A new recombinant factor VIII: from genetics to clinical use

Dear editor

The December 2014 issue of *Drug Design, Development and Therapy* included a review article by Santagostino entitled “A new recombinant factor VIII: from genetics to clinical use”. The article provided a timely review of recent advances and developments in the treatment of hemophilia A with recombinant factor VIII (rFVIII). However, when reviewing licensed rFVIII products, Santagostino did not include Human-cl rhFVIII (simoctocog alfa, Nuwiq®). Nuwiq® is a new-generation rFVIII protein produced in HEK 293 F cells that was approved by the European Medicines Agency in July 2014 for the prevention and treatment of bleeds in hemophilia A patients of all ages.

Santagostino described the gradual improvements made to rFVIII production/formulation and how these have coincided with the introduction of first-, second-, and third-generation rFVIII products, particularly in relation to the elimination of production-related additives from animal/human sources and viral removal/inactivation. These developments were summarized in Table 1 of the article, which is adapted here with an additional row providing the respective information for Nuwiq® (Table 1).

The Nuwiq® production process is entirely free of additives of animal or human origin. In addition, the purification process for Nuwiq® has incorporated technological advances into a multi-step process involving one centrifugation, two filtration, and five chromatography steps, including two dedicated virus clearance steps (solvent/detergent treatment and 20 nm nanofiltration).

Santagostino focused on the glycosylation patterns of turoctocog alfa (NovoEight®) and concluded that:...
The oligosaccharide structures of the novel rFVIII [NovoEight\textsuperscript{8}] and plasma-derived FVIII are very similar, with mainly small, quantitative differences, and heterogeneous glycosylation is present in both products.

Comparable glycosylation of Nuwiq\textsuperscript{8} and plasma-derived FVIII has also been reported.\textsuperscript{4} It is well documented that potentially antigenic non-human glycan epitopes, such as N-glycolyneuraminic acid (Neu5Gc) or Gal-\(\alpha\)-3Galβ1-(3)4GleNAc-R (\(\alpha\)-Gal), are present in recombinant products derived from hamster cells.\textsuperscript{1,6} Thus, a comparison of these epitopes in rFVIII products derived from hamster cells might have been of interest to your readers. As Nuwiq\textsuperscript{8} is produced in a human cell line, Neu5Gc or \(\alpha\)-Gal are not present.\textsuperscript{4}

In summary, the Santagostino article\textsuperscript{1} was a welcome addition to the literature that provided a timely update on recent advances and developments in rFVIII treatment of hemophilia A. However, the omission of data for the new-generation human cell derived rFVIII, Nuwiq\textsuperscript{8}, which have been summarized in this letter, was a major limitation of the article.\textsuperscript{1}

**Disclosure**

Christopher Kannicht, Guido Kohla, Maya Tiemeyer, Olaf Walter are employees of Octapharma. Helena Sandberg is a former employee of Octapharma. Editorial assistance was provided by nspm ltd, Meggen, Switzerland, with financial support from Octapharma.

**References**

1. Santagostino E. A new recombinant factor VIII: from genetics to clinical use. Drug Des Devel Ther. 2014;8:2507–2515.
2. Casademunt E, Martinelle K, Jernberg M, et al. The first recombinant factor VIII: from genetics to clinical use. Drug Des Devel Ther. 2014;8:2507–2515.
3. Kannicht C, Kohla G, Tiemeyer M, et al. The first recombinant factor VIII: from genetics to clinical use. Drug Des Devel Ther. 2014;8:2507–2515.

---

**Table 1** Licensed recombinant factor VIII products

| Generation | Product (manufacturer) | FVIII | Cell line | Culture medium | Stabilizer | Purification/viral inactivation |
|------------|------------------------|-------|-----------|----------------|------------|-------------------------------|
| First      | Recombinate\textsuperscript{5} (Baxter BioScience) | Full-length | CHO | Bovine serum albumin | Human albumin | IAC/IEC/SD/NF/Se |
| Second     | Kogenate\textsuperscript{5} FS (Bayer Healthcare) | Full-length | BHK | Human plasma protein solution | Sucrose | IAC/IEC/SD/UF |
| Second     | Helixate\textsuperscript{5} FS (CSL Behring) | Full-length | BHK | Human plasma protein solution | Sucrose | IAC/IEC/SD/UF |
| Third      | Advate\textsuperscript{5} (Baxter Healthcare) | Full-length | CHO | None | Trehalose | IAC/IEC/SD |
| Third      | Xyntha/Refacto\textsuperscript{5} AF (Pfizer) | B-domain-deleted | CHO | None | Sucrose | IAC/IEC/SD/UF |
| Third      | Turoctocog alfa (Novo Nordisk) | B-domain-truncated | CHO | None | Sucrose | IAC/IEC/SD/UF/SE |
| New        | Nuwiq\textsuperscript{8} (Octapharma AG) | B-domain-deleted | HEK | None | Sucrose/arginine | IAC/IEC/SD/UF/SE |

