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ORIGINAL ARTICLE *Clinical haemophilia*

Efficacy and safety of OBI-1, an antihaemophilic factor VIII (recombinant), porcine sequence, in subjects with acquired haemophilia A

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Summary. Acquired haemophilia A (AHA) is a rare bleeding disorder caused by autoantibodies against human factor VIII (hFVIII). OBI-1 is an investigational, B-domain deleted, recombinant FVIII, porcine sequence, with low cross-reactivity to anti-hFVIII antibodies. Efficacy can be monitored with FVIII activity levels in addition to clinical assessments. This prospective, open label, phase 2/3 study was designed to evaluate the efficacy of OBI-1 treatment for bleeding episodes in subjects with AHA. After an initial dose of 200 U kg^{-1}, OBI-1 was titrated to maintain target FVIII activity levels, in correlation with clinical assessments, throughout the treatment phase. All 28 subjects with AHA had a positive response to OBI-1 treatment 24 h after initiation despite inhibition of FVIII activity levels immediately after infusion in 10 subjects with baseline anti-porcine FVIII inhibitors. Control of the qualifying bleed was ultimately achieved in 24 of 28 subjects. No related serious adverse events, thrombotic events, allergic reactions or thrombocytopaenia occurred. The results of this study indicate that OBI-1 is safe and effective in treating bleeding episodes in subjects with AHA. The ability to safely and effectively titrate dosing based on FVIII activity levels in this study demonstrates that OBI-1 fulfils the unmet medical need to monitor the key coagulation parameter in AHA patients.

Keywords: acquired haemophilia A, bleeding episodes, recombinant FVIII porcine sequence, replacement therapy

Introduction

Acquired haemophilia A (AHA) is a rare bleeding disorder resulting from the formation of autoantibodies that inhibit the activity of endogenous human coagulation factor VIII (hFVIII) [1,2]. In approximately 50% of cases, autoantibody development is idiopathic [1,2]. Comorbidities, advanced patient age, location and severity of typical bleeding episodes have contributed to a historic mortality rate of 9–27% [3–5], decreasing in recent years to around 3% due to improved awareness and availability of effective haemostatic agents [6,7]. Bleeding often occurs spontaneously or in response to minimal injury to mucosal and deep soft-tissue or skin. Bypassing agents, the currently recommended first-line treatment [6,8], are limited due to the inability to monitor standard pharmacodynamic measures of coagulation (e.g. FVIII activity levels, activated partial thromboplastin time (aPTT), prothrombin time (PT)) [9]. Consequently, haemostatic efficacy of bypassing agents is determined mainly by clinical assessments alone, which can be challenging in bleeding events typical for AHA [10].

A modified FVIII that would not be inhibited by anti-hFVIII antibodies could provide an alternative to current treatments by allowing real-time monitoring

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of FVIII activity levels as an objective measure for evaluating haemostasis. Typically, anti-hFVIII inhibitors bind to the A2 and C2 domains of the hFVIII molecule. Porcine FVIII (pFVIII) has been used in patients with inhibitors due to the difference between the A2 and C2 domains of the pFVIII and hFVIII molecules (84% and 76% homologous with hFVIII respectively) [11]. A plasma-derived pFVIII (Hyate:C) was available on the market until 2004 when it was removed due to viral safety concerns [12–15].

A recombinant B-domain-deleted FVIII, porcine sequence (OBI-1) has been produced in a well-characterized baby hamster kidney (BHK) cell line and manufactured using two viral clearance steps to reduce the risk of potential pathogen transmission. Phase 1 and Phase 2 studies with OBI-1 demonstrated comparable pharmacokinetic parameters to the plasma-derived pFVIII [16] and a positive safety profile with efficacy in treating mild and moderate bleeds in patients with congenital haemophilia A with inhibitors [17,18]. Recently, management of bleeding with OBI-1 in one patient who developed AHA following stem cell transplantation, was described [19].

Presented here are the results of a prospective, multicentre phase 2/3 study that evaluated the efficacy and safety of OBI-1 for the treatment of serious bleeds in patients with AHA.

Methods

Study design

This prospective, phase 2/3 multicentre, international, open-label, single-cohort clinical study was conducted to assess the safety and efficacy of OBI-1 for bleed treatment in AHA patients. The trial (registered on clinicaltrials.gov: NCT01178294) was approved by relevant health authorities and ethics committees and conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice. An independent data and safety monitoring board (DSMB) reviewed the data at predefined times during the study. All subjects provided written informed consent.

