*Original article*

# Efficacy and safety of von Willebrand factor concentrate almost devoid of factor VIII (Wilfactin®) in paediatric patients under 6 years of age with severe von Willebrand disease

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**Background** - Plasma-derived von Willebrand factor (VWF) (Wilfactin®, LFB, France) was developed for prophylaxis and treatment of haemorrhages in both adults and adolescents with von Willebrand disease (VWD). Replacement therapy in paediatric patients is a key element of the clinical trial programme.
**Material and methods** - Patients aged <6 years with severe VWD were enrolled in a multinational, open-label study to evaluate the in vivo recovery for Wilfactin®, and its efficacy in preventing and treating bleeding episodes and during surgery. Overall haemostatic efficacy based on a 4-point scale was assessed by investigators. The treatment period ≥18 months investigated the long-term safety.
**Results** - Nine patients, including 7 with type 3 VWD were exposed to treatment with Wilfactin® for up to 4.2 years. Recovery of VWF in 7 patients (n=5 type 3, n=1 type 2, n=1 type 1) was 1.8±0.4 IU/dL *per* IU/kg. Of the 62 bleeds, 89% were controlled with one (73%) or two (16%) infusions of Wilfactin®. The median dose *per* infusion was 54 IU/kg. A factor VIII dose was co-administered in 1.6% of bleeds. "Excellent"/"Good" haemostatic efficacy was achieved in 90.3% of episodes. Six patients underwent 11 minor surgical interventions. Treatment duration was 1 day (range: 1-6 days) with a dose administered 30-60 minutes before procedure of 56 IU/kg (range: 41-106 IU/kg). Haemostasis was rated as "Excellent" in all surgeries. During 4-year prophylactic treatment in one patient, breakthrough bleeds were reported in 2.2% of infusions. No VWF inhibitors, thromboembolic events or allergic/anaphylactic-type reactions were observed following a total exposure of 770 days.
**Discussion** - The results show that Wilfactin® provides a safe and effective treatment in patients <6 years of age with severe VWD.

**Keywords:** *von Willebrand disease, paediatrics, bleeding, surgery, Wilfactin®.*

## INTRODUCTION

Von Willebrand disease (VWD), a congenital bleeding disorder, is caused by quantitative deficiency (types 1 and 3) or qualitative (type 2) defect of von Willebrand factor (VWF), a plasma protein that mediates the initial adhesion of platelets at sites of vascular injury and the stabilisation of factor VIII (FVIII) in the circulation[1,2]. Therefore, defects in VWF

can cause bleeding by impairing platelet adhesion and/or by reducing the concentration of FVIII. Type 1 is the most common form, and patients have mild bleeding symptoms. Type 2 has a variable impact on bleeding symptoms[3]. Type 3, the rarest form which accounts for 1-5% of cases, is characterised by the virtual absence of VWF and very low FVIII plasma levels (usually <5 IU/dL)[4]. Patients with type 3 VWD experience frequent episodes of mucocutaneous bleeding, as well as musculoskeletal bleeds with occurrence of spontaneous haemarthrosis and/or soft tissue haemorrhages. These patients are usually diagnosed in childhood, as they experience severe bleeding complications from an early age. Patients with less severe defects in VWF and FVIII are often identified later; some of them may only experience bleeding after surgical procedures such as tonsillectomy or tooth extraction[5]. Patients who are unresponsive to desmopressin treatment, or in whom desmopressin is contraindicated, have to be treated by replacement therapy.

In the context of the advent of specific plasma fractionation techniques, Wilfactin® (called Willfact® in some countries) (LFB-BIOMEDICAMENTS, Les Ulis, France) is a highly purified plasma-derived VWF (pdVWF) almost devoid of FVIII and containing high molecular weight (HMW) multimers with a distribution similar to normal plasma[6]. The percentage of HMW forms relative to plasma for 27 batches produced between 1998 and 2002 was 78±14% for multimers ≥10 mers and 60±18% for multimers ≥15 mers[7]. The viral inactivation/removal is achieved by three dedicated steps: solvent/detergent treatment, dry heating (72 hours at +80°C), and nanofiltration (35 nanometers). The choice of pure pdVWF product is a therapeutic option for treating patients with VWD, as they have normal FVIII synthesis[8]. Intravenous administration of the product immediately corrects VWF levels and triggers subsequent correction of FVIII a few hours later. Therefore, if an immediate haemostatic control is required in patients with low FVIII levels, a dose of FVIII has to be administered with the first infusion of Wilfactin®. Efficacy and safety of this concentrate have been previously reported in adult/adolescent patients during an extensive clinical trial programme involving more than 50 patients. The product has been approved for the clinical management of patients with VWD[9,10]. According to European guidelines on the clinical investigation of pdVWF products (CPMP/BPWG/220/02), data from paediatric patients aged <6 years represent a key element of the clinical development programme[11]. This phase III study was performed to further assess the *in vivo* recovery, efficacy and safety of Wilfactin® in paediatric patients with severe VWD. The study was registered in Eudra CT (n. 2004-005051-34).

