heat Stress Study in pH 5.5 Formulation Buffer at 37°C

- Large decreases in CDC, ADCC and FcγRIIIa binding (>50%)
- Small increases in N-terminal cyclization, Asp isomerization and HC Asn389/394 deamidation (<5%)
- Large increases in HC Asn330 deamidation & acidic peaks by cIEF
Unique pH Profile for HC Asn330 Deamidation
Comparison of 3 IgG1 mAbs at 37°C

- Asn330 deamidation occurs very slowly in mild acidic buffer (6%/mon at 37°C)
- Asn330 deamidation does not occur at neutral or higher pH. Manufacturing process variation has little impact on Asn330 deamidation.
Unique pH Profile for HC Asn330 Deamidation
Molecular Mechanism

- His sidechain imidazole ring has a pKa around 6.0.
- Below pH 6, protonation of His273 helps form salt bridge with Glu299, which increases the solvent accessibility of Asn330.
- Above pH 6, deprotonation of His273 breaks the salt bridge with Glu299 and reduces the solvent accessibility of Asn330.
- Below pH 3, deamidation occurs by acid hydrolysis at very slow rate.
HC Asn330 Deamidation is a CQA Based on Structural Modeling and Bioassay Data

HC Asn330 sits near regions involved in FcγRIIIa binding needed for ADCC activity and C1q binding needed for CDC activity.

Asn330 is asked to have analytical control even though deamidation rate on stability was very slow (0.4% / year at 5°C).
Asn330 deamidation correlates with loss of CDC, ADCC & FcγRIIIa binding

Set “Clinically Relevant” AC Based on S/F Correlations
4.4% Asn330 Deamidation has 95% FcγRIIIa Binding

Limit: 95% FcγRIIIa binding correlates with 4.4% Asn330 deamidation
Compared to ≤3.0% from statistical analysis of mfg batches
HC Asn330 Deamidation is Still Well Controlled
Based on Data from Heat Stress Study at pH 5.5

- 80% ADCC
- AC (S/F)
- AC (Statistics)
- Clinical Samples
- R&S Range

Buffer Zone
Assay Control

Time at 37°C (Days)
Control of Asn330 Deamidation by cIEF
Data from Heat Stress Study at pH 5.5

Asn330 Deamidation correlates with increase in acidic peaks by cIEF

Release / Stability Acceptance Criteria: 23-40% acidic peaks
Limit: 40% Acidic Peaks correlates with 3.7% Asn330 deamidation
Increase in Acidic Peaks Due Primarily to Asn330 Deamidation & Unique to Heat Stress at pH 5.5

<table>
<thead>
<tr>
<th>Attribute</th>
<th>T0</th>
<th>1M at 37C</th>
<th>2M at 37C</th>
<th>3M at 37C</th>
<th>6M at 37C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peptide Map</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HC Glu 1 Cyclization</td>
<td>1.1</td>
<td>1.7</td>
<td>2.4</td>
<td>3.2</td>
<td>5.2</td>
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<tr>
<td>HC Asp285 Isomerization</td>
<td>0.4</td>
<td>1.3</td>
<td>2.0</td>
<td>3.0</td>
<td>5.1</td>
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<tr>
<td>HC Asn320 Deamidation</td>
<td>6.9</td>
<td>6.9</td>
<td>7.2</td>
<td>6.9</td>
<td>7.4</td>
</tr>
<tr>
<td>HC Asn330 Deamidation</td>
<td>0.4</td>
<td>6.6</td>
<td>11.8</td>
<td>18.2</td>
<td>31.6</td>
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<td>HC Asn389/394 Deamidation</td>
<td>8.8</td>
<td>9.8</td>
<td>10.4</td>
<td>11.0</td>
<td>12.7</td>
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<td>HC Asn439 Deamidation</td>
<td>1.0</td>
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<td>1.0</td>
<td>1.1</td>
<td>1.1</td>
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<td><strong>cIEF</strong></td>
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<tr>
<td>Main Peak</td>
<td>62.1</td>
<td>48.2</td>
<td>40.6</td>
<td>32.1</td>
<td>21.3</td>
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<tr>
<td>Acidic peaks</td>
<td>33.9</td>
<td>44.0</td>
<td>50.4</td>
<td>59.4</td>
<td>71.0</td>
</tr>
<tr>
<td>Basic Peaks</td>
<td>3.9</td>
<td>7.8</td>
<td>9.0</td>
<td>8.5</td>
<td>7.7</td>
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Forced deg conditions

<table>
<thead>
<tr>
<th>Forced deg conditions</th>
<th>Glucose Stress</th>
<th>Peroxide Stress</th>
<th>Photo Stress</th>
<th>Heat Stress (pH 5.5) 6M at 37C</th>
<th>High pH Stress (8.5)</th>
<th>Low pH Stress (3.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asn330 Deamidation</td>
<td>0.7</td>
<td>0.5</td>
<td>0.8</td>
<td>31.6</td>
<td>1.7</td>
<td>1.3</td>
</tr>
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</table>
Control of HC Asn330 Deamidation
AC for cIEF and Peptide Map are Equally Effective

- 25°C Stability data for 36 DP batches (color coded)
- AC indicated by the red bars in the graphs
- AC for cIEF based on statistical analysis of batch results
Control of HC Asn330 Deamidation
AC for cIEF and Peptide Map are Equally Effective

- 40°C Stability data for 36 DP batches (color coded)
- AC indicated by the red bars in the graphs
- AC for cIEF based on statistical analysis of batch results
Methionine Oxidation is a Critical Deg Pathway
Hydrogen Peroxide Stress Study

- Large decreases in CDC and FcRn binding (>50%)
- Large increases in Met oxidation (>80%), but no impact on Trp oxidation or other structural attributes
Only HC Met257 and Met 433 Oxidation are CQAs Based on Structural Modeling and Bioassay Data
Met257 oxidation impacts FcRn binding and CDC activity

Set “Clinically Relevant” AC Based on S/F Correlations
8.0% HC Met257 Oxidation has 95% CDC Activity

Limit: 95% CDC correlates with 8.0% Met257 oxidation
Compared to ≤5.6% from statistical analysis of mfg batches
Glycosylation is a CQA
Single Point, Glycoform Enrichment Studies

Terminal galactose and core fucose impacted CDC, ADCC and FcγRIIIa binding


Set “Clinically Relevant” AC Based on S/F Correlations

Results of G1F Spiking Study

- Current AC for G1F is 15-27% based on statistical analysis of clinical batches
- mAb with up to 50% G1F met the AC for ADCC and CDC activity

<table>
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<tr>
<th>Acceptance Criteria</th>
<th>ADCC (%)</th>
<th>CDC (%)</th>
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<tbody>
<tr>
<td>15-27%</td>
<td>93-103</td>
<td>89-102</td>
</tr>
<tr>
<td>15-32%</td>
<td>93-107</td>
<td>89-107</td>
</tr>
</tbody>
</table>

5% increase in G1F
= 4% increase in ADCC
= 5% increase in CDC
Summary
Analytical Control Strategy for PTM CQAs

- Multi-point, forced degradation or glycoform enrichment studies provide a wealth of information
- Can clearly identify PTM CQAs (deamidation, oxidation and terminal galactose)
- Can set “clinically relevant” AC based on impact to function rather than statistical analysis of mfg batches
- Can develop robust analytical control strategies using acceptance criteria from multiple methods
THANKS!!

Analytical Development
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