Subjects

Male and female AHA patients aged ≥18 years suffering from a serious bleed (e.g. threatening vital organ function, requiring a blood transfusion, compromising muscle viability or neurovascular integrity, or impacting a major joint), were eligible for participation. Exclusion criteria included an anti–OBI-1 inhibitor titre >20 Bethesda Units (BU) and a platelet count <100 000 per μL. Prior treatment with recombinant activated FVII (rFVIIa) or activated prothrombin complex concentrate (aPCC) was not an exclusion criterion, provided that a 'washout period' was allowed (3 or 6 h respectively) before the initial OBI-1 infusion. A complete list of eligibility criteria is available on http://clinicaltrials.gov/ct2/show/record/NCT01178294.

Study product

OBI-1 is a glycosylated, B-domain deleted, recombinant FVIII, porcine sequence, produced in a well-characterized BHK cell line and manufactured using two viral clearance steps – solvent detergent and 15-nm nanofiltration [16,20].

Treatments

Treatment of the primary (qualifying) bleed was initiated with a single 200 U kg^{-1} dose of OBI-1; this dose level was determined based on previous experience in the Phase 2 study [17]. Subsequent OBI-1 dosing was assigned by the investigator based on clinical status and FVIII activity measured with either a standard one-stage clotting or chromogenic assay using the World Health Organisation human FVIII plasma standard. During the first 24 h, the target FVIII trough level (assessed 10–20 min pre- and post-infusion and repeated every 2–3 h post-infusion) was >80% for bleeds considered by the investigator to be of 'particular concern' (e.g. severe mucosal, intracranial, retro- or intra-abdominal, genitourinary, neck, traumatic, postoperative), and >50% for all other bleeds (e.g. joint, muscle, soft tissue). After the first 24 h, the OBI-1 dose was titrated to target post-infusion trough FVIII levels ≥50% for all bleeds.

Haemostatic efficacy assessment

OBI-1 efficacy assessments were performed throughout the patient's treatment phase using a well-defined, ordinal response scale based on FVIII activity levels and clinical assessments (Table S1). Evaluation criteria for bleeding 'effectively controlled' ('stopped' or 'significantly reduced'), by body system of anticipated bleed sites, are summarized in Table S2. Re-bleeding at a bleeding site deemed 'successfully controlled', that occurred within 2 weeks following the last OBI-1 dose, was considered a continuation of the same bleed.

Safety

Safety was evaluated through standard assessments (e.g. vital signs, physical examinations, clinical laboratory measurements). Adverse events (AE) were monitored, and relatedness to study drug was assessed.

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Antibody assessments

Neutralizing anti-hFVIII and anti-pFVIII antibodies were measured using the Nijmegen modification of the Bethesda assay [21] prior to treatment, at least every 5 days during treatment and at every follow-up visit up to 90 days post treatment. Primarily, central laboratory results prevailed for the analysis, however, when no central laboratory data were available, the local laboratory result was used. Non-neutralizing anti-FVIII antibodies were not assessed. Anti-BHK cell antibody levels were determined by ELISA before treatment and, at a minimum, at the last follow-up visit.

Sample size and statistical methods

This study was designed to evaluate 28 qualifying bleeds in at least 20 unique subjects. Assuming a positive response rate (R) at 24 h after initiation of 80%, a baseline low-response rate of 50%, and a two-sided alpha of 0.05, a study of 28 bleeds would have in excess of 90% power to test the null hypothesis that R = 50%, against the alternative hypothesis that R > 50%. Efficacy was expressed by the percentage (and 95% Clopper–Pearson confidence interval) of subjects with bleeds with a positive response to OBI-1 treatment at 24 h. Magnitude and statistical significance of the dose adjustment were determined using non-parametric statistics (signed rank test).

Results

Study population

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Twenty-nine subjects were enrolled and treated with OBI-1 (Figure S1) (19 male, 10 female; median age 70 years, Table 1). Significant comorbidities were identified in 13 subjects: six subjects had malignancies, three suffered from an autoimmune disorder, three from infections and one subject with an autoimmune disorder also had a malignancy. For 16 subjects, no underlying disease was identified. At screening, FVIII activity levels ranged from 0 to 30% (median: 3%, Table 1). Eleven subjects received haemostatic agents within 1 month prior to treatment with OBI-1: seven subjects reported receiving rFVIIa, three received aPCC and three received tranexamic acid. All subjects received immunosuppressive therapy during the study: corticosteroids alone or with cyclophosphamide or rituximab.

