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First Turkish interactive workshop on regulation and approval of similar biotherapeutic products/biosimilars, 2–3 March 2016, Ankara, Turkey

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This paper discusses the findings and summary of items covered in the First Turkish interactive workshop on regulation and approval of similar biotherapeutic products/biosimilars, in which structure–function was highlighted as a key issue.

Keywords: Glycosylation, immunogenicity, potency, receptor binding, serum half-life, sialic acid

Introduction

This interactive workshop was held on 2–3 March 2016 at the Hacettepe University in Ankara, Turkey using an almost identical format to what was used in prior educational workshops as reported in *GaBI Journal*. For more details of methods and case presentations, see the published report of the First Latin American educational workshop on similar biotherapeutic products [1] and the First MENA educational workshop on regulation and approval of similar biotherapeutic products/biosimilars [2].

The workshop was organized in collaboration with the Faculty of Pharmacy, Hacettepe University. Presentations were made by both international speakers as well as local academic faculty, clinicians and Turkish regulators. After the formal presentations (as done in the prior two workshops referenced above), the audience was presented with data for two semi-fictional follow-on biotherapeutic products, one a recombinant human erythropoietin (EPO) and the other a monoclonal anti-TNF antibody. The audience was divided into groups; each of which was asked to discuss specific questions about the potential approval of one of the two proteins under the direction of two to three local, university faculty and regulatory experts. The list of speakers and the slides they presented and the slides used to summarize the quality and clinical trial performance of the two products, are all available on the *GaBI Journal* website (www.gabi-journal.net/about-gabi/educational-workshops).

Summaries of parallel working group discussions are presented below.

Group 1 and 2 Summary

Groups 1 and 2, who were assigned to evaluate the EPO product, chose to work together rather than separately. The specific questions they were asked and an edited simplified summary of their responses are presented in Table 1.

General summary information of Group 1 and 2 discussions is presented in Table 2.

Table 1: Case study – recombinant human erythropoietin (EPO)	
Group 1 and 2 moderators: Assistant Professor Dr Devrim Demir Dora, PharmD, PhD; Dr Aydan Eratalay, PharmD, PhD **Co-moderators**: Fikriye Handan Çelikel, MSc; Bilgen Beldüz, MSc	
Do the data of similar biological products (SBPs) candidate EPO qualify for biosimilarity with reference product from a quality perspective with respect to any potential impact on efficacy	
Do the observed differences in post-translational modifications have potential impact on *in vivo* potency and pharmacokinetics? Is it possible to predict the impact of two offsetting differences in glycosylation?	The pharmacokinetic profile of the product could be estimated since the serum half-life of the product is known. But for potency, some bioassay parameters, such as receptor binding studies, cell proliferation studies, should be evaluated. Glycosylation increases blood half-life but decreases receptor binding.
Are the non-clinical *in vitro* and *in vivo* studies sufficient to conclude that there would be similar potency in patients? Could additional non-clinical studies answer this question?	Since sialic acid content differences may increase potency values, as even **small** differences can affect receptor binding, potency values in patients could not be presumed.
The data of SBP candidate EPO qualify for biosimilarity with reference product from a quality perspective with respect to any potential impact on safety/immunogenicity	
Do the observed differences in microscopic (sub-visible) particle levels have the potential to impact immunogenicity? If so, what additional analytical characterization studies might inform this question?	Yes, they have potential impact on immunogenicity. Additional analytical characterization studies such as size exclusion are needed.
Are the non-clinical rodent studies adequate to show comparable immunogenicity?	No, they are not sufficient. For evaluation of clinical safety, at least 12 months of human immunogenicity data should be obtained.

