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Importance of manufacturing consistency of the glycosylated monoclonal antibody adalimumab (Humira®) and potential impact on the clinical use of biosimilars

Professor Paul Declerck[1], PhD; Paul W Tebbey[2], PhD

The clinical performance of biological therapies is affected by their manufacturing processes. The advent of biosimilars increases the need to understand the clinical impact of potential differences versus originator products. This paper reviews important recently published technical data on adalimumab consistency and manufacturing experience from a clinical perspective, including the implications for immunogenicity when there are changes to a tightly controlled manufacturing process that may lead to variations in the epitopes of biological therapies.

Keywords: Adalimumab, biosimilar pharmaceuticals, consistency, glycosylation, immunogenicity, manufacturing

Introduction

Biological therapeutics are highly sensitive to formation of structural variants during manufacturing processes. These structural variants can affect clinical performance. Here, we review important recently published technical data on adalimumab consistency and manufacturing experience from a clinical perspective.

Adalimumab

Adalimumab (Humira®, AbbVie, Inc) is a recombinant human immunoglobulin (Ig) G1 anti-tumour necrosis factor-α (TNF-α) monoclonal antibody originally approved for the treatment of rheumatoid arthritis in the US in 2002, and subsequently approved for the management of Crohn's disease, ulcerative colitis, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, and hidradenitis suppurativa [1]. Since its approval in the US, many other countries have approved its use for multiple indications [2, 3].

Complex nature of biologicals

As is the case for many biologicals, adalimumab is a large glycoprotein that is a heterogenous mixture of structural isoforms [4-6]. Glycosylation, the addition of sugar residues by the host organism during manufacturing process, is a major post-translational protein modification resulting in different structural isoforms [7, 8]. In addition to glycosylation, enzymatic cleavage of C-terminal lysines contributes to the heterogeneity of monoclonal antibodies [9]. The formation and nature of these structural variants are highly sensitive to manufacturing processes, and changes in any of the manufacturing steps may lead to differences in the specific isoforms that are present.

Differences in glycosylation profile and cleavage of C-terminal lysines can affect the tertiary (i.e. the overall 3-dimensional shape of the protein) and quaternary structure (i.e. interaction of protein subunits) of a biological agent [10]. Thus, it is important to characterize the key quality attributes, e.g. physicochemical and structural features, of a biological drug substance and constantly monitor these during production, using robust analytical methods to help ensure product consistency, including the pattern and percentage of each structural isoform.

Consistency of adalimumab manufacturing

A recent product quality analysis evaluated 544 total batches of adalimumab manufactured between 2000 and 2013 [6]. Molecular charge, as measured by the presence of acidic species and C-terminal lysine variants, and glycosylation pattern were the key properties used to demonstrate the comparability and consistency of the production process, see Table 1, [6]. The quantitation of the sum and overall lysine variant profile demonstrated consistency between the batches over the course of 13 years [6]. Additionally, a high degree of consistency for the sum of lysine variants was observed between five different bioreactor sizes used for production [6]. Comparison of the identity and quantity of adalimumab oligosaccharides profile from 381 different batches indicated a high level of consistency between the batches over time and throughout the production scale changes, see Table 1 [6]. Furthermore, the consistency of the charged species and glycosylation patterns of adalimumab was supported by biological data, demonstrating that its interaction with and intrinsic binding affinity for soluble TNF-α ligand remained stable during this period [6].

Importance of consistency of adalimumab manufacturing

It has been clearly demonstrated that the manufacturing process for adalimumab produced a consistent product over an extended (> 10 years) period of time [6],

Author for correspondence: Professor Paul Declerck, PhD, Department of Pharmaceutical and Pharmaceutical Sciences, KU Leuven, University of Leuven, O & N II, 49 Herestraat, BE-3000 Leuven, Belgium

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even as necessary changes to the manufacturing processes and production scale were introduced. It is paramount for manufacturers of biological products to maintain tight control of the drug substance and its production to ensure effective and safe use in patients. In the case of adalimumab, the need for a high level of consistency and product quality may be magnified, in part, because of the diverse patient populations being treated, and the underlying etiologies of the different chronic immune-mediated inflammatory diseases for which adalimumab is indicated.

