PAVING THE WAY FOR ASSESSING IN VIVO DYNAMICS OF MULTIPLE QUALITY ATTRIBUTES FOR PROTEIN THERAPEUTICS

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Outline

• Workflow for measuring multiple quality attributes in vivo
  – Key drivers
  – Challenges
  – Method development considerations
• Proof of concept studies
• Differential Analysis
In Vivo Dynamics of Quality Attributes: Bioanalytical Multi-Attribute Method

Impact of PQAs on product safety and efficacy

Drug (antibody, fusion protein, peptide, nanobody, etc)

In Vivo Dosing

Blood Collection

Affinity Purification

Elution/Digestion

High-resolution LC/MS Characterization

In Vivo Dosing

Blood Collection

Affinity Purification

Elution/Digestion

High-resolution LC/MS Characterization

Differential Analysis

Peptide Identification

Attribute Identification and Quantification

Key Drivers

- Help evaluate criticality of the quality attributes and facilitate manufacturing process development
- Assess the effect of product quality attributes on safety and efficacy
- Establish better understanding of PK/PD relationship
Challenges in Quantifying Multiple Quality Attributes In Vivo

Bioanalytical

Determine drug concentration (PK)

In Vivo
- Limited volume
- Low concentration
- Complex matrix
- May need purification

LC/MS – QQQ
- Simple processing steps (Monitor one or two peptides)
  - Absolute quantitation
  - High throughput
  - Known

In Vitro

LC/MS – High Resolution
- Complex processing steps (Monitor all peptides)
  - Relative quantitation
  - Low throughput
  - Known & unknown

MAM

Detect changes in multi-attributes in vitro (CMC)

In Vitro
- Volume not limited
- High concentration
- Buffer
- No need for purification

Bioanalytical MAM

Detect changes in multi-attributes in vivo (metabolism)

In Vivo
- Limited volume
- Low concentration
- Complex matrix
- Require purification

LC/MS – High Resolution
- Complex processing steps (Monitor all peptides)
  - Relative quantitation
  - Low throughput
  - Known & unknown

In Vivo
- Volume not limited
- High concentration
- Buffer
- No need for purification

Bioanalytical MAM

Detect changes in multi-attributes in vitro (CMC)
Proof of Concept Using an IgG1 mAb

Starting point: CMC MAM

Drug in Buffer
Direct analysis

Drug in Buffer
Immuno-enrichment

Drug Spiked in Serum
Immuno-enrichment

Drug in Serum ex vivo
Immuno-enrichment

Drug in Serum in vivo
Immuno-enrichment

Control for sample processing induced modifications
Method Development Considerations

• Sensitivity requirement
  – Concentrations in PK profile
  – Available sample volume

• Affinity purification
  – Choice of the capture antibody
  – Affinity purification media (binding capacity, nonspecific binding, ease of automation, etc.)
  – Optimization on yield and purity

• Digestion conditions
  – Choice of protease

• LC/MS instrumentation
  – Low flow vs high flow chromatography
  – Low resolution vs high resolution mass spectrometer

• Overall method reproducibility
Method Performance

- Reproducibility
- Sequence coverage
- Control for sample processing induced modifications
Consistent recovery of all the quality attributes with and without affinity purification and with different digestion methods indicates that

1. There was no bias in the affinity purification step toward any specific quality attribute
2. Overall sample processing procedure did not alter quality attributes
Bioanalytical MAM Study Design

**In Vivo**
IV infusion at 200 mg/kg

- Predose
- D0 (0.5hr)
- D2
- D4
- D7

**Ex Vivo**
0.5 mg/mL spiked in cynomolgus monkey serum; incubate at 37°C

- D0
- D2
- D4
- D7
N-terminal Modification: HC Glutamine to Pyro-glutamate

- A complete conversion of N-terminal glutamine residue into N-terminal pyroglutamate was observed in vivo.

Relative Percentage (%) = \( \frac{\text{Peak Area (Peptide mod1)}}{\text{Peak Area (Peptide mod1)} + \text{Peak Area (Peptide modx)} + \text{Peak Area (Peptide unmod)}} \)
Deamidation: HC N385/N390

- A conserved site in the Fc domain commonly found in humanized monoclonal antibodies exhibited a rapid increase in deamidation.

Deamidation Rate:

In vivo: 0.9%/day (1.1%\(^1\))
Ex vivo: 1.5%/day (1.7%\(^1\))

C-terminal Modification: HC Lysine Processing

- C-terminal lysine processing was likely due to carboxypeptidase activity
High-mannose Glycans

- High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance
**Example of Attribute Exposure Profile**

**HC N385 & N390 Deamidation**

**PK Profile**

**% Attribute**

**Attribute Exposure Profile**

![Graphs showing PK profile, % attribute, and attribute exposure profile over post-dose days (0 to 8)].

- **Serum Concentration (µg/mL)**
- **Relative Percentage (%)**
- **Serum Concentration (µg/mL)**

**Legend**

- Cyno-1
- Cyno-2
- Cyno-3
Looking for Unknown Changes in an Unbiased Manner

Ex Vivo Day 0  Ex Vivo Day 7

Monkey Serum

Affinity Purification

LC/MS/MS

Differential Analysis

Identification

Quantification

• SIEVE (ThermoFisher)
• BioPharma Finder (ThermoFisher)
• Progenesis QI (Waters-Nonlinear Dynamics)
SIEVE

- Ex Vivo Day 0 vs Ex Vivo Day 7
  - SIEVE was able to pick up most of the known changes in the sample
Low abundant deamidation was missed by SIEVE with threshold of 1e6

Lower threshold (eg, 1e4) allowed SIEVE to detect HC76-86 deamidation. However, more false positives were observed when using lower threshold.
Low abundant attribute changes were detected, and the percent attribute was calculated automatically.
Progenesis QI

- Low abundant HC76-86 deamidation was also detected using Progenesis
Differential Analysis for Bioanalytical MAM

• All three algorithms can pick up known attribute changes
  – BioPharma Finder has better identification capability and calculates attribute percentage automatically when peptide identity is known

• Challenges for all three algorithms include:
  – Setting up the appropriate threshold to minimize the number of false positives
  – Statistical analysis tools to help reduce false positives
  – Filtering strategies and visualization tools to help distinguish false positives from true positives
  – The ability to normalize overall intensity, align retention time, and perform peak picking when molecule concentration differs several folds across multiple time points
Summary

• We demonstrate that it is feasible to track changes in multiple quality attributes for a IgG1 monoclonal antibody in cynomologus monkey serum

• We present a general strategy on how to identify and quantify changes in multiple quality attributes of protein therapeutics in vivo

• Further developmental efforts are needed to improve
  – Overall sample processing sensitivity and reproducibility
  – Algorithms for differential analysis can be modified to streamline the data analysis workflow (eg, comparison with multiple time points and better visualization tools)
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