## MATERIALS AND METHODS

### Clinical study design

This prospective, multinational, multicentre, open-label, non-controlled phase III study was set up to evaluate the efficacy and safety of Wilfactin® for the prevention and treatment of bleeding episodes, and in surgery in paediatric patients under 6 years of age. The study was conducted from 2006 to 2014. Patients were enrolled from five sites: Tunisia (4 patients from 1 site), Poland (3 patients from 2 sites), Belgium and Greece (1 patient from 1 site each). In addition, the study was designed to evaluate baseline and control incremental recoveries. Efficacy and safety parameters were monitored and assessed for at least 18 months after the first treatment. Vials of 1,000 international units (IU) of VWF: ristocetin cofactor activity (VWF:RCo) in 10 mL of reconstituted product were used in this study. A bleed was considered major if it leads to a decrease in haemoglobin levels by more than 2 g/dL or if it represented a life-threatening condition (e.g., intracranial or gastrointestinal haemorrhage); all other episodes were considered minor. Major surgical procedure was defined as one with a high risk of haemorrhage according to the investigator's opinion. All other surgeries were considered minor.

### Patients' characteristics

Previously-treated or treatment-naïve patients aged <6 years with severe inherited VWD (VWF:RCo <20 IU/dL) were eligible. Exclusion criteria included present or past inhibitor activity against FVIII and/or VWF:RCo, platelet count <100×10⁹/L (except for type 2B VWD) or confirmed kidney and liver disease. Parents/legal guardians of all children enrolled in the study provided written informed consent.

### Dosing

For on-demand treatment of acute bleeding episodes, surgical and invasive procedures, the first dose was based on individual recovery with the aim of reaching the target VWF:RCo level of 100 IU/dL. This dose was calculated

**Table I** - *Dosing recommendations during the paediatric study*

| Indications | Target VWF:RCo plasma level | Wilfactin® dosing regimens | Co-administration of FVIII |
|---|---|---|---|
| **Acute bleeding episodes** | | | |
| **All bleeds** | Peak: 100 IU/dL | 1st dose as soon as possible after the onset of bleeding, based on recovery determination (otherwise 60-100 IU/kg) | 20-40 IU/kg at 1st Wilfactin® dose to immediately reach haemostatic FVIII:C levels (40 IU/dL) if needed |
| **Minor** | Not stated | Subsequent dose if needed 12 or 24 h later | |
| **Major†** | Trough: ≥60 IU/dL | Subsequent doses every 12-24 h for 3-4 days | |
| | then ≥40 IU/dL | until the risk of haemorrhage was no longer present | |
| **Surgical and invasive procedures** | | | |
| **All surgeries** | Peak: 100 IU/dL | 1st dose 30-60 min (loading dose) before procedure based on recovery determination (otherwise 60-100 IU/kg) | 20-40 IU/kg at 1st Wilfactin® dose to immediately reach haemostatic FVIII:C levels (40 IU/dL) if needed § |
| **Minor** | Trough: ≥40 IU/dL | Subsequent doses every 12-24 h for 2-4 days or until complete healing | |
| **Major‡** | Trough: ≥60 IU/dL | Subsequent doses every 8-12 h for 3-4 days | |
| | then ≥40 IU/dL | for at least 7 additional days until complete healing | |
| **Invasive procedures** | Peak: 100 IU/dL | One dose 30-60 min (loading dose) before procedure based on recovery determination (otherwise 60-100 IU/kg) | |
| **Prophylaxis** | Not stated | By stepwise escalation according to a dosing regimen ranging from 50 IU/kg once *per* week to 30 IU/kg every other day with a potential adjustment every 3 months or as needed according to clinical outcome | No co-administration |

†Major bleeding episode was defined by a decrease in haemoglobin levels by more than 2 g/dL or a life-threatening condition. ‡Major surgical procedure was defined as that with a high risk of haemorrhage according to the investigator's opinion. §Alternatively, in case of non-urgent procedure, it was possible to start the VWF treatment the day before surgery (12-24 h prior to the procedure) allowing the natural increase of patient's endogenous FVIII:C levels and to proceed with the pre-operative dose of Wilfactin® 30-60 min before the procedure.
Min: minutes; H: hours; FVIII:C: factor VIII:coagulant activity; IU: International Unit; VWF: von Willebrand factor; VWF:RCo: VWF:ristocetin cofactor activity.

according to the formula: *dose (IU) = body weight (kg) × desired VWF:RCo rise (IU/dL) / individual incremental recovery at 15 min (IU/dL per IU/kg)*.

