Efficacy and safety of simoctocog alfa (Nuwiq®) in patients with severe hemophilia A: a review of clinical trial data from the GENA program

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Abstract: Simoctocog alfa (human-cl rhFVIII, Nuwiq®) is a 4th generation recombinant FVIII (rFVIII), without chemical modification or fusion with any other protein/fragment. Nuwiq® is produced in a human embryonic kidney cell line (HEK293F), which ensures human-specific post-translational protein processing. Nuwiq® was evaluated in seven prospective clinical studies in 201 adult and pediatric previously treated patients (PTPs) with severe hemophilia A. The NuProtect study in 110 previously untreated patients (PUPs) is ongoing. The mean half-life of Nuwiq® was 15.1–17.1 h in PTP studies with adults and adolescents, and 12.5 h in children aged 2–12 years. Clinical trials in PTPs demonstrated the efficacy and safety of Nuwiq® in the prevention and treatment of bleeds and as surgical prophylaxis. In the NuPreviq study of pharmacokinetic (PK)-guided personalized prophylaxis in 66 adult PTPs, 83% of patients had no spontaneous bleeds during 6 months of personalized prophylaxis and 57% were treated ⩽2 per week. No FVIII inhibitors were detected in PTPs after treatment with 43,267 injections and >80 million IU of Nuwiq®. Interim data for 66 PUPs with ⩾20 exposure days to Nuwiq® in NuProtect demonstrated a low cumulative high-titer inhibitor rate of 12.8% [actual incidence 12.1% (8/66)] and convincing efficacy and safety.

Keywords: clinical trials, coagulation disorders, Nuwiq®, simoctocog alfa

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Introduction

Hemophilia A is treated by infusion of exogenous plasma-derived FVIII (pdFVIII) or recombinant FVIII (rFVIII), either prophylactically to prevent bleeds or as on-demand treatment after a bleed has occurred.[1,2] Concerns about virus transmission from blood products have largely been ameliorated by integrating multiple viral inactivation and attenuation steps into the manufacturing process of pdFVIII products and the development of rFVIII products.[3–5] Since the introduction of the first-generation rFVIII produced in Chinese hamster ovary (CHO) cells in the early 1990s, there have been incremental improvements in rFVIII production and formulations, particularly in relation to the elimination of additives from animal/human sources and virus removal/inactivation.[4] The newer products were classified as 2nd and 3rd generation rFVIII products, and were derived from CHO and baby hamster kidney (BHK) cell lines. More recently, 4th generation products produced in human cell lines (HEK293F) have become available for the treatment of hemophilia A.[5–7]

Primary prophylaxis has emerged as the standard of care for maintaining hemostasis and preserving joint function in children with severe hemophilia A.[8–10] There is also strong evidence for the benefits of prophylaxis (termed 'secondary') versus on-demand treatment in adults with severe hemophilia A.[11–14] However, a 'one size fits all' approach

to prophylaxis is not ideal, as this potentially leads to over-treatment in some individuals and under-treatment in others. Furthermore, a generic plan fails to take into account a patient's lifestyle and personal preferences.[15] Personalized, patient-tailored prophylaxis based on pharmacokinetic (PK) data has the potential to optimize patient care and enable fewer infusions by matching the dosing regimen to the PK of each patient to ensure that all patients achieve a predetermined FVIII trough level and protection from bleeding.[15–20]

The development of neutralizing alloantibody inhibitors to FVIII replacement therapy, which neutralize the coagulation effects of FVIII replacement therapy, is generally considered the most serious complication in the current treatment of hemophilia A patients in economically developed countries due to major adverse implications for bleeding rates, morbidity, mortality, quality of life, and treatment costs.[21–24] Inhibitors are estimated to develop in ~35% of previously untreated patients (PUPs)[25,26] and 1% of previously treated patients (PTPs).[27] Inhibitor development is mediated by a complex interaction of unmodifiable host-related factors, such as hemophilia severity, family history, ethnicity, and *F8* genotype, and potentially modifiable treatment-related factors, such as treatment intensity, FVIII dose, treatment regimen, and product type.[25,26,28,29] Inhibitors can arise in patients with hemophilia A at any time throughout life with a bimodal risk, with peak incidence in early childhood [after a median of ~15 exposure days (EDs)] and a smaller peak in old age.[30,31] Intensive treatment, for example for surgical procedures, has been shown to be a risk factor for FVIII inhibitor development in PTPs.[30,32]

Simoctocog alfa (human-cl rhFVIII, Nuwiq®; Octapharma AG, Switzerland) is a 4th generation rFVIII, without chemical modification or fusion with any other protein/fragment, produced in a human cell line.[6,33–36] The purification process involves 10 stages: one centrifugation, two filtration, five chromatography, and two dedicated pathogen clearance steps (solvent/detergent treatment and 20 nm nanofiltration).[37] The purification process ensures a virus-free product, and effectively removes process-related (proteins and DNA) and product-related (ratio active/inactive FVIII) impurities.[37]

The production of Nuwiq® in a human cell line results in human-specific post-translational protein processing, such as glycosylation and sulfation, which closely mimic those of endogenous FVIII.[34,35,38] Glycosylation alters the structural, functional, and immunogenic properties of a protein[7,39] and the presence of glycans of nonhuman origin may have immunogenic potential.[40] Nuwiq® has a glycosylation pattern similar to that of pdFVIII and is devoid of potentially antigenic, nonhuman glycan epitopes that are present in rFVIII products derived from hamster cell lines, and, thus, may be less immunogenic.[35,41,42]