The diagnosis of AHA was based in part on anti-hFVIII antibody levels prior to treatment. One subject had a neutralizing anti-hFVIII antibody titre as determined before treatment at the local laboratory (baseline FVIII activity level: 30%) and began OBI-1 treatment. However, central laboratory results did not confirm the local laboratory result (Figure S1). This subject was therefore excluded from the efficacy

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analyses (n = 28). Among patients with AHA, the median anti-hFVIII neutralizing antibody titre prior to treatment was 31 BU (Table 1). Ten subjects had measurable levels of anti-pFVIII antibodies prior to treatment (median: 4 BU). The mean cross-reactivity of the inhibitory antibody to pFVIII vs. hFVIII at baseline among AHA patients was 8.7%; 64% of subjects had no measurable cross-reactivity. For all subjects, the date of first diagnosis of AHA excluded the possibility of a prior exposure to a plasma-derived pFVIII.

Efficacy within 24 h after infusion and haemostatic efficacy

The response to OBI-1 treatment at 24 h was used to predict eventual bleed control. At 24 h after the first OBI-1 infusion, all 28 subjects with AHA (100–95% CI: 88.1–100) had a positive response to treatment of the primary bleed according to predefined criteria (Table S1), and the majority showed a positive treatment response within 8 h post infusion (Fig. 1a).

Control of the primary bleed at the time of final treatment dosing (deemed 'successful treatment') was achieved in 24 of 28 (85.7%) subjects with AHA (Fig. 1b). Control of the primary bleed was achieved in 16 [illegible] subjects whose bleed was treated 'first-line' with OBI-1 and in 8 of 11 (73%) subjects whose bleed had first been treated with another haemostatic agent (Fig. 1b).

Four subjects were withdrawn from the study after the 24-h assessment and therefore the overall control of the primary bleed at the last OBI-1 dose was not assessed as successful. One subject, whose primary bleed was controlled, discontinued due to lack of efficacy in controlling a third bleed (Subject 7); one subject was withdrawn from medical support by family due to a potential haemorrhagic conversion/extension of the initial neurological insult (Subject 8); after four successfully treated bleeds, one subject suffered from sepsis following emergent endoscopic retrograde cholangiopancreatography for biliary decompression and stent replacement (Subject 15); one subject discontinued due to the development of anti-pFVIII antibodies after successful control of the primary bleed (Subject 18).

Amount of OBI-1 required to treat the primary bleed

In the 24 subjects with AHA whose primary bleed was ultimately controlled, the median cumulative OBI-1 dose administered in the first 24 h post infusion was 458.7 U kg^{-1} with 3.5 infusions (median) at an interval of 7.4 h between doses (median, Table 2). The median dose after the first 24 h of treatment until bleed control (100 U kg^{-1}) was substantially lower than the initial dose of 200 U kg^{-1}: the median dose

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Table 1. Demographic and baseline characteristics for all patients treated with OBI-1.

