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DRUG EVALUATION

Andexanet alfa for the reversal of factor Xa inhibitors

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ABSTRACT

Introduction: Andexanet alfa is a recombinant modified factor Xa protein that has been developed to reverse factor Xa inhibitors. Since May 2018, the FDA has approved its utilization in patients treated with apixaban and rivaroxaban in case of life-threatening or uncontrolled bleeding. On 28 of February 2019, the Committee for Medicinal Products for Human Use adopted a positive opinion, recommending the granting of a conditional marketing authorization for andexanet alfa in Europe.

Area covered: The authors provide an overview of andexanet alfa development and its pharmacokinetic and pharmacodynamic properties. The results of the clinical phase III trial ANNEXA as well as current limitations related to andexanet alfa are also discussed.

Expert opinion: Although phase I and II studies have proven that andexanet alfa can be effective in reversing the effect of factor Xa inhibitors, its efficacy in major bleeding patients has only been shown for apixaban and rivaroxaban, without any comparator group. Well-designed studies comparing the efficacy and safety of andexanet alfa to other reversal strategies are required to confirm preliminary data. The benefit of andexanet alfa in specific settings needs to be investigated and its use in clinical practice needs to be facilitated by the implementation of international guidelines.

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Andexanet alfa; antidote; reversal agents; factor Xa; DOACs; ANNEXA; major bleeding

1. Background

Direct oral anticoagulants (DOACs), including direct anti-IIa (dabigatran etexilate) and direct anti-Xa anticoagulants (apixaban, edoxaban, and rivaroxaban), have been used worldwide since their approval in several thromboembolic disorders, including the treatment and secondary prevention of venous thromboembolism (VTE) as well as the prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation (NVAF) [1–4].

DOACs are increasingly used in therapeutic practice thanks to their proven efficacy and their favorable safety profiles [5–12]. DOACs also exhibit more predictable pharmacokinetic and pharmacodynamic profiles than vitamin K antagonists (VKAs). Routine coagulation monitoring is therefore not required [13,14]. Currently, most guidelines in Europe, the United States, and Canada prefer DOACs over VKAs for VTE treatment and prophylaxis in patients without active cancer and for stroke prevention in NVAF [15–17].

However, assessment of drug exposure and its anticoagulant effect may be helpful in some clinical settings including patient with bleeding or thrombosis, urgent invasive procedures, extreme body weight, drug–drug interactions, gastrointestinal (GI) malabsorption, acute renal or liver failure, and the initiation and follow-up of antidote administration [13,18].

Other concerns including higher drug costs and increased bleeding risk (i.e. GI hemorrhage) have also been raised [7,8,19]. Clinical trials and patient registries data have shown a major bleeding rate of 2.1–3.5% per year in patients treated with DOACs [20–22]. The number of patients taking DOACs and presenting with hemorrhage (including life-threatening ICH) is also growing [7,23]. Therefore, an antidote is required in case of life-threatening bleeding or urgent surgery carrying a high risk of bleeding [18,24,25]. In such settings, prothrombin complex concentrates (PCCs) and activated PCCs may be used to reverse direct oral factor Xa (FXa) anticoagulant in clinical practice [26–29]. However, evidence of such nonspecific strategies is limited to *in vitro* studies, animal models, plasma analysis of healthy human volunteers, and preliminary data in patients with major bleeding [6,28–40]. Among these small observational studies without appropriate control groups, the hemostatic efficacy of PCCs ranged from 65.0% to 83.8% in patients with major bleeding associated with the use of DOACs (mostly apixaban or rivaroxaban) and the risk of thromboembolic events were present in up to 8.0% [28–30,35,39,41]. Despite the lack of robust data, an Expert Consensus Decision Pathway issued by the American College of Cardiology [42] and the European Heart Rhythm Association

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Box 1. Drug Summary Box

Name: Andexanet alfa (Ondexxya)

Phase: Phase I, II, III and IV

Indication: Since May 2018, the FDA has approved its utilization in patients treated with apixaban and rivaroxaban in case of life-threatening or uncontrolled bleeding.

Pharmacology description/mechanism of action: This antidote is a recombinant truncated form of human FXa that binds FXa inhibitors in the intravascular space with high affinity in a 1/1 stoichiometric fashion. These latter are therefore sequestered and the inhibition of endogenous FXa is prevented.

Route of administration: Intravenous

Pivotal trials:

- Phase I: The 'first-in-man' randomized, double-blind, placebo-controlled, single-center phase I study included 32 healthy human volunteers receiving a single IV dose of andexanet alfa (Crowther et al. 2013).
- Phase II: Randomized, double-blind, placebo-controlled studies for apixaban, betrixaban, edoxaban, rivaroxaban (Crowther et al. 2014, Siegal et al. 2017).
- Phase III: Randomized, double-blind, placebo-controlled trials ANNEXA-A and ANNEXA-R were undertaken to determine the ability of andexanet alfa to reverse anticoagulation with apixaban or rivaroxaban as well as its safety in a total of 145 subjects (Siegal et al. 2015).
- Phase IIIb-IV: Multicenter, prospective, open label, unblinded, single arm study named ANNEXA-4 that evaluated the clinical efficacy and safety of andexanet alfa in patients with FXa inhibitor–associated acute major bleeding (Connolly et al. 2016, Connolly et al. 2019).