Sulfated residues play an important role in FVIII activation in the coagulation pathway and in the interaction between FVIII and von Willebrand factor (VWF).[7,43] In human plasma, FVIII is non-covalently bound to VWF: a larger carrier protein that stabilizes FVIII by preventing proteolytic degradation and considerably prolongs FVIII survival.[38,44] Sulfation of tyrosine 1680 impacts significantly on the VWF-binding affinity to FVIII and, consequently, on FVIII stability.[38,45] It has been suggested that bound VWF acts as an immune modulating chaperone molecule for FVIII, reducing the immunogenicity of therapeutic FVIII.[46] Nuwiq® is fully sulfated at all tyrosine binding sites, including tyrosine 1680,[35] and has a high binding affinity for VWF.[34] These properties suggest that Nuwiq® might be less likely to induce the development of alloantibody inhibitors to FVIII, and have an extended circulating half-life *in vivo* compared with hamster cell line-derived rFVIII products.

Here, we describe key findings from prospective clinical trials in PTPs and PUPs with severe hemophilia A treated with Nuwiq® as part of the GENA clinical trial program.

Overview of the GENA clinical trial program

The GENA clinical trial program for Nuwiq® was developed with consideration of European Medicines Agency (EMA) guidelines[47] and after discussion with the US Food and Drug Administration (FDA). Five pre-registration clinical studies in PTPs were conducted in Europe and the USA: GENA-01, GENA-08, GENA-03, GENA-09, and its extension GENA-04. Studies GENA-01,[48] GENA-08,[48,49] and GENA-03[50] were multinational pivotal studies (Table 1). Data relating to surgical prophylaxis and safety from supportive studies (GENA-09/GENA-04) are also described here. GENA-09 was a

single-center Russian study in 22 adult PTPs with longstanding, poorly controlled hemophilia A. Upon completion of GENA-09, 18 of the 22 patients entered the GENA-04 extension study. Following the approval of Nuwiq®, two additional PTP studies have been completed: GENA-13,[51] a long-term extension of the pediatric GENA-03 study, and GENA-21 (NuPreviq),[20] a study of PK-guided personalized prophylaxis in 66 adult PTPs (Table 1). In total, 201 PTPs (190 individuals) were enrolled across the seven PTP studies.[7,20,51]

All seven PTP studies enrolled patients with severe hemophilia A, who had been previously treated (⩾150 EDs in patients ⩾12 years of age, ⩾50 EDs in patients <12 years of age). All patients were to be treated for at least 6 months and at least 50 EDs. Identical objective measures were used to assess prophylactic efficacy, hemostatic efficacy of on-demand (and breakthrough) bleeds, and surgical prophylaxis across all studies. The safety variables were practically identical across all studies. Key laboratory parameters were measured in the same certified central laboratory using the same validated methods. Plasma FVIII:C activity assays were used for PK assessment, and activity was measured by both a one-stage coagulation and a chromogenic assay (indirectly measuring FVIII activity through its ability to generate factor Xa). Inhibitory antibodies were measured by the Bethesda assay (Nijmegen modification) method, as suggested by the EMA.[47,53] An inhibitor was defined as an inhibitor titer ⩾0.6 (⩾0.6 to <5 BU [Bethesda units]/mL for a 'low titer' inhibitor and ⩾5 BU/mL for a 'high-titer' inhibitor).

The ongoing NuProtect study (GENA-05; NCT01712438) was initiated in 2013 to assess the immunogenicity, safety, and efficacy of Nuwiq® in PUPs. NuProtect is a prospective, multinational, open-label, non-controlled, phase III study in 110 PUPs with severe hemophilia A, that is, those at highest risk of developing inhibitors. PUPs of any age and ethnicity are under observation over their first 100 EDs or a maximum study participation of 5 years. The patient population is considered to be 'true' PUPs as patients with any previous treatment containing FVIII are excluded. Intensive screening for inhibitors is scheduled every 3–4 EDs until ED20, then every 10–12 EDs until ED100 or every 3 months (whichever occurs first) until study completion. Interim data for 66 PUPs who were treated for ⩾20 EDs, the time by which the majority of inhibitors would be expected to arise,[25,26,31,54] were published recently and final data are expected in 2019.[55]

Clinical data in PTPs

Half-life

The half-life of Nuwiq® was assessed in 20 adults and two adolescents ($N=22$) in GENA-01, 66 adults in NuPreviq and 26 children aged 2–12 years in GENA-03 ($N=13$ aged 2–5 years and $N=13$ aged 6–12 years) (Table 2).[20,50,52] In GENA-01 and GENA-03, half-life of a 50 IU/kg infusion of Nuwiq® was calculated using a non-compartmental PK model. In the NuPreviq personalized prophylaxis study, half-life of a 60 ± 5 IU/kg infusion of Nuwiq® was calculated using a one- or two-compartment PK model (as individually appropriate); a non-compartmental model was chosen in cases of uncertainty. The FVIII PK profile (one-stage assay) was best described by a two-compartment PK model for 36 (54.5%) patients, and by a one-compartment model for 23 (34.8%) patients. For the remaining seven patients (10.6%), a non-compartment model was used as neither a two- nor a one-compartment model appeared to be appropriate.

