Platform Analytical Approaches Enabling Expedited Development of Therapeutic mAb Products

Bay Area Discussion Group

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Abstract

• A common challenge faced in biopharma is the ability to advance numerous projects throughout development while minimizing cost and timelines, and maximizing the robustness of the CMC strategy. The Pfizer Biotherapeutics portfolio includes multiple therapeutic monoclonal antibodies (mAbs) along with several other therapeutic modalities in development. With the number of monoclonal antibody programs increasing and rapidly advancing through development, it is imperative to apply state-of-the-art analytics and to implement streamlined/efficient work processes without compromising product quality and safety.

• At Pfizer we have implemented a platform analytical approach to rapidly advance our therapeutic mAb programs. This analytical approach monopolizes on the structural homology across mAbs yet provides modular options to address mAbs with unique characteristics.

• State-of-the-art analytics and streamlined work processes have enabled the rapid advancement of our monoclonal antibody therapeutics without compromising product integrity.

• This presentation will describe our mAb platform analytical approach towards methods development, methods qualification, and product characterization.
Outline

- Introduction to Biotherapeutic diversity at Pfizer
- Drivers for streamlining mAb development
- Platform strategies to enable efficient mAb development
  - Why focus on monoclonal antibodies?
    - Conserved Product Quality Attributes
  - Components of the platform
  - Analytical Strategy
    - Platform analytical tools
    - Characterization tools to de-risk platform approach
    - Approaches to confirming method suitability
    - Specifications
- Platform ≠ No change
  - Comparability strategies
Diversity of Pfizer Biotherapeutics; Marketed Products

• Delivery of a diverse portfolio has resulted in an extensive technology and strategy “Tool-Kit”

| >500 kDa | Glycoconjugates | Virus-like Particles | Carbohydrates |
| 100-350 kDa | mAb / mAb Conjugates / ADCs | Recombinant Proteins | Native Proteins |
| 20-100 kDa | Recombinant Proteins | Conjugates |
| <20 kDa | Peptides | Oligonucleotides | Heparins |
Diversity of Pfizer Biotherapeutics; Development Experience

- Development of an array of modalities
- Extensive experience advancing mAb programs (n>50)
- Tool-Kit leveraged to create “platform” strategies

<table>
<thead>
<tr>
<th>10-50 kDa</th>
<th>50-100 kDa</th>
<th>150-200 kD</th>
<th>&gt;1000 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptides, Peg-peptides: insulin, growth hormone, cytokines, oligonucleotides</td>
<td>Fusion Proteins: TTP4000 mIFN Transferrin-fusion</td>
<td>PEGylation: PEG-hGH PEG-peptide PEG-Fab PEG-ON</td>
<td>Monoclonal Antibodies: IgG1 IgG2 IgG4 ADCs</td>
</tr>
<tr>
<td>Protein-Phospholipid Complex: ApoA1-M</td>
<td></td>
<td></td>
<td>Conjugates: KLH-Peptide VLP-Peptide Antibody-Peptide Antibody-Protein DT-peptide Polysaccharide Conjugated Vaccines</td>
</tr>
</tbody>
</table>
Drivers for Implementation of a Platform Development Strategy; The Decline in R&D Productivity: Increased Spending, Lower Output

**Worldwide Total Pharmaceutical R&D Spend**

- **+10.6% CAGR 2002-08**
- **+2.3% CAGR 2009-16**

**FDA Approval Count (NMEs & Biologics)**

- Total no. of molecules approved
- NMEs
- Biologics

Source: EvaluatePharma April 30, 2010

CGAR: Compound Growth Annual Rate
Drivers for Implementation of Platform Strategies

• **Current State:**
  - Questionable sustainability of current drug development costs
  - Hefty investments may drive focus on **fewer** “safe” targets
  - Resource constraints inhibit innovation

• **Future State:**
  - Platform approaches decrease development timeline, minimize cost, while maintaining safety and efficacy
  - Facilitates the increase of “shots on goal”
  - Decreased resource burn enhances innovation investments
Identifying Opportunities to Streamline Development

- Investments vary throughout mAb development
- Focus areas for streamlining development; both cost and speed are noted (*)

**Diagram:**
- Early Development
- FIH-Start
- PIIIb-Start
- PIII Readiness
- BLA Readiness
- BLA-Filed
- Launch
- Final Launch Preparations