[43,44] recommend PCCs for treating acute hemorrhage in patients receiving direct oral FXa anticoagulants.

On the other hand, the lack of specific and well-designed studies assessing the efficacy and safety of current reversal agents in patients taking DOACs in case of life-threatening bleeding or surgery led to the development of specific reversal agents [6,7,19,26,32]. Since 2015, a specific reversal agent for dabigatran (idarucizumab, Praxbind®, Boehringer Ingelheim Pharmaceuticals, Ingelheim am Rhein, Germany) has been approved by the Food and Drug Administration (FDA) [7,45,46]. In May 2018, a specific reversal agent for apixaban and rivaroxaban (andexanet alfa, Andexxa®, Portola Pharmaceuticals, San Francisco, CA) has also been approved by the FDA in the United States [6,7]. In this review, we present the latest data regarding andexanet alfa and highlight the current limitation of this newly FDA-approved antidote.

2. Andexanet alfa: from development to introduction in clinical practice

2.1. Development and mechanism of action

Andexanet alfa ('andexanet' or r-antidote, PRT064445) has been developed to reverse both direct (apixaban, betrixaban, edoxaban, rivaroxaban) and indirect (unfractionated heparin (UFH), low molecular weight heparin (LMWH) and fondaparinux) FXa anticoagulants and is therefore considered as a universal antidote for this class of drugs [6,7,27,32,47].

This antidote is a recombinant truncated form of human FXa composed of two units: a light chain (LC) of ~11 kDa and a heavy chain (HC) of ~39 kDa. Andexanet alfa binds FXa inhibitors in the intravascular space with high affinity in a 1/1 stoichiometric fashion. These latter are therefore sequestered and the inhibition of endogenous FXa is prevented [6,32,48,49]. Andexanet alfa is catalytically inactive due to the replacement of an active-site serine (S419) with alanine (A419), and to the removal of a 34-residue fragment containing the 11-residue membrane-binding domain N-terminal γ-carboxyglutamic acid (GLA) eliminating the protein's ability to adhere to the surface membrane of platelets and therefore to assemble into the prothrombinase complex [6,32,50,51] (Figure 1). The replacement of the activation peptide to form the RKRRKR linker permits the connection between the LC and the HC [32]. Box 1 presents the drug summary of andexanet alfa.

2.2. Pharmacokinetics and pharmacodynamics

Andexanet alfa has a short initial half-life of approximately 15 min corresponding to the distribution phase. After intravenous infusion, the terminal half-life ranges from 5 to 7 h [7,48,52–54]. The C_{max} and the AUC of andexanet alfa increase proportionally with increasing doses and its volume of distribution is equivalent to the blood volume of 5 L with a plasma clearance of 4.3 L/hour [54,55]. However, the elimination mechanisms of this antidote are not yet well established [6]. It has been shown that pharmacokinetics parameters of andexanet alfa are not influenced by age or by the presence of orally given apixaban (5 mg twice daily for 6 days) or rivaroxaban (20 mg once daily for 6 days) [52,54]. As compared to apixaban, higher doses of andexanet alfa are required for rivaroxaban due to both higher peak drug concentrations and larger volume of distribution [28,51]. Moreover, andexanet alfa has the ability to displace direct FXa inhibitors from the extravascular to the intravascular compartment [32]. A rebound effect of direct FXa inhibitors is therefore possible. The relative potency (K_d) of binding is in line with the inhibition constants (K_i) found in literature for apixaban, betrixaban, and rivaroxaban [32].

2.2.1. Preclinical experience

In human plasma samples, the prolongation of prothrombin time (PT) due to rivaroxaban (1 μM) was corrected by the addition of equimolar concentration of andexanet alfa. The antidote did not change the PT in the absence of rivaroxaban, even at the highest concentration of antidote tested. The formation of thrombin as assessed by a thrombin generation assay in human plasma samples remained unchanged up to the highest concentration of andexanet alfa tested [32].

In rats infused with apixaban, betrixaban, and rivaroxaban, the twofold increase of whole-blood international normalized ratio (INR) was rapidly and sustainably reversed after the administration of an intravenous (IV) bolus injection of andexanet alfa (4 mg) followed by a continuous infusion (4 mg/h). These results were correlated with the decrease of unbound concentration of FXa inhibitor to very low levels following the IV administration of andexanet alfa [32].

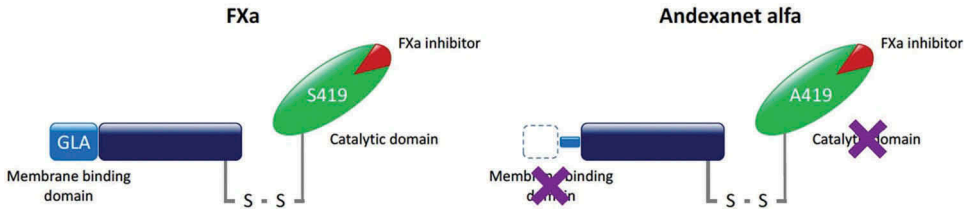

Figure 1. Mechanism of action of andexanet alfa [32,48,50].