The mean ± SD half-life (one-stage assay) of Nuwiq® in adults and adolescents was 17.1 ± 11.2 h and 15.1 ± 4.7 h in the GENA-01 and NuPreviq studies, respectively (Table 2). In GENA-03,[50] the mean ± SD half-life (one-stage assay) of Nuwiq® was 11.9 ± 5.4 h in younger children (2–5 years), 13.1 ± 2.6 h in older children (6–12 years), and 12.5 ± 4.2 h overall (Table 2). The shorter half-life in children compared with adults is well documented for rFVIII products, and may result from higher plasma volumes per unit weight in children compared with adults.[56,57] Across the studies, half-life was shorter when using the chromogenic compared with the one-stage assay (Table 2).

An international comparative field study assessed the performance of one-stage and chromogenic assays in measuring FVIII activity of Nuwiq® in routine clinical practice.[58] Data for Nuwiq® and Advate®, a hamster cell line-derived rFVIII, from

Table 1. Overview of Nuwiq® pivotal pre-registration and post-approval clinical trials in PTPs with severe hemophilia A.

	Pivotal pre-registration studies			Post-approval studies	
	Adults		Children	Children	Adults
	GENA-01[52] (on-demand)	GENA-08[49] (prophylaxis)	GENA-03[50] (prophylaxis)	GENA-13[51] (long-term prophylaxis)	GENA-21 (NuPreviq)[20] (personalized prophylaxis)
Development phase	II	III	III	IIIb	IIIb
Trial period	May 2010–Sep 2012	Jun 2010–Jan 2012	Dec 2010–Nov 2012	Oct 2011–May 2016	Aug 2013–Jan 2015
Number of centers	9	11	15	10	20
Number of countries	Three: Bulgaria, Germany, USA	Four: Austria, Bulgaria, Germany, UK	Seven: Czech Republic, France, Poland, Romania, Russia, Turkey, UK	Six: Czech Republic, France, Poland, Romania, Russia, UK	Eight: Austria, Bulgaria, Germany, Hungary, Poland, Romania, Slovakia, UK
Number of patients	22	32	59	49[$]	66[‡]
Previous FVIII treatment	⩾150 EDs	⩾150 EDs	⩾50 EDs	⩾100 EDs	⩾150 EDs
Age	12–65 years*	⩾18 years	2–12 years	3–13 years	⩾18 years
PK assessment	Yes	IVR only	Yes	IVR only	Yes
Treatment	On demand; surgical prophylaxis	Prophylaxis; breakthrough bleeds, surgical prophylaxis	Prophylaxis; breakthrough bleeds, surgical prophylaxis	Prophylaxis; breakthrough bleeds, surgical prophylaxis	Prophylaxis; Breakthrough bleeds, surgical prophylaxis
Duration of treatment	⩾6 months and ⩾50 EDs	⩾6 months and ⩾50 EDs	⩾6 months and ⩾50 EDs	Mean (range) months: 29.4 (9.6–53.2); mean (range) EDs: 415 (145–802)	~7–9 months; including ⩾6 months of PK-guided personalized prophylaxis

*Includes two adolescents aged 12–17 years.
[$]Study GENA-13 was an extension of study GENA-03; therefore, all of these patients participated in study GENA-03.
[‡]A total of 11 patients had previously participated in GENA-01 or GENA-08.
PTP, previously treated patient; ED, exposure day; PK pharmacokinetic, IVR, *in vivo* recovery.

49 laboratories in nine countries were analyzed. Mean absolute FVIII:C was comparable for both products at all concentrations and for both assays, with interproduct ratios (Nuwiq®:Advate®) of 1.02–1.13. Chromogenic to one-stage ratios based on overall means ranged from 0.99 to 1.17 for Nuwiq®, and from 1.01 to 1.17 for Advate®, which indicates similarly higher FVIII:C with the chromogenic assay for both products. These data demonstrate that the FVIII:C of Nuwiq® can be accurately measured using both one-stage and chromogenic assays in routine laboratory practice, without the need for a product-specific reference standard. The majority of laboratories use one-stage assays for monitoring in the clinical setting.[59]

Table 2. Nuwiq® half-life in previously treated patients (PTPs) at study start.

Study	*N*	Age (years)	Half-life, h (mean ± SD)	
			One-stage	Chromogenic
GENA-01* (adolescents/adults)[52]	22	12–65	17.1 ± 11.2	14.7 ± 10.0
GENA-21 (NuPreviq)[$] (adults)[20]	66	18–65	15.1 ± 4.7	Not reported
GENA-03[‡] (children)[50]	26	2–12	12.5 ± 4.2	9.7 ± 2.7[¶]
	13	2–5	11.9 ± 5.4	9.5 ± 3.3[¶]
	13	6–12	13.1 ± 2.6	10.0 ± 1.9[¶]

*FVIII plasma level was measured at 0.25, 0.5, 0.75, 1, 3, 6, 9, 12, 24, 30, and 48 h post injection (96 h washout period). Nominal dose: 50 international units (IU)/kg.
[$]FVIII plasma level was measured at 0.5, 1, 3, 6, 9, 24, 30, 48, and 72 h (72 h wash-out period). Nominal dose: 60 ± 5 IU/kg.
[‡]FVIII plasma level was measured before and 0.5, 2, 5, 10, 24, and 48 h post injection (96 h wash-out period). Nominal dose 50 IU/kg.
[¶]One value was missing for the chromogenic assay.

