Supplement

Subcutaneous concizumab prophylaxis in hemophilia A and hemophilia A/B with inhibitors: Phase 2 trial results

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Supplementary methods

Exclusion criteria for explorer4 and explorer5

Patients were excluded if they had a known inherited or acquired bleeding disorder other than hemophilia, underwent major surgery within one month prior to the initiation of trial activities or had a planned surgical procedure during the trial, a previous history or current signs of thromboembolic disease, significant infection or a known systemic inflammatory condition requiring systemic treatment at screening, hepatic dysfunction (alanine aminotransferase [ALT] >3 times the upper limit of normal) and/or renal impairment (estimated glomerular filtration rate [eGFR] ≤60 ml/min/1.73m^2) at screening; platelet count ≤100 x 10^9/L and/or fibrinogen level less than the lower limit of normal at screening, ongoing or planned immune tolerance induction (ITI) therapy or prophylaxis with factor VIII (FVIII) or factor IX (FIX) (explorer4 only), antithrombin levels below the normal reference range at screening (explorer4 only) or a previous history or presence of FVIII inhibitors at screening (explorer5 only).

Tissue factor pathway inhibitor (TFPI) assay for concizumab anti-drug antibody-positive samples in explorer4 and explorer5

Samples positive for anti-concizumab antibodies were further characterized for neutralizing activity using a modified TFPI functionality assay. The assay principle is based on activation of FX to FXa when functional TF/FVIIa complex is present; this is inhibited by TFPI and further addition of concizumab restores the signal. If anti-concizumab neutralizing antibodies are present in a trial sample, TFPI is not inhibited and the FXa generation in the mixture is decreased. In brief, Protein A purified-antibodies from quality controls and study samples were mixed and incubated with concizumab for 5 min. Following this, FX, TFPI and TF/FVIIa were added to the mixture and after 15 min of incubation EDTA and a chromogenic reagent (p-nitroanilin) were added. After a 10-min incubation, acetic acid was added and the absorption at 405 nm was measured.

Thrombin generation assay in explorer4 and explorer5

The Calibrated Automated Thrombogram (CAT) method (by Thrombinoscope BV) was used to measure thrombin generation. The analyses were performed on patients' platelet-poor plasma. PPP-Reagent LOW (1 pM TF) was used as a trigger, added to the platelet-poor plasma to initiate thrombin generation. PPP-Reagent LOW is particularly recommended to be used in hemophiliac plasma in order to increase sensitivity to factors VIII, IX and XI. This method uses a slow-acting fluorogenic substrate that allows continuous measurement of thrombin generation in double-centrifuged citrated plasma. In this assay set-up, thrombin generation is initiated by low dose tissue factor that is combined with phospholipid. The result is obtained by comparison to a constant known thrombin activity in a parallel non-tissue factor initiated sample. The assay has been validated fit-for-purpose.

Supplementary results

Pharmacokinetics and pharmacodynamics

In explorer5 (non-inhibitor trial), the baseline mean (SD) free TFPI was 96.3 (11.1) ng/mL and was reduced to 30.1 (15.6), 64.4 (35.3) and 12.4 (2.2) ng/mL in the concizumab 0.15, 0.20

and 0.25 mg/kg groups, respectively. Of interest, there were two patients in the 0.20 mg/kg dose group with very low concizumab exposure who did not show a decrease in free TFPI. In explorer4 (inhibitor trial), concizumab lowered free TFPI from a mean (SD) of 100.7 (12.8) ng/mL at baseline to 26.9 (12.2) ng/mL prior to/at the last dose administration at 24 weeks. As expected, free TFPI was unchanged after 24 weeks of on-demand treatment with rFVIIa in explorer4.

Peak thrombin generation (TG) potential at 24 weeks was within the normal reference range (26−147 nmol/L) across all concizumab dose levels in explorer4 (mean [SD]: 65.0 [34.0] nmol/L) and explorer5 (mean [SD]: 88.6 [34.5], 67.5 [35.0] and 83.4 [10.6] nmol/L for the 0.15, 0.2 and 0.25 mg/kg groups, respectively).