The injection of andexanet alfa (~1 mg) in a mouse model of blood loss 2 h following the administration of rivaroxaban corrected the increased blood loss by ~84% and the anti-Xa activity by >80%. In a rabbit model of liver laceration, the administration of andexanet alfa as a bolus injection (75 mg) resulted in a decrease of blood loss (>85%), anti-Xa activity (98%), PT (74%), activated partial thromboplastin time (aPTT) (66%), and nonprotein bound rivaroxaban concentration (from 26% to 0.5%) [32].

In human or rat plasma, the anti-Xa activities of enoxaparin (1 IU/mL) and fondaparinux (2 µg/mL) were dose-dependently reversed following the administration of the antidote. In a rat tail transection model treated with enoxaparin (4.5 mg/kg), a bolus of andexanet alfa (4 mg) followed by a sustained infusion (4 mg/h) completely corrected the increase of blood loss. Accordingly, the cessation of blood loss was observed after the administration of fondaparinux (25 mg/kg) followed by the antidote administration (6 mg bolus plus sustained infusion of 6 mg/h). Moreover, andexanet alfa alone had no impact on blood loss [32].

The initiation of blood loss in the above-mentioned animal model studies was performed while andexanet alfa was already administered [32]. In order to more closely mimic the clinical situation of bleeding, a more recent study evaluated the efficacy of andexanet alfa given after the induced hemorrhage [56]. In rivaroxaban-anticoagulated rabbits with active visceral hemorrhage, a dose-dependent decrease of the anti-FXa activity (99%, 96.6%, 75%, and 46.7%) and of the unbound rivaroxaban concentrations (99.9%, 95.1%, 67%, and 21.6%) were observed immediately following the administration of 75, 35, 15, and 5 mg of andexanet, respectively. PT and aPTT also proportionally decreased immediately following the administration of the antidote. Blood loss (net weight of blood, in grams) following the administration of a single IV bolus of 35 or 75 mg of andexanet alfa decreased by 75% and 63%, respectively as compared to controls.

Reduction of blood loss and anti-Xa activity for apixaban (porcine model) and for betrixaban (rabbit model) have also been observed in two recent studies [57,58].

Recently, a similar study was designed to evaluate the efficacy of andexanet alfa to reverse the effect of edoxaban [59]. In edoxaban-anticoagulated rabbits with surgically induced bleeding, a rapid and dose-dependent reduction of anti-Xa activity (82%) by andexanet alfa was objectified. The blood loss decreased by 80% and the unbound edoxaban concentrations by ~80%. The prolongation of PT was also corrected by the administration of andexanet alfa (shortening by 31% at the end of antidote administration) [59].

The toxicity of repeated administrations of andexanet alfa in cynomolgus monkeys was also investigated (up to 60 mg kg^{-1} day^{-1}, corresponding to approximately twofold to threefold higher plasma concentration as compared to those observed in Phase III trials) [56]. No deaths occurred in the study and clinical observations were not considered to be adverse. Even if a transient increase in plasma levels of thrombin-antithrombin complex and D-dimer was observed, no evidence of clot formation or fibrin deposition was visualized by histopathological examination of monkeys at necropsy. PT and aPTT were not influenced. Andexanet alfa was also well tolerated in rats, with no evidence of prothrombotic events. The generation of antibodies to andexanet alfa following its administration has been described in monkeys in a dose and time-dependent manner. However, no neutralizing antibody activity was observed [56].

2.2.2. Phase I clinical data

The 'first-in-man' randomized, double-blind, placebo-controlled, single-center phase I study included 32 healthy human volunteers receiving a single IV dose of andexanet alfa (n = 24; 30, 90, 300, or 600 mg) or placebo (n = 8) [53,54]. No thrombotic event was reported during the 28-day follow-up, even if a transient rise of D-dimer, thrombin-antithrombin complex, and prothrombin fragment 1 + 2 (F1 + 2) was noticed. One case of pneumonia and three nonserious infusion-related reactions (two patients receiving 90 mg of antidote, and one placebo) were observed. The addition of exogenous rivaroxaban (50 ng/mL) in plasma samples also permitted the evaluation of the efficacy of andexanet alfa [53,54]. As observed in preclinical studies, the anti-Xa activity decreased in a dose-depend manner. These observations reinforced the potential use of andexanet alfa and paved the way for further studies in humans.

2.2.3. Phase II clinical data

Results of phase II randomized, double-blind, placebo-controlled studies were also published for different anticoagulants [6,54,60–65]. A rapid (within 2 min) and dose-dependent reversal of anticoagulant (i.e. unbound FXa inhibitor concentration, anti-Xa activity, thrombin generation) following IV administration of andexanet alfa in more than 100 healthy volunteers (18–45 years) receiving apixaban (5 mg twice daily for 5.5 days), betrixaban (80 mg four times a day for 7 days), edoxaban (60 mg daily for 6 days), enoxaparin (40 mg daily for 6 days), and rivaroxaban (20 mg daily for 6 days) was observed. Anti-Xa levels returned to placebo levels ~2 h after the bolus or after the end of infusion of andexanet alfa

[6,54,61–64]. This latter observation is explained by the short half-life of andexanet alfa [47,51,66].