If no recovery data was available, a dose of 60-100 IU/kg was recommended; subsequent doses were applied according to medical needs. When an immediate rise in FVIII was needed to reach haemostatic levels (≥40 IU/dL) in patients with low basal FVIII, a factor VIII dose (20-40 IU/kg) was co-administered with the first VWF infusion. In patients entering long-term prophylaxis (LTP), dosing started with an escalation schedule (**Table I**).

In the recovery assessment performed at study entry (baseline recovery), patients were administered a single dose of 100 IU/kg. The administered dose was aligned to the dose given in adults during previous pharmacokinetics studies[9]. After at least six months of follow up, a second recovery evaluation (control recovery) was only performed for patients with type 3 VWD. Blood samples were taken pre-dose and 15 minutes after the end of infusion to measure VWF levels in a central laboratory (Caen, France). Incremental *in vivo* recovery was determined for

activity and antigen. The VWF:RCo was run in an optical aggregometer (APACT 4004 analyser; Helena Laboratories, Beaumont, TX, USA), using lyophilised human normal stabilised platelets (VW Reagent; Siemens, Marburg, Germany), and 1 mg/mL ristocetin. Measurements of VWF antigen (VWF: Ag) were performed using automated latex immunoassay reagent (STA® - LIATEST® VWF: Ag) and automatic coagulometer (STA-R®) by Diagnostica Stago, Asnières-sur-Seine, France.

**Efficacy assessments**

The primary efficacy endpoint was the overall rating of haemostatic efficacy by the investigator using a 4-point scale (Excellent, Good, Moderate, None) at the last infusion to control bleeding episodes and at hospital discharge for surgical and invasive procedures (**Table II**). Efficacy of the prevention of bleeding during LTP was estimated by the number of breakthrough bleeding episodes occurring over the study period.

The patients had a check-up with the investigators every three months until the end of the study. All events were documented in detail, including date and type of clinical

**Table II** - *Haemostatic efficacy rating scale*

| Rating | Efficacy rating criterion | |
|---|---|---|
| | **Bleeding episodes** | **Surgical and invasive procedures** |
| **Excellent** | • Bleeding stopped immediately | • Normal haemostasis |
| **Good** | • Bleeding stopped within the expected time period | • Mild bleeding at surgery site |
| **Moderate** | • Bleeding controlled with difficulty | • Moderate, but controlled bleeding |
| **None** | • Bleeding not controlled | • Severe, uncontrolled bleeding |

event, time elapsed between bleeding and treatment, dosage of products, and concomitant treatments such as possible transfusions. In case of surgery, perioperative blood loss was also documented.

### Safety assessments

The secondary endpoints of the study were the safety assessments. Every three months, standard laboratory parameters (complete blood count, alanine aminotransferase, aspartate aminotransferase) were assessed locally. Inhibitors to VWF:RCo and FVIII: C, and non-neutralising anti-VWF-binding antibodies (assessed using a sandwich enzyme-linked immunosorbent assay ELISA[12]) were monitored in two central laboratories (Caen, France and Lille, France). Regarding viral infections, blood samples were drawn at baseline and at the end of the study for viral infection markers (antibodies to human immunodeficiency virus [HIV], hepatitis C virus [HCV], hepatitis A virus [HAV] (IgG, IgM), Parvovirus B19 (IgG, IgM), hepatitis B virus [HBV] (anti-HBs/anti-HBc and HBV surface antigen [HBsAg]). Safety was also evaluated through clinical assessments, including allergic/anaphylactic-type reactions. The occurrence of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), and their relationship with the treatment were also recorded.

### Statistical analysis

Statistical analysis was descriptive for all the study endpoints. No hypothesis was tested. Undetectable pre-dose levels of VWF:RCo and VWF: Ag measured the day of the recovery study were set to zero for type 3 and decreased by 0.5 for other types of VWD. The efficacy and safety analysis included all patients who received at least one treatment dose. The success rate was given with a confidence interval (CI) of 95% using an exact binomial method.

## RESULTS

A total of 10 patients with inherited and severe VWD were screened: one patient was excluded due to screening failure and 9 patients were enrolled, including 7 with type 3 VWD. All patients received at least one dose and completed at least 18 months (up to 4 years) of follow up. Demographic characteristics of the study population are summarised in **Table III**. Basal levels of VWF:RCo and FVIII: C were <10% and <20%, respectively. None of the patients had a history of VWF or FVIII inhibitors before treatment. Six patients had been previously treated with pdVWF/FVIII concentrates (n=5) or fresh frozen plasma (n=1). Three patients (all with type 3 VWD) had not received any prior treatment with VWF, but one was administered red blood cells for gastrointestinal bleeding. All patients were vaccinated against HBV, and one patient against HAV, prior to study enrolment. Parvovirus B19 serology was positive in 2 patients. Serological markers for HIV and HCV were negative in all patients.