List of explorer4 investigators

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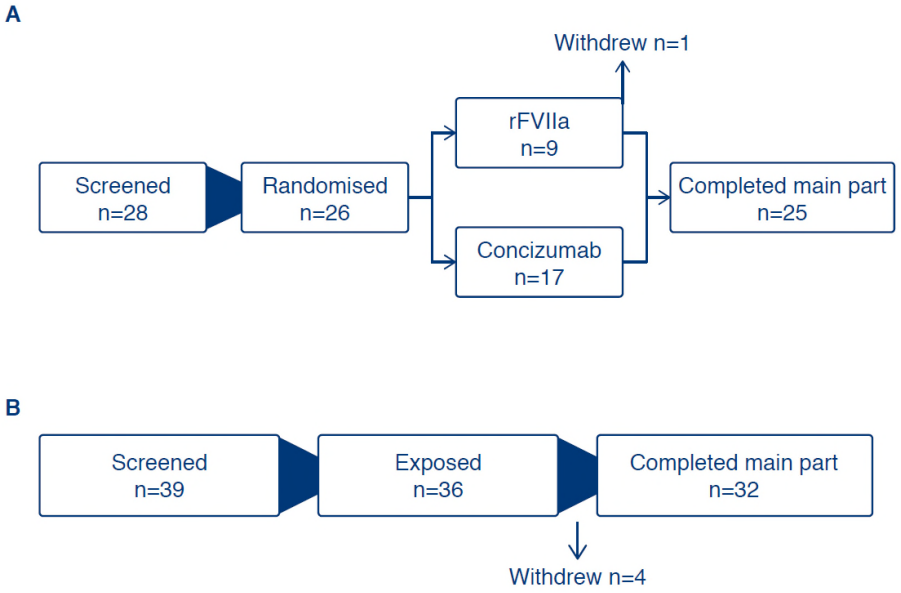
Supplementary table and figures

Table S1. Patient baseline characteristics and treatment and bleed history by treatment arm in explorer4 (HAwI/HBwI).

	Concizumab	rFVIIa	Total
N	17	9	26
Mean age at baseline, y (SD)	34.1 (11.1)	41.1 (15.0)	36.5 (12.7)
Mean body weight, kg (SD)	71.5 (12.6)	70.6 (17.0)	71.2 (13.9)
Mean time from diagnosis, y (SD)	33.5 (11.5)	40.3 (15.3)	35.8 (13.0)
Patients on-demand, n (%)	17 (100)	9 (100)	26 (100)
On-demand mean ABR (min; max)*	25.3 (6−120)	18.6 (7−38)	23.0 (6−120)

*During the treatment regimen. Patients were permitted to be treated with more than one treatment regimen during the 12 months prior to screening.
HAwI, hemophilia A with inhibitors; HBwI, hemophilia B with inhibitors; kg, kilogram; rFVIIa, recombinant activated factor VII; SD, standard deviation; y, years

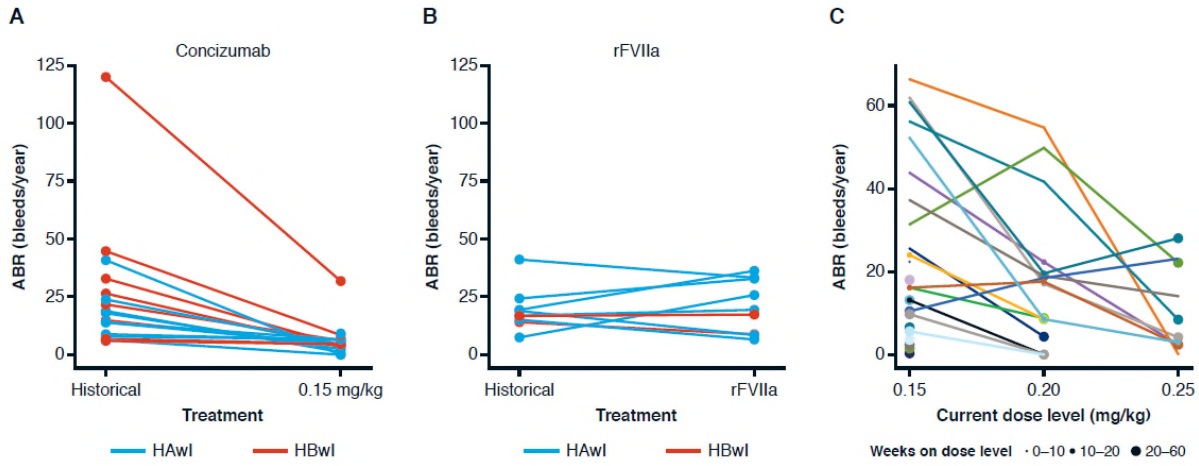
Figure S1. Patient disposition in (A) explorer4, a concizumab multicenter, open-label randomized controlled phase 2 trial (HAwI/HBwI); and (B) explorer5, a concizumab multicenter, single-arm, open-label phase 2 trial (HA without inhibitors).