In more details, the study of Siegal *et al*. enrolled 54 healthy volunteers receiving apixaban (5 mg given orally twice daily) for 5.5 days followed by andexanet alfa (n = 36) or placebo (n = 18) 180 min after the last dose of apixaban (C_{max}) [54]. A total of six different cohort combinations were tested: (1) 90 mg bolus over 3 min, (2) 210 mg bolus over 7 min, (3) 420 mg bolus over 15 min, (4) 420 mg over ~14 min followed by 4 mg/min over 45 min, (5) 420 mg over ~14 min followed by a second bolus of 180 mg 45 min later, and (6) 420 mg over ~14 min followed by 4 mg/min over 120 min. A dose-dependent reduction of unbound apixaban (up to ~90%) was observed within 2 min after the end of the andexanet alfa bolus dose. No change in apixaban concentration was observed in the placebo group. Depending on the andexanet alfa administration scheme, the concentration of apixaban returned to placebo levels within ~10 to 210 min. A significant dose-dependent reduction of anti-Xa activity was also observed for every scheme within 2 min following the bolus infusion. The greatest effect of andexanet alfa on FXa activity was observed in cohorts 3, 4, 5, and 6 (92.8–95%). Restoration of thrombin generation was also observed in a dose-dependent manner within 2 min following andexanet alfa bolus administration in each cohort as compared to placebo ($P < 0.01$). Nonsignificant PT, aPTT and activated clotting time (ACT) changes were observed following the end of andexanet alfa bolus dose in all cohorts.

A dose-dependent increase in D-dimer and F1 + 2 was observed after andexanet alfa infusion [54]. Interestingly, levels of D-dimer and F1 + 2 were lower when andexanet alfa was administered in the presence of apixaban as compared to andexanet alfa alone [53,54]. Out of the 54 healthy volunteers included, no serious adverse event was recorded [54]. Only infusion reactions classified as treatment related were reported. No neutralizing antibodies to andexanet alfa were recorded. In a pooled analysis gathering clinical data in healthy volunteers, adverse events reported in the andexanet alfa cohort (n = 223) were similar (54% vs. 57%) as compared to placebo (n = 94). Only infusion-related reactions had a higher incidence as compared to placebo (18% vs. 6%) and no serious adverse events were recorded [48,52]. The data from these phase II studies were useful for the dose selection for phase III studies [6,54].

2.2.4. Phase III clinical data

Two parallel randomized, double-blind, placebo-controlled, phase III trials (Andexanet Alfa, a Novel Antidote to the Anticoagulation Effects of FXA Inhibitors Apixaban (ANNEXA-A) and Rivaroxaban (ANNEXA-R)) were undertaken to determine the ability of andexanet alfa to reverse anticoagulation with apixaban or rivaroxaban as well as its safety in a total of 145 subjects [51]. In the ANNEXA-A study, 48 healthy adult volunteers (50–75 years) were given apixaban (5 mg twice daily for 3.5 days). Three hours after the last intake of apixaban (~C_{max}), andexanet alfa was administered as a bolus alone (400 mg) or followed by a continuous infusion (4 mg/min over 120 min). In the ANNEXA-R study, 53 healthy adult volunteers (50–75 years) were given rivaroxaban (20 mg twice daily for 3 days). Three hours after the last intake of rivaroxaban (~C_{max}), andexanet alfa was administered as a bolus alone (800 mg) or followed by a continuous infusion (8 mg per minute for 120 min). A total of 44 healthy older volunteers were also randomly assigned to receive placebo. A rapid decrease in anti-Xa activity (within 2–5 min) was observed following andexanet alfa bolus administration for both apixaban (94.0%) and rivaroxaban (92.0%) as compared to placebo (21% and 18%, respectively) and a sustained action on anti-Xa activity was observed for 120 min. The same degree of decrease was observed when the antidote was administered as a bolus followed by a continuous infusion (92 and 97%, for apixaban and rivaroxaban) as compared to placebo (33% and 45%, respectively). Moreover, a rapid restoration of thrombin generation (within 2–5 min) was also observed for both apixaban (1,323 nM*min) and rivaroxaban (1,314 nM*min) as compared to placebo (88.2 and 173.9, respectively). Thrombin generation returned to normal within 30 min following the antidote administration. The mean unbound concentration of apixaban (9.3 ng/mL) and rivaroxaban (23.4 ng/mL) was also rapidly reduced (within 2–5 min) as compared to placebo (1.9 and 4.2 ng/mL, respectively). Concentrations of unbound apixaban and rivaroxaban returned to placebo levels within 1 to 3 h [51].

Neither thrombotic nor serious adverse events were recorded. Neutralizing antibodies against andexanet alfa were not detected. Of note, D-dimer and F1 + 2 were transiently increased and returned within normal range within 24–72 h [51]. These elevations may be explained by the formation of a complex between andexanet alfa and the natural anticoagulant tissue factor pathway inhibitor (TFPI) that fails to inhibit the factor VIIa-tissue factor (TF) complex and reducing the TFPI activity [7,51].

