FDA U.S. FOOD & DRUG ADMINISTRATION

Memorandum

To: **File (STN BL 125586/0)**

From: **Mikhail Ovanesov, PhD, Research Biologist, Hemostasis Branch (HB), Division of Plasma Protein Therapeutics (DPPT), OTAT/CBER**

Through: **Tim Lee, PhD, Chief, HB/DPPT/OTAT/CBER**

Basil Golding, MD, Director, DPPT/OTAT/CBER

Subject: **An addendum to CMC review (17 August 2016) of Portola's BLA for coagulation factor Xa (recombinant), inactivated [ANDEXXA] & review of Portola's 3 August 2017 responses to the *Complete Response Letter***

Contents

1. Executive Summary

This memorandum is an addendum to my Chemistry, Manufacturing and Controls (CMC) review dated 17 August 2016 for Portola Pharmaceuticals Inc. (Portola)'s original biologics license application (BLA) STN 125586/0 for *coagulation factor Xa (recombinant), inactivated* with the proprietary name ANDEXXA, and International Nonproprietary Name (INN) *andexanet alfa*. The purpose of this memorandum is to summarize my review of Portola's 3 August 2017 responses to the FDA Complete Response Letter (CRL), and associated amendments.

ANDEXXA is presented as a lyophilized cake for intravenous administration after reconstitution with sterile Water for Injection. Portola proposed two dosing regimens for ANDEXXA. The lower dosing regimen consists of an initial bolus of 400 mg followed by a 2-hour infusion of 480 mg of the product, totaling 880 mg. The higher dosing regimen consists of an initial bolus of 800 mg followed by a 2-hour infusion of 960 mg, totaling 1760 mg. The safety and effectiveness of longer and higher doses, or repeat treatment with ANDEXXA have not been evaluated out of concerns for increased risks of thrombotic adverse events.

The active ingredient of ANDEXXA is a genetically modified variant of human coagulation Factor (F)Xa produced by recombinant DNA technology in a Chinese Hamster Ovary (CHO) cell line. ANDEXXA was designed to bind small-molecule anticoagulant drugs that inhibit FXa, e.g., apixaban and rivaroxaban. In the ANDEXXA molecule, the serine residue responsible for the FXa proteolytic activity was replaced with alanine, and the gamma-carboxyglutamic acid (Gla) domain responsible for binding to procoagulant lipid was genetically removed. The aim was to prevent the activation of blood coagulation, but retain the protein's ability to bind FXa inhibitors. Since FXa and ANDEXXA bind to FXa inhibitors with comparable affinities, ANDEXXA competes with FXa for these inhibitors, and increases the activity of FXa in blood.

It is important to note that ANDEXXA has two independent mechanisms of action. ANDEXXA's second physiological target, like that of FXa, is Tissue Factor Pathway Inhibitor (TFPI), an endogenous protein which, in complex with FXa, FVIIa and Tissue Factor, acts as the only known inhibitor against the initiation of blood coagulation via the Tissue Factor (extrinsic) pathway. Because FXa's Gla domain is needed for the formation of the quaternary complex to exert inhibition, the binding of ANDEXXA to TFPI interferes with TFPI's inhibitory action, and thereby allows the activation of blood coagulation to proceed.

Portola is conducting a program to demonstrate reversal of the anticoagulant effect of direct FXa inhibitors as measured by an anti-FXa activity assay in patient plasma. Portola proposed that the reduction of anti-FXa activity by ANDEXXA is reasonably likely to predict clinical benefit, and could be used as a surrogate endpoint in clinical studies. Controlled pre-licensure clinical studies using clinical endpoints, such as cessation of bleeding or decrease in bleeding-associated mortality, were deemed not feasible. FDA had agreed with this proposal, and determined that an *Accelerated Approval* pathway was appropriate for a BLA for the reversal of anticoagulation in patients who are taking direct FXa inhibitors.

CMC Issues Resolved during BLA Review

The scope of this CMC review doesn't include stability studies (reviewed by Dr. Yideng Liang), safety regarding adventitious agents (reviewed by Dr. Ze Peng), validation of immunogenicity

assays (reviewed by Dr. Zuben Sauna), structural characterization studies by (b) (4) (reviewed by Dr. Wojciech Jankowski), Final Drug Product (FDP) release methods and development of associated reference standards (reviewed by a team from OCBQ/DBSQC), justification for in-process and release specifications, and extractables and leachables studies (reviewed by Dr. Andrey Sarafanov).

Specific CMC issues were conveyed to the sponsor as information requests (IRs) in the course of the review as well as in a CRL at the completion of review cycle. At the request of the FDA, Portola:

- investigated the sources of (b) (4) activity responsible for degradation of ANDEXXA intermediates;
- provided additional validation of the manufacturing process including validation of (1) (b) (4) impurities clearance, (2) in-process control parameters and hold times for process intermediates, (3) equipment cleaning, (4) control strategy for the critical process parameters discovered post process validation, and (5) Container/Closure System Integrity Testing (CCIT);
- performed additional experiments and analyses to complete the validation of the analytical methods, and revised release specifications for the Bulk Drug Substance (BDS) and FDP and stability studies;
- provided additional information on extractables and leachables;
- developed a reference standard for the potency of ANDEXXA, and demonstrated comparability between the previous (b) (4) and the proposed commercial (b) (4) ;
- addressed deviations from CGMP requirements during the FDP process performance qualification (PPQ) studies;
- provided stability data to support the proposed shelf-lives of the commercial product;
- removed the Comparability Protocol (CP) which was inadequate for the proposed post-approval changes in the planned scaled-up process; and
- addressed observations from the Pre-License Inspection.

At the request of the FDA, Portola provided additional information on the bioanalytical methods used in the clinical trials, which include:

- development and validation of the assays used to measure neutralizing antibodies against endogenous Factors X and Xa, and antibodies against (b) (4) ;
- development and validation of TF-independent thrombin generation (TG) assay to assess TFPI-independent action of ANDEXXA;
- data on bridging between TG assay methods used in Phase 1, 2, and 3 clinical trials;
- validation of TFPI activity assay; and
- data on bridging between the TFPI activity assay used in the Phase 1 study, and the TFPI antigen assays used in the Phase 2 and 3 studies.

Unresolved issues not directly related to CMC

I also reviewed several sections in other modules of the BLA related to the mechanisms of action of ANDEXXA, and have the following concerns regarding the efficacy and safety of the product.

These issues are as follows:

1. **The clinical data collected to date do not support the assumption that reversal of anti-FXa activity by ANDEXXA is reasonably likely to predict clinical benefit.** The clinical reviewer found poor correlation between Portola's surrogate marker and clinical outcomes in patients who are taking FXa inhibitors in the ongoing confirmatory trial. These results confirm the long-standing concerns expressed by the FDA since the beginning of our review in 2009 about the use of anti-FXa activity reversal as a surrogate marker to support the *Accelerated Approval* pathway of ANDEXXA.
2. **The duration of the inhibition of TFPI following administration of ANDEXXA has not been established, therefore, the risk associated with the exposure of patients to potentially thrombogenic conditions is not known**. Portola provided incomplete data on the changes of the level of TFPI activity over time, and no data on when TFPI activity would return to either the pre-ANDEXXA treatment level, or the normal range. Underestimation of the effect of TFPI inhibition by ANDEXXA may increase the risk of thrombosis, particularly, if ANDEXXA is used off-label at higher doses, with longer infusions, or used repeatedly, or in patients without major bleeds.
3. **The Thrombin Generation (TG) biomarker failed to correlate with the clinical effect of ANDEXXA in the intended patient population.** In most patients, contrary to the observations in healthy volunteers, and theoretical expectations, TG was not inhibited below normal prior to ANDEXXA treatment. In many patients, TG was not improved after ANDEXXA treatment. These observations question the current understanding of the risks and benefits of ANDEXXA for its proposed indications.
4. **The interpretation of confirmatory clinical trials is confounded by the fact that Portola had not established comparability between investigational material used in confirmatory trials (Generation 2 material) and ANDEXXA product that has been described in the BLA.** Results from analytical and manufacturing studies indicate a lack of comparability between the ANDEXXA product described in the BLA, and the product manufactured using a non-validated (b) (4) GEN 2 process; GEN 2 is not described in the BLA. In the absence of results from a human PK/PD study that demonstrate comparability between the two materials, data from patients who received the GEN 2 product in the confirmatory study should be excluded from analysis, which will result in the need for additional enrollment. As a result, the conduct of confirmatory studies, required under the *Accelerated Approval* pathway, should be considered pending.
5. **Inappropriate promotion of ANDEXXA may result in its unnecessary and unsafe use**. The new data presented in Portola's response to the CRL confirm that ANDEXXA has a significant procoagulant effect mediated by the sustained inhibition of TFPI. However, based on Portola's submitted advertising materials, ANDEXXA will be promoted as a *specific* antidote to FXa inhibitors, ignoring unintended and confirmed procoagulant risk of the product. This despite the data indicating that the duration of anti-TFPI action far exceeds the duration of anti-FXa reversal.
6. **Based on the concerns delineated above, a randomized controlled trial (RCT) is needed to evaluate the risk/benefit of ANDEXXA**. A post-licensure RCT may not be feasible for logistical reasons.