A. explorer4: One patient in the rFVIIa arm withdrew his consent on Day 60. B. explorer5: Two patients withdrew consent on Days 21 and 80 while on 0.15 and 0.25 mg/kg concizumab, respectively; one patient withdrew from treatment on Day 49 while on 0.15 mg/kg concizumab; and one patient withdrew due to a lack of treatment efficacy on Day 11 while on 0.15 mg/kg concizumab. The “lack of efficacy” that led this patient to withdraw was at the discretion of the treating investigator who provided this explanation for the withdrawal. Because the bleeding pattern of this patient did not fit the definition of “lack of efficacy” as defined in the protocol (i.e., 3 spontaneous bleeding episodes within 12 weeks, which also triggered a dose escalation), the grounds for this withdrawal constituted a protocol deviation. There was no evidence of anti-drug antibodies in these patients up to the time of withdrawal.

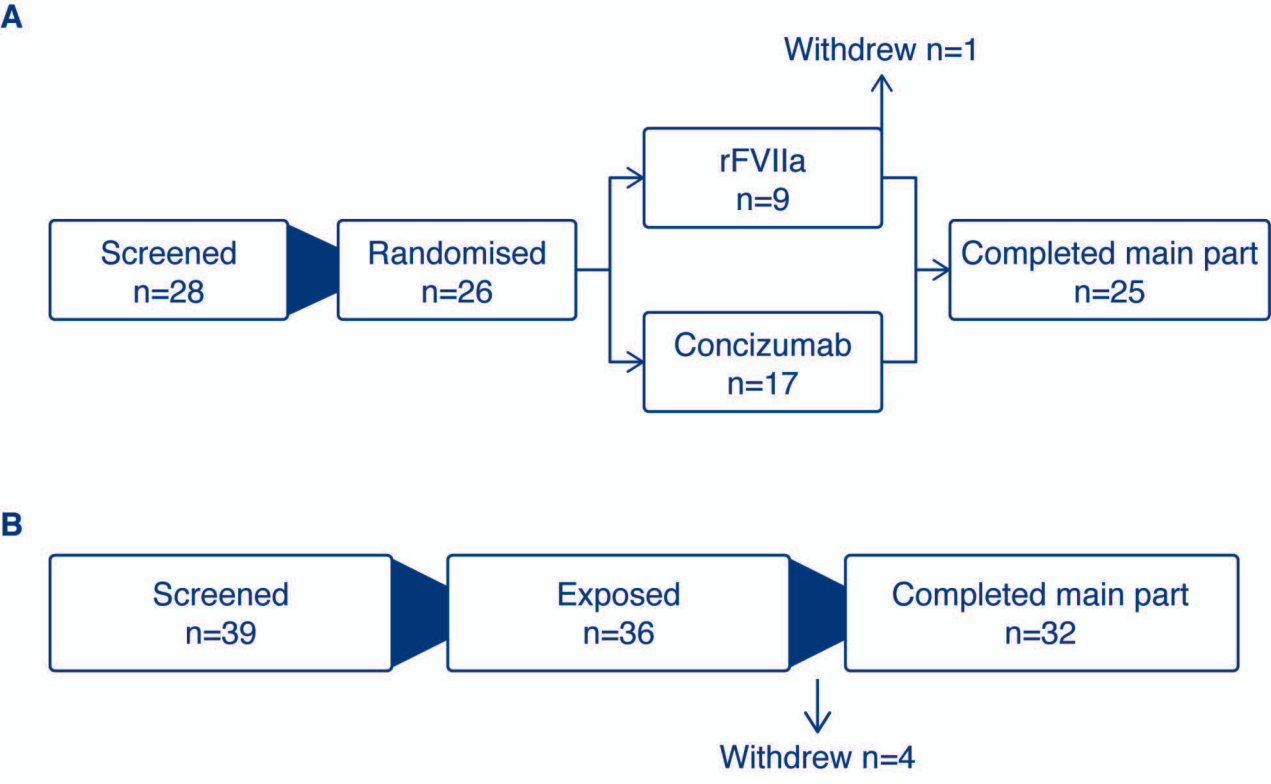
HA, hemophilia A; HAwI, hemophilia A with inhibitors; HBwI, hemophilia B with inhibitors
rFVIIa, recombinant activated factor VII

Figure S2. Historical and on-trial ABRs for all bleeding episodes in patients treated with (A) 0.15 mg/kg concizumab or (B) rFVIIa in explorer4 (HAwI/HBwI) and (C) ABR when assessing each patient's last concizumab dose level in explorer5 (HA without inhibitors).



ABR, annualized bleeding rate; HA, hemophilia A; HAwI, hemophilia A with inhibitors; HBwI, hemophilia B with inhibitors rFVIIa, recombinant activated factor VII

Supplementary Figure S1



Supplementary Figure S2