**Legend:**
- Project Cost / Snapshot FTE Burn(*)
- Candidate
Why Focus on Monoclonal Antibodies?

- Structural homology between mAbs (IgG1s, IgG2s, and etc.) enables application of platform strategies
Diverse mAb Development Experience Facilitates Platform Approaches

• Pfizer has developed >50 mAbs over the last ~10 years
• Structural homology and broad development experience has enabled implementation of platform approaches
• Platform process, formulation, and methods have evolved
  – Platform expression systems
  – Upstream and downstream process standardization
  – Same raw materials, media, filters, buffers and resins
  – Platform formulations e.g. histidine/sucrose and standardized DP configurations
  – Standardized analytical methods and specifications
• Platform approaches are not unique to Pfizer
Representative Antibody Manufacturing Process

mAb Platform Manufacturing Process

1. CHO Culture
   - 1-4 gm/L titer, > 10^7 cells/mL

2. Clarification
   - Centrifugation

3. Protein A/Low pH Virus inactivation
   - Capture column
     - Single polishing step
       - “Weak partitioning mode”

4. AEX
5. Virus Filter
   - Assurance of viral safety

6. UF/DF
   - High concentration DS (> 50 mg/mL)

Phase I Purity Targets
- < 10 ppm Protein A
- < 100 ppm HCP
- < 5% HMW
- < 10 ng/dose DNA
Conserved Quality Attributes for mAbs Enable Implementation of a Platform Analytical Strategy

Key deliverable of mAb development is to define product profile and demonstrate consistency of control

Quality Attributes
- Molecular mass and size
- Primary structure
- Product isoforms
- Disulfide bonds
- N-linked oligosaccharides
- Charge heterogeneity
- Size heterogeneity (HMMS)
- Degradation products
- Higher order structure
- Potency / biological activity
Platform Analytical Toolbox;
Defining Product Profile/Elucidating Structure

**Attribute**

**Primary Structure Characterization**
- Molecular mass
- Amino acid sequence
- Amino acid composition

**Methods**
- Mass spectrometry – intact mass
- LC/MS - peptide mapping
- AAA
- Edman sequencing

**Variants & PTM’s**
- Disulfide bonds, Free thiols
- Glycosylation: N- and O-linked
- Amino acid modifications

**Methods**
- LC/MS - subunit analysis
- LC/MS - peptide mapping
- Glycan size and charge profiling
- Ellman’s

**Higher-order Structure**
- Secondary and tertiary structure

**Methods**
- CD spectroscopy, DSC
- Fluorescence spectroscopy, AUC, MALS, FFF
Platform Analytical Toolbox; Controlling Quality

**Attribute**

**Product Purity**
- Size Heterogeneity
- Charge Heterogeneity
- Identity

**Methods**
- SEC, CGE, SDS-PAGE
- IEX-HPLC, iCE
- Proteolytic mapping

**Product Variants/Degradants**
- Aggregation/Fragments/Clips
- Disulfide Isoforms
- Deamidation
- Oxidation
- C-terminal/N-Term modifications

**Methods**
- SEC, CGE, SDS-PAGE
- RP-HPLC
- IEX-HPLC, iCE
- LC/MS - peptide mapping
- AUC, LS, FFF

**Biological Activity**

**Methods**
- In Vitro Binding
- Cell Based Bioassay
- In Vivo assays
- SPR

*Not inclusive*
# Product Quality Assessment: Platform Analytical Tools

<table>
<thead>
<tr>
<th><strong>Product Quality Attributes</strong></th>
<th><strong>Platform Analytical Methodology</strong></th>
<th><strong>Assessment Criteria</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Size Heterogeneity</td>
<td><strong>Release/Stability</strong>&lt;br&gt;Size Exclusion Chromatography</td>
<td>Ensure low levels of HMMS</td>
</tr>
<tr>
<td>N-Linked Glycosylation</td>
<td><strong>Release</strong>&lt;br&gt;HILIC-HPLC</td>
<td>Ensure typical glycosylation profiles and minimal levels of atypical glycan structures</td>
</tr>
<tr>
<td>Charge Heterogeneity</td>
<td><strong>Release/Stability</strong>&lt;br&gt;Imaged Capillary Electrophoresis</td>
<td>Assess levels of total acidic and basic variants</td>
</tr>
<tr>
<td>Amino Acid Sequence</td>
<td><strong>Characterization</strong>&lt;br&gt;mAb subunit/domain mapping&lt;br&gt;Peptide Mapping&lt;br&gt;cDNA sequencing</td>
<td>Monitor for amino acid variants</td>
</tr>
</tbody>
</table>
Size Exclusion Chromatography