Reviewer's Conclusion & Recommendation for BLA STN 125586/0:

I conclude that Portola has satisfactorily addressed all the major CMC issues raised in the 17 August 2017 CR Letter. There are no outstanding CMC issues associated with this BLA.

However, I have unresolved concerns about the product's potentially unsafe and unnecessary use, including off-label use, if the ANDEXXA BLA is approved.

2. Background

2.1. Scientific background

ANDEXXA was developed to address an unmet medical need for patients treated with direct FXa inhibitors when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding. These small-molecule drugs bind and inhibit FXa activity directly, without the involvement of antithrombin III. In the United States (U.S.), these FXa inhibitor products are approved for the prevention of stroke in patients with nonvalvular atrial fibrillation, prevention of deep vein thrombosis (DVT) in hip or knee replacement surgery, and treatment and secondary prevention of venous thromboembolism (VTE) including DVT. These direct oral FXa inhibitors, together with direct oral thrombin inhibitors, belong to a class of anticoagulants known as direct oral anticoagulants (DOACs) or novel oral anticoagulants (NOACs). Since the approval of the first product in 2010, DOACs have been adopted rapidly reaching 4.21 million treatment visits in 2014, matching the use of the older oral anticoagulant drugs, such as vitamin K antagonists[1].

Direct FXa inhibitors are associated with an increase in bleeding events, some of which are life-threatening or fatal. There are no products licensed for the reversal of these direct FXa inhibitors. Administrations of Prothrombin Complex Concentrates (PCCs), activated PCC, or recombinant activated Coagulation Factor VII (rFVIIa) are currently used off-label for emergency care of patients receiving direct FXa inhibitors[2,3,4].

The active ingredient in ANDEXXA is a genetically modified variant of human FXa. Portola has designed ANDEXXA to bind the direct FXa inhibitors. FXa inhibitors reduce the ability of FXa to activate prothrombin to thrombin (Figure 1B). Similar to FXa, *coagulation factor Xa (recombinant),*

[1] Barnes GD et al. National Trends in Ambulatory Oral Anticoagulant Use. *Am J Med*. 2015 Dec;128(12):1300-5.e2.
[2] Eikelboom JW et al. Emergency care of patients receiving non-vitamin K antagonist oral anticoagulants. *Br J Anaesth*. 2018 Apr;120(4):645-656
[3] Tomaselli GF et al. 2017 ACC Expert Consensus Decision Pathway on Management of Bleeding in Patients on Oral Anticoagulants: A Report of the American College of Cardiology Task Force on Expert Consensus Decision Pathways. *J Am Coll Cardiol*. 2017 Dec 19;70(24):3042-3067
[4] Majeed A et al. Management of rivaroxaban- or apixaban-associated major bleeding with prothrombin complex concentrates: a cohort study. *Blood*. 2017 Oct 12;130(15):1706-1712

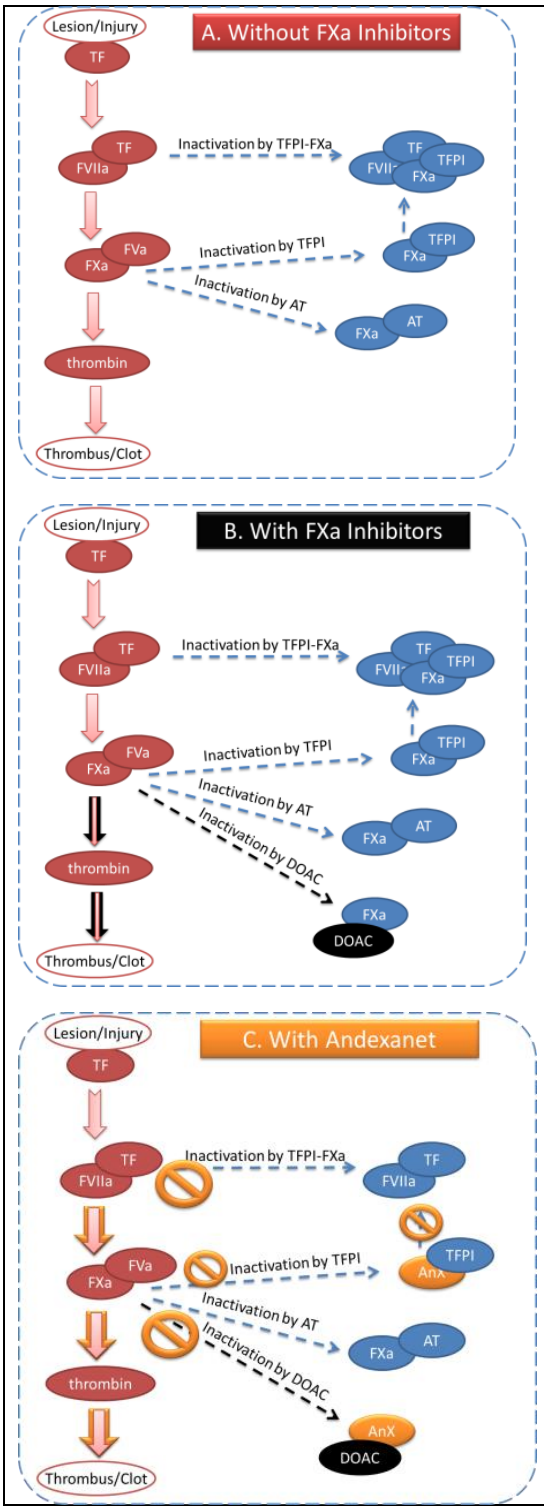


Figure 1: Mechanisms of action of direct FXa inhibitors and ANDEXXA.

A. Coagulation process without FXa inhibitors and ANDEXXA. Coagulation is initiated by exposure of Tissue Factor (TF) at the site of a vascular lesion followed by the formation of the TF-FVIIa complex (extrinsic FX-ase), activation of FX by TF-FVIIa (i.e., FXa generation), formation of FXa-FVa (prothrombinase complex) and activation of prothrombin to generate thrombin. Thrombin activates platelets and fibrinogen to fibrin, which leads to a hemostatic plug or thrombus formation. This process is inhibited by TFPI and antithrombin III (AT). TFPI inhibits FXa and TF-FVIIa in two stages: first, TFPI binds FXa to form a TFPI-FXa complex, and second, to form a stable inactive complex of TFPI-FXa-FVIIa-TF.

B. Direct Oral Anticoagulants (DOACs) rivaroxaban and apixaban facilitate FXa inhibition leading to reduced thrombin generation and reduced thrombus formation.

C. ANDEXXA (AnX) blocks rivaroxaban and apixaban leading to restoration of thrombin generation. In addition, AnX inactivates TFPI preventing its inhibition of TF activity. This leads to elevated TF-activated generation of FXa and thrombin.

inactivated forms a 1:1 inactive complex with FXa inhibitors leading to their sequestration from plasma. ANDEXXA lacks the FXa catalytic activity due to the replacement of the active site serine with alanine, and is therefore unable to cleave and activate prothrombin. ANDEXXA also lacks the γ-carboxyglutamic acid (Gla)-containing domain of FXa, thus preventing its incorporation into the prothrombinase complex. The lack of interference in the formation of the prothrombinase

complex is important for normal thrombin generation because the prothrombinase complex is ~300,000 fold more active than FXa alone in the activation of prothrombin to thrombin. Treatment with ANDEXXA is designed to reduce the concentration of FXa inhibitors, which should result in the restoration of normal thrombin generation needed to stop bleeding (Figure 1C).

ANDEXXA's 's second mechanism of action, binding and inactivation of TFPI, may also contribute to its procoagulant and/or thrombogenic activity. TFPI is the only known inhibitor of tissue factor (TF) which is a transmembrane glycoprotein responsible for the initiation of thrombin generation at the site of vascular lesions. Activation of coagulation starts with the formation of a complex between TF and FVIIa. The TF-FVIIa complex activates FX to FXa. TF-mediated activation of coagulation is down-regulated by the formation of TFPI-FXa complex, which leads to the formation of an inactive quaternary complex of TF, FVIIa, FXa and TFPI, thereby inhibiting coagulation. Like FXa, ANDEXXA binds TFPI, but the absence of the Gla domain prevents the formation of the inactive quaternary complex[5], rendering the TFPI inactive. The result is the acceleration of the generation of FXa and thrombin, as described in Portola's 2013 patent application[6] (Figure 1C), which would, in turn, promote thrombosis.