Variations in glycosylation pattern or other post-translational modifications can result in subtle changes in the conformation of a biological that can potentially alter its solubility, stability, efficacy, or immunogenicity [8, 10]. Studies have demonstrated that variations in glycosylation can lead to significant changes in the circulating levels of the biological subsequent to altered pharmacokinetics, and alter its distribution to specific tissues and organs [7]. For example, therapeutic IgGs containing high-mannose glycans in the Fc region are cleared more rapidly in humans than other glycan forms [11]. Furthermore, some residues, such as galactose-containing glycans, can induce conformational changes that expose portions of the molecule previously hidden from the immune system and possibly lead to an antigenic response [8, 12].

Table 1: Manufacturing consistency of Adalimumab

Attribute	Mean	SD	Relevance	References
Glycans				
Agalactosylfucosylated biantennary oligosaccharides (G0F + G0F-GlcNAc)	74.28	1.75	Glycoforms containing terminal N-acetylglucosamine residues may activate pattern recognition receptors, such as mannan binding lectin and mannose receptor. Afucosylation enhances affinity to FcγRIIIa, ADCC activity and *in vivo* efficacy.	[12, 20-22]
Galactosyl-containing glycans (G1F + G2F)	18.45	1.80	Galactosylation may affect IgG1 conformation, bioavailability and activity; highly galactosylated mAbs show increased *in vitro* C1q-binding and CDC activity, increased FcγRII and FcγRIIIa binding, and enhanced ADCC activity.	[12, 23-27]
Mannose-containing glycans (M5 + M6)	7.29	0.86	High mannose glycans may increase ADCC activity, stimulate enhanced clearance of mAbs, and elicit immunogenicity.	[11, 24, 28, 29]
Charge variants				
C-terminal lysines (sum of K0, K1, K2)	86.33	1.10	Alterations in acidic species show decreased binding affinity and potency, reduced half-life, and immunogenicity. C-terminal lysine processing may affect complement C1q binding and thus effector functions such as CDC.	[30-35]
Acidic species (AR1)	2.41	0.52		
Acidic species (AR2)	10.20	0.71		

Glycan mapping of adalimumab (Humira®, AbbVie, Inc) proceeded via assessment of oligosaccharides (G0F + G0F-GlcNAc; G1F + G2F; M5 + M6) using NP-HPLC to separate fluorescent-labelled glycan species. Adalimumab batches (n = 381) produced from 2001 to 2013 (scale ranges from 3,000 to 20,000 liters) were analysed. The relative amount of each glycan species was calculated as a per cent of total area from NP-HPLC chromatograms.
Charge variant profiles of adalimumab were analysed using WCX-HPLC on batches (n = 525) that derived from manufacturing scales ranging from 3,000 to 20,000 liters over the time period 2001–2013. The relative amounts of the acidic species (AR1 and AR2) and c-terminal lysine isoforms (K0, K1, K2) was calculated from the chromatograms as a per cent of total area.
ADCC: antibody-dependent cell-mediated cytotoxicity; AR: acidic region; C1: complement component 1; CDC: complement dependent cytotoxicity; FcγR: Fc gamma receptor; G0F: agalactosyl fucosylated biantennary glycan; G0F-GlcNAc: agalactosyl fucosylated biantennary glycan without a terminal N-acetyl glucosamine; G1F: galactosylated fucosylated biantennary glycan; G2F: di-galactosylated fucosylated biantennary glycan; Ig: immunoglobulin; K: lysine; M: mannose; mAb: monoclonal antibody; NP-HPLC: normal phase high-performance liquid chromatography; SD: standard deviation.

Immunogenicity is a primary area of safety for biologicals and is often carefully monitored [13]. Immunogenic response results in the development of non-neutralizing (sometimes termed *binding*) or neutralizing anti-drug antibodies against the biological product. Non-neutralizing antibodies bind biological molecules on sites unrelated to target binding; however, non-neutralizing antibodies can reduce drug bioavailability through increased clearance, indirectly decreasing efficacy [14]. Neutralizing antibodies, on the other hand, bind biologicals on sites that interfere with target binding, reducing or sometimes halting efficacy [14]. Immunogenic response can have a number of outcomes ranging from adverse events, such as hypersensitivity reactions, i.e. reactions to drug administration, to reduced serum levels and neutralized activity of the biological; in other cases, it has no apparent effect on efficacy or safety of the product [9].

