Biopharmaceuticals - Current FDA & EMAs Regulations on glycan analysis

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Hope and Risk

Arthritis drugs offer fresh hope at a price

NEWS

This Week

DURAL TRIALS

Violent Reaction to Monoclonal Antibody Therapy Remains a Mystery

CAMBRIDGE, UK—It took only minutes to see that something had gone seriously wrong. On 13 March, in a busy location in central Cambridge, two men collapsed and passed out, one became blazingly hot, and even all of them became unconscious. A rescuer, who is a fellow patient person, had noted the strange sensations of the two patients. This situation was to participate in the trial according to the plan. The first seven patients, all with different symptoms, had a large number of side effects: multiple sclerosis and rheumatoid arthritis. They were given a single, monoclonal antibody TGN1412 designed by Tel-Genus in Whitby, Canada, and manufactured by Genzyme. Initial results from the trial suggest there is no evidence of biological activity of TGN1412 and no evidence of any significant long-term effects. However, the results from one patient suggest that TGN1412 may have caused a severe allergic reaction, leading to a serious adverse event. The patient had been given TGN1412 as part of a trial, and after receiving the drug, he developed a severe allergic reaction, requiring emergency medical treatment. He was taken to the hospital, where he was treated for a severe allergic reaction. The patient was later discharged, and it is unclear whether he will be able to continue with the trial.

MEGA, Tel-Genus, and the company that managed the trial, Inail, have both issued statements. However, it remains unclear whether the patient will be able to continue with the trial. It is possible that the patient will be able to continue with the trial if the company that managed the trial is able to demonstrate that the patient was not exposed to any risk.

MEGA has stated that the patient was not exposed to any risk, and the company that managed the trial has stated that the patient was not exposed to any risk. However, it remains unclear whether the patient will be able to continue with the trial. It is possible that the patient will be able to continue with the trial if the company that managed the trial is able to demonstrate that the patient was not exposed to any risk.
The total number of genes does not define complexity
Post translational modifications explain the complexity of protein function

- DNA
- mRNA & Protein translation machinery
- Glycosyltransferases
- Glycosylation
- Oligosaccharides
- Proteins
- Glycoproteins

Role of glycosylation:
- Glyco-biomarkers
- Molecular trafficking
- Cell-cell adhesion
- Immune modulation
- Tumour growth
- Metastasis

Level of complexity

- (~ 20-25,000 genes) Genomics
- (~ 100,000 variants) Transcriptomics
- (~ 1,000,000 variants) Proteomics
- (~ 10,000,000 variants) Glycomics
Glycan Biosynthesis is a complex process

- Complex process No genetic code for glycosylation
- Glycan synthesis occurs in ER and Golgi
- Main types are N linked and O Linked glycans
- All proteins need not go thru the whole pathway makes the glycans highly heterogeneous
- Macro heterogeneity
- Micro heterogeniety
- Size of the glycans can vary
- Glycan are involved in protein function in terms of binding, safety, Efficacy, immune response, half life,
EPO glycosylation

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Glycan micro heterogeneity affects protein function

Desialylation of IVIg abrogates anti-inflammatory properties in K/N mice

Sialylation increases the half life of protein

Involved with placental transport of IgG
IgG galactosylation increased in pregnant women

IgG G0 interacts with MBL to activate complement

Loss of core α(1,6) fucose on IgG results in enhanced ADCC activity

Highly mannosylated proteins can be cleared out the system within minutes by mannose binding lectin
Glycosylation Functions:
Risks and Regulatory Concerns

- Mediates biological activity
- Glycans impact safety and efficacy
  - Correct and consistent structure of the glycans
  - Obtaining the desired medical effect
  - Avoid adverse immunological reaction
- Alteration in glycans may eliminate or alter activity
- Immune response triggered by unrecognized glycans
- Consistent glycan distribution indicates process stability


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Biopharmaceuticals

- $163 billion dollar market
- 20% are biopharmaceuticals
- 8% Annual Growth
- 50% of top drugs are biomolecules
- Was 28% in 2008
- Biopharmaceuticals are more complex in structures
The Biologics Market