2.2. Regulatory history

The product is developed in the U.S. under Investigational New Drug application (IND 15089) for the proposed indication in patients who use FXa inhibitors when reversal of anticoagulant effect is needed due to serious uncontrolled bleeding events, (b) (4) . ANDEXXA, if approved, will be the first recombinant FXa product, and the first product indicated for the reversal of the FXa inhibitors, rivaroxaban and apixaban.

On 22 November 2013, ANDEXXA received *Breakthrough Therapy* designation under IND 15089. On 23 February 2015, ANDEXXA also received *Orphan* designation for the proposed indication of "*reversing the anticoagulant effect of direct or indirect factor Xa inhibitors in patients experiencing a serious uncontrolled bleeding event* (b) (4) ".

The BLA was submitted as a rolling review. The Nonclinical sections in Module 2 (2.4 and 2.6) and Module 4 were received on 6 November 2015. The remaining Modules 1, 2, 3 and 5 were received on 17 December 2015. The original application was reviewed under a *Priority Review* schedule, and subject to PDUFA V requirements. During the first review cycle, CBER reviewers found significant deficiencies in the CMC and clinical information, which resulted in the issuance of a *Complete Response Letter* (CRL) to Portola on 17 August 2016.

On 3 August 2017, Portola responded to the CRL with additional CMC and clinical information. The clinical team determined that Portola's response was incomplete, and requested additional information. On 18 December 2017, Portola submitted a BLA amendment containing revisions to

[5] Marlu R & Polack B. Gla-domainless factor Xa: molecular bait to bypass a blocked tenase complex. *Haematologica* 2012 Aug;97(8):1165-72

[6] Patent WO 2014116275 A1 "Inhibition of tissue factor pathway inhibitor with factor Xa derivatives". Publicly available source: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2014116275

the safety data related to thrombotic events. The data presented in this amendment were part of a series of amendments, submitted between October 16 and December 1 of 2017, that were meant to provide the clinical data needed by the FDA to complete the risk assessment in relation to the CRL. On 22 December 2017, FDA determined that the 18 December 2017 submission is a major amendment, because it contained a substantial amount of new data not previously submitted to, or reviewed by the Agency, that added an additional three months to the review clock. Therefore, the new action due date is 4 May 2018.

Since ANDEXXA received *Breakthrough Therapy* designation, FDA has provided Portola frequent guidance on its development. The CBER CMC review team for ANDEXXA remained unchanged since the first meeting with Portola in 2009, and the primary clinical reviewer was changed once. The BLA was submitted in accordance with 21 CFR, Part 601.40, Subpart E *Accelerated Approval of a Biological Product for a Serious or Life-threatening Illness*. The data used to support *Accelerated Approval* came from 5 pharmacokinetic (PK) and pharmacodynamic (PD) studies of healthy volunteers in which the reversal of anti-FXa activity was used as a surrogate endpoint. In these studies, an anti-FXa activity assay was used to measure the concentration of FXa inhibitors in plasma. Because the concentration of FXa inhibitor has been shown to correlate with its anticoagulant action, Portola proposed that the reduction of anti-FXa activity by ANDEXXA is reasonably likely to predict clinical benefit, and could be used as a surrogate endpoint in clinical studies.

In the healthy volunteer clinical studies, a short-term transient reversal of anti-FXa activity, and a more sustained procoagulant effect of inactivation of the TFPI activity, as evidenced by sustained elevated levels of thrombin generation and elevation of thrombogenicity markers TAT, PF1.2 and D-dimer, were observed. Consequently, throughout the ANDEXXA development program, FDA raised several concerns about the suitability of the proposed surrogate endpoint, some of which remain unresolved, e.g.,

- the level to which the anti-FXa activity should be reduced to achieve clinical benefit remains unknown;
- ANDEXXA's reversal of anti-FXa activity is incomplete in some patients on rivaroxaban and apixaban, as well as in most patients on other FXa inhibitors;
- the duration of FXa inhibitor reversal at the proposed doses may be too short for indications like ICH where a sustained reversal of anticoagulation is needed;
- ANDEXXA delays the clearance of FXa inhibitors, and is associated with a rebound in anti-FXa activity after ANDEXXA infusion; and anti-FXa activity reversal is not sensitive to the second mechanism of action, inhibition of TFPI activity.

To support the application, Portola submitted the results from an ongoing single-arm Phase 3b/4 clinical study (ANNEXA 4) in the intended patient population. In this study, ANDEXXA demonstrated anti-FXa reversal, but the changes in FXa inhibitory activity did not predict the clinical outcomes, while the risk of thrombosis in these patients was elevated. Therefore, FDA recommended Portola to conduct a Usual Care Cohort (UCC) study and a Randomized Control Trial (RCT) to evaluate ANDEXXA's clinical benefit versus the available standard of care.

3. CMC summary

a) Product Quality

Manufacturing Process

The Bulk Drug Substance (BDS) and Final Drug Product (FDP) of ANDEXXA are manufactured at two FDA-licensed manufacturing facilities. The BDS is manufactured at (b) (4) in (b) (4); and the FDP at (b) (4) in (b) (4).

Bulk Drug Substance

The recombinant FXa variant of ANDEXXA, *coagulation factor Xa (recombinant), inactivated*, is expressed in a CHO cell line, (b) (4)

(b) (4)

(b) (4)

Final Drug Product

Between (b) (4) batches are used to manufacture (b) (4) batch of FDP which may consist of approximately (b) (4) vials, sufficient to deliver an approximate (b) (4) low doses or (b) (4) high doses. There is a (b) (4). ANDEXXA is provided as a sterile, non-pyrogenic, white to off-white lyophilized cake in single-use glass vials, each containing about 100 mg of the recombinant protein. After reconstitution with 10 mL of sterile Water for Injection (sWFI), ANDEXXA forms a clear, colorless solution of the following composition: 100 mg (10 mg/mL) *coagulation factor Xa (recombinant), inactivated*, 12.2 mg tromethamine, 94.8 mg L-arginine hydrochloride, 200 mg sucrose, 500 mg mannitol, and 1 mg polysorbate 80. sWFI is not provided with ANDEXXA.

The drug product is filled into a 20-mL clear (b) (4) glass vial with a 20-mm finish ((b) (4)) and stoppered with a gray 20-mm (b) (4) and (b) (4)

chlorobutyl rubber stopper ((b) (4)). The vial with stopper is capped with a 20-mm aluminum flip-off seal with a blue polypropylene flip-off cap ((b) (4)). (b) (4) conducted the container closure integrity testing on the vials using a (b) (4) method; all acceptance criteria were met.

Reviewer's comment: The Container Closure Integrity Testing data were reviewed by Dr. Christine Harman, DMPQ. Please refer to her review memorandum for details.

Manufacturing process development

Portola has thus far used (b) (4) manufacturing processes to produce ANDEXXA (b) (4) at the (b) (4) scale. (b) (4) was the GMP process to produce the first (b) (4) FDP batches used in clinical trials. The subsequent (b) (4) included the addition of (b) (4) Polysorbate 80 in the final formulation so that the (b) (4) could be concentrated from (b) (4) to 10 mg/mL during FDP manufacture. (b) (4) (b) (4) batches were produced and used to support the Phases 2 and 3 trials with healthy volunteers. (b) (4) is the proposed commercial manufacturing process. The most significant changes from (b) (4) to (b) (4) are the introduction of the (b) (4) . (b) (4) also incorporates the final (b) (4) and formulation steps using the FDP (b) (4), instead of it being part of the FDP process.

The FDP manufacturing process has been changed (b) (4) concurrent with the respective changes in the (b) (4) manufacture from (b) (4) to (b) (4) . The FDP of (b) (4) was presented as a (b) (4) . (b) (4) introduced a lyophilized dosage form formulated at higher concentration (10 mg/mL) with mannitol manufactured at (b) (4) in (b) (4) . The FDP of (b) (4) used the same formulation as (b) (4) , but is manufactured at an approximately (b) (4) in scale than (b) (4) , at the new FDP contract manufacturer, (b) (4) in (b) (4) .

Only (b) (4) materials were used in the pivotal Phase 3 clinical studies intended to provide evidence to support the ANDEXXA BLA through the *Accelerated Approval* pathway. (b) (4) materials were introduced in the ongoing confirmatory Phase 3b/4 clinical study in February 2016.