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Table 1: Case study – recombinant human erythropoietin (EPO) (*Continued*)

How could 'residual uncertainty' concerning impact on potency be addressed in the preclinical and/or clinical studies?	
A. Impact on potency	
Would a greater than 20% difference in potency be relevant to similarity of efficacy for a therapy that is titrated at the individual patient level?	A 20% difference in potency is not an acceptable result; it should be no more than 8%.
If so, how could clinical comparability studies exclude the possibility of a 20% potency difference?	The number of the subject should be corrected for an 8% maximum difference or else extra clinical studies should be done.
B. Impact on immunogenicity	
It is difficult to address 'residual uncertainty' concerning impact on immunogenicity. This could be addressed in preclinical and/or clinical studies since low-titer, i.e. clinically benign, anti-EPO binding antibodies may be detected with about a 1% incidence in patients treated with epoetins. High-titer, neutralizing antibodies are very rare, and pure red cell aplasia (PRCA) has an incidence of < 1 per 10,000 patient-years.	
Given these observations, what questions about similarity with respect to immunogenicity can be answered in a reasonably sized pre-approval clinical study, i.e. with 100–300 patients treated with the candidate biosimilar?	100–300 patients may be enough but because of the low incidence (1 per 10,000 patient-years) expected, a statistical power analysis is required.
Can such a pre-approval study rule out a 10-fold increased risk of PRCA relative to background? A 100-fold increased risk?	We are not sure.
How could the risk of PRCA be addressed in a post-marketing risk management plan?	Haemoglobin and haematocrit levels should be followed at different time intervals.
Conclusion: It may be a biosimilar but some further data/studies are required.	

Discussion/Conclusion of Group 1 and 2

EPO is one of the important glycosylated therapeutic proteins. Glycosylation patterns have effects on immunogenicity and half-life of therapeutic proteins by influencing the active clearance of a protein. Glycosylation is dependent on the number of sialic acid residues attached to the protein molecules. Glycans and sialic acids have effects on receptor binding and serum half-life. Serum half-life of the therapeutic proteins increase when the number of sialic acid residues increases. Glycosylation increases blood half-life but decreases receptor binding.

After manufacturing changes such as manufacturing site and method, the influence of *in vitro* potency activity should be evaluated. As sialic acid content and lactosamine extension is increased, serum half-life of the product is extended but receptor-binding affinity is decreased. Although the pharmacokinetic profile of the product could be estimated since the serum half-life of the product is known; potency values in patients could not be presumed because of the small differences which have an effect on receptor binding. Some bioassay parameters such as receptor binding and cell proliferation studies should be evaluated for estimating potency values in patients. Sialic acid content differences may increase potency values. Thus, increasing the degree of sialylation and glycosylation decreases the renal clearance rate and can increase EPO *in vivo* activity.

Table 2: Case study – recombinant human erythropoietin (EPO): summary information

Group 1 and 2 moderators: Assistant Professor Dr Devrim Demir Dora, PharmD, PhD; Dr Aydan Eratalay, PharmD, PhD **Co-moderators:** Fikriye Handan Çelikel, MSc; Bilgen Beldüz, MSc
rHuEPO is a 165 amino acid protein with 1 O-linked and 3 N-linked carbohydrates. When an N-linked carbohydrate has up to 4 sialic acid residues, O-linked carbohydrate has up to 2 sialic acids each. Similar biological products (SBP) has got 14 sialic acids in total. But commercial product (reference standard) has got 9–14 sialic acids. Normal RBC Homeostasis is maintained by erythropoietin-stimulated erythropoiesis. rHuEPO stimulates erythropoiesis by activating EPO receptors. Glycans have effect on receptor binding and serum half-life. Serum half-life is 5–8 hours. Serum clearance is the primary determinant of *in vivo* activity of EPO. Serum clearance: while sialic acid content is getting closer to 14, receptor binding is decreasing and serum half-life is getting longer. Haematocrit: while sialic acid content is getting closer to 14, haematocrit is increasing. As they were not manufactured by SBP pathway, there is a different isoelectric pattern for 11 products. As two isoform patterns of a biosimilar product have same sialic acid content, but their biological activity is different. After manufacturing changes, influence of *in vitro* potency activity is evaluated. As sialic acid content and lactosamine extension is increased, serum half-life of the product is extended but receptor-binding affinity is decreased. In this case study, glycosylation and product-related impurities are affecting quality. Glycosylation can affect potency and bioavailability (efficacy) and potentially immunogenicity. Product-related impurities, such as aggregates, can impact immunogenicity with potentially life-threatening consequences. The potential clinical impact of these quality attributes (glycosylation and impurities) may be difficult to assess non-clinically.

