Case Study

Level 2: Regulatory Scientific Concepts

Introduction

Biological products (biologics) have been used as therapies in the United States for several decades. They also represent a growing proportion of treatment options and costs in the U.S. health care market. Biologics treat a number of chronic illnesses like cancer, rheumatoid arthritis, and diabetes. In this case study, you will learn about FDA's Comparative Analytical Assessment and use of Quality Attributes that can help characterize a proposed biosimilar product and determine whether it has any clinically meaningful differences from the reference product.

Topics

- Terms related to biological products
- Comparative Analytical Assessments and how they may demonstrate safety and efficacy
- Quality Attributes and how they are analyzed

As You Read...

How do manufacturers and the FDA know which attributes are most important to support a demonstration of biosimilarity?

What are post-translational modifications?

Why are post-translational modifications important to consider when assessing a product's biosimilarity to its reference product?

What types of physicochemical and functional characteristics are assessed and reviewed for biosimilar product approval?

Where can you find more details on FDA-approved biological products?

Where can you find product labeling and review information for reference products (i.e., originator biologics), biosimilar, and interchangeable biosimilar products?

Comparative Analytical Assessment: Data Supporting Biosimilarity

A pharmacist-in-training gains knowledge about the evaluation of biosimilar products during her clinical rotation

Introduction

Natalie, a pharmacist-in-training, recently finished an experiential rotation that provided insights into drug development and regulation. As part of this rotation, Natalie learned about how the FDA evaluates biosimilar and interchangeable biosimilar biological products. Now completing her clinical rotation, Natalie encounters a patient diagnosed with rheumatoid arthritis. This prompts an interest in tumor necrosis factor-alpha (TNFα) inhibitors, which she knows are a group of recombinant monoclonal antibody products commonly used to treat rheumatoid arthritis. Natalie has recently seen announcements about biosimilar products for TNFα blockers and is interested in learning more about these in addition to their approved reference products. Familiar with the FDA's Purple Book Database of Licensed Biological Products, Natalie accesses the resource to search for information about available adalimumab products as she knows her patient is prescribed a biosimilar product to Humira (adalimumab). While looking at the information, she also discovers that there are several adalimumab biosimilars approved as an interchangeable biosimilar product. Natalie wants to learn more about the information the FDA evaluated to approve this medication as an interchangeable biosimilar product. She remembers from one of her courses that the Drugs@FDA online database includes the FDA review information and other documents for many approved drug and biological products that she can review.

Natalie knows from her training that therapeutic proteins like TNFα inhibitors (as well as other types of biological products) are distinct from small molecule drugs in that they are more complicated to manufacture and analytically characterize. Because these proteins are usually manufactured from living organisms (e.g., microorganisms, animal cells), there is inherent variation introduced by the manufacturing process, such as post-translational modifications. As she reads through the resources available for adalimumab-wxyz* on Drugs@FDA, Natalie encounters the comparative analytical assessment in the product quality review document. It includes a long list of molecular properties, also referred to as "quality attributes," that were compared between adalimumab-wxyz and Humira (adalimumab). After looking over the data, Natalie noted a few questions to ask her preceptor, Dr. Xan, who has prior experience working in biological product development.

Post-Translational Modi ications

Some quality attributes are a product of the manufacturing process. For example, recombinant DNA technology is used to program cells to make many copies of a therapeutic protein with the same amino acid sequence, and very small changes can occur to one or more amino acids through a process called post-translational modification. During protein production, cells may add sugar molecules (glycans) to specific amino acids of the protein. This type of post-translational modification is called glycosylation. Because these glycan structures have been shown to affect binding between an antibody's Fc region and its cognate Fc receptor, among other potential effects, it is important to understand the nature of and control for the variation in these modifications during the manufacturing process.

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Quality Atrributes

During office hours, Natalie explains to Dr. Xan, "I was exploring the review documents for adalimumab-wxyz and other adalimumab products on Drugs@FDA. There are a lot of quality attributes listed, but I'm not completely clear on what those are or what they tell us about the product."