In-Process Controls

In-process controls (IPC) for the commercial process were developed using a risk-based approach to ensure the consistency of the manufacturing process and product quality. Portola proposed IPC parameters that are most likely to affect product quality attributes. The limits for the IPC parameters were based on prior manufacturing experience rather than prospective process optimization studies, which are normally done to define the edge-of-failure boundaries for critical and non-critical IPC parameters. Because Portola has limited experience with commercial (b) (4) , the IPC limits were developed using data collected from (b) (4) rather than (b) (4)

(b) (4). The use of data from (b) (4) was supported by the results of analytical studies that demonstrated comparability of (b) (4) FDP batches manufactured by all (b) (4) processes.

Process Performance Qualification

Process Performance Qualification (PPQ) covers the two major stages of production - (b) (4) (b) (4) PPQ batches) and FDP (b) (4) PPQ batches). Although the data support a successful PPQ, Portola experienced several deviations post-PPQ, which resulted in the development of (b) (4) new IPCs and adjustment of the existing IPC limits. These changes were validated to demonstrate adequate control over the manufacturing process.

In addition to the PPQ studies, several ancillary validation studies were performed to support the consistency of the manufacture of ANDEXXA BDS. The studies included the validation of *Impurity Clearance, In-Process Hold Time,* (b) (4), and (b) (4), as well as *Shipping Qualification*.

Portola developed Continued Process Verification (CPV) plans for both (b) (4) and (b) (4) to ensure that the ANDEXXA manufacturing processes are in a state of control throughout the product lifecycle. The CPV program is designed to collect process data and perform statistical evaluation of the dataset to routinely confirm the processes be in a state of control, and to identify and evaluate planned and unplanned changes in the manufacturing processes.

Potency

ANDEXXA is dosed by mass, therefore, it is important to establish a meaningful potency assay to assess the structure and function of the protein for the control of manufacturing process, product quality, and dosing consistency. Three assays were developed for the activity of ANDEXXA based on its effect on the (i) direct FXa inhibitor, (ii) indirect FXa inhibitor, and (iii) TFPI: (i) The assay using a direct FXa inhibitor, (b) (4), is designed to measure the activity of ANDEXXA based on its ability to reverse the inhibition of FXa activity by (b) (4). The assay mixture is composed of ANDEXXA, FXa and (b) (4). (ii) Similarly, the assay using an indirect FXa inhibitor, (b) (4), is performed with (b) (4) in place of (b) (4). (iii) The assay using TFPI measures the ability of ANDEXXA to reverse the inhibition of the human TF-FVIIa complex by TFPI. (b) (4)

In all three assays, the remaining FXa activity is measured by an FXa-specific (b) (4). The potency of ANDEXXA is determined by comparing the response of the test sample to that of the reference standard.

The assay for the reversal by ANDEXXA of the activity of direct FXa inhibitor is relevant to the biomarker, anti-FXa activity. Its reversal was proposed as the reasonably likely surrogate endpoint in the Phases 1, 2 and 3 clinical studies. However, the ability of this surrogate endpoint to predict the clinical outcome has yet to be established.

The activity assays used for the control of product manufacture and quality, and the ones used in the clinical studies differ in the source of FXa. The product activity assays use human FXa, and the clinical assays use (b) (4) FXa.

Release specifications

The specifications of (b) (4) FDP are summarized in Tables 1 and 2 below. The methods and specifications are established based on manufacturing experience and theoretical safety considerations. *Reviewer's comment: Release specifications were reviewed by Dr. Andrey Sarafanov. Dr. Sarafanov concluded that Portola has satisfactorily addressed all the major issues raised in the 17 August 2017 CR letter, and recommends approval of the original BLA for ANDEXXA. Please refer to his review memorandum and to section 5 "Review of CRL responses" for details.*

(b) (4)

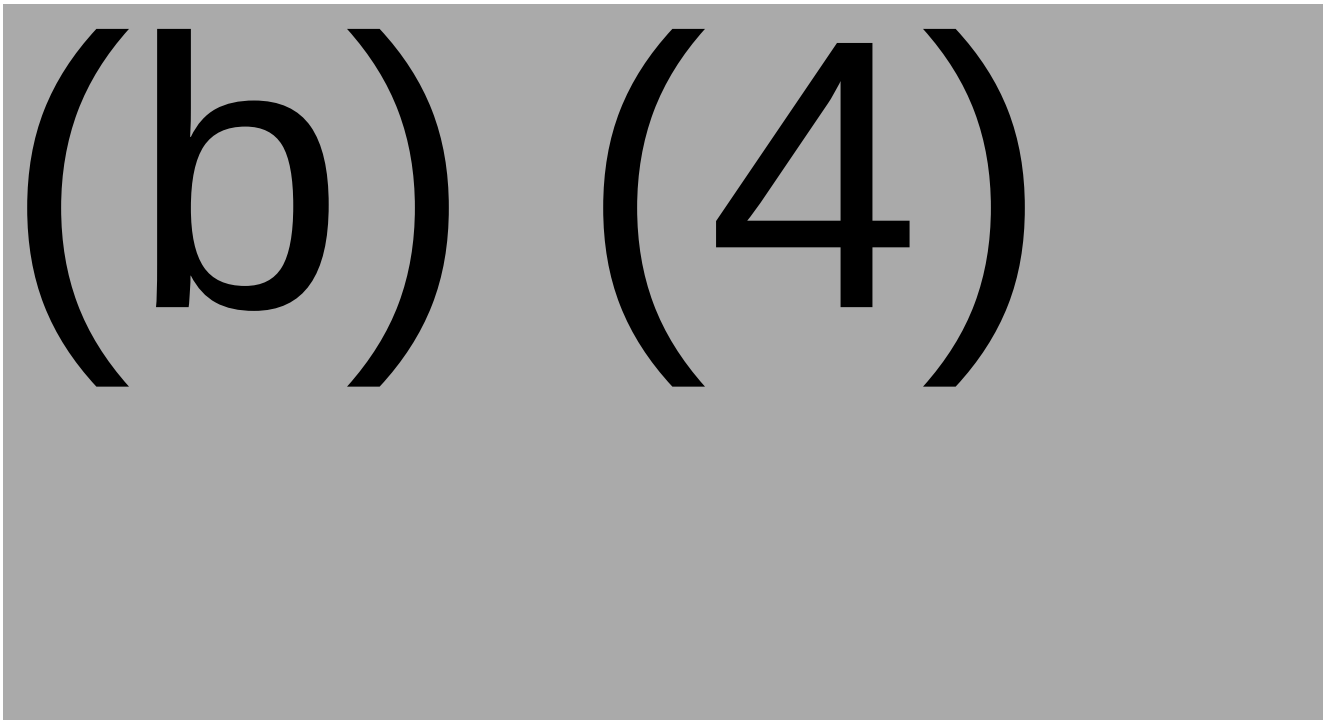


Table 2: **FDP Specifications**

Test Method	**Parameter monitored**	**Acceptance Criteria**
Tests Performed on Lyophilized Product:		
Visual Appearance	Characteristics	White to off-white lyophilized cake
Reconstitution Time	Characteristics	(b) (4)
Moisture Content per (b) (4)	Characteristics	(b) (4)
Tests Performed on Product after Reconstitution with sWFI		
Sterility [a] per (b) (4)	Purity	Sterile
Endotoxin per (b) (4)	Purity	(b) (4)
Appearance after Reconstitution	Characteristics	Clear, colorless to slightly yellow solution, essentially free from visible particulates
pH per (b) (4)	Characteristics	(b) (4)
Osmolality per (b) (4)	Characteristics	(b) (4)
Sucrose Content	Characteristics	(b) (4)
Mannitol Content	Characteristics	(b) (4)
Polysorbate 80 Content	Characteristics	(b) (4)
Direct Potency	Identity and Potency	(b) (4)
Indirect Potency	Potency	(b) (4)
(b) (4) TFPI Inhibition	Potency	(b) (4)
Protein Concentration by (b) (4)	Potency	(b) (4)

Test Method	Parameter monitored	Acceptance Criteria
Purity by (b) (4)	Purity	(b) (4)
(b) (4)	Purity	
(b) (4)	Purity	
(b) (4)	Purity	
Particulate Matter per (b) (4)	Purity	
(b) (4)	Purity	

Abbreviations and Notes:

(b) (4)

[a] Sterility per (b) (4) is performed for batch release. Container closure integrity testing per (b) (4) is performed instead of the (b) (4) sterility test at (b) (4) stability time points.