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The structural characteristics of the therapeutic product such as glycosylation and product or process related impurities have effects on quality and immunogenicity of the protein product. Clinical immunogenicity is a key factor to determining safety and efficacy of biosimilars. It is important to note that only clinical studies are appropriate for detecting immunogenicity and for evaluation of clinical safety, at least 12 months of human immunogenicity data should be obtained.

Group 3, 4 and 5 Summary

The three groups who evaluated the IgG1 anti-TNF monoclonal antibody SBP candidate did so separately. The specific questions they were asked followed by the edited, simplified summaries of their individual group responses are presented in Table 3.

Group 4 included further summary information, see Table 4.

Discussion/Conclusion of Group 3, 4 and 5

Despite the large and growing number of products being approved and marketed worldwide as 'biosimilars', there continues to be a lack of consensus concerning the best practices for the evaluation, approval, use and post-marketing surveillance of follow-on biologicals. While there were areas of general agreement in the case evaluations done by the participants

Table 3: Case study – monoclonal antibody IgG 1

Group 3 Moderator: Professor Dr Sevda Şenel, MSc, PhD **Co-moderators:** Professor Dr Semra Şardaş; Gökçe Yildirim	**Group 4 Moderator:** Professor Dr R Neslihan Gürsoy, PhD **Co-moderators:** Dr Enes Karabulut, MD; Professor Dr Nefise Ozlen Şahin, PhD	**Group 5 Moderator:** Professor Dr Türkan Eldem, Dr Nat Sci **Co-moderators:** Dr İsmail Burak Bal; Professor Dr Nazan Bergişadi, PhD
Do the data of SBP candidate IgG1 qualify for biosimilarity with the reference product from quality perspective and why?		
No, because potency would be affected by the differences in charge profile (acidity) and deamidation and because the differences in aggregates and particulates will affect immunogenicity.	No, assuming the data are from the final product, the data are not sufficient to come to the conclusion that this product might be biosimilar; needs more structural analysis, functional analysis, non-clinical and clinical data for comparability. Quality is not by itself a determinant of biosimilarity.	No, the physicochemical properties are different. The difference in acidic profile, deamidation and dimerization will all have an impact on potency and the differences in aggregation and particulates will impact immunogenicity. There are important glycosylation pattern differences: more galactosylation (affects CDC), more fucolyslation (affects ADCC), more mannose (affects PK/clearance) and different range of sialylation (clearance may be different). Functional characteristics also differ; while TNF binding capacity is equal, the effector functions are not equal (probably as a result of the altered glycosylation profile), and both the Fc-gamma R3 binding and ADCC are reduced.
What steps would you recommend to remediate the differences?		
Steps should be taken to prevent aggregation and dimerization such as adjusting charge and attempts could be taken to modify the production process related to N-linked glycans in the mAb Fc domain, e.g. for selected glycan attribution: fucosylation, high mannose (ADDC and PK affected).	Extensive stability studies, e.g. accelerated, stress. Experimental data on formulation attributes.	a) Select a new and better expression system including a vector for improving glycosylation issues b) Change in upstream and downstream processes c) Prevention of dimerization by changing formulation design and quality controls d) Proper storage condition which minimizes stress e) Appropriate container
How could residual uncertainty be addressed in the preclinical and/or clinical studies?		
After manufacturing modifications, physicochemical biological tests should be performed and preclinical studies redone in regard to the ADCC evaluation. Clinical studies should also be done in regard to the PK evaluation.	Extensive studies on safety (preclinical, clinical).	a) Preclinical *in vivo* studies are needed (PK/ pharmacodynamics [PD]) b) Clinical studies (phase I, PK and immunogenicity)

(*Continued*)

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Table 3: Case study – monoclonal antibody IgG 1 (*Continued*)