The clinical efficacy and safety of andexanet alfa in patients with FXa inhibitor–associated acute major bleeding has been investigated in a phase IIIb–IV, multicenter, prospective, open label, unblinded, single arm study named ANNEXA-4) [51,66,67]. Results of an interim analysis of 67 patients (mean age 77 years) have been published [66]. Among these patients, 32 received rivaroxaban (mostly 20 mg daily), 31 received apixaban (mostly 5 mg daily), and 4 received enoxaparin within the last 18 h prior to acute major bleeding. Gastrointestinal (49%) and IC (42%) bleedings were most frequently seen. All patients had a history of cardiovascular disease and thrombotic events and most of them (70%) received anticoagulant treatment for AF. Each patient received an IV bolus of andexanet alfa followed by a 2-h infusion. However, the dosing of andexanet alfa varied with the anticoagulant type and the timing of the last dose. If the last intake of apixaban or rivaroxaban was more than 7 h before the administration of the antidote, the IV bolus dose and the infusion dose were 400 mg and 480 mg, respectively. Higher doses of 800 mg for the IV bolus and 960 mg for the infusion were required if the delay since the last intake of enoxaparin or rivaroxaban was 7 h or less (or when the last intake was unknown). On average, patients received the IV bolus of andexanet within 4.8 ± 1.9 h following the presentation in the emergency department (ED). A follow-up period of 30 days (or until death) was respected for the collection of

all adverse events in every patient (n = 67). The efficacy population only included patients in whom the baseline anti-Xa activity was at least 75 ng/mL (or ≥ 0.5 IU/mL for enoxaparin) and for whom the acute bleeding episode was confirmed at adjudication (n = 47; 26 rivaroxaban, 20 apixaban, and 1 enoxaparin) [66]. The safety population included all the patients who received andexanet alfa [66].

At the end of the IV bolus administration, the median anti-Xa activity decreased by 89% (from 277 ng/mL to 16.8 ng/mL) and by 93% (from 149.7 ng/mL to 10.3 ng/mL) for rivaroxaban and apixaban, respectively. At the end of the 2-h infusion, the median anti-Xa activity decreased by 86% (from 277 ng/mL to 30.6 ng/mL) and by 92% (from 149.7 ng/mL to 12.5 ng/mL) for rivaroxaban and apixaban, respectively. After 4 h, a decrease of 39% and 20% was observed for rivaroxaban and apixaban respectively. Based on only one patient that received enoxaparin, a decrease from 0.61 IU/mL to 0.15 IU/mL was seen at the end of the IV bolus injection. At the end of the 2- and 4-h infusion, anti-Xa activities were 0.19 and 0.46 IU/mL respectively. The unbound concentration of rivaroxaban (ng/mL) also decreased with median percent changes of −80%, −65%, and −36% at the end of the IV bolus, at the end of the infusion, and 4 h after the end of the infusion, respectively. The median percent changes of the unbound concentration of apixaban (ng/mL) fell by −88%, −86%, and −39% at the end of the IV bolus, at the end of the infusion, and at 4 h, respectively [66].

A rating system was also used by an independent adjudication committee for the determination of the hemostasis efficacy in each case (i.e. visible cessation of bleeding, % increase in hematoma volume, % decrease in hemoglobin/hematocrit) (Table 1) [66]. Those criteria developed for andexanet alfa were based on the publication of Sarode *et al*. that evaluated the efficacy and safety of PCC in patients on VKA presenting with major bleeding [6,68,69]. Out of the 47 patients included in the efficacy population and 12 h after the antidote infusion, 31 were judged as having excellent hemostasis, 6 as having good hemostasis, 9 as having poor or no hemostasis efficacy, and one in which the hemostatic state could not be evaluated due to an administrative issue [68]. Interestingly, hemostatic efficacy was similar in patients taking rivaroxaban or apixaban. However, the percentage of patients that would have achieved hemostatic efficacy without the antidote administration could not be determined in this study given the lack of control group.

Safety data gathered in the 67 patients revealed that neither infusion reaction nor neutralizing antibodies to andexanet alfa were observed. However, 12 patients (1 myocardial infarction, 5 strokes and 8 VTE with some patients having more than one event; 18%) developed thrombotic events (i.e. myocardial infarction, stroke, deep-vein thrombosis, and PE) of which four developed a thrombotic event within 3 days and 8 between days 4 and 30 following andexanet alfa administration. Therapeutic dose of anticoagulation was only resumed before a thrombotic event in one patient out of 12 with thrombotic events. In the 67 patients, 27% resumed anticoagulation within 30 days and 15% died [68].

The final study report of the ANNEXA-4 study evaluating safety outcomes in 352 patients and efficacy in 254 patients was just released [67]. Only patients with baseline anti-Xa

Table 1. Rating system for effective hemostasis used in the ANNEXA-4 study [66]).