Subject	Age (years)/ Gender	Primary bleed site	Baseline anti-hFVIII (BU)	Baseline anti-pFVIII (BU)†	Cross-reactivity (%)¶	Baseline FVIII activity level** (%)
1	82/F	Haematoma (R arm)	5	Not detectable	0	1
2	43/M	Compartment syndrome (R arm)	12*	Not detectable*	0	18
3	90/M	Haematoma (R shoulder)	20	10	50.0	1
4	68/M	Periorbital bleed (R)	46	Not detectable	0	29
5	69/M	Haematoma (L arm)	51	Not detectable	0	22
6	66/M	Haematoma (R hand)	80	Not detectable	0	1
7	74/F	Compartment syndrome (R thigh)	142	3	2.1	0
8	70/F	Haematoma (subdural)	5*	Not detectable*	0	29
9	66/M	Soft tissue & PICC site (R arm)	65	Not detectable	0	1
10	86/M	Surgical incision (L arm)	17	Not detectable	0	9
11	79/F	Haemorrhage (retro-peritoneal)	651	4	0.6	1
12	81/F	Haematoma (R pelvic)	21	29§	138.1	0
13	81/M	Haematoma (R thigh)	33	1	3.0	0
14	61/M	Haematoma (R hip)	4	Not detectable	0	5
15	87/F	Scheduled surgery (melaena)	80	Not detectable	0	NA
16	84/F	Haematoma (neck)	98	3	3.1	1
17	70/M	Haematoma (R arm)	203	Not detectable	0	6
18	61/M	Surgical Haemicolectomy	26	Not detectable	0	7
19	51/M	PEG tube insertion (stomach)	24	Not detectable	0	7
20	63/M	Haematoma & PICC line (R arm)	87	10	11.5	1
21	64/F	Haematoma (L arm)	11*	Not detectable	0	3
22	79/F	Haemarthrosis (L knee)	10	Not detectable	0	14
23	82/F	Tracheotomy	29	4	13.8	6
24	84/M	Haematoma (R thigh)	21	0.8	3.8	1
25	54/M	Haematoma (L ankle)	49	Not detectable	0	2
26	51/M	Intracranial bleed	Not detectable	Not detectable	Not applicable	30
27	61/M	Haematoma (hip)	10	Not detectable	0	3
28	42/M	Haematoma (hip)	79	15	19.0	4
29	76/M	Haematoma (hip)	467	Not detectable	0	1

hFVIII, human FVIII; pFVIII, porcine FVIII; BU, Bethesda units; NA, not available.
*Local laboratory result used in absence of central laboratory.
†'Not detectable' was defined as anti-OBI-1 titre <0.6 BU.
‡Subject did not meet the inclusion criteria of diagnosed AHA.
§Subject did not meet the inclusion criterion of anti-pFVIII antibody titre ≤20 BU yet the subject responded positively to OBI-1 treatment prior to the anti-pFVIII antibody level being available and was allowed to complete the study.
¶Percent cross-reactivity: Proportion of anti-pFVIII to anti-hFVIII antibodies = (anti-pFVIII titre/anti-hFVIII titre) ×100.
**Local laboratory results.

reduction between the initial dose and the subsequent median dose in the treatment phase was 41.2% (Q1–Q3: 0–50.0%; $n = 26$, $P < 0.01$, Table S4). Furthermore, between the first 24 h of treatment, and all subsequent 24-h periods of treatment, the median dose reduction was 65.4% (Q1–Q3: 47.4–79.9; $n = 24$, $P < 0.0001$, Table S4). Throughout the treatment phase, total dose infused, dose per infusion, and exposure duration (all median values) were 1580 U kg^{-1}, 116.5 U kg^{-1}, and 6.5 days respectively (Table 2). Individual exposure data within the first 24 h of treatment are provided in Table S3.

FVIII activity levels

After the initial 200 U kg^{-1} dose, further dose and regimen decisions were based on the maintaining of target FVIII activity trough levels. The median rise in FVIII activity immediately post loading dose was 203% (Table 3). The highest FVIII activity measured in the first 24 h of treatment was 255% (median). At 24 h post-infusion (time-point selected to assess primary response to treatment), the median FVIII activity was 108%, demonstrating the ability to monitor FVIII activity and subsequently titrate dose and regimen to achieve clinically relevant FVIIII activity levels.

Haemostatic efficacy in subjects with anti-pFVIII antibodies

Immediately after the first OBI-1 dose, the rise in FVIII activity levels in most of the subjects with baseline anti-pFVIII inhibitor was below 100% (Fig. 2a). With repeated OBI-1 dosing, all subjects with baseline

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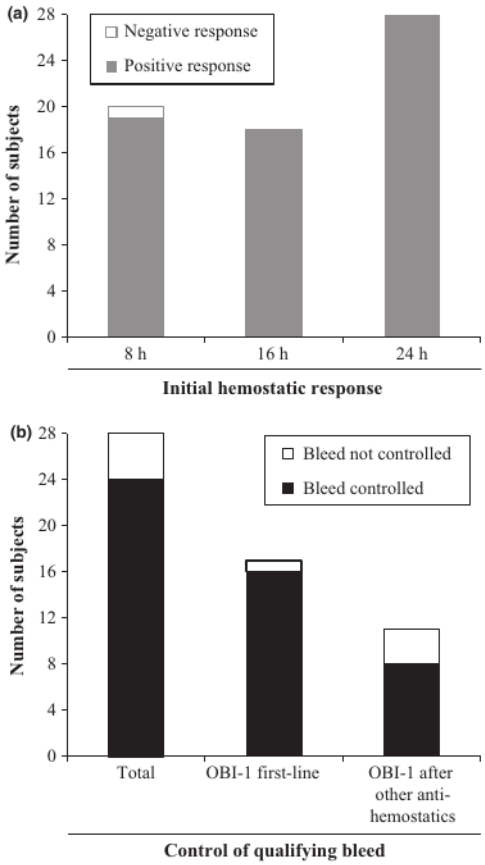