### Recovery

At study entry, incremental recovery (mean±SD) for VWF:RCo from 7 patients was 1.8±0.4 IU/dL *per* IU/kg infused (median 2.0 IU/dL *per* IU/kg) after a mean dose of 101±5 IU/kg. After at least six months of follow up, the control recovery from 6 patients with type 3 VWD was 1.5±0.2 IU/dL *per* IU/kg (median 1.5 IU/dL *per* IU/kg).

**Table III** - *Demographic and baseline characteristics of patients with von Willebrand disease (VWD)*

| Parameter | Baseline recovery population N=7 | Efficacy/Safety population N=9 |
|---|---|---|
| **VWD type according to investigators' opinion** | | |
| **1**[†] | 1 (14.3) | 1 (11.1) |
| **2 (unclassified)**[‡] | 1 (14.3) | 1 (11.1) |
| **3**[§] | 5 (71.4) | 7 (77.8) |
| **Sex (male/female)** | 4/3 | 5/4 |
| **Age (years)** | 3.0 (0.9-5) | 2.0 (0.9-5) |
| **Body weight (kg)** | 15.5 (10.0-22.0) | 15.5 (10.0-22.0) |
| **Body mass index (kg/m²)** | 15.6 (13.8-18.3) | 15.8 (13.8-18.3) |

**Data are presented as number (%) or median (min-max).**
[†]VWF:RCo: 7 IU/dL, VWF:Ag: 24 IU/dL; FVIII:C: 18 IU/dL. [‡]VWF:RCo: <10 IU/dL, VWF:Ag: 38 IU/dL; FVIII:C: 1 IU/dL. [§]FVIII:C ranging from <1 to 8 IU/dL.
N: number.

There was no notable difference in the results of the 4 patients who completed both the baseline and control recovery study: mean ratio was 0.9±0.1 and 1.1±0.2 for VWF:RCo and VWF: Ag, respectively (**Table IV**).

### Bleeding episodes

A total of 92 bleeding episodes were documented, of which 62 in 7 patients (6 with type 3 VWD and 1 with type 2 VWD) who required treatment with the study drug. Among the 62 treated bleeding episodes (including 2 major episodes), 40 (64.5%) were mucosal, 14 (22.6%) were musculoskeletal, and 8 (12.9%) occurred in other sites. Bleeding characteristics according to bleeding site are shown in **Table V**.

Of the 62 bleeding episodes (48 in 6 patients treated on-demand and 14 in one patient under LTP), 56 (90.3%) were rated as "Excellent" or "Good" (95% CI: 80.1-96.4) in their response to treatment, and 9.7% (6/62) had a "Moderate" response. A "Moderate" response was reported for 4 epistaxis in one patient, one calf haematoma, and one knee haemarthrosis (treated 3 days after trauma) in two other patients, all treated on demand. Overall efficacy rating was "Good" for the 2 major bleeding episodes observed in one patient under LTP: 2 epistaxis each requiring 400 mL blood transfusion at hospital entry.

A total of 104 infusions were given to resolve these 62 bleeding episodes. Most of them (89%) were controlled with one (73%) or two (16%) infusions. Median dose *per* infusion was 54 IU/kg (min-max: 44-114). Major bleeding episodes (2 epistaxis) required a higher median total dose (222 IU/kg) than minor bleeding episodes (61 IU/kg). There was no difference in the total dose administered between the different types of minor bleeds. To ensure a prompt haemostatic level of FVIII: C, FVIII was co-administered with the first infusion of Wilfactin® in only one minor bleeding episode (refractory epistaxis), i.e., 1.6% of all bleeding episodes. The dual treatment in this patient allowed a partial efficacy ("Moderate" response). Tranexamic acid was used as adjunctive therapy in 11.3% of bleeding episodes (7/62) in 42.9% of patients (3/7).

### Surgical and invasive procedures

Of the 9 patients enrolled, 6 underwent 11 surgical procedures. All were minor procedures, documented as scheduled and described in **Table VI**. Haemostasis was rated as "Excellent" for 100% of procedures (95% CI: 71.5-100.0). No blood transfusion was required. The median loading dose administered immediately before the procedure was 56 IU/kg (min-max: 41-106).