Prevention of bleeds

Standard prophylaxis. The efficacy of standard prophylaxis with Nuwiq® was assessed in pivotal studies of adults and children aged 2–12 years and in the GENA-13 extension study in children.[49–51] The recommended dose for prophylaxis was 30–40 IU FVIII/kg administered every other day in GENA-08 and every other day or three times per week in GENA-03 and GENA-13.

In GENA-08, the median (mean) annualized bleeding rates (ABRs) during Nuwiq® prophylaxis were 0.9 (2.28) for all bleeds and 0 (1.16) for spontaneous bleeds (Table 3).[49] For all joint bleeds (ankle, elbow, knee), the median (mean) ABR was 0 (1.14). The ABR (negative binomial regression estimate) during prophylaxis with Nuwiq® in GENA-08 was 2.30 compared with 57.74 during on-demand treatment in GENA-01, which equates to a 96% lower ABR during prophylaxis.[48] The ABR for all joint bleeds was 1.15 during prophylaxis, compared with 35.26 during on-demand treatment, which equates to a 97% lower ABR for joint bleeds during prophylaxis.[48]

In GENA-03, the median (mean) ABRs in children were 1.9 (4.12) for all bleeds and 0 (1.50) for spontaneous bleeds (Table 3).[50] For all joint bleeds, median (mean) ABR was 0 (1.53). In six patients who had previously received on-demand treatment, a 97% reduction in mean ABR from 35.9 to 0.96 was observed.

A total of 49 children from GENA-03 continued Nuwiq® prophylaxis in GENA-13 for a mean of 2.5 years.[51] The mean ± SD dose per prophylactic infusion was comparable between GENA-03 and GENA-13 (38.6 ± 6.7 and 38.6 ± 7.4 IU/kg, respectively). The median (mean) ABR was 1.72 (2.91) for all bleeds and 0.34 (0.67) for spontaneous bleeds (Table 3). Younger children (2–5 years) had lower ABRs than children aged 6–12 years. For all joint bleeds, median (mean) ABR was 0.36 (0.85). ABRs were markedly reduced in GENA-13 *versus* GENA-03, especially for spontaneous bleeds in younger children, in whom a 71% reduction was observed (Figure 1). During 2.5 years of prophylaxis treatment, 45% of children had no spontaneous bleeds (Octapharma, data on file).

PK-guided personalized prophylaxis. PK-guided personalized prophylaxis with Nuwiq® was assessed in the NuPreviq (GENA-21) study.[20] The study consisted of three phases: an initial PK evaluation phase; a 1–3 month standard prophylaxis treatment phase; and a 6-month PK-guided personalized prophylaxis phase.

The prophylactic dose and dosing interval recommended for the personalized prophylaxis phase were based on the analysis of individual PK data obtained using the one-stage assay at the initial evaluation. As patients with FVIII:C >1% experience fewer spontaneous bleeds and consequential damage, the main aim of prophylaxis is to

Table 3. Annualized bleeding rates (ABRs) during Nuwiq® prophylaxis.

Study	*N* Age	ABR				Patients without bleeds (%)	Monthly prophylaxis dose (IU/kg)*	
		All bleeds		Spontaneous bleeds				
		Median (range)	Mean ± SD	Median (range)	Mean ± SD		Median (range)	Mean ± SD
Adults								
GENA-08 Standard prophylaxis[49]	*N* = 32 ⩾18 years	0.9 (0–14.7)	2.28 ± 3.73	0 (0–8.6)	1.16 ± 2.57	50	468.7 (208.4–582.6)	466.1 ± 65.5
GENA-21 (NuPreviq) Personalized prophylaxis[20]	*N* = 65 ⩾18 years[$]	0 (0–17.5)	1.45 ± 3.51	0 (0–11.7)	0.79 ± 2.31	74	407.2 (173.1–663.2) (397.6 in last 2 months)	416.7 ± 98.5 (405.8 in last 2 months)
Children								
GENA-03 Standard prophylaxis[50]	*N* = 59 2–12 years	1.90 (0–20.7)	4.12 ± 5.22	0 (0–13.8)	1.50 ± 3.32		521.9 (332.3–888.5)	527.7 ± 112.3
	N = 29 2–5 years	0 (0–12.2)	2.60 ± 3.57	0 (0–9.5)[‡]	1.10 ± 2.68[‡]	34	513.4 (359.0–888.5)	525.0 ± 120.4
	N = 30 6–12 years	3.63 (0–20.7)	5.59 ± 6.13	0 (0–13.8)[‡]	1.95 ± 3.90[‡]		533.9 (332.3–809.5)	530.4 ± 105.9
GENA-13 Standard prophylaxis[51]	*N* = 49 2–12 years	1.72 (0–27.8)	2.91 ± 4.66	0.34 (0–5.42)	0.67 ± 1.05	16	519.0 (368.4–791.8)	531.2 ± 100.8
	N = 26 2–5 years	0.82 (0–6.3)	1.46 ± 1.53	0 (0–2.49)	0.34 ± 0.55		559.9 (373.1–791.8)	557.3 ± 98.2
	N = 23 6–12 years	2.6 (0–27.8)	4.54 ± 6.28	0.85 (0–5.42)	1.05 ± 1.33		488.3 (368.4–774.0)	501.7 ± 97.4

*Octapharma, data on file are given to provide monthly mean and median values for all studies.
[$]Data for one patient who was a major outlier are excluded.
[‡]Calculated from mean monthly (30-day) values multiplied by 12.195 to obtain the ABR based on 365.25 days per year.
IU, International units.

keep the FVIII plasma level >1%.[15] The goal in NuPreviq was to determine the maximum regular prophylactic dosing interval that could be achieved with a single dose of not more than 60–80 IU/kg, while maintaining a trough FVIII:C level of ⩾1% and not exceeding a maximum plasma FVIII:C of 200%.