Fig. 1. Initial haemostatic response to OBI-1 treatment and successful control of bleeding event. (a) Positive response to OBI-1 treatment, defined as effective or partially effective control of bleeding (see also Table 1), was assessed at 8, 16 and 24 h after the first infusion. Not all subjects were assessed at every time point. (b) Successful treatment outcome was defined as the bleed being controlled at the time of final treatment dosing. Treatment success data are presented overall, for the total subject population with AHA whose presenting bleed was successfully controlled, for those AHA subjects who received OBI-1 as 'first-line' treatment (i.e. no haemostatic agents reported immediately prior to the first OBI-1 treatment) and for AHA subjects whose bleeding episode was treated with other haemostatic agents prior to OBI-1. Four subjects discontinued prematurely and the final treatment outcome of the primary bleed was not assessed.

pFVIII inhibitors achieved a rise in FVIII activity greater than 100% within the first 24 h after treatment (Fig. 2b) and all had a positive treatment response at 24 h (Table 4).

All subjects received immunosuppressive therapy to treat their underlying autoimmune disease during the study. Among the subjects who had anti-pFVIII inhibitors at baseline, 2 of 10 subjects continue to exhibit detectable anti-pFVIII inhibitors at the final assessment (Table 4). In one subject the primary bleed and a subsequent bleed were controlled, a third bleed was not (Subject 7); in the other subject, control of the primary bleed was achieved (Subject 28). Five subjects developed *de novo* anti-pFVIII antibodies 8, 18, 22, 35 and 85 days after the first infusion (Table 4). Bleed control was not assessed as successful in two of those subjects: one subject died of cholangitis and sepsis after a fourth bleed had been controlled (Subject 15); another subject (Subject 18) experienced a fatal re-bleed at the qualifying site 10 days after discontinuation from OBI-1 (Table S5).

Safety

No related serious AEs occurred. Positive anti-pFVIII inhibitor test results led to study discontinuation in two subjects and were considered to be non-serious AEs related to OBI-1 in accordance with predefined criteria. No subjects developed anti-BHK antibodies and no thrombotic events, thrombocytopaenia or hypersensitivity reactions related to OBI-1 were observed. Seven deaths occurred during the study: three cases of sepsis; three deaths following haemorrhages (one intestinal and two intracranial) and one case of renal failure in a subject with a history of renal insufficiency (Table S5). Although 3 of 7 deaths were due to bleeding, nor to study treatment or due to ent. Each case was also assess MB (Table S5). Two of these three subjects were receiving a haemostatic treatment other than OBI-1 at the time of death: one subject (Subject 16), with a history of hypertension was administered rFVIIa to stop an intracranial haemorrhage; another subject (Subject 18), withdrawn from OBI-1 treatment due to pFVIII inhibitor development, was treated with aPCC for a re-bleed at the primary site.

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3 deaths from 28 patients = 10.7%

1. **Although 3 of 7 deaths were due to bleeding, Anchor Name: 10.7% mortality rate** *[Agency W2O - Claudia Alves]*

Discussion

A recombinant porcine FVIII such as OBI-1 could offer a novel option for the treatment of AHA as it is less susceptible to inactivation by hFVIII inhibitors and facilitates objective assessment of safety and efficacy by enabling real-time monitoring of FVIII activity levels.

Bypassing agents, the recommended first-line treatment to control bleeds in AHA patients [6,8], are associated with a risk of thromboembolic events in a population with significant comorbidities and enhanced risk of cardiovascular disease [7]. Bypassing agent efficacy cannot be monitored through routine laboratory assessments [9], nor predicted by validated laboratory assays. Therefore, clinicians rely on subjective clinical observation alone to determine haemostatic efficacy [22]. In contrast, objective FVIII

Table 2. Exposure to OBI-1 throughout the study.