**Table IV -** *Individual recovery investigations for VWF:RCo and VWF:Ag measured 15 minutes after single infusion of Wilfactin® in patients <6 years of age*

| Patient | Age at screening | VWD type* | Baseline recovery study | | | Control recovery study | | | Control/baseline | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Dose infused (IU/kg) | VWF:RCo recovery (IU/dL *per* IU/kg) | VWF:Ag recovery (IU/dL *per* IU/kg) | Dose infused (IU/kg) | VWF:RCo recovery (IU/dL *per* IU/kg) | VWF:Ag recovery (IU/dL *per* IU/kg) | VWF:RCo recovery (ratio) | VWF:Ag recovery (ratio) |
| 1 | 0.9 | 3 | 104 | 1.50 | 1.88 | 86 | 1.46 | 2.02 | 0.97 | 1.07 |
| 2 | 1 | 1 | 97 | 1.14 | na | na | na | na | na | na |
| 3 | 2 | 3 | na | na | na | 52 | 1.37 | 2.30 | na | na |
| 4 | 2 | 3 | na | na | na | 49 | 1.65 | 2.77 | na | na |
| 5 | 2 | 3 | 100 | 2.03 | 2.21 | 94 | 1.83 | 2.17 | 0.90 | 0.98 |
| 6 | 3 | 3 | 104 | 1.60 | 2.00 | 56 | 1.44 | 2.82 | 0.90 | 1.41 |
| 7 | 4 | 3 | 109 | 1.97 | 2.60 | 56 | 1.37 | 2.52 | 0.70 | 0.97 |
| 8 | 4 | 3 | 99 | 2.02 | 2.16 | na | na | na | na | na |
| 9 | 5 | 2 | 94 | 2.01 | 1.72 | na | na | na | na | na |
| Mean (SD) | | | 101 (5) | 1.8 (0.4) | 2.1 (0.3) | 66 (19) | 1.5 (0.2) | 2.4 (0.3) | 0.9 (0.1) | 1.1 (0.2) |
| Median | | | 100 | 2.0 | 2.1 | 56 | 1.5 | 2.4 | 0.9 | 1.0 |
| N. patients | | | 7 | 7 | 6 | 6 | 6 | 6 | 4 | 4 |

*Patients with type 3 VWD who entered the recovery study at baseline re-performed the pharmacokinetics study after at least 6 months.
VWD: von Willebrand disease; VWF:RCo: ristocetin cofactor activity; VWF:Ag: VWF antigen; na: not available; SD: standard deviation; N: number.

**Table V** - *Bleeding characteristics, dosing and overall haemostatic efficacy rating by bleeding site*

| Parameter | Minor bleeds | | | | Major bleeds | All bleeds N=62 |
|---|---|---|---|---|---|---|
| | Nose N=28 | Oral N=10 | Musculoskeletal† N=14 | Other‡ N=8 | Nose N=2 | |
| N. patients by bleeding site | 4 | 4 | 6 | 4 | 1 | 7 |
| **Bleeding characteristics** | | | | | | |
| **Distribution by VWD type** | | | | | | |
| Type 2 | 0 (0) | 5 (50) | 1 (7) | 0 (0) | 0 (0) | 6 (9.7) |
| Type 3 | 28 (100) | 5 (50) | 13 (93) | 8 (100) | 2 (100) | 56 (90.3) |
| **Aetiology** | | | | | | |
| Spontaneous | 23 (82.1) | 5 (50.0) | 3 (21.4) | 1 (14.3) | 1 (50.0) | 33 (54.1) |
| Post-traumatic | 5 (17.9) | 5 (50.0) | 11 (78.6) | 6 (85.7) | 1 (50.0) | 28 (45.9) |
| Missing | 0 | 0 | 0 | 1 | 0 | 1 |
| **Time elapsed between the start of bleed and the 1st dose** | | | | | | |
| <2 hours | 2 (7.1) | 1 (11.1) | 3 (21.4) | 2 (28.6) | 1 (50.0) | 9 (15.0) |
| 2-6 hours | 19 (67.9) | 5 (55.6) | 3 (21.4) | 5 (71.4) | 1 (50.0) | 33 (55.0) |
| 6-24 hours | 6 (21.4) | 2 (22.2) | 6 (42.9) | 0 (0.0) | 0 (0.0) | 14 (23.3) |
| >24 hours | 1 (3.6) | 1 (11.1) | 2 (14.3) | 0 (0.0) | 0 (0.0) | 4 (6.7) |
| **Missing** | 0 | 1 | 0 | 1 | 0 | 2 |
| **Transfusion before treatment** | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 2 (3.2) |
| **Overall haemostatic efficacy rating *per* bleed** | | | | | | |
| **Excellent** | 10 (35.7) | 7 (70.0) | 0 (0.0) | 7 (87.5) | 0 (0.0) | 24 (38.7) |
| **Good** | 14 (50.0) | 3 (30.0) | 12 (85.7) | 1 (12.5) | 2 (100.0) | 32 (51.6) |
| **Moderate** | 4 (14.3) | 0 (0.0) | 2 (14.3) | 0 (0.0) | 0 (0.0) | 6 (9.7) |
| **None** | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **Success§ [Exact 95% CI]** | 24 (85.7) [67.3-96.0] | 10 (100.0) [69.2-100.0] | 12 (85.7) [57.2-98.2] | 8 (100.0) [63.1-100.0] | 2 (100.0) - | 56 (90.3) [80.1-96.4] |
| **Dosing *per* bleed** | | | | | | |
| **Dose *per* infusion (IU/kg)** | 54 (47-104) | 55 (44-114) | 52 (48-62) | 71 (45-91) | 79 (64-95) | 54 (44-114) |
| **N. infusions** | 1 (1-9) | 1 (1-1) | 1 (1-8) | 2 (1-3) | 3 (2-4) | 1.0 (1-9) |
| **N. exposure days** | 1 (1-7) | 1 (1-1) | 1 (1-8) | 2 (1-3) | 3 (2-4) | 1.0 (1-8) |
| **Total dose (IU/kg)** | 73 (47-556) | 55 (44-114) | 53 (48-396) | 119 (45-273) | 222 (190-254) | 66 (47-556) |

