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

Professor Philip D Walson, MD; Robin Thorpe, PhD, FRCPath

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.

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**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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Submitted: 4 June 2016; Revised: 7 September 2016; Accepted: 15 September 2016; Published online first: 28 September 2016

GaBI Journal | www.gabi-journal.net

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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 Çelik, 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) have 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 clearance is the primary determinant of in vitro 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. |
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

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**Table 3: Case study – monoclonal antibody IgG1**

<table>
<thead>
<tr>
<th>Group 3 Moderator: Professor Dr Sevda Şenel, MSc, PhD</th>
<th>Group 4 Moderator: Professor Dr R Neslihan Günsoy, PhD</th>
<th>Group 5 Moderator: Professor Dr Türkan Eldem, Dr Nat Sci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-moderators: Professor Dr Semra Şardaş, Gökçe Yıldırım</td>
<td>Co-moderators: Dr Enes Karabulut, MD; Professor Dr Nefise Ozlen Şahin, PhD</td>
<td>Co-moderators: Dr Ismail Burak Bal; Professor Dr Nazan Bergiadi, PhD</td>
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**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. | 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. |

| Quality is not by itself a determinant of biosimilarity. | There are important glycosylation pattern differences: more galactosylation (affects CDC), more fucosylation (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. |

**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? | Extensive studies on safety (preclinical, clinical). | a) Preclinical in vivo studies are needed (PK/ pharmacodynamics [PD]) b) Clinical studies (phase I, PK and immunogenicity) |

| 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. | |

(Continued)
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 biologicals 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.

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

<table>
<thead>
<tr>
<th>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?</th>
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<tr>
<td>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.</td>
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Table 4: Case Study – monoclonal antibody IgG1: summary information

<table>
<thead>
<tr>
<th>Group 4 Moderator: Professor Dr R Neslihan Gürsoy, PhD</th>
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<tbody>
<tr>
<td>Co-moderators: Dr Enes Karabulut, MD; Professor Dr Nefise Ozlen Şahin, PhD</td>
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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; CDC: complement dependent cytotoxicity; PD: pharmacodynamics; PK: pharmacokinetics; TNF: tumour necrosis factor.
**Moderators and Co-moderators**
Dr Aydan Eratalay, PharmD, PhD
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Dr İsmail Burak Bal
Professor Dr Nazan Bergişadi, PhD
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Assistant Professor Dr Devrim Demir Dora, PharmD, PhD
Professor Dr Türkan Eldem, Dr Nat Sci
Professor Dr R Neslihan Gürsoy, PhD
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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.

**Acknowledgements**
The Generics and Biosimilars Initiative (GaBI) wishes to thank Professors Bülent Gümüşel and Neslihan Gürsoy of the Hacettepe University for their strong support through the offering of advice and information towards the development and preparation of this interactive workshop.

The authors would like to acknowledge the help of all the workshop speaker faculty and participants, each of whom contributed to the success of the workshop and the content of this report, as well as the support of the moderators and co-moderators: Dr Aydan Eratalay, Bilgen Beldüz, Dr İsmail Burak Bal, Professor Dr Nazan Bergişadi, Fikriye Handan Çelikel, Assistant Professor Dr Devrim Demir Dora, Professor Dr Türkan Eldem, Professor Dr R Neslihan Gürsoy, Dr Enes Karabulut, Professor Dr Nefi se Ozlen Şahin, Professor Dr Semra Şardaş, Professor Dr Sevda Şenel, Gökçe Yıldırım, in facilitating meaningful discussion during the parallel case study working session, presented the discussion findings at the workshop and contributed in the finalization of this Meeting Report.

**Competing interests:** The workshop was sponsored by an unrestricted educational grant to GaBI from Amgen Inc.

**Provenance and peer review:** Commissioned; internally peer reviewed.

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**References**

DOI: 10.5639/gabij.2016.0503.034

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