Bleed type	Excellent (effective)	Good (effective)	Poor/none (not effective)
Visible	Cessation of bleeding ≤ 1 h after end of infusion and no plasma, coagulation factor or blood products (excludes packed red blood cells)	Cessation of bleeding between > 1 and ≤ 4 h after end of infusion and ≤ 2 units plasma, coagulation factor or blood products (excludes packed red blood cells)	Cessation of bleeding > 4 h after end of the infusion and/or >2 units plasma, coagulation factor or blood products (excludes packed red blood cells)
Muscular/skeletal	Pain relief or no increase in swelling or unequivocal improvement in objective signs of bleeding ≤1 h after the end of infusion; and the condition has not deteriorated during the 12-h period	Pain relief or no increase in swelling or unequivocal improvement in objective signs of bleeding >1 and ≤4 h after end of infusion; and the condition has not deteriorated during the 12-h period	No improvement by 4 h after end of infusion and/or condition has deteriorated during the 12-h period
Intracerebral hematoma	≤20% increase in hematoma volume compared to baseline on a repeat computerized tomography (CT) or magnetic resonance imaging (MRI) scan performed at both the 1 and 12 h postinfusion time points	>20% but ≤35% increase in hematoma volume compared to baseline on a repeat CT or MRI scan at +12-hour time point	>35% increase in hematoma volume on a CT or MRI compared to baseline on a repeat CT or MRI scan at +12-h time point
Subarachnoid bleed	≤20% increase in maximum thickness using the most dense area on the follow-up vs. baseline at both the 1 and 12 h post infusion time points	>20% but <35% increase in maximum thickness using the most dense area on the follow-up at +12 h vs. baseline	>35% increase in maximum thickness using the most dense area on the +12 h vs. at baseline
Subdural hematoma	≤20% increase in maximum thickness at both the 1 and 12 h post infusion assessments compared to baseline	>20% but < 35% increase in maximum thickness at +12 h compared to baseline	>35% increase in maximum thickness at +12 h compared to baseline
Pericardial	No increase in the size of pericardial effusion on repeat echocardiogram done within 12 h of the end of infusion	<10% increase in the size of pericardial effusion on repeat echocardiogram done within 12 h of the end of infusion	10% or more increase in the size of pericardial effusion on repeat echocardiogram done within 12 h of the end of infusion
Intraspinal	No increase in hematoma size on repeat CT or MRI scan done within 12 h of the end of infusion	<10% increase in hematoma size on repeat CT or MRI scan done within 12 h of the end of infusion	10% or more increase in hematoma size on repeat CT or MRI scan done within 12 h of the end of infusion
GI, urinary or nonvisible bleeding not described above	≤10% decrease in both corrected hemoglobin/hematocrit at 12 h compared to baseline	>10% to ≤20% decrease in both corrected hemoglobin/hematocrit at 12 h compared to baseline	>20% decrease in both corrected hemoglobin/hematocrit

activity of ≥75 ng/mL (or ≥0.25 IU/mL for those receiving enoxaparin) were included in the efficacy analysis. All patients that have received andexanet alfa were included in the safety population. Excellent or good hemostasis were observed in 82% of patients (mean age 77 years) with a median decrease in anti-Xa activity of 92%, 92%, and 75% in patients receiving rivaroxaban (n = 100), apixaban (n = 134), and enoxaparin (n = 16), respectively. Most patients (80%) received anticoagulation for stroke prevention in NVAF. Gastrointestinal and IC bleedings accounted for 26 and 64%, respectively. The prevalence of thrombotic events and deaths at 30 days were 10% and 14%, respectively. In the patients who restarted oral anticoagulation (n = 100, 28%), no patient had a thrombotic event during the 30-day follow-up. As observed in the interim study, neither infusion reaction nor neutralizing antibodies to andexanet alfa were observed [67].

Three other trials (NCT03330457, NCT03310021, and NCT03083704) are currently recruiting subjects for further evaluation of the efficacy and safety of andexanet alfa [52,70]. The NCT03330457 trial is recruiting healthy adults exposed to betrixaban or placeboThe NCT03310021 trial is recruiting healthy adults exposed to apixaban, edoxaban, rivaroxaban, or placebo. The NCT03083704 trial is recruiting healthy adults treated with FXa inhibitors and aims at demonstrating safety and tolerability of second-generation andexanet alfa as compared to the first generation [52,70]. A recent study found that the second-generation andexanet alfa was well tolerated and as effective as the first generation in reducing blood loss and improving survival in a porcine polytrauma model with apixaban anticoagulation [71]. Finally, a randomized phase IV trial (NCT03661528) is also expected to begin in 2019 [67].

2.3. Current status of andexanet alfa

Since May 2018 and based on the results of the ANNEXA-A/R and ANNEXA-4 trials, the FDA has approved the use of andexanet alfa in adults treated with apixaban or rivaroxaban and presenting life-threatening or uncontrolled bleeding [47,51,52,66,72]. The lack of robust clinical evidence of other reversal strategies (i.e. PCC) also concurred to add andexanet alfa to formulary [26]. Andexanet alfa is however not indicated for the treatment of bleeding related to any other FXa inhibitors than apixaban and rivaroxaban given the lack of available data [48].

Andexanet alfa has only been approved in the United States and a black box warning has been added for thromboembolic risks, ischemic risks, cardiac arrest, and sudden death.

On 28 of February 2019, the Committee for Medicinal Products for Human Use reported a favorable consensus on its use in Europe only in patients treated with apixaban and rivaroxaban as well. This opinion will now be sent to the European Commission for the adoption of a decision on an EU-wide marketing authorization [73].

Meanwhile, alternative reversal strategies (i.e. PCC or aPCC) should be considered in countries in which andexanet alfa is not approved (i.e. Canada, Europe, and Asia) [47].

The average price of a 100 mg andexanet alfa vial is $3,300 US dollars (USD) [74]. Given that multiple administrations are required, the total price related to its use varies from $29,700 (9 vials for the low-dose regimen) to $59,400 USD (18 vials for the high-dose regimen). The cost of andexanet alfa is much more expensive than the cost of idarucizumab for the reversal of dabigatran ($4,200 USD) (which is similar to the cost of PCC 50U/kg IV [23]) and will likely be a major limiting problem [6,27]. Nevertheless, a recent analysis showed that the total healthcare costs may be significantly higher in the absence of a specific reversal agent in patient with major bleeding event [75,76]. Additional data are however required in real-world settings regarding the use of andexanet alfa [75].