- Separation based on hydrodynamic radius;
- Larger species elute faster
- Detection is by UV(280nm)

> 99 % monomer

< 1% HMMS
Imaging Capillary Isoelectric Focusing (iCE)

- iCE separates species based on their charge differences (pI value) in a pH gradient.
- The protein is focused in a capillary column under high voltage.
- The focusing is monitored in real-time mode with whole column imaging detection (UV280).
Glycan Profiling by HILIC (NPLC)

PNGaseF → Oligosaccharides released and fluorescently labeled with 2-aminobenzamide

HPLC (amide column) with FLD

Oligosaccharides released and fluorescently labeled with 2-aminobenzamide

Glycans

G0F - GlcNAc

G1F - GlcNAc

Man5

G2F

G2F + SA

Sialylated glycans
Platform Risk Mitigation; Application of State-of-the-art Characterization Tools

1. **Intact protein mass analysis**
   - Confirm 4-chain architecture via mass; identify major and minor product isoforms

2. **LC/MS – Subunit analysis**
   - Quick and reliable “sequence” verification via accurate mass with 100% coverage
   - Localize major/minor/trace modifications to subunits/domains; rapid scFc N-glycan profiling

3. **LC/MS – Peptide mapping**
   - Confirm sequence and localize/confirm modifications at peptide level with ≤ 100% coverage
   - Detect and quantitate low-level species (N- & C-terminal processing, oxidation, deamidation)

4. **Released N-glycan profiling via LC/MS**
   - Confirm subunit/domain and peptide mapping peak assignments and profiles
   - Detect and quantitate low-level N-glycans
Antibody Subunit/Domain Fragmentation via IdeS

**Advantages**
- Rapid – IgG is fully cleaved within 30 minutes
- No optimization of reaction conditions needed
- Homogenous fragments – site specific cleavage

**IdeS**, Immunoglobulin G degrading enzyme of *Streptococcus pyogenes*; a novel cysteine protease that cleaves H-chains of human IgG below the hinge region at the conserved consensus sequence motif between amino acid residues Gly\(^235\) and Gly\(^236\)

![Diagram of antibody subunit/domain fragmentation via IdeS](Image)
# A New Generation QTOF: Bruker maXis

<table>
<thead>
<tr>
<th></th>
<th>Previous Generation</th>
<th>New Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (m/Δm)</td>
<td>10,000 to 15,000</td>
<td>30,000 to 60,000</td>
</tr>
<tr>
<td>Mass accuracy</td>
<td>15-25 ppm</td>
<td>1-2 ppm (&lt; 0.05 Da @ 25 kDa)</td>
</tr>
<tr>
<td>Fragmentation methods</td>
<td>CID</td>
<td>CID and ETD</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Moderate</td>
<td>High</td>
</tr>
</tbody>
</table>

- Platform strategy continues to evolve based on the new QTOF ultrahigh resolution capabilities
Platform Risk Mitigation; Characterization
3-part Subunit/Domain Analysis with Ultra-high resolution Mass Spectrometry

Define Product Profile and Support Bioprocess Development:
Sequence Variant detection and characterization

Define Product Profile:
Charge Isoform characterization, ie Deamidation
Characterization of Deamidation

- Asparagine (N) to Aspartic Acid (D)
- Process is spontaneous, influenced by pH and sequence environment
- Mass change = +0.984 Da (-NH$_3$, +H$_2$O)
- Traditionally identified by isolation of charge isoform and peptide mapping

Assessing Method Suitability Throughout Product Lifecycle

- Platform analytics are integrated early during bioprocess development to enable an efficient development lifecycle and to establish a foundation of product/process knowledge.

1. **Platform Method Assessment:**
   - Performed during early Development prior to RegTox

2. **Platform Method Verification:**
   - Performed prior to Phase I
   - or
   - Method Development/Qualification:
   - Performed prior to Phase I for non-platform analytics

3. **Method Validation:**
   - Product specific
   - Performed prior to PV

- Platform analytics are integrated early during bioprocess development to enable an efficient development lifecycle and to establish a foundation of product/process knowledge.
What is meant by “Method Suitability”?