Can approval be extrapolated to all indications approved for the reference product based on studies done only in one condition (psoriasis and psoriatic arthritis) based on having a single, similar mechanism of action?		
No, not for Crohn's and ulcerative colitis (UC) because of the differences in effector functions and glycosylation profile as well as the reduced FcRIII binding and ADCC responses.	If the Mechanism of Action (MoA) is the same, provided that the product is proven biosimilar, then extrapolation is possible. If the MoA is different extrapolation cannot be made; studies on efficacy and safety should be done.	No, because the ADCC, which seems to be relevant for the MoA for Crohn's and UC, differs. The SBP candidate was shown to be less effective in this regard according to these assays (increased fucosylation seems to play a role). Also, there are no published clinical data for inflammatory bowel disease indications.
Provided that the immunogenicity and aggregation problems are resolved could the product be approved and if not, why not?		
Yes, but only for psoriasis and psoriatic arthritis indications.		
Post-marketing survey is necessary for immunogenicity in rheumatoid arthritis (RA) and ankylosing spondylitis (AS) patients who received this product. For this purpose: tests should be performed at specified time intervals.	Question not answered.	No, because of the ADCC results, which seem to be relevant for the MoA for Crohn's and UC, differs. The SBP candidate was shown to be less effective in this regard according to these assays (increased fucosylation seems to play a role). Also, there are no published clinical data for inflammatory bowel disease indications.

ADCC: antibody-dependent cell-mediated cytotoxicity; CDC: complement dependent cytotoxicity; PD: pharmacodynamics; PK: pharmacokinetics; TNF: tumour necrosis factor.

Table 4: Case Study – monoclonal antibody IgG1: summary information

Group 4 Moderator: Professor Dr R Neslihan Gürsoy, PhD
Co-moderators: Dr Enes Karabulut, MD; Professor Dr Nefise Ozlen Şahin, PhD

Objective 1 – Evaluation of biosimilarity from analytical perspective for a given set of physicochemical and functional data
Objective 2 – Evaluation of whether the data provided is sufficient for extrapolation
Assumption: this product is in its final formulation; therefore, data on formulation attributes should also be presented

Physicochemical characteristics
Acceptability limits for deamidation and dimer formation should be presented as a change in these attributes may cause immunogenicity.
Additional data should be presented on:
- Acidic charge profile
- Basic charge profile and oxidation – charge and oxidation status definitely have an impact at certain point, like stability of the product
- Aggregates and particulates due to apparent out limit data

Glycan and biological attributes
Additional data required on galactosylation, fucosylation, high mannose and ADCC activity.
A clinician should also evaluate this set of data.

ADCC: antibody dependent cell mediated cytotoxicity.

in this workshop, there were many differences in participants' responses and opinions from the participants concerning whether these two fictitious follow-on biologicals were qualified to be called biosimilars. This has also been seen in GaBI's similar workshops.

These differences are likely a reflection of the fact that this is a relatively new area of pharmaceutical science and both the number of products and the information becoming available about the proper definition, approval, monitoring, substitution and switching of follow-on biological products are increasing rapidly. The lack of consensus suggests that academics and regulators, as well as prescribers and patients, need to be provided with the training and unbiased information needed for them to properly approve or regulate, prescribe, or use follow-on biological products, i.e. biosimilars.

Speaker Faculty and Moderators

Speakers

Dr Elwyn Griffiths, DSc, PhD, UK
Professor Dr Ibrahim C Haznedaroğlu, Turkey
Dr Sundar Ramanan, PhD, USA
Dr James S Robertson, PhD, UK
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Editor's comment

Assistant Professor Dr Devrim Demir Dora of Faculty of Pharmacy/Department of Pharmaceutical Biotechnology at Hacettepe University and Dr Aydan Eratalay from the Turkish Medicines and Medical Devices Agency had read the report, provided the discussion/conclusion of Group 1 and 2 and revised content of Table 2.

Professor Dr Sevda Şenel of Faculty of Pharmacy/Department of Pharmaceutical Technology at Hacettepe University had reviewed Group 3 discussions detailed in Table 3, and confirms that the summary reflects perfectly what has been discussed and decided.

Professor Dr R Neslihan Gürsoy of Faculty of Pharmacy at Hacettepe University had read Group 4 report detailed in Table 3, and commented that it was well prepared.

Professor Dr Türkan Eldem of Faculty of Pharmacy/Department of Pharmaceutical Biotechnology at Hacettepe University had reviewed Group 5 discussions detailed in Table 3 and made a few updates.

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