The Integration of Mass Spectrometry to the Process Development of Fusion Protein Therapeutics

Chun Shao, Ph.D.

2021 CASSS Northeast Regional Forum
Outline

• Introduction
• Site-Specific Glycosylation Monitoring with LC-MS
• Summary
• Acknowledgement
Bioprocess Development Analytical Support

- High-throughput Platform Assays
- Sample Automation
- PAT for Real time Analytics
- Mass Spectrometry for In-Process Support
Fc-fusion Protein Therapeutics

- Fc-fusion protein therapeutics are one of the most successful classes of IgG-based products;

<table>
<thead>
<tr>
<th>Product</th>
<th>Sales in 2019 (billion, USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eylea</td>
<td>7.5</td>
</tr>
<tr>
<td>Enbrel</td>
<td>7.2</td>
</tr>
<tr>
<td>Trulicity</td>
<td>4.3</td>
</tr>
<tr>
<td>Orenicia</td>
<td>3.2</td>
</tr>
<tr>
<td>Elocta</td>
<td>1.2</td>
</tr>
</tbody>
</table>

- Fc-fusion protein combine the pharmacological properties of biological ligands with the additional stability and inherent properties of IgG Fc domain;

- Fc-fusion protein can significantly improve the clinical potential of active protein drugs such as extend the plasma half life as well as engage immune-mediated effector functions;

- To date, ~37 therapeutic fusion proteins are in clinical development and 13 products have been approved by the FDA, CFDA, and EMA;
  - Enbrel (TNFR-Fc fusion, FDA approval in 1998) and Orenicia (CTLA4-Fc fusion, FDA approval in 2005) for treatment of rheumatoid arthritis;

Major Groups of Fc-fusion Proteins

- Due to highly **heterogenous** structure (the presence of sialic acid, complex glycan structure, etc.), the analysis of Fc-fusion proteins is more challenging and complex than monoclonal antibodies;
- **Product-specific** methods over conventional generic or platform methods are often desirable to support process development;

Therapeutic Fc-fusion proteins: Current analytical strategies
Fab Site-Specific Glycosylation Monitoring

- A Fc-fusion protein with four \( N \)-glycosylation sites in Fab region and two \( N \)-glycosylation sites in the Fc region;

- A LC method was developed to monitor **Fab site-specific glycoforms** (G0F, G1F, G2F, G2FS1, and G2FS2) as the understanding about site-specific glycosylation as pCQA is continuously evolving during product development lifecycle;

- Mass spectrometry is coupled with LC for the peak identification of the complex chromatogram and the optimization of LC method parameters;
  - Mobile phase screening
  - Fluorescence tag screening
  - LC gradient optimization
Glycopeptide Chemical Labeling

Fab

Fc

N-glycan

Fluorescence tag

Denature

Reduce

Label

Trypsin

Fc Glycopeptide

Fab Glycopeptide A

Fab Glycopeptide B
Workflow Overview

Label
- Denature
- Reduce
- Label Cys with fluorescence tag (Alkylation)

Desalt
- Sample desalt with SEC spin column

Proteolysis
- Trypsin digestion

LC Separation
- HILIC for glycopeptide separation

MS Identification & FLR Quantitation
- MS for peak identification
- FLR for Fab glycopeptide relative quantitation
Ammonium Acetate vs Ammonium Formate as Mobile Phase

- Significant improvement in separation resolution with **ammonium formate** for IASD FLR tag;

- Glycopeptide A and B coelution

- Only Glycopeptide A + G0F and Glycopeptide B + G1F coelutes
**IASD vs IAEDANS as Fluorescence Tag**

- Different glycoforms of Fab glycopeptide A and B are fully resolved with **IAEDANS** FLR tag;

- Only Glycopeptide A + G0F and Glycopeptide B + G1F coelutes

- Glycopeptide A + G0F and Glycopeptide B + G1F were separated

- Better resolution between Glycopeptide A+ G2FS1 and Glycopeptide B+ G2FS2
Quantitation of Fab Site-Specific Glycopeptides through Fluorescence Peak Intensity

- Reproducible quantitation for glycopeptides;
Summary

• A HILIC LC-FLR-MS method was established to separate glycopeptides and quantify Fab site-specific glycosylation for a Fc-fusion protein;

• Molecule specific methods are often required to support the development of complex protein therapeutics;

• Creativity and collaboration cross functional areas is essential to develop innovative methodology for problem solving;
Acknowledgement

Partnership among Process Development Analytics, Upstream, and Downstream

- Hangtian Song
- Diane Worrell
- Li Tao
- Yunping Huang
- Chris Chumsae
- Julia Ding
- Henrik Andersen

Thank You for Your Attention!