(b) (4)

<u>Reviewer's comment:</u> I agree with the proposed list of methods used for release of (b) (4) FDP and proposed specification limits. The proposed limits for each of ANDEXXA (b) (4) assays are relatively wide, e.g., FDP Direct Potency: (b) (4). This approach is acceptable for control of ANDEXXA activity because (b) (4) FDP batches are controlled by (b) (4) independent (b) (4) assays, with (b) (4) specification limits for each assay ((b) (4)).

<u>Analytical Methods</u>

Release methods were validated for their suitability for the intended use. The results of in-support testing for potency of the FDP were within the proposed specifications. In-support testing by (b) (4) identified the existence of (b) (4) within the (b) (4) of ANDEXXA.

<u>Reviewer's comment:</u> Analytical method validations for FDP release methods and development of associated reference standards were reviewed by a review team from OCBQ/DBSQC. These

reviewers concluded that Portola has satisfactorily addressed all the major issues raised in the 17 August 2017 CR letter, and recommend approval of the original BLA for ANDEXXA. Please refer to their review memorandums and to section 5 "Review of CRL responses" for details.

Elucidation of Structure and Product-Related Impurities and Substances

ANDEXXA is expressed in CHO cells as a functional protein, i.e., it does not require either *in vitro* or *in vivo* cleavage of the activation peptide, which is necessary for converting native FX to its activated form FXa. This is accomplished by (b) (4) . ANDEXXA has (b) (4) amino acid residues and an approximate molecular weight of 41 kDa based on the cDNA sequence. Portola submitted data to confirm the primary, secondary, and higher order structures of ANDEXXA.

At least (b) (4) variants have been identified in ANDEXXA. They result from (b) (4) . A variant present in a significant level in the FDP contains (b) (4) . Other variants present in smaller fractions are resulted from (b) (4) . Another minor variant contains the (b) (4) . Common (b) (4) variation was due to (b) (4) at positions (b) (4) . This grouped product variation represents the (b) (4) variants of ANDEXXA and is controlled at release by (b) (4) .

The dominant (b) (4) and full-length *coagulation factor Xa (recombinant), inactivated* species were purified and shown to be functionally active by the direct and indirect potency assays. The remaining protein variants are expected to be functionally active as well because they have the same active site domain needed for binding to the FXa inhibitors.

Reviewer's comment: During the review of original BLA, I noted time-dependent increases in the content of the beta forms in multiple studies including hold time validation, accelerated and real-time (b) (4) FDP stability studies and (b) (4) vs. (b) (4) comparability studies, raising concerns about the (b) (4) impurities that may negatively affect stability of the product and product intermediates. Please refer to my original review memorandum and CRL dated 17 August 2016 for details. In the CRL response, Portola provided the root-cause investigations into the formation of the (b) (4) in each of the studies I mentioned, implemented additional process controls to improve control over (b) (4) formation, and conducted new PPQ studies to demonstrate adequate process control. Please refer to section 5 "Review of CRL responses" below for details.

Characterization of Process-Related Impurities

The safety of process-related impurities in the FDP are evaluated in clinical studies, and the levels at which they are present have not been directly associated with adverse events. These impurities are derived from the cell line, cell culture medium, and materials used in the purification process. Risk assessment considered the number and capacity of the purification steps, amount per (b) (4)

dose (a conservative estimate above the maximum dose of 1760 mg that would be administered to a patient), toxicological risk of the potential impurities, and information in the literature.

Reviewer's comment: *During the first review cycle in 2016, Portola submitted an amendment in which they suggested that* (b) (4) *formation can be mediated by* (b) (4) *. In the CRL, I requested that the sources of these impurities be investigated. In addition, because* (b) (4) *increase was observed in* (b) (4) *FDP stability studies, FDA had concerns about the immunogenic potential of* (b) (4) *in the FDP, and therefore I requested that an* (b) (4) *assay be developed for evaluation of retained clinical study samples. Portola's responses to these requests were submitted in the CRL response, and I found them acceptable.*
Please refer to section 5 "Review of CRL responses" below for details.

Evaluation of Safety Regarding Adventitious Agents

For non-viral adventitious agents including bacteria, fungi, and mycoplasma, the potential of contamination of these agents is well controlled through the use of: (1) appropriate environmental monitoring in the manufacturing process; (2) in-process controls, e.g., testing for (b) (4) ; and (3) filtration steps including (b) (4) sterile filtration. The potential of ANDEXXA to be contaminated with non-viral adventitious agents is further reduced by testing the FDP for sterility, endotoxins, and particulate matter. Portola and its contract manufacturers manufacture ANDEXXA according to GMP regulations.

No human- or animal-derived raw materials are used in the manufacture of ANDEXXA. No raw materials or ingredients of human or animal origin are used in the formulation of ANDEXXA FDP. Thus, the potential risk of adventitious viruses or transmissible spongiform encephalopathy (TSE) agents is minimized.

The potential of contamination by viruses in cell culture is well controlled in the manufacture of ANDEXXA, which is produced in a genetically modified CHO cell line. (b) (4) , a contract testing facility for Portola, performed viral testing on the (b) (4) for ANDEXXA that are consistent with the International Conference on Harmonization (ICH) Q5A(R1) guideline. All test results for endogenous and adventitious viruses were negative except for the presence of (b) (4) found through (b) (4) , and cells that were at the limit of established cell age used for production ((b) (4)). (b) (4) are considered non-pathogenic. (b) (4) routinely tests the cell cultures for adventitious viruses and (b) (4) to ensure that these viruses are below the levels of detection of the assays.

Additionally, the potential risk of viral contamination of ANDEXXA is further mitigated through two dedicated, (b) (4) viral clearance steps: (b) (4) . The (b) (4) steps also contribute to virus removal. Portola has evaluated these viral clearance steps in down-scale studies using model viruses of a wide range of physico-chemical properties. These studies on the relevant steps

resulted in the following overall log reduction factors, in parentheses, for these viruses: (b) (4) . These results are supportive of the effectiveness of the manufacturing process in viral clearance.

Reviewer's comment: Evaluation of safety regarding adventitious agents was reviewed by Dr. Ze Peng. No issues related to adventitious agents were raised in the 17 August 2017 CR letter or during the review of CRL response. Please refer to his review memorandum for details.

Stability

Portola proposed that the BDS can be stored at (b) (4), and the FDP can be stored at +2°C to +8°C for 24 months. Thus far, the available stability results support the proposal. The stability studies are ongoing.

Reviewer's comment: Stability studies were reviewed by Dr. Yideng Liang. Dr. Liang concluded that Portola has satisfactorily addressed all the major issues raised in the 17 August 2017 CR letter, and recommends approval of the original BLA for ANDEXXA. Please refer to her review memorandum and to section 5 "Review of CRL responses" for details.

b) CBER Lot Release

Under the provision described in Federal Register (FR) 58:38771-38773 and the 60 FR 63048-63049 publication (December 8, 1995), routine lot-by-lot CBER release is not required for ANDEXXA because it is a well-characterized recombinant product.