Dr. Xan nods saying, "A product's molecular properties, also known as quality attributes, are physical, chemical, or biological characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality. In biosimilar development, the most important attributes that research has shown to impact safety or effectiveness are core components of determining whether a proposed product is biosimilar to its reference product—and therefore, we can feel confident prescribing them to our patients."

Taking a moment, Natalie thinks through the list of quality attributes she noted for adalimumab-wxyz, remembering that they included multiple physicochemical and functional characteristics of the product. She wondered how the biosimilar manufacturer knew what specific properties of adalimumab-wxyz were important attributes they needed to assess. Natalie asks Dr. Xan, "How do manufacturers know which quality attributes are important to measure for demonstrating biosimilarity?"

Dr. Xan explains, "Quality attributes compared between a proposed biosimilar and its reference product are generally associated with the characteristics that impact a product's structure and function. Manufacturers use state-of-the-art technology to compare these kinds of characteristics. There are also attributes that influence the quality of a protein-based product (such as its sterility, purity, and stability), which are not evaluated by direct comparison to the reference product, but are evaluated to determine whether the proposed biosimilar product meets FDA's rigorous quality standards for sterility, purity, and stability for approval.

Comparative Assessment of Product Quality Attributes

Nodding, Natalie continued, "That makes sense, but it seems like post-translational modifications and other sources of biological variability could lead to a list of attributes too long to keep track of and test. Using adalimumab as the example I've been thinking about, how are the most important quality attributes to characterize during development determined?"

Dr. Xan explains, "The FDA publishes guidance documents, including one on comparative analytical assessments for manufacturers and stakeholders that provide them with recommendations about best practices. The specific quality attributes evaluated to support a demonstration of biosimilarity depend on a few things such as the structure and function of the therapeutic protein, its mechanisms of action, and the extent to which the mechanisms are known, among other factors. Using this information, the molecular properties (**Figure 1**) are evaluated according to their risk and degree of uncertainty for impacting clinical performance, including biological activity, pharmacokinetics and pharmacodynamics (PK/PD), safety, efficacy, or immunogenicity." As he speaks, Dr. Xan looks up the product quality review and points to the extensive table of attributes evaluated for adalimumab-wxyz (**Table 1**). "For example, the therapeutic activity of TNFα inhibitor antibody products used to treat chronic conditions like rheumatoid arthritis is mediated via Fc-mediated effector function, complement-dependent cytotoxicity (CDC), and antibody-dependent cell-mediated toxicity (ADCC), which are known to be impacted by N-linked glycosylation. Therefore, manufacturers conduct a thorough assessment of the glycan profile (e.g., fucose, mannose) using state-of-the-art oligosaccharide profiling methods to detect potential differences in glycosylation that can impact the product's biological activity.

Following up, Natalie asks, "What happens if there is a difference in one or more of the attributes that are assessed? Does that mean the new product is not biosimilar to the reference product?"

Dr. Xan responds, "Minor differences in quality attributes between a biosimilar and its reference product does not necessarily mean the product doesn't meet the standards for a biosimilar. Depending on the attribute, an identified difference may require additional testing so that the manufacturer can understand and appropriately address any impact for the FDA's review and decision making. Once a difference has