The majority of patients (62%, 41/66) received only on-demand treatment in the 6 months prior to the study. Prior mean ± SD ABRs were 38.9 ± 27.59, 45.6 ± 23.71, and 27.8 ± 30.33 for all patients, prior on-demand patients and prior prophylaxis patients (at least one dose of regular or irregular prophylactic treatment), respectively, during which the weekly doses were 34.3 ± 28.64, 21.1 ± 8.78, and 56.6 ± 36.19 IU/kg, respectively. Mean ± SD Hemophilia Joint Health Score was 41.4 ± 25.2 in patients who were previously treated on-demand, and 30.3 ± 24.3 in patients who received previous prophylaxis.

The median dosing interval during personalized prophylaxis was 3.5 days, with 57% of patients on

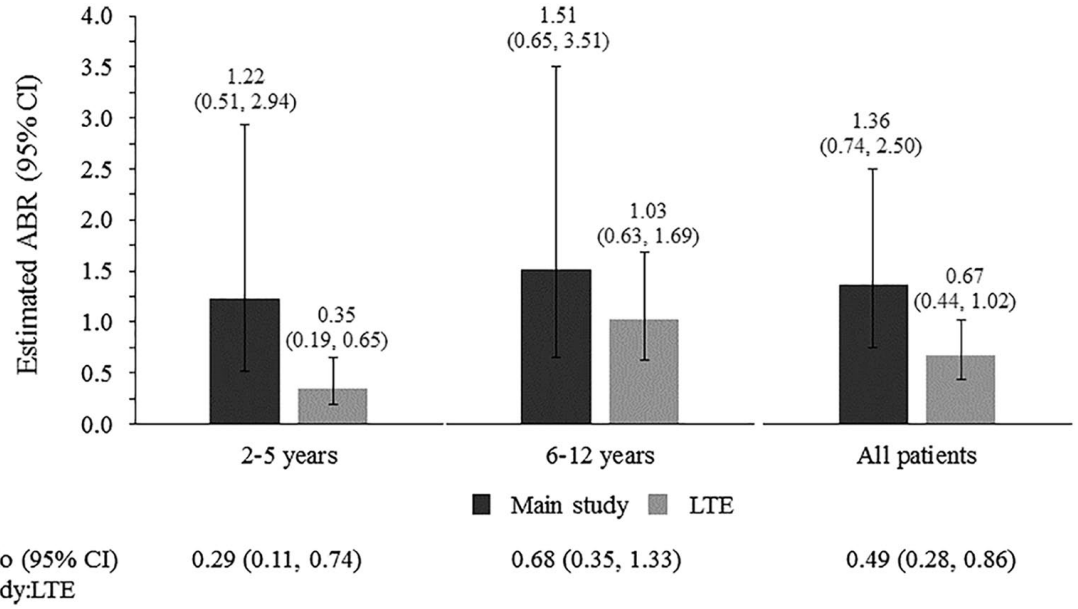

Figure 1. Estimated annualized bleeding rates (ABRs) (95% CIs) for spontaneous bleeds in the main pediatric study (GENA-03) and its long-term extension (GENA-13). Only patients enrolled in both studies were considered. ABRs were estimated based on calculations using a negative binomial counting regression model and considering only the time under prophylaxis. BE, bleeding episode; LTE, long-term extension. Data from Klukowska and colleagues.[51]

⩽2 weekly dosing. During personalized prophylaxis, 83% of patients had no spontaneous bleeds, and 74% had no bleeds of any type. Median (mean) ABRs during personalized prophylaxis were 0 (1.45) for all bleeds, 0 (0.79) for spontaneous bleeds, and 0 (0.91) for joint bleeds (Table 3). Compared with the preceding standard prophylaxis regimen, median weekly prophylaxis dose was reduced by 7.2% from 100.0 to 92.8 IU/kg during the last 2 months of personalized prophylaxis.

In patients with available measurements, mean ± SD FVIII trough levels after 2, 4, and 6 months of personalized prophylaxis were 3.4% ± 4.0 ($n = 19$), 4.2% ± 7.0 ($n = 24$), and 2.7% ± 2.4 ($n = 20$), respectively. Thus, trough levels were consistent throughout personalized prophylaxis with Nuwiq® and higher than the target level of 1%. In clinical practice, target FVIII trough levels can be set according to individual needs to provide greater bleed protection when needed (e.g. patients with high physical activity levels and/or with bleeding phenotype even at 1–5% troughs). A recent Delphi Consensus Statement recommended the use of different target plasma FVIII levels tailored to individual people with hemophilia A.[60]

Treatment of bleeds

The efficacy of Nuwiq® in the treatment of bleeds was assessed in all three pivotal studies and the GENA-13 and NuPreviq studies. Efficacy was assessed during on-demand treatment in GENA-01[48] and in the treatment of breakthrough bleeds during prophylaxis in the other studies[20,48–51] (Table 4). Efficacy was assessed as excellent, good, moderate or none at the end of each bleed according to predefined objective criteria (Table 4), including the number of infusions and improvement in signs of bleeding and pain.