2.4. Guidance for andexanet alfa administration

Andexanet alfa is available as a lyophilized drug supplied in single-use vials of 100 mg. Each vial is stable for 2 years between 2 and 8°C. For the reconstitution, it is recommended to slowly inject 10 mL of sterile water in each vial and to mix gently. The solution is then transferred into an IV bag to match the total volume needed. Reconstituted vials are stable at room temperature (RT) for up to 8 h and may be stored at 2–8°C for up to 24 h. Intravenous bags are also stable at RT for up to 8 h but only 16 h at 2–8°C [48].

Based on the type of FXa inhibitor, the last dose of FXa inhibitor and the time since last FXa inhibitor dose, two different dosing regimens exist (low- and high-dose regimens). These dosing regimens directly come from the ANNEXA-4 study and are presented in Table 2 [48].

Several strategies have been suggested for the administration of andexanet alfa as compared to other alternatives [23,47,77]. Sheikh-Taha *et al*. [29], Steiner *et al*. [47], and Weitz *et al*. [23] suggest the use of andexanet alfa for the reversal of apixaban or rivaroxaban in severe or life-threatening bleeding as a first line agent and only consider the use of PCC or aPCC (50 U/kg IV) in case of unavailability of andexanet alfa (outside the United States). The use of fresh frozen plasma is however not recommended [47]. In case of minor bleeding or moderate to severe bleeding, the use of andexanet alfa as well as other specific reversal agents has not been retained [23,24].

The ACC Expert Consensus Decision Pathway on Management of Bleeding in Patients on Oral Anticoagulants document suggests to administer PCC (50 U/kg IV) as first choice and aPCC (50 U/kg IV) if PCC is not available. Activated charcoal should be considered for known ingestion within 2–4 h. However, they do not consider the potential utilization of andexanet alfa [42]. However, this expert consensus was published before andexanet alfa was available and may need updates. The European Heart Rhythm Association Practical Guide on the use of nonvitamin K antagonist anticoagulants in patients with NVAF published in 2018 recommend the use of andexanet alfa in case of life-threatening bleeding (if available and approved) [78]. Otherwise, the use of PCC (50 U/kg IV, + 25 U/kg IV if indicated) or aPCC (50 U/kg IV, max 200 U/kg/day IV) has to be considered. The recent release of the full study report of the ANNEXA-4 study [67] and the approval of andexanet alfa in USA (and probably soon in Europe) will probably trigger the redaction of update guidelines.

Table 2. Dosing regimen for andexanet alfa administration [48,66].

		< 8 h or unknown			≥ 8 h
FXa inhibitor	Last dose of FXa inhibitor	Dose	Initial IV bolus	Follow-on IV infusion	Dose
Apixaban	≦ 5 mg	Low Dose	400 mg at a target rate of 30 mg/min (4 vials)	4 mg/min for up to 120 min (5 vials)	Low dose
	> 5 mg or unknown	High dose	800 mg at a target rate of 30 mg/min (8 vials)	8 mg/min for up to 120 min (10 vials)	
Rivaroxaban	≦ 10 mg	Low Dose	400 mg at a target rate of 30 mg/min (4 vials)	4 mg/min for up to 120 min (5 vials)	
	> 10 mg or unknown	High Dose	800 mg at a target rate of 30 mg/min (8 vials)	8 mg/min for up to 120 min (10 vials)	

In trauma patients receiving direct FXa inhibitors the use of PCC (25–50 IU/kg IV) is preferred in life-threatening bleeding events despite poor supporting evidence [79,80]. Alternatively, andexanet alfa could be considered but will be used off-label [27].

3. Expert opinion

The use of andexanet alfa has been approved by the FDA for adults treated with apixaban or rivaroxaban and presenting life-threatening or uncontrolled bleeding [47,51,52,66,67,72]. However, the efficacy of andexanet alfa has not been evaluated against a comparator group and questions the phase III study results [66,67,81]. A randomized trial (NCT03661528) is expected to begin in 2019 [67]. PCC may also be more useful as compared to specific antidote given that PCC play a double duty by reversing the action of DOACs and by treating trauma-induced coagulopathy [82], the initiation of placebo-controlled or PCC-controlled trials is thus needed to confirm the preliminary results [66,70,81].

A concern has also been raised regarding the 10% of patients who developed an ischemic event during the 30 days following the infusion of andexanet alfa [67,83]. The fact that only 28% of individuals resumed oral anticoagulation within the 30-day follow-up period in the ANNEXA-4 study could be related to the higher rate of thrombotic events observed [67,83,84]. In the patients who restarted oral anticoagulation (n = 100, 28%), no patient had a thrombotic event during the 30-day follow-up [67].