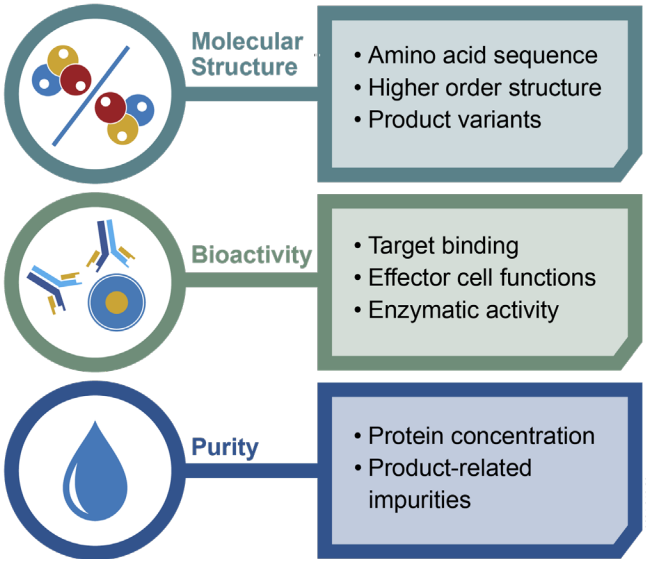

Figure 1: Examples of Attributes Measured for in the Comparative Analytical Assessment

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been identified, the FDA examines it carefully to determine whether it is of concern or not. The FDA then works with the manufacturer to determine what additional information or tests can provide the data needed to determine whether the difference may have implications for the safety and effectiveness."

Dr. Xan continued, "Take the example of a monoclonal antibody and the glycan fucose. The proportion of antibody molecules that don't have fucose at the glycosylation site can impact potency as measured by ADCC activity. Therefore, if differences are detected in levels of afucosylation (absence of fucose), it is important to evaluate potential downstream differences, which can be assessed by an in vitro cytotoxicity assay."

"Wow. That's a lot to consider, but I'm glad to hear that there is a well-established approach for analyzing the attributes, resolving issues, and ultimately demonstrating biosimilarity." Natalie continues, "I have one more question. After all the data have been collected and provided to FDA by manufacturers, how does FDA make the final determination about biosimilarity?"

Dr. Xan responds, "When manufacturers begin collecting data, they identify many quality attributes, from which a critical subset are selected for inclusion in the comparative analytical assessment. Selection of quality attributes depends on the reference product and its known mechanisms of action, as well as an attribute's risk for impacting clinical performance in terms of safety and efficacy. The data package submitted by the manufacturer includes an extensive analytical comparison to show that the proposed biosimilar product and the reference product are highly similar in structure and function and that there are no clinically meaningful differences. Since there is no one 'pivotal' study that demonstrates biosimilarity and given the number of tests and attributes assessed, FDA uses this totality-of-the-evidence approach to evaluate whether a biological product is biosimilar to its U.S.-licensed reference product. I would like to also note that even though this product is an interchangeable adalimumab product, the same analytical methods for determining biosimilarity apply. An interchangeable product isn't more similar, safer, or more efficacious than a biosimilar that is not approved as interchangeable," emphasized Dr. Xan.

"Thanks for your time, Dr. Xan. This has really helped me understand how to apply the concepts I learned in class the last few years!"

Terms

Reference Product
The FDA-approved biological product against which a proposed biosimilar product is evaluated.

Biosimilar Product
A biological product that is highly similar to and has no clinically meaningful differences as compared to the reference product.

Interchangeable Biosimilar Product
A biosimilar product that may be substituted at the pharmacy for the reference product without the intervention of the prescriber, subject to state laws.

351(k) Biologics Licensure Application
The abbreviated approval pathway that allows manufacturers to seek approval of biosimilar and interchangeable products, including insulin products.

Product-Related Impurities
Molecular variants with properties different from those of the desired product formed during manufacture and storage.

Comparative Analytical Assessment
The data analysis from comprehensive, robust comparative physicochemical and functional studies (biological assays, binding assays, and enzyme kinetics) conducted by the manufacturer to evaluate and compare quality attributes identified in the proposed biosimilar product and the reference product..

Quality Attributes
The physical, chemical, biological, or microbiological properties/characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

Post-Translational Modification
The processes that change the properties of a protein by proteolytic cleavage and adding a modifying group, such as acetyl, phosphoryl, glycosyl and methyl, to one or more amino acids. These modifications regulate protein folding, interaction, and signaling.