	Within first 24 h of treatment initiation*				After first 24 h of treatment initiation to bleed controlled*				From treatment initiation until bleed controlled*				Overall exposure			
Statistic	Total dose (U kg^{-1})	Median dose per subject (U kg^{-1})	Dosing interval (h)	Number of infusions	Total dose (U kg^{-1})	Median dose per subject (U kg^{-1})	Exposure days	Number of infusions	Total dose (U kg^{-1})	Median dose per subject (U kg^{-1})	Exposure days	Number of infusions	Total dose (U kg^{-1})	Median dose per subject (U kg^{-1})	Exposure days	Number of infusions
N	24	24	20†	24	21‡	21‡	21‡	21‡	24	24	24	24	29	29	29	29
Mean	741.1	192.8	10.2	3.5	2057.5	133.3	6.1	13.3	2541.4	143.1	6.6	15.2	3123.0	145.8	7.1	18.9
SD	616.4	82.9	6.6	2.0	2529.5	84.4	4.6	11.6	2902.7	80.0	4.7	12.9	4365.9	78.8	5.6	26.2
Median	458.7	200.0	7.4	3.5	1050.0	100.0	6.0	11.0	1580.0	116.5	6.5	12.5	1637.0	133.0	7.0	13.0
Min,	100,	88,	3,	1,	100,	34,	1,	1,	100,	34,	1,	1,	100,	34,	1,	1,
Max	2100	400	23	7	10 394	400	22	51	12 194	400	23	57	20 660	400	25	140

*OBI-1 exposure in AHA subjects whose primary bleed was controlled ($n = 24$).
†Four subjects received only one dose within the first 24 h of treatment (see Table S3).
‡Three subjects did not receive any infusion to treat the primary bleed after 24 h.

measurements can allow the clinician to assess haemostasis in bleeds that cannot be monitored clinically, and to adjust the treatment regimen accordingly or proceed with alternative therapeutic options. Unlike bypassing agents, OBI-1 replaces the missing coagulation protein and enables measurement of FVIII activity using readily available standard FVIII assays, thereby, guiding dosing and enhancing treatment efficacy and safety. In this study, the initial OBI-1 dose (200 U kg^{-1}), which was selected based on results of the Phase 2 study [17], achieved 'supra-physiologic' postinfusion FVIII activity levels, nonetheless, no related thrombotic event was reported in the initial 24 h of treatment or afterwards. The total dose of OBI-1 required to achieve control of bleeding in this study was highly variable. Dose adjustment was effective throughout the treatment as indicated by a significant dose reduction between the first 24-h period and the subsequent treatment periods. These findings support the concept of individually tailored dose regimens of OBI-1 through monitoring of FVIII activity.

The primary bleed was deemed to have been treated successfully in 86% of subjects in this study. A higher rate of treatment success was achieved among subjects receiving OBI-1 as 'first-line' therapy in comparison with subjects treated with another haemostatic agent prior to OBI-1 treatment. The difficulty in assessing the efficacy beyond the first 24 h is evident; in 4 of 28 subjects the investigator was unable to assess the initial qualifying bleed for eventual successful treatment because patients were prematurely withdrawn. Nonetheless, in most cases, a positive response to treatment at the 24-h assessment was consistent with eventual bleed control.

Similar to observations in AHA patients receiving a plasma-derived pFVIII [23], anti-pFVIII antibodies were detected prior to OBI-1 infusion in 35.7% of AHA patients in the present study. Baseline anti-pFVIII titres have been attributed to cross-reactive anti-hFVIII inhibitors that also inhibit pFVIII, as previously reported [24,25]. Pretreatment anti-pFVIII antibodies appear to have affected the immediate FVIII activity level rise after the first dose, particularly if the titre was greater than 5 BU. However, therapeutic FVIII activity levels could be achieved using a tailored dosing regimen even in the presence of high inhibitor titres. These findings are consistent with previous reports, recommending continuation of plasma-derived pFVIII treatment despite the presence of pFVIII inhibitors if incremental FVIII activity and clinical response are indicative of efficacy [26,27].