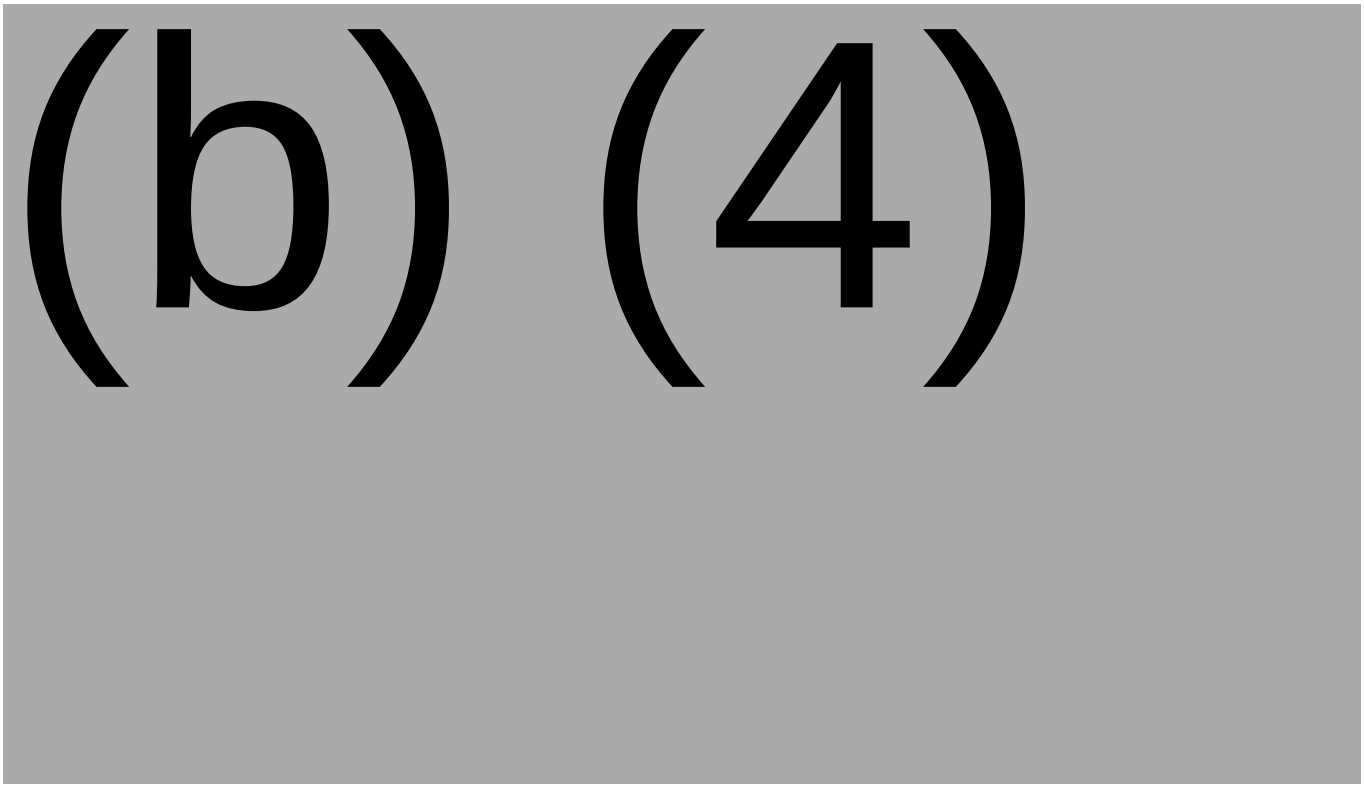
Reviewer's comment: During the first review cycle, a proposal to place ANDEXXA on formal lot release program was considered because of the lapses in CGMP compliance of the firm. However, our final decision was that the approval is not possible until the CGMP deficiencies are resolved. A CRL was therefore recommended for this BLA.

c) Facilities review/inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facilities involved in the manufacture of *coagulation factor Xa (recombinant), inactivated* are listed in the table below. The activities performed and inspectional histories are noted in Table 3 and are further described in the paragraphs that follow.

Reviewer's comment: The facilities information was reviewed by Dr. Christine Harman, DMPQ. Please refer to her review memorandum for details.

CBER performed a Pre-License Inspection (PLI) of (b) (4) from (b) (4) and a Form FDA 483 was issued at the end of the inspection. The firm responded to the observations and the corrective actions were reviewed and found to be adequate. All inspectional issues have been satisfactorily resolved.



<u>Reviewer's comment:</u> The CRL dated 17 August 2016 included requests to address the deficiencies related to observations I had made during the (b) (4) *inspection, including the insufficient control over* (b) (4) *in hold time and other process validation studies, and the lack of comparability between the* (b) (4) *and* (b) (4) *materials. Portola's responses to these requests were submitted in the CRL response, and I found them acceptable. Please refer to section 5 "Review of CRL responses" for details.*

d) Product Comparability

ANDEXXA received *Breakthrough* designation in 2013 because it was developed to address an unmet medical need. To accommodate accelerated clinical development and in anticipation of the demand, several process modifications were introduced, including changes in dosage presentation ((b) (4) lyophilized powder), formulation, protein concentration of FDP from (b) (4) to 10 mg/mL, excipient concentrations, new facilities and scale-up of the (b) (4) FDP processes. To evaluate the impact of these changes on product quality and process performance, Portola conducted extensive comparability studies for the clinical-scale and commercial-scale processes and provided sufficient process and analytical data to demonstrate that the material used in clinical trials is representative of the material intended for commercial distribution. These studies included comparisons between:

1. BDS manufacturing (b) (4)
2. FDP (b) (4) materials
3. (b) (4) FDP (b) (4) materials

Lack of comparability between the existing and future (GEN 2) materials

Portola stated that the existing (b) (4) manufacturing process designated as Generation 1 (GEN 1) is the only commercial process under this BLA. However, Portola believes that the GEN 1 process is not capable of meeting the projected demand for ANDEXXA. Portola therefore plans to permanently (b) (4) the commercial ANDEXXA process with a larger scale and more efficient GEN 2 process through a manufacturing BLA supplement in 2018. The manufacturing changes introduced in the GEN 2 process, (b) (4) are classified as major because they are likely to have an impact on product quality, safety and efficacy. *In vitro* analytical comparative studies demonstrated a lack of comparability between the materials manufactured using the commercial ANDEXXA and GEN 2 processes as evidenced by the differences in (b) (4). Therefore, Portola and FDA had agreed that comparability of the GEN 1 and GEN 2 materials should be demonstrated in a PK/PD comparative study in healthy volunteers treated with FXa inhibitors[7]. For the same reasons, FDA did not agree to Portola's proposal of the immediate introduction of the GEN 2 product in the ANNEXA 4 study.

4. Overview of substantive CMC issues resolved during the BLA review

The table below briefly groups and identifies the major areas of CRL issues and supporting evidence provided in the response.

Table 4: Overview of major CRL issues

CRL issue	Supporting evidence provided in CRL response
Complete validation studies	Process Hold & Cleaning validation Supplemental PPQ, establishing (b) (4) CPP
(b) (4) impurity clearance studies	Characterization of (b) (4) and (b) (4) Activity Clearance of (b) (4)
Reference Standard (RS): • Establishment of Primary RS (PRS) • Standardization of PRS to (b) (4) standards • Link current and previous RS	PRS qualification data and RS Replacement Protocol
Comparability of (b) (4) vs. (b) (4)	Testing of all available lots with new and revised methods, updated side-by-side accelerated stability data for comparability report
Validation of new (b) (4) analytical methods	Validation of (b) (4) methods; establishment of new reporting criteria for (b) (4) methods
Validation of new FDP analytical methods	Validation of Polysorbate 80, sucrose, mannitol, TFPI activity methods; establishment of new reporting criteria for (b) (4) methods

[7] Post-meeting comments, face-to-face meeting CRMTS 10547 minutes dated February 15, 2017

CRL issue	Supporting evidence provided in CRL response
Analytical specifications for (b) (4)	New specifications on (b) (4) and (b) (4) (if poolable) or set specifications on (b) (4)
FDP PPQ (b) (4) OOS	Revised PPQ series consisting of (b) (4) FDP lots; (b) (4) OOS report, and QA System enhancements and corrective measures
Stability trending	Assess stability data for trends; incorporate newly validated test methods into current stability studies
Development of clinical immunogenicity assays and testing of retained clinical samples	(b) (4) antibodies Neutralizing antibodies to FX or FXa

(b) (4); CPP = Critical Process Parameter; CRL = Complete Response Letter; (b) (4); OOS = Out-of-Specification; PPQ = Process Performance Qualification; PRS = Primary Reference Standard; QA = Quality Assurance; (b) (4); TFPI = Tissue Factor Pathway Inhibitor; WRS = Working Reference Standards

The sections that follow describe the substantive issues that were resolved during the review of the ANDEXXA BLA.

4.1. Deficient process validation, process comparability, and (b) (4) impurity clearance studies

The data on process development and validation were deficient, including those on the validation of the proposed commercial (b) (4) FDP (b) (4), in-process hold times, process control strategy, impurity evaluation and clearance, batch consistency, comparability of (b) (4) and (b) (4) batches, and stability. Specifically, repeated elevation of the (b) (4) of ANDEXXA over the upper acceptance limits were observed in (b) (4) lots manufactured using (b) (4), but not (b) (4), at release, in stability studies, in-process intermediates, and in extended process hold time studies. The increase in (b) (4) was linked to product (b) (4) due to the presence of (b) (4) impurities in the ANDEXXA intermediates and (b) (4), but no data on the identification and clearance of these impurities were provided in the original BLA.

Portola satisfactorily addressed these review concerns by demonstrating effective measures that control the formation of the (b) (4) forms of ANDEXXA (b) (4) and (b) (4) forms, as evidenced by the consistent levels of these (b) (4) species in the (b) (4) FDP; and in stability samples under recommended storage conditions. Additional impurity clearance and process validation studies demonstrated that:

- A (b) (4) is responsible for (b) (4) variants formation in process intermediates upstream to the (b) (4) step. This (b) (4) is removed during the (b) (4) step, and no additional (b) (4) variants formation is observed downstream.
- (b) (4) and longer hold times were responsible for the increase in the (b) (4) in early (b) (4) batches. The introduction of a critical process parameter (CPP) that controls the (b) (4) step, and

shorter hold times, provided control of the (b) (4) as demonstrated in the supplemental PPQ study.