**Data are presented as number (%) or median (min-max).**
†Haematoma (11), knee haemarthrosis (1), bruises (2). ‡Other included head injury (1), forehead injury (2), nose tear (1), foot sole injury (1), post-circumcision bleed after scratching the wound scab (1), undefined bleed (1), loss of scab 9 days after circumcision (1). §Success of the treatment was defined as a score of "Excellent" or "Good" responses.
VWD: von Willebrand disease; CI: Confidence Interval; N: number.

Post-operative doses (2-5 doses) were required for 3/11 surgeries (stye excision, placement of catheter, and dental surgery). To account for the low FVIII baseline level, a first dose of Wilfactin® (median 77 IU/kg; min-max: 41-106) was administered 12-24 hours before surgery for 4 (36%) procedures (3 circumcisions and 1 stye excision). One patient with type 3 VWD on regular prophylaxis did not require FVIII replacement therapy for 2 procedures (decidual tooth extraction and port-a-cath insertion) as the last prophylactic infusion was administered 2 and 3 days before surgery, respectively. No treatment to increase FVIII: C was judged necessary before 4 decidual tooth extractions and one root tooth extraction in one type 2 VWD patient.

**Table VI** - *Surgery characteristics, dosing and overall haemostatic efficacy rating by type of surgery*

| Parameter | Tooth extraction N=6 | Circumcision N=3 | Other† N=2 | All surgeries N=11 |
|---|---|---|---|---|
| **N. patients** | 2 | 3 | 2 | 6 |
| **Surgery characteristics** | | | | |
| **Distribution by VWD type** | | | | |
|   Type 1 | 0 (0) | 0 (0) | 1 (50) | 1 (9) |
|   Type 2 | 5 (83) | 0 (0) | 0 | 5 (45) |
|   Type 3 | 1 (17) | 3 (100) | 1 (50) | 5 (45) |
| **Mean duration (hh:mm)** | 00:05 | 00:25 | 00:27 | 00:18 |
| **Overall haemostatic efficacy rating *per* surgery** | | | | |
| **Excellent** | 6 (100.0) | 3 (100.0) | 2 (100.0) | 11 (100.0) |
| **Good** | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **Moderate** | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **None** | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **Success‡ [Exact 95% CI]** | 6 (100.0) [54.1-100.0] | 3 (100.0) [29.2-100.0] | 2 (100.0) [15.8-100.0] | 11 (100.0) [71.5-100.0] |
| **Dosing *per* surgery** | | | | |
| **N. surgeries with 1st VWF infusion 12-24h before surgery** | 0 (0) | 3 (100) | 1 (50) | 4 (36) |
| **Dose 12-24h before surgery (IU/kg)** | - | 69 (41-85) | 106 (106-106) | 77 (41-106) |
| **Pre-operative loading dose (IU/kg)** | 56 (45-104) | 69 (41-85) | 92 (77-106) | 56 (41-106) |
| **Dose *per* infusion (IU/kg)§** | 56 (45-104) | 69 (41-85) | 83 (77-88) | 56 (41-104) |
| **N. infusions§** | 1 (1-6) | 2 (2-2) | 4 (4-4) | 2 (1-6) |
| **N. exposure days§** | 1 (1-6) | 2 (1-2) | 4 (4-4) | 1 (1-6) |
| **Total dose (IU/kg)§** | 56 (45-626) | 139 (82-169) | 331 (309-353) | 82 (45-626) |

**Data are presented as number (%) or median (min-max).**
†Other included the insertion of venous access device (port-a-cath) and a stye excision. ‡Success of the treatment was defined as a score of "Excellent" or "Good" responses. §The parameters such as dose *per* infusion, number of infusions, exposure days, and total dose included the VWF infusion administered 12-24 h before surgery , if any.
VWD: von Willebrand disease; CI: Confidence Interval; h: hours; N: number.