The presence of anti-adalimumab antibodies after treatment varied between 3% and 28% among different disease states, see Table 2 [1, 2, 15-17]. Because the measured incidence of anti-drug and neutralizing antibodies is highly dependent on the assay type and because multiple factors (such as assay sensitivity and specificity, methodology, disease state, sample handling, and timing of sample collection) affect the results, comparison of the incidence of antibodies between studies or between different biologicals is misleading [14]. Nevertheless, presence of anti-adalimumab antibodies, even at moderate concentration, can impact patients' response to treatment. In studies of rheumatoid arthritis and Crohn's disease, anti-adalimumab antibodies were associated with lower adalimumab serum levels [16, 17]. Furthermore, a higher percentage of treatment non-responders had anti-adalimumab antibodies compared with treatment responders in one study [16]. Because adalimumab is used for the treatment of patients with chronic immune-mediated inflammatory diseases, it may be important to establish and maintain a long-term immunologic equilibrium in patients treated with adalimumab. By providing a consistent product with key attributes maintained within a narrow window of variability from batch to batch, the risk of potential changes in the

Table 2: Anti-adalimumab antibody response in different disease states [1, 2]

Disease state	Overall rate of anti-adalimumab antibodies*
Rheumatoid arthritis	5–6%†
Enthesitis-related arthritis	11%‡
Polyarticular juvenile idiopathic arthritis	16%§
Crohn's disease	3%
Paediatric Crohn's disease	3–10%
Ulcerative colitis	5–21%
Psoriasis	8–21%
Paediatric psoriasis	13%¶
Hidradenitis suppurativa	7–28%

*Patients with ankylosing spondylitis or with psoriatic arthritis have rates of anti-adalimumab antibodies comparable to rates in patients with rheumatoid arthritis (overall rates for ankylosing spondylitis, and rates with monotherapy for psoriatic arthritis).
†Approximately 5% of patients developed low-titer antibodies to adalimumab at least once during treatment, which were neutralizing *in vitro*; levels range from 1% in patients receiving concomitant immunotherapy to 12% in patients receiving adalimumab as monotherapy.
‡Ranges from 8% in patients receiving concomitant immunotherapy to 14% in patients receiving adalimumab as monotherapy.
§Ranges from 6% in patients receiving concomitant immunotherapy to 26% in patients receiving adalimumab as monotherapy.
¶Adalimumab received as monotherapy.

antigenicity of adalimumab over time is minimized.

Changes to a tightly controlled manufacturing process that may lead to variations in epitopes could disrupt the immunologically tolerated state of a patient to their initial therapeutic. Likewise, switching a stable patient to a related but different molecule, as would be the case when using a biosimilar of the initial therapeutic made by a different manufacturer, may increase antigenicity. Well-established mechanisms of tolerance include clonal deletion, receptor editing, clonal anergy, blockade of memory response, and competitive tolerance [18]. Overall, immune responses are unpredictable and immunological response to treatment with biological therapies, such as adalimumab, can vary greatly between individual patients and disease states. Furthermore, patients can develop anti-drug antibodies with clinical impact ranging from no effects to secondary loss of response. The immune response to adalimumab is a dynamic process in which anti-drug antibodies can develop and then disappear over time. Whether a stable patient who undergoes a non-medical switch between an initial therapeutic and its biosimilar (which would expose the immune system to distinct antigens) can maintain or subsequently regain tolerance is unknown. Appropriate investigation is recommended because of the paucity of available clinical data that pertain to the phenomenon of switching [19]. Importantly, data regarding the use of multiple biosimilar molecules in a single patient are also lacking.

Conclusions

The comparison of over 500 batches of adalimumab from 2000 to 2013 demonstrated that the key physicochemical and functional quality attributes of adalimumab have remained within a narrow range during this long time frame. Thus, patients who have been or are currently treated with adalimumab have received a consistent product during the course of their treatments, have developed immune tolerance to the antigens consistently delivered, and ultimately have achieved stability in terms of clinical response.

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Authors

Professor Paul Declerck, PhD
Department of Pharmaceutical and Pharmaceutical Sciences, KU Leuven, University of Leuven, O & N II, 49 Herestraat, BE-3000 Leuven, Belgium.

Paul W Tebbey, PhD
AbbVie, Inc, US Medical Affairs, Biotherapeutics, AP32-2, 1 N Waukegan Road, North Chicago, IL 60064, USA.

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