- An additional (b) (4) lot release assay, (b) (4), is validated to assure accurate and consistent quantification of the (b) (4) in the (b) (4).
- The levels of the (b) (4) in the (b) (4) are now comparable between (b) (4) and (b) (4), and consistent in (b) (4) manufacture.
- Formation of the (b) (4) variant was also observed in process intermediates downstream to the (b) (4) step. (b) (4) analysis implicated (b) (4) including (b) (4). However, (b) (4) activity has not been detected in the final formulation matrix as the level of the (b) (4) variant does not increase in the (b) (4) in the current formulation under routine storage conditions.
- Compared to ANDEXXA (b) (4) batches, ANDEXXA preparations (b) (4) in the (b) (4) and (b) (4) forms have similar potency and FXa inhibitor binding properties, confirming that these forms are functionally active product-related substances.

These newly developed validation data were used to confirm the validity of the data from process development, qualification and verification, and comparability studies.

4.2. CGMP requirements were not followed during FDP PPQ studies

Review of evidence collected during the PLI of the (b) (4) manufacturing facility revealed that an out-of-specification (OOS) (b) (4) batch (b) (4) was mixed with conforming (b) (4) batches to manufacture the (b) (4) PPQ FDP batch (b) (4). Because of this (b) (4), FDP batch (b) (4) met the predetermined acceptance criteria and was reported in final FDP PPQ study report and listed in BLA section 3.2.P.5.4 Batch Analyses. (b) (4) OOS batches with in-specification ones is not acceptable from CGMP perspective. Although Portola directly approved the use of a non-compliant (b) (4) batch, no information on the no-compliant status was reported in quality documents submitted in the BLA.

At the request of the FDA, Portola provided a detailed explanation of the events that led to this CGMP excursion, acknowledged the Agency's concerns, and has worked with the (b) (4) FDP contract manufacturers to enhance the controls over the batch release and shipment procedures. An additional report using data from a total of (b) (4) FDP lots was provided detailing the consistency of the FDP manufacturing process.

4.3. Deficient characterization of ANDEXXA potency standard

The ANDEXXA potency standard was not properly qualified and the consistency of product potency in the event of future standard replacement was not assured. To ensure the consistency of the potency, stability and integrity of the ANDEXXA primary product-specific standard (PRS), Portola developed product-specific activity units relative to international reference standards to allow for traceability of potency for the PRS. Portola also developed a program to monitor the stability of

ANDEXXA PRS and continuity of potency unitage for the working reference standards. Since inhibition of TFPI by ANDEXXA was observed in the clinical trials, Portola developed a potency assay to control anti-TFPI activity in released FDP batches.

4.4. Deficiencies in specifications

The release specifications of the (b) (4) FDP were insufficiently justified and lacked several important parameters to ensure product safety and efficacy.

- For the (b) (4) specifications, FDA requested Portola to include (b) (4) .
- For the FDP specifications, FDA requested Portola to include contents for *Sucrose*, *Mannitol*, *Polysorbate 80*, (b) (4) *TFPI Inhibition* and *Purity by* (b) (4) *;* and to tighten the acceptance criteria for (b) (4) and (b) (4) .
- For both (b) (4) FDP specifications, FDA requested Portola to replace expression of Potency (both Direct and Indirect) in percent of a reference standard into units of the standard.

Portola successfully addressed these concerns by re-assessing the manufacturing data, and revising and justifying the specifications. As part of the specification-setting exercise, all available (b) (4) and (b) (4) lots were tested with the new, validated release test methods. These lots were found comparable to the more recently manufactured lots, demonstrating that the old batches meet the release and stability specifications.

4.5. Deficiencies in stability studies

The available stability data were not sufficient to support the proposed shelf-life because only 6 months of real-time data for (b) (4) FDP were provided using an incomplete set of analytical methods. Portola has now established new and revised methods for the release and stability studies of the (b) (4) FDP. These fully validated methods have been used for the analysis of primary stability lots of (b) (4) FDP ((b) (4) lots each) manufactured by the proposed commercial process. After 18 months, no adverse trends have been observed under long-term storage conditions.

4.6. Inadequate characterization of manufacturing risks in (b) (4) Comparability Protocol

A comparability protocol (CP) was submitted in the BLA to support the introduction of a new manufacturing suite at (b) (4) and scale-up of the (b) (4) FDP manufacturing processes. Portola had originally planned to include these changes in the BLA but was advised by the FDA to report these changes in a post-licensure supplement. In the CP, Portola requested a

downgrade of the reporting category of the supplement from a prior approval to a Changes Being Effected in 30 days (CBE-30) category. However, the CP was found to be deficient and unacceptable because repeated process failures have been observed with the (b) (4) process. These failures demonstrated that the scope of manufacturing changes was too significant to be addressed through a CBE-30 category. Portola informed the FDA that (b) (4) is not being considered for the BLA or for future use.

4.7. Gaps in validation of bioanalytical methods used in the clinical studies

The methods used to assess immunogenicity in patient samples did not quantify (b) (4) P antibodies or anti-ANDEXXA antibodies that can inhibit activities of endogenous FX and FXa and did not permit the adequate assessment of safety of ANDEXXA in the clinical trials. Portola addressed these deficiencies by developing and validating required immunogenicity methods and testing retained clinical samples for (b) (4) antibodies or FX/FXa neutralizing antibody activity.

Portola also failed to properly qualify and bridge different versions of the assays for TFPI activity and TFPI antigen, and the TG methods used in the Phases 1, 2 and 3 studies. These deficiencies allowed for incorrect conclusions about ANDEXXA activity to be included in clinical study reports, as these conclusions seemed plausible within the gaps of assay qualifications[8]. Because of the deficiencies in the qualification of the methods used in the clinical studies, the magnitude and duration of the inhibition of TFPI activity by ANDEXXA was underestimated, and the action of anti-FXa reversal was overestimated.

Portola has now validated all TFPI and TG methods, and confirmed that the role of the inhibition of TFPI activity by ANDEXXA in the elevation of thrombin generation was underestimated. These conclusions are now described in the BLA file and in the revised *Prescribing Information*.

4.8. Deficient in vitro studies of anti-TFPI mechanism of action

In the original BLA, results from *in vitro* mechanism of action studies were presented as theoretical evidence for the clinical insignificance of the TFPI inhibition by ANDEXXA. In my review, I found that several critical *in vitro* studies were incorrectly conducted or interpreted. Although the *in vitro* studies cannot be used in lieu of clinical evidence, Portola chose to rely on these *in vitro* studies in the various sections of the BLA as well as in meetings with the FDA when the insufficient relevance of the chosen surrogate, anti-FXa activity reversal, was discussed. Because the incorrect *in vitro* data could offer a plausible deniability of the anti-TFPI mechanism of action, I proposed a series of experiments to examine the validity of the following statements:

1. That ANDEXXA has no procoagulant effect on thrombin generation in the absence of FXa inhibitors,
2. That FXa inhibitors can block ANDEXXA binding to TFPI on endothelial cells,

[8] 1.11.3 CLINICAL INFORMATION AMENDMENT Response to the Agency's IR dated 01 June 2016

3. That TFPI inhibition observed in systems of purified proteins is not reproduced when effect is studied in whole blood or plasma,
4. That ANDEXXA does not induce elevation of thrombin generation markers TAT and F1.2 in whole blood *in vitro*.

In the CRL response, Portola provided results of the requested experiments, which invalidated their previous conclusions. Specifically,

1. ANDEXXA has substantial procoagulant effect on the TG assay *in vitro*, when the assay is analyzed through the readouts other than the ETP, a parameter that was presented in the BLA originally.
2. At pharmacological concentrations, FXa inhibitors are not able to block ANDEXXA binding to TFPI on endothelial cells.
3. Plasma proteins have no effect on TFPI-ANDEXXA-FXa interaction on endothelial cells.
4. ANDEXXA induces TAT and F1.2 elevation in whole blood and plasma, when the artificial contact activation is blocked.

5. Review of CRL responses

In the Table 5, the review of the CRL responses is organized by listing each CRL item, followed immediately by the review of Portola's responses and data. Note that CRL items 9-12 were reviewed by DMPQ.