• The analytical result must be reliable enough that decisions based upon the result can be made with confidence

• The error associated with the measurement must be known and understood within the context of the methods intended use

• The requirements of the method are often defined by the specification acceptance criteria for the given test
  • Validation must demonstrate that the method can support the specification

• Definition of “suitable” evolves throughout the development lifecycle
Analytical Method Lifecycle

- **Method Development** – Non cGMP process of defining the conditions of operation that will deliver specified performance parameters
  - Begins at candidate selection

- **Verification** - Demonstration that a previously qualified method behaves as expected for a different analyte
  - Platform method performed on “new” mAb; confirm performance parameters and profile are as expected

- **Qualification** – cGMP testing and documentation of the performance of a method at a point in time
  - All methods are qualified prior to testing of Ph1 Clinical supplies
  - Components of qualification continue from Ph1 to Ph3

- **Validation** – cGMP confirmation as per ICH Q2, that a method is suitable for its intended purpose
  - Platform methods are converted to molecule specific methods
  - All methods are ICH validated prior to execution of process validation
  - Method Validation confirms suitability to support specification
## Platform Specification Tests

<table>
<thead>
<tr>
<th>Category</th>
<th>Analytical Procedure</th>
<th>Quality Attribute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>Appearance</td>
<td>Clarity, Coloration, and Visual Particulates</td>
</tr>
<tr>
<td>Characteristics</td>
<td>pH</td>
<td>pH</td>
</tr>
<tr>
<td>Characteristics</td>
<td>UV Spectroscopy</td>
<td>Concentration</td>
</tr>
<tr>
<td>Characteristics</td>
<td>iCE</td>
<td>Charge Heterogeneity</td>
</tr>
<tr>
<td>Characteristics</td>
<td>HPLC of released glycans</td>
<td>Glycan Fingerprint</td>
</tr>
<tr>
<td>Identity</td>
<td>Peptide Mapping</td>
<td>Peptide Profile (primary sequence confirmation)</td>
</tr>
<tr>
<td>Purity &amp; PR*-Impurity</td>
<td>SEC-HPLC</td>
<td>Monomer, HMMS, LMMS</td>
</tr>
<tr>
<td>Purity &amp; PR-Impurity</td>
<td>Reducing CGE</td>
<td>HC+LC-related, Fragments</td>
</tr>
<tr>
<td>Purity &amp; PR-Impurity</td>
<td>Non-Reducing CGE</td>
<td>Intact IgG, Fragments</td>
</tr>
</tbody>
</table>

*Product Related Impurity*
<table>
<thead>
<tr>
<th>Category</th>
<th>Analytical Procedure</th>
<th>Quality Attribute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process Related Impurities</td>
<td>ELISA</td>
<td>HCP</td>
</tr>
<tr>
<td>Process Related Impurities</td>
<td>qPCR</td>
<td>DNA</td>
</tr>
<tr>
<td>Process Related Impurities</td>
<td>ELISA</td>
<td>rProtA</td>
</tr>
<tr>
<td>Safety</td>
<td>Bioburden</td>
<td>Safety</td>
</tr>
<tr>
<td>Safety</td>
<td>Endotoxin</td>
<td>Safety</td>
</tr>
</tbody>
</table>
Platform ≠ No Changes Throughout Development
Process 1 to Process 2: Case Study for Late-Stage Projects

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2500-L Scale</td>
<td>12000-L Scale</td>
<td>Commercial scale</td>
<td>Primary structure, PTM, isoforms, purity, stability</td>
</tr>
<tr>
<td>MCB</td>
<td>MCB</td>
<td>WCB</td>
<td>Introduce working cell bank</td>
<td>Primary structure, PTM, isoforms, purity, stability</td>
</tr>
<tr>
<td>High Seeding Density</td>
<td>Increased Titer Process</td>
<td>Increased Titer Process</td>
<td>Increase process titer; nutrient delivery based on process understanding, increased amounts of nutrients delivered to sustain productivity</td>
<td>Primary structure, PTM, isoforms, purity, stability</td>
</tr>
<tr>
<td>Production Process</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein A Chromatograph y</td>
<td>Protein A Chromatography – new resin</td>
<td>Capture mechanism does not change; improved capability due to resin properties</td>
<td>Purity/impurities</td>
<td></td>
</tr>
<tr>
<td>1X conc.</td>
<td>1.5X conc.</td>
<td></td>
<td>DS concentration increase to manage commercial scale DS volumes (formulation remains constant between supplies)</td>
<td>Higher order structure</td>
</tr>
<tr>
<td>EVA bags</td>
<td>Stainless Steel Cryovessel</td>
<td>Batch volume, scale appropriate, improve logistics</td>
<td>Stability</td>
<td></td>
</tr>
</tbody>
</table>
Comparability Expectations and Strategy

- Comparable does not mean “identical” - ICH Q5E
  “The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change products are identical; but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety and efficacy of the drug product.”