Efficacy of Nuwiq® for on-demand treatment of bleeds or treatment of breakthrough bleeds during prophylaxis was assessed for 1530 bleeds across the five studies. The majority of the bleeds were as expected in the GENA-01 study, which was specifically designed to assess the efficacy of on-demand treatment. In GENA-01, efficacy in the treatment of bleeds was rated as excellent or good for 94.5% of 985 evaluated bleeds, with efficacy in 5.5% of bleeds rated as moderate.[48] The vast majority (97.2%) of bleeds were treated with 1 (91.4%) or 2 (5.8%) infusions. The mean ± SD number of infusions and dose per bleed were 1.1 ± 0.59 and 36.6 ± 27.64 IU/kg, respectively.

Table 4. Efficacy of Nuwiq® in the treatment of bleeds.

Study (population)	Treatment	No. of treated bleeds	Mean ± SD dose per bleed (IU/kg)	% of bleeds treated successfully*	% of bleeds managed with one or two infusions
GENA-01[48] (adolescents/adults)	On-demand	986	36.6 ± 27.64	94.5[$]	97.2
GENA-08[49] (adults)	Prophylaxis	30	60.4 ± 73.4	100[‡]	88.9
GENA-21 (NuPreviq)[20] [§,‖] (adults)	Prophylaxis	95	63.9 ± 81.1	90.5	88.4
GENA-03[50] (children)	Prophylaxis	108	95.9 ± 169.3	82.4	81.3
GENA-13[51] (children)	Prophylaxis	311	68.5 ± 54.0[‖]	83.0[¶]	84.9

*Excellent or good efficacy rating. Excellent: Abrupt pain relief and/or unequivocal improvement in objective signs of bleeding within approximately 8 h after a single infusion; Good: Definite pain relief and/or improvement in signs of bleeding within approximately 8–12 h after an infusion requiring up to two infusions for complete resolution; Moderate: Probable or slight beneficial effect within approximately 12 h after the first infusion, requiring more than two infusions for complete resolution; None: No improvement within 12 h, or worsening of symptoms, requiring more than two infusions for complete resolution.
[$]*N* = 985 treated and evaluated bleeds.
[‡]*N* = 28 treated and evaluated bleeds.
[§]Includes bleeds treated during the standard prophylaxis phase that preceded the personalized prophylaxis phase.
[‖]Octapharma, data on file.
[¶]*N* = 305 treated and evaluated bleeds.
IU, International units.

Across the prophylaxis studies, the majority of bleeds were treated successfully with one or two infusions (Table 4).

Surgical prophylaxis

Across all seven GENA studies, 36 patients aged 3–55 years received surgical prophylaxis with Nuwiq® for 60 surgeries (28 major and 32 minor), and efficacy was evaluated for 52 surgeries (25 major and 27 minor).[61] The success rate of Nuwiq® treatment was 98.1% [95% confidence interval (CI): 89.7%, 100.0%]; hemostatic efficacy (see Zozulya *et al.* for criteria[61]) was assessed as excellent or good in all but one major surgery (assessed as moderate). The moderate rating, for a joint arthroscopy, had an intraoperative efficacy of good; however, during the postoperative period the patient experienced two minor nose bleeds unrelated to surgery. Treatment was successful (excellent) in all 21 (14 major and 7 minor) evaluated procedures in children.

The number of infusions ranged from 1 to 19 for minor surgeries and from 3 to 76 for major surgeries. The median daily doses were 42.0 IU/kg for minor surgeries and 69.3 IU/kg for major surgeries. The median total dose administered per surgery was 591.2 IU/kg for major surgeries and 50.0 IU/kg for minor surgeries.

Safety and immunogenicity

The safety of Nuwiq® has been evaluated in 201 PTPs across the seven GENA studies (190 individuals), with emphasis on immunogenicity (FVIII inhibitors) and development of FVIII antibodies (antibodies were measured in all studies except GENA-21). Patients received a total of 81,478,132 IU of Nuwiq® *via* 43,267 infusions. There were no FVIII inhibitors in any patient irrespective of their previous treatment. Of 190 PTPs, 97 (51.1%) switched from pdFVIII products, 74 (38.9%) from rFVIII, 16 (8.4%) from both pdFVIII and rFVIII, and 3 (1.6%) switched from an unknown FVIII.

Across the seven studies, 12 adverse drug reactions (ADRs) occurred once each in eight patients. Ten ADRs were mild, and two were severe (malaise, dizziness). Both severe events occurred in the same patient during the standard

prophylaxis phase of the NuPreviq study and resolved without sequelae. One ADR, mild pyrexia in a child in GENA-13, was classified as serious because the patient was hospitalized. The pyrexia resolved without sequelae. The remaining nine mild, non-serious ADRs were: vertigo, dry mouth, paresthesia, and injection site inflammation (all in the same GENA-08 patient during the first infusion); injection site pain (GENA-08); back pain (GENA-03); headache (GENA-03); dyspnea (GENA-13); and FVIII antibody positive (non-neutralizing) (GENA-04).