While the development of anti-pFVIII inhibitors is a potential complication of therapy, no correlation has been reported between the presence of baseline anti-pFVIII antibodies and treatment efficacy [23,27]. In this study, *de novo* anti-pFVIII antibodies were observed within 8–85 days following the first OBI-1

Table 3. Individual FVIII activity levels and assessment of response to OBI-1 in AHA patients.

	FVIII activity levels* (%)				
Subject	Pre-dose	Immediate post -first dose	Highest in 24 h	At 24 h‡	Response assessment at 24 h
1	1	258	258	46	Partially effective
2	18	540	601	352	Effective
3	1	38	248	169	Effective
4	26	426	426	270	Effective
5	22	270	270	106	Effective
6	1	224	224	74	Effective
7	0	76	246	220	Partially effective§
8	29	200	200	98	Partially effective§
9	1	119	356	280	Effective
10	9	417	417	184	Effective
11	1	77	340	91	Effective
12	0	20	297	74	Effective
13	0	73	231	231	Effective
14	5	163	171	105	Effective
15	NA	195	230	86	Effective§
16	1	116	213	61	Effective
17	6	163	173	98	Effective
18	7	296	296	156	Effective§
19	7	240	240	70	Effective
20	1	22	160	2	Effective
21	3	288	369	369	Partially effective
22	14	439	439	221	Effective
23	6	209	209	64	Effective
24	1	158	410	109	Effective
25	2	206	252	114	Effective
27	3	401	401	111	Effective
28	4	72	173	26	Effective
29	1	342	775	227	Effective

NA, not available.
*Local laboratory result used in absence of central laboratory.
†Rise in FVIII activity level immediately post first dose = FVIII activity immediately post first dose – FVIII immediately prior to first OBI-1 dose.
‡Time point selected for assessment of initial treatment response.
§Subjects withdrawn from study before assessment of overall bleeding control of the qualifying bleed could be determined.

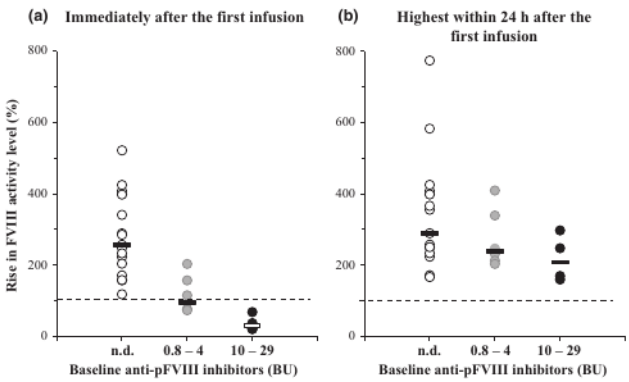

Fig. 2. Effect of baseline anti-pFVIII antibodies on FVIII activity level increase upon OBI-1 treatment in AHA patients. (a) Rise in FVIII activity over baseline immediately after the first OBI-1 dose in subjects with no detectable anti-pFVIII antibody titre [n.d.; n = 18, median (range): 257 % (118–522)], and in subjects with low [0.8–4 BU; n = 6; median (range): 96 % (73–203)] or high [10–29 BU; n = 4; median (range): 29 % (20–68)] anti-pFVIII antibody titre. (b) Rise in FVIII activity over baseline at 24 h after the first OBI-1 dose in subjects with no detectable anti-pFVIII antibody titre[n.d.; n = 18, median (range): 289 % (166–774)], and in subjects with low [0.8–4 BU; n = 6; median (range): 239 % (203–409)] or high [10–29 BU; n = 4; median (range): 208 % (159–297)] anti-pFVIII antibody titre. Rise in FVIII activity = (FVIII activity level at indicated time−baseline FVIII activity). Dotted line indicates 100% FVIII activity. Solid horizontal bar indicates median FVIII activity rise for each subject group.

[no notes on this page]

Table 4. Subjects with neutralizing antibodies against porcine FVIII.