One patient underwent one invasive dental procedure and received one infusion of 91 IU/kg. Haemostasis was rated as "Excellent".

## Long-term prophylaxis

Two patients with type 3 VWD, aged 2 and 4 years, received LTP with Wilfactin® to prevent bleeding. In both cases, the reasons for initiating prophylaxis were multiple bruises and traumatic bleeding of soft tissues, explained by the increased physical activity in these growing children. A port-a-cath was placed in the first patient after the start of prophylaxis therapy due to poor venous access. He received a total of 624 infusions over the four years of study participation at a mean dose *per* infusion of 81 IU/kg. A clear reduction in the intensity and number of

bruises was observed. In this patient, 30 bleeding episodes were reported, 14 of which (2.2% of infusions) required additional treatment with Wilfactin®. The majority of these breakthrough bleeds were minor (86%, 12/14) and two were major. Bleeds were mostly post-traumatic (64%, 9/14). All were treated successfully. No haemarthrosis was observed. The second patient started prophylaxis one month before the end of the study and received once weekly infusions of Wilfactin® 52 IU/kg. No bleeding episodes occurred during this short period (5 infusions).

## Safety

Patients were followed for 18-51 months (median 19 months) after the first dose. Four patients had a follow up longer than 3 years. A total of 1,109,065 IU

of Wilfactin® were administered in 775 infusions over 770 EDs. Distribution of EDs by clinical events showed that 81.7% (629/770) were related to LTP. A total of 139 TEAEs in 9 patients, including 5 SAEs, were reported. None of the TEAEs were considered to be related to Wilfactin®. Most (83.5%) reported events were considered mild in severity, and none were severe. The most common AE was nasopharyngitis (incidence 9.4%), followed by traumatic haematoma (8.6%) and bronchitis (6.5%). There were no deaths or AE that led to study drug withdrawal. No post-treatment VWF or FVIII inhibitors, or non-neutralising antibodies were observed among any of the patients, including 3 type 3 VWD patients who had not previously received VWF. No allergic/anaphylactic-type reactions or thromboembolic event were recorded. No suspicion of transmission of infective agents was reported. The only serologic change resulted from HAV vaccination in 7 patients. Parvovirus B19 serology remained negative at the end of study for the 7 patients with a negative or unknown test on enrolment; these 7 patients received a total of 751 infusions from 15 different batches. Abnormal clinically significant haemoglobin and haematocrit were reported in 5 patients, but 3 of them reported a history of anaemia with iron deficiency. Anaemia was responsive to iron supplementation and was attributed to common complications related to VWD.

## DISCUSSION

The results of this prospective, open-label study conducted specifically in children under 6 years of age with severe VWD, demonstrate the efficacy and safety of Wilfactin® in this patient population. With regard to sample size, regulatory guidelines (CPMP/BPWG/220/02) recommend that a minimum of 8 patients be enrolled in a clinical trial[11]; this requirement was met in the present study with the enrolment of 9 children. Baseline recovery for VWF:RCo determined 15 minutes after the end of infusion is numerically lower to that previously reported in an adult population (1.8±0.4 IU/dL *per* IU/kg *vs* 2.1±0.3 IU/dL *per* IU/kg)[9]. This observation might be explained by a larger volume of distribution in children than in adults[13], but results are similar. In addition, recovery evaluation in this study remained higher than data from studies conducted in the same population with two different VWF/FVIII concentrates. Median recovery was

0.6 IU/dL *per* IU/kg in 7 children in the first study[14] and 1.2 IU/dL *per* IU/kg in 9 children in the second study[15]. Based on these heterogenous results in children, it appears that the assessment of patient recovery is a key element when designing appropriate dosing/scheduling regimens and personalised treatment in VWD.

In our study, Wilfactin® was used to treat 62 bleeding episodes in 7 patients with VWD, including 6 with type 3. The overall haemostatic efficacy rating was "Excellent" or "Good" in 90.3% of bleeding episodes; this is in line with paediatric studies performed using VWF/FVIII concentrates[14,15]. Co-administration of FVIII was recommended as *per* protocol in case of non-haemostatic levels of FVIII:C at the time of treatment. However, despite basal FVIII:C levels <20% in all patients, a dose of FVIII was co-administered for only one bleeding episode (1/62). The reason to omit FVIII co-administration may result from the mucocutaneous nature of bleeding episodes reporting a defective primary haemostasis outcome, and for which VWF only may be sufficient, or due to the fact that some patients were under LTP, thus resulting in increased FVIII activity levels. Furthermore, in the majority of cases, bleeds were minor, therefore reducing the urgency for restoring haemostatic FVIII levels. Most bleedings responded to a single infusion of Wilfactin® at a median dose of 54 IU/kg. This is in line with the recommended doses for on-demand treatment and control of bleeding episodes in the Wilfactin®/Willfact® Summary of Product Characteristics (SmPC)[16] and the literature[17,18,19,20]. In addition, the median treatment duration of 1 day for bleeding episodes in this study was similar to the treatment durations already reported in children[14,15].