A non-neutralizing anti-FVIII antibody was detected in one adult patient in GENA-04 (completion visit) that was judged to be related to treatment. The sample was tested by the central laboratory at eight dilutions. The result was positive only at dilution factor 1, and the antibody titer was very low (0.34). Inhibitory activity, as measured by the modified Bethesda assay, was not detected in this patient. The antibody was undetectable at a subsequent examination. Efficacy in this patient did not seem to be affected, as he had no breakthrough bleeds during the study, and, on the day the positive anti-FVIII antibody was detected, his *in vivo* recovery was unaffected.

Clinical trial data in PUPs

An interim analysis was performed on data from 66 PUPs who had been treated with Nuwiq® for at least 20 EDs,[55] the time by which most inhibitors develop.[25,26,31,54] *F8* gene mutation analysis data were available for 59 patients. Mutations were identified in 58 of these 59 patients; 44/58 patients (75.9%) had known high-risk mutations and 47/58 (81.0%) had null mutations associated with inhibitor development.[62]

Immunogenicity

Inhibitors developed in 13 of 66 (19.7%) patients; 8 (12.1%) developed high-titer inhibitors, and 5 (7.6%) developed low-titer inhibitors, which were transient in 4 of these patients. Inhibitors developed within the first 20 EDs in 11 of the 13 patients who developed inhibitors (one high-titer inhibitor developed after 24 EDs, and one low-titer inhibitor developed after 25 EDs). The cumulative incidence of all inhibitors was 20.8% (95% CI: 10.7%, 31.0%); 12.8% (95% CI: 4.5%, 21.2%) for high-titer inhibitors, and 8.4% (95% CI: 1.3%, 15.6%) for low-titer inhibitors. None of the patients with non-null *F8* mutations developed inhibitors. In patients with null *F8* mutations, the cumulative incidence of all inhibitors was 26.7% (95% CI: 13.7%, 39.7%), and of high-titer inhibitors was 17.8% (95% CI: 6.5%, 29.0%).[63]

Efficacy and safety

A total of 45 patients received continuous prophylactic treatment up to the interim analysis.[63] The mean prophylactic dose was 39.1 IU/kg per ED, and the median (range) number of EDs for prophylaxis was 70.8 (5–115) over 9.2 months (1.1–22.3). During inhibitor-free periods, the median (mean) ABRs during prophylaxis were 0 (1.57) for spontaneous bleeds and 2.40 (3.94) for all bleeds.

While inhibitor-free, patients experienced 354 bleeds that required treatment with 329 infusions (infusions given to treat parallel bleeds were counted once). Patients received a mean ± SD dose of 47.3 ± 41.1 IU/kg per bleed with 1.3 ± 0.95 infusions. In most cases (304/329, 92.4%), one (82.1%) or two (10.3%) infusions were administered for controlling bleeds. Efficacy of treatment was rated as 'excellent' or 'good' for 92% of rated bleeds during inhibitor-free periods. Of the nine surgical procedures during inhibitor-free periods with available efficacy assessments, efficacy was rated as 'excellent' or 'good' in eight procedures and 'moderate' in one procedure.

Three patients experienced adverse events (AEs) assessed to be related to treatment (other than inhibitor development): one experienced mild fever; one experienced mild allergic reactions after three consecutive Nuwiq® administrations (his other 105 infusions were without complication); and the third patient developed a rash, which, although mild, was considered serious due to hospitalization. He continued treatment until completion of the study with no other related AEs. No venous or arterial thromboembolic complications or severe allergic reactions were recorded.

Discussion

Use of a human cell line and state-of-the-art production and purification processes has resulted in a 4th generation rFVIII with human-specific post-translational protein processing that is virus-free and of high purity.

Clinical trials in PTPs have demonstrated the efficacy and safety of Nuwiq® in the prevention and treatment of bleeds in adults and children with severe hemophilia A.[20,36,48–51] The mean half-life of Nuwiq® was 15.1 to 17.1 h in PTP studies with adults and adolescents and 12.5 h in children aged 2–12 years.[20,50,52] The NuPreviq study in 66 patients confirmed the considerable interpatient variation in Nuwiq® half-life, ranging from 6.2 to 31.9 h; this variation is common to other rFVIII products and provides a strong rationale for PK-guided personalized prophylaxis.[20] No FVIII inhibitors have been observed in clinical trials in 201 PTPs (190 individuals) switched to Nuwiq®.