The update of decision-making guidelines regarding the timing of anticoagulation resuming in case of life-threatening bleeding is therefore needed [42–44]. In animal models and in healthy volunteers, a transient elevation in D-dimer, thrombin-antithrombin complex, and F1 + 2 has been observed following the administration of andexanet alfa [51,53,54,56]. Such markers can rise without necessarily being related to a prothrombotic state and no evidence of any thrombotic event has been observed in these populations [68,85–88]. The increase of anticoagulant levels after 4 h following the andexanet alfa administration may compensate this increase. Safety data in patients with previous thromboembolic events or disseminated intravascular coagulation, or having received nonspecific reversal agents prior to andexanet alfa are still required [55,89]. Andexanet alfa also transiently decreases TFPI activity [51]. Low TFPI levels have been associated with an increased risk of thrombotic events [89] and as such, an increased risk cannot be excluded on this basis with andexanet alpha. However, the temporal association between the transient decreased of TFPI due to its inhibition by andexanet alpha and a permanent decrease seen in other settings preclude firm conclusions. Of note, less thrombotic events occurred in FXa anticoagulated patients receiving PCC or aPCC as compared to andexanet alfa in case of major bleeding [29,35,37–39,41,77,90]. However, the level of evidence of such strategies are limited [28–30,35,39,41], optimal dosing regimens of PCC or aPCC are not yet known (possibly exposing the patient to unnecessary prothrombotic risks) [38,82,91,92], and it is not possible to assess the efficacy of these strategies with the anti-Xa activity [93]. Recent data from the French pharmacovigilance database suggest that idarucizumab was not superior to aPCC or PCC in terms of fatality rate (17.6% vs. 18.6%) [94].

Another major problem arises from the availability and the cost of andexanet alfa. Eligible patients will not be able to benefit from this drug [6].

The median delay of 4.8 h from ED presentation to the antidote administration was probably due to the complexity of participation in the clinical trial but could also complicate the interpretation of the results in real life settings [66,68,81]. Nevertheless, an earlier administration could improve the efficacy of andexanet alfa [68]. In the recent study of Culbreth, the time from order to andexanet alfa delivery and administration was comprised between 43 min and 66 min [72]. The time for antidote preparation was long because multiple vials had to be reconstituted.

Moreover, there are many situations in which the administration of andexanet alfa has not been evaluated or approved by the FDA (Table 3) [48,55,66,72]. In case of renal insufficiency, effects of the FXa inhibitors may return after stopping the infusion of andexanet alfa. The possibility of reaching optimal duration of infusion may therefore be useful but need to be evaluated. In case of off-label criteria, an approval from hemostasis experts is required to possibly use andexanet alfa [72].

In the recent study of Culbreth *et al*., 15 patients received andexanet alfa since the approval of the antidote in their institution [72]. Out of these 15 patients (apixaban = 7, rivaroxaban = 8), many would have been excluded from the ANNEXA-4 study because anticoagulants taken more than 18 h before presentation (n = 4), pulmonary embolism within two weeks (n = 1), PCC utilization prior to transfer (n = 7), and surgery within 12 h following the antidote administration (n = 3) [72]. The time from order to andexanet alfa delivery and administration was 43 min and 66 min, respectively [72]. The time for antidote preparation was long because multiple vials had to be reconstituted (Table 2). The communication between acute or emergency care specialists, hemostasis experts, pharmacy, and nursing staff is mandatory for the management of bleeding anticoagulated patients [18,72,77].

Table 3. Situations in which the administration of andexanet alfa has not been evaluated or approved by the FDA [48,66,72].

- Expected survival less than 1 month
- ICH with GCS < 7
- Intracerebral hematoma volume > 60 mL
- Last anticoagulant dose > 18 h prior administration
- Less severe bleeding
- Thrombotic event within 2 weeks before administration
- Other FXa inhibitors than apixaban and rivaroxaban (i.e. betrixaban, edoxaban)
- Patient requiring surgery (less than 12 h before administration), excluding minor intervention
- Pediatric patients
- Pregnancy/lactation
- Renal/hepatic failure
- Traumatic bleeding
- VKA, dabigatran, PCC, aPCC, rVIIa, whole blood, or plasma within 7 days before administration
- Planned administration of PCC, fresh frozen plasma, or rVIIa within 12 h following the administration of andexanet alfa

Another problem arises when an unconscious trauma patient taking DOAC is referred to the ED but without the correct identification of the type of DOAC [77]. Specific assays for DOAC measurement can be implemented to discriminate between thrombin and FXa inhibitors [13]. These assays must be available 24/7 with short turn-around times (TAT) to be consistent with the urgency of clinical decision making for DOAC reversal [54,77]. This includes faster centrifugation processes [95] or the use of point-of-care testing [77]. Rapid determination of the DOAC concentration may be helpful to guide the andexanet alfa administration (if information (Table 1) is not provided by the patient or relatives (i.e. time since last intake, type of drug, dose regimen) and to follow the efficacy of andexanet alfa administration [77]. In case of serious bleeding, a drug concentration > 50 ng/mL has been proposed to warrant antidote administration while a drug concentration > 30 ng/mL should be considered in case of planned urgent intervention [18].

Identification of drug–drug interactions and adapted dosing regimens in some situations is also needed since the package insert does not list any interaction [6,48,55]. Most patients (78%) included in the phase III study received an anticoagulant in the context of NVAF. The same level of efficacy remains to be confirm for other indications with a higher level of evidence [6,52].

Finally, other antidotes are in development including UHRA (Universal Heparin Reversal Agent) for the reversal of all heparin anticoagulants; ciraparantag (PER977), a small-molecule antidote for UFH, LMWH, and DOACs; chimeric FX-C, a FXa variant antidote for direct FXa inhibitors; and FXa^{I16L} (PF-05230907), a recombinant FXa variant for direct FXa inhibitors [6,7,47,49,96–99].

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•• **Initial proof-of-concept study on andexanet alfa.**

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