- Process improvements are likely to impact the product

- Risk-based strategy to ensure no adverse effects on product quality, safety or efficacy
  - Focus on changes to quality attributes (critical or non-critical)
  - Understand the magnitude of the process changes
  - Leverage platform manufacturing experience with the product
  - Rely on analytical capacity to detect significant product quality changes
  - Build on overall clinical experience – the likelihood of impact on safety and efficacy
Comparability Toolkit

➢ **Release methods**
- measure quality attributes that define identity, purity, potency, safety
- compare batch release data pre- and post-change, potential statistical analysis
- if assay has changed may repeat side-by-side analysis of comparability samples

➢ **Heightened characterization methods**
- similar to methods used for reference material characterization (ICH Q6B) with some exceptions/additions
- assess potential changes based on process changes
- in-process testing may be used to compare process changes
- typically 3 batches from each process, tested side-by-side

➢ **Stability studies**
- accelerated stability studies may be used to establish degradation profiles and provide additional direct comparison of pre- and post-change product
- if no accelerated stability data available, side-by-side forced degradation studies: thermal stability at accelerated temperatures and photo-degradation
Comparability Assessment – Acceptance Criteria

- **Release methods**
  - assess quantitative assays results for Process 2 against both pre-established Process 2 specification and Process 1 historical ranges

- **Heightened characterization methods**
  - primary structure, post-translational modifications, higher order structure results will be assessed qualitatively in a side-by-side comparison

- **Stability studies**
  - quantitative assays results for Process 2 will be assessed against historical ranges of Process 1 for accelerated stability studies, if available (n>=4)
  - if side-by-side forced degradation study is performed, compare ranges for Process 2 and Process 1
  - assess qualitatively heightened characterization data in both cases
Comparability; Late Stage Change in the Host Cell
Significant Difference in Glycan Profile Observed

Glycan profile of IgG2 mAb

NS0

CHO
Comparability Risk Assessment Performed

- Both processes have same major N-linked glycans;

- Some difference in the abundance of major and minor species;

- CHO derived glycans are less complex and more aligned with those in human mAbs:
  - lower levels of Man5;
  - absence of Gal-α(1-3)-Gal (alpha-Gal) species;
  - absence of N-glycolyl neuraminic acid (NeuGc) moieties;

- Slight variations in the relative abundance of major glycans and elimination of the minor species is not expected to have any impact to the clinical safety

- Composition of the Fc N-glycan is not essential for MOA of the IgG₂ subclass of antibodies - impact on clinical efficacy is not expected

- Literature suggests a possible impact on PK – non-clinical PK study to mitigate the risk was recommended

- Risk to safety/efficacy assessed as low
Platform Strategies Reduce mAb Development Timelines

- Platform strategies reduce mAb cycle times and cost (FTEs and $$)
- Allows companies to take “more shots on goal”
- More shots on goal should lead to more drugs to patients!

*The patient is waiting for us to put more years in their life and more life in their years!*

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<table>
<thead>
<tr>
<th>Year</th>
<th>Months</th>
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<tbody>
<tr>
<td>2004/2005</td>
<td>15.0</td>
</tr>
<tr>
<td>2006</td>
<td>14.6</td>
</tr>
<tr>
<td>2007</td>
<td>10.9</td>
</tr>
<tr>
<td>2008</td>
<td>9.6</td>
</tr>
<tr>
<td>2009</td>
<td>8.7</td>
</tr>
<tr>
<td>2010</td>
<td>5.2</td>
</tr>
</tbody>
</table>

- Platform Processes
- Platform Analytics
- State-of-the-Art Characterization Tools
- Platform Specs
- Method lifecycle management
Summary

- Structure of monoclonal antibodies make them amenable to platform development approaches
- Pfizer’s vast mAb development experience has enabled them to institute a platform manufacturing process, platform formulation, and platform analytical/characterization toolkit
- Suitability of platform methods must be assessed for each new mAb
- Platform specifications leveraged for each mAb project and modified as needed
- State of the art characterization techniques de-risk platform approach and facilitate robust development strategies with high probability of technical and regulatory success
- Strategy evolves with lessons learned on each mAb project
- Although structural homology of mAbs facilitates execution of platform strategies, strategies do change throughout development and thus comparability studies are often required
- Platform strategies result in reduced development cycle times and cost (FTEs and $$)
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