	Anti-pFVIII					Anti-hFVIII	
Subject	Baseline (BU)	First detected after first infusion (BU) [days after first infusion]	Peak after treatment (BU)	Final (BU)	Response assessment at 24 h	Baseline (BU)	Final (BU)
	With baseline inhibitors						
3	10	N/A	2	Not detectable	Effective	20	Not detectable
7	3	N/A	6	6	Partially effective*	142	77
11	4	N/A	Not detectable	Not detectable	Effective	651	98
12	29	N/A	Not detectable	Not detectable	Effective	21	Not detectable
13	1	N/A	Not detectable	Not detectable	Effective	33	Not detectable
16	3	N/A	Not detectable	Not detectable	Effective	98	49
20	10	N/A	Not detectable	Not detectable	Effective	87	0.6
23	4	N/A	3	Not detectable	Effective	29	0.6
24	0.8	N/A	1†	Not detectable	Effective	21	Not detectable
28	15	N/A	183	183	Effective	79	47
	With *de novo* inhibitors						
6	Not detectable	28 [35 days]	51	51	Effective	80	Not detectable
15	Not detectable	0.6 [85 days]	0.6	0.6	Effective*	80	NA
18	Not detectable	8† [8 days]	8†	8†	Effective*	26	128†
19	Not detectable	22† [18 days]	108	108	Effective	24	169
27	Not detectable	1 [22 days]	166	42	Effective	10	0.07

BU, Bethesda units; NA, not available; N/A, not applicable.
'Not detectable', is defined as titre <0.6 BU.
*Subjects without control of bleeding.
†Local laboratory result used in absence of central laboratory.

dose in 5 of 28 subjects. As two of these subjects had a concomitant increase in anti-hFVIII antibody titres, the observed anti-pFVIII titres may be attributable to either: (i) an immunogenic response to pFVIII treatment, or (ii) an increase in cross-reactive anti-hFVIII antibodies. Another two of five subjects had a concurrent decrease in anti-hFVIII antibody titres, suggesting that the observed increase in anti-pFVIII titre resulted from an immunogenic response to pFVIII. Thus, the immunosuppressive therapy used to treat the underlying autoimmunity appears not to have consistently suppressed the alloimmune response.

Safety analyses raised no concerns with OBI-1 treatment in AHA patients. There were no related serious AEs, and only two non-serious AEs related to treatment. The seven deaths were not considered to have been associated with OBI-1 or lack of efficacy to control the qualifying bleed based on a review by the DSMB. Plasma-derived pFVIII has been associated with hypersensitivity and thrombocytopaenia especially with high doses or prolonged treatment, due presumably to contamination with porcine von Willebrand factor [12–15]. In our study, no hypersensitivity reactions, and no treatment-related thrombocytopaenia occurred, as OBI-1, a highly pure recombinant FVIII, does not contain von Willebrand factor.

In summary, results of this study support findings that OBI-1 is sufficiently similar to hFVIII to support haemostasis yet different enough in structure to render it less susceptible to inactivation by hFVIII inhibitors [13,16,18]. The ability to measure and monitor increments in FVIII activity with OBI-1 could afford the treating physician an objective surrogate measure of safety and efficacy, indicating that OBI-1 would be an important treatment option in patients with AHA.

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Author contribution

E.G. and R.K-.J. contributed to study conception and design; E.G. and H.F. supervised the research; H.F., A.N., R.K-.J, A.S, P.C, B.E. and C.B. wrote the manuscript; R.K.-J., J.St-L, A.G., A.S., H.S., P.C. and A.D. contributed to study conduction, as well as acquisition and interpretation of data; H.F., A.N., M.M. B.E. and C.B. interpreted the data; M.M. performed the statistical analysis; and all authors reviewed the manuscript and approved the final version.

Disclosures

R.K.-J. served as an investigator in the trial and received honoraria from Baxter for consultation work. J.St-L. and A.G. served as investigators and declare not having received honoraria. A.D., A.S. and H.S declare having no conflict of interest. P.C. served as an investigator in the trial and declares receiving honoraria from Baxter, Bayer, Biogen, CSL Behring, Novo Nordisk and Pfizer; served on advisory boards for Baxter, Biogen, CSL Behring, Novo Nordisk, Pfizer; and received funding from CSL Behring, Novo Nordisk and Pfizer. B.E., H.F., A.N., M.M. and C.B are employees of Baxter. E.G. is a former Baxter employee and a consultant to Baxter.

[no notes on this page]

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Efficacy assessment criteria.

Table S2. Investigator assessment of control of bleeding by body system of anticipated sites of bleeding.

Table S3. Exposure per subject within the first 24 hours (n = 28).

Table S4. Dose reduction from initial dose and from the first 24- hour period to treat primary serious bleed in AHA patients.

Table S5. Subject deaths.

Figure S1. Subject enrolment and disposition