The NuPreviq study has shown that individual PK-guided personalized prophylaxis with Nuwiq® in adult PTPs provides excellent bleed protection, with 83% of patients free from spontaneous bleeds during 6 months of treatment and 74% of patients having no bleeds of any type; 57% of patients were on ⩽2 weekly dosing, and there was a 7.2% reduction in median dose compared with standard prophylaxis.[20]

The results of the NuPreviq study compare favorably with published data for other rFVIII products, although differences in study designs and patient populations prevent direct comparison of results from different studies. In studies of similar duration (~6 months), the percentage of patients with no bleeds was 39.6% for twice weekly PEGylated full-length rFVIII derived from CHO cells,[64] 43% for twice or three times weekly single-chain rFVIII derived from CHO cells,[65] and 74% during personalized prophylaxis with Nuwiq®, which is derived from human HEK293F cells.[20] In longer studies, 45.3% of patients treated with individualized rFVIII-Fc (67% ⩽2 infusions per week) derived from the human HEK293F cell line had no bleeds over ~8 months,[19,66] 26.5% of patients treated PK-tailored with full-length rFVIII derived from CHO cells had no bleeds over 1 year,[17] and 27% of patients treated with full-length rFVIII derived from BHK cells had no bleeds over 1 year.[67] The high percentage of patients with no bleeds during personalized prophylaxis with Nuwiq® is reflected in the very low mean ABR of 1.45 (median 0).[20] Mean ABRs reported in other studies ranged from 1.9 to 4.9 and median ABRs ranged from 1.1 to 2.0.[17,19,64,65,67]

In Italy, a modified NuPreviq approach that includes six sampling points and at-home sampling has been used successfully in routine clinical practice.[68,69] A population PK model for Nuwiq® is also being developed and validated in partnership with the Web-Accessible Population Pharmacokinetic Service –Hemophilia (WAPPS-Hemo; www.wapps-hemo.org), a multicentric prospective project led by McMaster University, Hamilton, Ontario, Canada.[70,71] The Nuwiq®-specific WAPPS model aims to provide a reliable estimation of individual PK based on fewer samples and provide an additional option for personalized prophylaxis with Nuwiq®.

Low bleeding rates can also be achieved with Nuwiq® in adults and children using a standard prophylaxis regimen.[49,50] The results of 6-month studies compare favorably with published data for standard prophylaxis with other rFVIII products in PTPs,[11,17,72–75] although, again, differences in study designs and patient populations limit direct comparisons between studies. In adult populations, the median ABR for all bleeds during standard prophylaxis was 0.9 (mean 2.28) for Nuwiq®,[49] median not reported (mean 5.3) for full-length rFVIII from CHO cells and 3.62 (mean 6.68) for B-domain-truncated rFVIII derived from CHO cells.[74,75] In children treated with standard prophylaxis, the median ABR was 1.9 (mean 4.12) for Nuwiq® in children aged 2–12 years of age[50] and 3.02 (mean 5.33) for B-domain-truncated rFVIII derived from CHO cells in children aged 0–11 years.[76] Median ABRs for full-length rFVIII from CHO cells were 4.0 in children aged 1–6 years,[77] and 5.2 in children aged 7–12 years.[78]

Interim data from the NuProtect study show that PUPs treated with Nuwiq® for ⩾20 EDs had a cumulative inhibitor rate of 20.8% (12.8% high-titer inhibitors).[55] In the 'Study on Inhibitors in Plasma-Product Exposed Toddlers' (SIPPET), 251 PUPs randomized to pdFVIII/VWF or hamster-cell-derived rFVIII treatment were evaluated.[26] Patients were to be treated for 50 consecutive EDs or 3 years or until inhibitor development was confirmed by a central laboratory. Of the 251 PUPs, 216 (86%) completed the trial according to the study protocol. The cumulative incidence of inhibitors for pdFVIII/VWF was 26.8% (18.6% high-titer) and 44.5% (28.4% high-titer) for rFVIII.[26]

A *post hoc* analysis of SIPPET data reported that *F8* genotype had an important influence on the

risk of inhibitor development with rFVIII compared with pdFVIII/VWF.[79] Among patients classified at high risk for inhibitor development (null *F8* mutations), the cumulative incidence of inhibitors was 31% in 101 patients treated with pdFVIII/VWF, and 47% in 96 patients treated with rFVIII. In contrast, among patients classified as low risk (non-null *F8* mutations), no inhibitors developed in 16 patients receiving pdFVIII/VWF treatment, whereas the cumulative incidence of inhibitors was 43% in 22 patients treated with rFVIII. Based on these findings, a *post hoc* analysis of the interim data from the NuProtect study was performed to investigate the influence of *F8* genotype on inhibitor development.[63] Of the 47 patients with null *F8* mutations, 12 developed FVIII inhibitors (cumulative incidence 26.7%), whereas none of 11 patients with non-null *F8* mutations developed inhibitors with Nuwiq®.

These *F8* genotype results suggest that Nuwiq® appears to follow the pattern exhibited by pdFVIII/VWF concentrates rather than that of the hamster-cell derived rFVIII concentrates. Nuwiq® was designed with the aim of reducing inhibitor development by replicating the native human FVIII protein and avoiding/minimizing potential immunogenic elements of rFVIII produced in hamster cell lines.[33–35] Nuwiq® is fully sulfated at tyrosine 1680, which is critical for FVIII binding to VWF.[35] In functional studies, Nuwiq® had a higher VWF binding compared with hamster-cell derived rFVIII products tested,[34] which suggests that it can efficiently bind to the endogenous VWF and that this may contribute to its low immunogenicity when interacting with antigen-presenting cells in the immune system.

In conclusion, comprehensive data from the GENA clinical trial program demonstrate the excellent efficacy and safety of Nuwiq® in PTPs and PUPs. Nuwiq® represents an important advance in the prevention and treatment of bleeds in patients with severe hemophilia A and can facilitate personalized treatment and reduce the risk of inhibitors.

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