The product is almost devoid of FVIII; however: 1) the ability of the protein to bind to FVIII allows sufficient FVIII levels to be generated; and 2) the well-preserved multimeric profile ensure haemostatic control. These achievements were illustrated by the results of a large multicentre observational post-marketing study including children[20].

In the present study, all surgical and invasive procedures were minor and planned, leading to the use of Wilfactin® as monotherapy. No FVIII concentrate was co-administered at the first infusion of VWF, but a first pre-operative Wilfactin® dose was administered 12-24 hours before

surgery in order to initiate the increase in endogenous FVIII and achieve an appropriate level of FVIII activity in patients with types 1 or 3 VWD (4 surgical procedures). This was judged unnecessary by the physician for two procedures in a patient with type 3 under LTP and 5 dental procedures in another patient with type 2 VWD. Overall haemostatic efficacy was rated as "Excellent" for all procedures, and dosing was consistent with the recommended VWD dosing guidelines for Wilfactin® of 40-80 IU/kg *per* day.

Long-term prophylaxis with plasma-derived FVIII/VWF or VWF concentrates may be administered to prevent haemarthrosis, haematomas, and frequent mucosal bleedings in patients with severe forms of VWD[20,21,22]. The effectiveness of LTP was assessed in two patients with type 3 VWD. One of them was treated three times a week for four years and received 624 infusions of Wilfactin®. LTP was considered to be favourable in this child who experiences bleeds in 2.2% of prophylactic infusions, despite the increase in physical activity in this growing child. The data obtained in the second patient were limited to draw any conclusion.

The safety profile of Wilfactin® was consistent with that observed in former studies involving adults and children[10,20]. The major potential safety concerns with blood products are hypersensitivity reactions, transmission of infective agents, immunogenicity, and thromboembolic events[23]. In our study, no AEs related to Wilfactin® and no allergic/anaphylactic-type reactions were observed over 770 EDs with a total dose of more than one million IU and a mean of participation of 3.1 years. There were also no cases of VWF or FVIII inhibitors. *In vivo* recovery over time was consistent during the treatment period, as no evidence of a decrease in activity was found in four type 3 patients, including 3 treatment-naïve patients. No thromboembolic event or suspicion of transmission of infective agents were reported. It is noteworthy that the unchanged negative status for the parvovirus B19 in 7 patients at the end of the study added new data that reinforce the known favourable viral safety profile of Wilfactin®.

## CONCLUSIONS

In summary, results from this prospective study are an addition to the limited data on VWF replacement therapy in paediatric patients. The study supports the conclusion that Wilfactin®, a pdVWF almost devoid of FVIII, has an excellent efficacy and safety profile in children under 6 years of age with severe VWD.

## AUTHORSHIP CONTRIBUTIONS

EG, AK, PM, and HP performed the research and acquisition of data. JG contributed to designing the study and analysed the data. SP and CH contributed to the data analysis and wrote the manuscript. FB designed the study, analysed the data, and wrote the manuscript. All Authors critically revised the manuscript and approved the final version.

## DISCLOSURE OF CONFLITS OF INTEREST

*EG, AK, HP declare no conflicts of interest. PM received consulting fees, payment or honoraria from Bayer Biological, Takeda, Novo Nordisk, Roche, CSL-Behring, CAF-DCF Sanofi, Novartis for Institution UZA or BVDA Dr P Maes Company; received support for attending meetings and/or travel from Bayer Biological, Takeda, Roche, CSL-Behring for BVDA Dr P Maes Company, grants or contracts from Bayer Biological, Takeda, Novo Nordisk, Roche, Sobi, CSL-Behring, Pfizer for Institution UZA or non-profit organization vzw/asbl Extra Small; participated*

on a data safety monitoring board or advisory board from Bayer Biological, Roche, CSL-Behring Institution UZA; and is a board member of BSTH. JG received support for attending scientific meetings and honoraria from Baxalta-Shire, Bayer Healthcare, CSL-Behring, LFB, Novo-Nordisk, Roche-Chugai, and Sobi. SP and CH are employees of LFB and FB is a former employee of LFB.

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