**Note:** Copyright © 2014. Dove Medical Press. Adapted from Santagostino E. A new recombinant factor VIII: from genetics to clinical use. Drug Des Devel Ther. 2014;8:2507–2515. Additional data added for Nuwiq\textsuperscript{8,1,2}.

**Abbreviations:** FVIII, factor VIII; IAC, immunoaffinity chromatography; IEC, ion exchange chromatography; NF, nanofiltration; SD, solvent/detergent treatment; SE, size exclusion; UF, ultrafiltration.

**Table 2** Levels of non-sulfated tyrosine in rFVIII

| Product | Origin | Non-sulfated tyrosine 1680 (%) |
|---------|--------|-----------------------------|
|         |        | LC-MS/MS                   | LC-MS/MS                   | MS-FT          |
| Turoctocog alfa | CHO | – | – | Below detection limit |
| Full-length, third-generation rFVIII | CHO | 2.6–16.7 | 5.0–8.0 | >9.0 |
| Full-length, second-generation rFVIII | BHK | 1–6.5 | 1.5 | – |
| BDD third-generation rFVIII | CHO | 4.5–13.9 | 4.0–5.0 | – |
| Simoctocog alfa, BDD new-generation rFVIII | HEK | – | Below detection limit | – |

**Notes:** Levels of non-sulfated tyrosine in rFVIII included Advate\textsuperscript{5} (full-length third-generation rFVIII), Kogenate FS\textsuperscript{5} (full-length second-generation rFVIII), and Xyntha/Refacto AF\textsuperscript{5} (B-domain-deleted third-generation rFVIII). The sulfated form dominated for all proteins, with the proportion of non-sulfated tyrosine 1680 being highest for some third-generation rFVIII products. A small peak for the non-sulfated isoform was also observed for second-generation rFVIII, while no non-sulfated species were detected for turoctocog alfa or Nuwiq\textsuperscript{8} (B-domain-deleted new-generation rFVIII). Below detection limit means negligible signal, <1%, or <0.5%, trace. Copyright © 2014. Dove Medical Press. Adapted from Santagostino E. A new recombinant factor VIII: from genetics to clinical use. Drug Des Devel Ther. 2014;8:2507–2515.

**Abbreviations:** BDD, B-domain-deleted; LC-MS/MS, liquid chromatography tandem mass spectrometry; rFVIII, recombinant factor VIII; MS-FT, mass spectrometry high resolution Fourier transform scan.
A new recombinant factor VIII: from genetics to clinical use

3. Sandberg H, Kannicht C, Stenlund P, et al. Functional characteristics of the novel, human-derived recombinant FVIII protein product, human-cl rhFVIII. *Thromb Res.* 2012;130(5):808–817.

4. Kannicht C, Ramstrom M, Kohla G, et al. Characterisation of the post-translational modifications of a novel, human cell line-derived recombinant human factor VIII. *Thromb Res.* 2013;131(1):78–88.

5. European Medicines Agency [homepage on the Internet]. Nuwiq simoctocog alfa (rFVIII); 2014 [updated December 19, 2014]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002813/human_med_001781.jsp&mid=WCO01ac058001d124. Accessed April 16, 2015.

6. Hokke CH, Bergwerff AA, van Dedem GW, et al. Sialylated carbohydrate chains of recombinant human glycoproteins expressed in Chinese hamster ovary cells contain traces of N-glycolyneuraminic acid. *FEBS Lett.* 1990;275(1–2):9–14.

7. Hironaka T, Furukawa K, Esmon PC, et al. Comparative study of the sugar chains of factor VIII purified from human plasma and from the culture media of recombinant baby hamster kidney cells. *J Biol Chem.* 1992:267(12):8012–8020.