Blood Proteins
Hormones
Growth Factors
Cytokines
Vaccines
Monoclonal Antibodies
Bone Proteins
Other

190 EMA/FDA Approved

Walsh (2010). Nature Biotech; 28(9):917-924
127 are Glycoproteins (>66%)

Walsh (2010). *Nature Biotech*; 28(9):917-924
Biopharma Attempts to Replicate Human Glycosylation


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First Plant made antibody

First plant-made biologic approved

Jeffrey L Fox

Published online 07 June 2012
Negative Attributes to Mammalian Cell Culture Glycosylation

50% of non-allergic blood donors contain antibodies against β(1,2)-xylose and α(1,3)-core fucose

N-glycolyNeuraminic acid is an oncofetal antigen in humans

Presence of gal-α(1,3)-gal can induce anaphylaxis
The concept promotes industry's **understanding** of the product and manufacturing process starting with product development, basically building quality in, not testing it. Under the concept of QbD, when designing and developing a product, a company needs to define desired product performance and identify CQAs.
**Bioprocessing Conditions Can Influence Glycosylation**

**Cell Line**
Critical in affecting glycosylation
(Raju et al. 2000)

**Dissolved O₂**
Variable effect, cell line specific
(Restelli et al. 2006)

**Ammonia**
High concns affect terminal glycosylation
(Yang and Butler 2000, 2002)

**Temperature**
Low temps (30°C) decrease sialylation
(Trummer et al. 2006)

**pH**
Galactosylation, sialylation microheterogeneity vary
(Muthing et al. 2003)

**Manufacturing Mode**
Perfusion increases sialylation over fed-batch
(Lipscomb et al. 2005)
Process analytical technology (PAT) has been defined by the United States Food and Drug Administration (FDA) as a mechanism to design, analyze, and control pharmaceutical manufacturing processes through the measurement of Critical Process Parameters (CPP) which affect Critical Quality Attributes (CQA).

It is absolutely essential to tightly control the levels of glycan critical quality attributes during the manufacturing process. Eg your Desired glycoforms that is involved in activitating ADCC Maintain the levels of immunogenenic epitopes Mannose containing glycans and so on
IN brief

FDA balks at Myozyme scale-up

Genzyme ran into a snag in April when the US Food and Drug Administration (FDA) rejected its application to produce Myozyme (alpha-glucosidase alfa, rhGAA) in its 2,000-liter-scale facility under the same approval authorization given for its 160-liter-scale plant. The FDA says the carbohydrate structure of the products manufactured at each scale differs and thus the 2,000-liter product requires a new biologic license application. Myozyme was

Myozyme raises awkward questions for would-be biogenerics manufacturers.

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The carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), the glycosylation site(s) and occupancy should be analysed.

Typically, monoclonal antibodies have one N-glycosylation site on each heavy chain located in the Fc region. The light chain is usually not glycosylated. However, additional glycosylation site(s) in the heavy chains may occur, and thus their presence or absence should be confirmed. Glycan structures should be characterised, and particular attention should be paid to their degree of mannosylation, galactosylation, fucosylation and sialylation. The distribution of the main glycan structures present (often G0, G1 and G2) should be determined.

Higher-order structure of the monoclonal antibody should be characterised by appropriate physicochemical methodologies.
FDA Drug application pathway

Developmental discussion

IND

BLA
Hierarchy of Protein Structure

**Primary structure**

- Lys
- Lys
- Gly
- Gly

**Secondary structure**

- α Helix

**Tertiary structure**

- Polypeptide chain

**Quaternary structure**

- Assembled subunits

All need to be evaluated as part of analytical similarity studies
Protein Heterogeneity

- Amino Acid Substitution
- N- and C-terminal mods
- Mismatched S-S bonds
- Folding
- Truncation
- Aggregation
- Multimer Dissociation
- Denaturation
- Acetylation
- Fatty acylation
- Deamidation
- Oxidation