Totality of the Evidence
The approach used by FDA to evaluate the applicant's structural, functional, and clinical data collectively to determine whether no clinically meaningful differences exist in a proposed biosimilar product's quality, safety, or efficacy as compared with the reference product.

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Table 1: Common Product Characteristics, Quality Attributes, and Analytical Methods for Measurement

Physicochemical and Functional Characteristics**	Quality Attribute (Molecular Property)	Analytical Methods
Primary Structure	Amino acid sequence	Peptide mapping by LC-MS
	N- and C-terminal sequencing	Edman degradation Peptide mapping by LC-MS
Higher Order Structure	Secondary structure	Far-UV CD FTIR
	Tertiary structure	Near-UV CD Intrinsic fluorescence
	Disulfide bonds	Non-reduced peptide mapping
	Free thiol analysis	Ellman's assay
Post-translational Modifications	Deamidation	Peptide mapping by LC-MS
	Oxidation	Peptide mapping by LC-MS
	N-terminal variants	Peptide mapping by LC-MS
	C-terminal variants	Peptide mapping by LC-MS IEC-HPLC
Glycosylation	Afucosylation	HILIC
	High mannose	HILIC
	Sialyation	HILIC
	Galactosylation	HILIC
Size Variants	Monomer/Intact IgG	Non-reduced CE-SDS SEC AUC
	Light chain + heavy chain	Reduced CE-SDS
	Non-glycosylated heavy chain	Reduced CE-SDS
	Fragments/LMW variants	SEC AUC CE-SDS
	Aggregates/HMW variants	SEC AUC
Charge Variants	Isoelectric point (pI)	icIEF
	Main peak	IEC
	Acidic species	IEC
	Basic species	IEC
Biological Activity	Target and Fc receptor binding (e.g., TNFα, FcRn, FcγRs)	SPR ELISA
	Potency	Apoptosis inhibition assay NF-kβ reporter gene assay HUVEC anti-proliferation assay
	ADCC activity	NK cells-mediated cytotoxicity assay
	CDC activity and C1q binding	Cytotoxicity assay ELISA
	Regulatory macrophage induction/T-cell antiproliferation	Mixed lymphocyte reaction (MLR)
	Reverse signaling	Apoptosis induction assay
Drug Product Attributes	Protein concentration	UV280

Figure 2: Please Visit: https://purplebooksearch.fda.gov

Biosimilar Resources

FDA Pages

- Biosimilar Materials
- Provider Materials
- Patient Materials
- Purple Book
- Drugs@FDA (Drug Information)
- Search Page for FDA Guidances
- Advisory Committee Materials for Biosimilars
- Biosimilar Approval Process Information

Additional Resources

Slide Decks

- Foundational Concepts
- Regulatory and Scientific Concepts

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- What Is a Biosimilar?
- Biosimilars in Patient Care
- Interchangeable Biosimilars
- Insulins/Interchangeable
- Comparative Analytical Assessment

Info Sheets

- Biological Products, Biosimilar Products, and Interchangeable Biosimilar Products
- Generics and Biosimilars
- Manufacturing and Variation
- Biosimilar Regulatory Approval Pathway
- Variation in Biological Products
- Comparative Clinical Studies
- Prescribing Biosimilar and Interchangeable Biosimilar Products
- Purple Book
- Comparative Analytical Assessment
- Quality Attributes
- Labeling/Package Insert
- Insulins/Interchangeable

Explanatory Videos

- Biosimilars: Manufacturing and Inherent Variation
- Biosimilars: Approval Process
- Biosimilars: Critical Quality Attributes
- Biosimilars: Interchangeability
- Purple Book
- Procedural Purple Book
- Totality of the Evidence
- Comparative Analytical Assessment

Discussion Questions

- Foundational Concepts
- Regulatory and Scientific Concepts
- Exercises (Provided in the Resource Guide for Teaching Faculty)