- Carbamylation
- Carboxylation
- Formylation
- $\gamma$-Carboxyglutamylolation
- O-linked Glycosylation
- N-linked Glycosylation
- Methylation
- Phosphorylation
- Sulphation
- PEGylation
Antibody Glycans

Gomord et al. Plant Biotechnology Journal 2010
Analytical Tools to Evaluate Proteins

- Amino acid sequence and modifications:
  - MS, peptide mapping, chromatographic separations
- Folding:
  - S-S bonding, calorimetry, HDX and ion mobility MS, NMR, dyes, circular dichroism, Fourier transform spectroscopy, fluorescence
- Subunit interactions:
  - Chromatography, ion mobility MS
- Heterogeneity of size, aggregates, charge, hydrophobicity:
  - Chromatography resins; gel & capillary electrophoresis, light scatter, IM-MS, Analytical ultracentrifugation, size-exclusion chromatography, field flow fractionation, light scatter, microscopy
- Glycosylation
  - Anion exchange, enzymatic digestion, peptide mapping, CE, MS
- Bioactivity
  - Cellular and animal bioassays; ligand & receptor binding (ELISA, surface plasmon resonance), signal transduction
- Impurities
  - Proteomics, immunoassays, metal & solvents analysis
Definition of Biosimilar/Biosimilarity in BPCI Act

*Biosimilar* or *biosimilarity* is defined in Section 351 of the PHS Act to mean that “the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components,” and that “there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product”.

Section 7002(b)(2) of the Affordable Care Act, amending section 351(i) of the PHS Act.
Highly Similar Analytical and PK/PD Data = Lower Risk of Clinical Differences

Two approaches to achieve biosimilarity

- Additional Clinical Studies
- Clin Pharm
- Nonclinical
- Analytical

351(k) package

Additional Clinical Studies

Clin Pharm

Nonclinical

Analytical
Fingerprinting

• It may be useful to compare products using a meaningful fingerprint-like analysis algorithm

  – that covers a large number of additional product attributes and their combinations with high sensitivity using orthogonal methods.

• Advances in manufacturing science and Quality-by-Design approaches may allow a better match to a reference product’s fingerprint.

• May allow a more selective and targeted approach to subsequent animal and/or clinical studies.
Fingerprinting

Sequence & Modifications
Higher Order Structure
Bioactivity
Glycoforms
Impurity Profile

Micro & Macro Heterogeneity

Keith Webber, FDA
Preferred Biosimilar Product Quality Development Process

Development Decision

Biosimilar Initial Advisory Meeting

IND Enabling

Analytical and functional similarity studies
Qualified/validated release and stability assays

Initial Clinical Studies

Continuous characterization
Specification setting
Final Ms scale
Stability
Viral Clearance

Additional Clinical Studies

Final analytical and functional similarity studies
Specification setting
Stability

BPD Type 1/2/3

BPD Type 4

BLA

Developmental Research

Purchase reference product lots
Analyze reference product lots
Develop biosimilar construct and cell line
Manufacturing process development

In depth characterization assay development
Preliminary analytical/functional similarity studies
Formulation studies
Development Framework:
Comparative Analytical Characterization Continuum

- Cannot be biosimilar

- Similar
  - Needs additional information to determine if highly similar (e.g., additional analytical data, or other studies to determine if minor differences are “clinically inactive components”)

- Highly similar
  - Permits a selective and targeted approach to determine if biosimilar

- Highly similar with fingerprint-like similarity
  - Permits a more selective and targeted approach to determine if biosimilar
Summary

- Biopharmaceuticals are complex molecules
- Glycan synthesis is a complex process
- Glycosylation plays a critical role in protein function
- Glycan complexity increases due to its micro and macro heterogeneity
- Glycans are involved in safety and efficacy of the protein
- Companies are genetically engg several expression systems to produce proteins with more human like glycosylation
- Glycosylation is an important aspect of QbD
- Glycosylation important in PAT
- Companies are required to do indepth Glycosylation analysis before regulatory submission