Table 5: Review of Portola's responses to CMC items in the CRL

#	*FDA CRL Comments* & My Review of Portola's Responses
1.	***The data you provided in your responses to the Form FDA 483 issued on*** (b) (4) ***do not adequately address the deficiencies in the validation of the ANDEXXA manufacturing process that were identified during the Pre-License Inspection (PLI) of the*** (b) (4) ***facility. The ANDEXXA process is not validated to assure reasonable control of sources of variability that could affect production output and to assure that the process is capable of consistently delivering a product of well-defined quality. Current good manufacturing practice (CGMP) requires that manufacturing processes be designed and controlled to assure that in-process materials and the finished product consistently and reliably meet pre-determined quality requirements. Please address the following deficiencies:***
1a.	***Complete the validation studies for the clearance of all impurities and submit the final study reports to demonstrate identification and control of these impurities. This is needed to assure process consistency and establish a process control strategy which will ensure the quality of the commercially manufactured product.*** ***You provided incomplete information regarding proteolytic impurities. In the final report for the deviation investigation DEV-1632 submitted on 30 June 2016, you stated that "*** (b) (4) ***would be more likely to promote*** (b) (4) ***reactions including the*** (b) (4)

#	*FDA CRL Comments* & My Review of Portola's Responses
	that may lead to (b) (4) ***product percentage." In the 17 July 2016 amendment to the BLA, you explained that several investigations on*** (b) (4) ***are ongoing and acknowledged that "As of yet, we have not identified the source of the*** (b) (4) ***activity in the upstream process." Please note that*** (b) (4) ***studies are considered critical to the process qualification stage of process validation (reference is made to the 2011 FDA Guidance on Process Validation) and therefore prior to submission to FDA these studies should be reviewed and approved by your quality assurance unit to document the use of sound scientific methodology and principles with adequate data to support the conclusions.***
	Review of Portola's Response to CRL Item No. 1a: New (b) (4) characterization and clearance studies demonstrated control over (b) (4) . These studies are illustrated by Figures 2 and 3 below. The impact of intermediate hold times and (b) (4) (in the presence and absence of several (b) (4)) on the levels of (b) (4) variants was reported in a development study CR-070 and described in Section 3.2.S.2.6. In another study CR-076, the classes of (b) (4) with ANDEXXA were identified using (b) (4) kits. Results indicate that a (b) (4) is likely responsible for (b) (4) variant formation and shown to be active at a processing pH of (b) (4) , but is cleared by the (b) (4) . Therefore, the (b) (4) variants content remains stable in the (b) (4) and downstream throughout the remainder of the purification process. (b) (4) During these studies, it was observed that an increase of the (b) (4) ((b) (4) variant) was predominantly observed after the (b) (4) , and this formation of (b) (4) variant correlates with (b) (4) . Further characterization suggested the presence of (b) (4) responsible for the (b) (4) in (b) (4) variant. It was also demonstrated that an (b) (4)

[9] STN 125586 Seq 077 - TR.030/0: Definition of Intermediate Hold Times in Andexanet Alfa Drug Substance Process 3

#	*FDA CRL Comments* & My Review of Portola's Responses
	(b) (4), identified in the (b) (4) studies, does not have activity in the ANDEXXA formulation buffer which is at pH 7.8. (b) (4) were shown to impact the (b) (4) step yield and (b) (4) variants content. These studies showed that (b) (4) had no impact on (b) (4) step yields and (b) (4) variant content. The responses are acceptable. (b) (4)
1b	***Demonstrate that the trends in the purity and stability attributes of the*** (b) (4) ***Final Drug Product (FDP) do not adversely affect the quality, safety, purity, or potency of the product as they relate to its safety and effectiveness. These trends were observed after the introduction of the proposed commercial*** (b) (4) ***. Demonstrated lack of analytical comparability between the materials manufactured using the previous*** (b) (4) ***and the proposed commercial*** (b) (4) ***is of concern because Phase 3 clinical studies were exclusively supported by*** (b) (4) ***materials. Please also address the following evidence of the reduced capacity of*** (b) (4) ***in clearing*** (b) (4) ***:***
	Review of Portola's Response to CRL Item No. 1b: The (b) (4) comparability reports have been updated to include additional side-by-side stability data from a (b) (4) storage condition study at (b) (4) that further support the comparability. No differential trends were noted in real time stability data either. The statistical distributions for (b) (4) release data were comparable, and therefore the data could be pooled to develop the specification limits applicable to both processes. I agree with Portola that (b) (4) lots from (b) (4) are now shown to be comparable to those from (b) (4).
1b (i)	***Analysis of consecutive*** (b) (4) ***batches in Figure 5b of the Investigation Final Report for DEV-1632 (submitted in your 30 June 2016 amendment) demonstrates that both the levels of the*** (b) (4) ***and batch-to-batch variability in the*** (b) (4) ***were increased when*** (b) (4) ***was replaced with*** (b) (4) ***.***

#	*FDA CRL Comments* & My Review of Portola's Responses
	Review of Portola's Response to CRL Item No. 1b(i): Portola acknowledged the apparent increase in, and batch-to-batch variability of, the (b) (4) found in Figure 5b of the Investigation Final Report for DEV-1632 [10]. This variability can be attributed to (b) (4) at the (b) (4) step. Further comparability analysis, as described in Response to 1b, shows that the (b) (4) levels are comparable between (b) (4) and (b) (4) materials, and are controlled by the revised Process Control Strategy. Characterization of the (b) (4) in (b) (4) and (b) (4) also supports comparable clearance of (b) (4) between the (b) (4) processes[11].
1b (ii)	*Results of the accelerated stability studies indicated an increase in* (b) (4) *in* (b) (4) *batches as evidenced by the adverse trends observed in* (b) (4) *and* (b) (4) *. Results from both methods demonstrated a* (b) (4) *rate of formation of the* (b) (4) *and a faster rate of reduction in the* (b) (4) *when comparing materials from* (b) (4) *to those from* (b) (4) *.*
	Review of Portola's Response to CRL Item No. 1b(ii): The updated comparability study and the revised (b) (4) stability summary show that (b) (4) variants levels, including stability trends, are comparable between (b) (4) and (b) (4) materials. The data to support this conclusion were collected after implementation of the new and revised analytical methods (including the (b) (4) method) and associated acceptance and reporting criteria. In addition, previous (b) (4) method was inadequate at accurately monitoring the (b) (4) variants and has been replaced by the validated (b) (4) method which provides better resolution and quantitation of the (b) (4) variants. Portola also provided the response to the request to assess the effects of (b) (4) on purity and potency of ANDEXXA. These data were requested per FDA meeting minutes dated 17 Oct 2016, and are submitted in Sections 3.2.S.7.1 and 3.2.S.7.3.
1b (iii)	*Adverse trends in real-time stability for the* (b) (4) *were observed for* (b) (4) *batch* (b) (4) *and the FDP batch* (b) (4) *(which was manufactured using this* (b) (4) *batch).*
	Review of Portola's Response to CRL Item No. 1b(iii): Portola acknowledged that the first (b) (4) of data submitted in the BLA for batch (b) (4) suggested an apparent adverse stability trend. However, the (b) (4) stability points have now been completed, and the additional data show that there is no adverse stability trend beyond expected analytical variability of the (b) (4) method. With regards to the sources for the previous trends, Portola noted that the data were within the expected precision in the assay validation report.
1b (iv)	***Data on*** (b) (4) ***modifications provided on 29 July 2016 indicated that*** (b) (4) ***batches were*** (b) (4) ***in*** (b) (4) ***content and*** (b) (4) ***in*** (b) (4) ***when compared to*** (b) (4) ***batches.***

[10] BLA 125596; SN0048 Section 1.11.1, dated 30 June 2016

[11] Section 3.2.S.3.2.6.6.3

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	Review of Portola's Response to CRL Item No. 1b(iv): The updated comparability report demonstrates that (b) (4) materials are comparable with respect to (b) (4) *content* and (b) (4). Statistical analysis of data is reported in *Justification of Specification*. This analysis supports the (b) (4) of (b) (4) and (b) (4) lots for the (b) (4) attribute, i.e., levels of (b) (4) across both (b) (4) and (b) (4) lots were comparable. Statistical analysis of the purity by (b) (4) assay (monitors the (b) (4) content of ANDEXXA via (b) (4)) also showed no discernable (b) (4) difference between (b) (4) and (b) (4) lots.
1c	***Submit the final reports of process validation studies to demonstrate the effectiveness of the control strategy for the newly established critical process parameter -*** (b) (4) ***- in assuring the consistency of*** (b) (4) ***performance and*** (b) (4) ***quality. Provide a timeline for the completion of the associated process validation activities.***
	Review of Portola's Response to CRL Item No. 1c: Effective control over new CPP for (b) (4) at the (b) (4) process step was successfully demonstrated in a supplemental PPQ study. (b) (4) lots were produced, with the last (b) (4) lots considered as the supplemental PPQ lots. (b) (4) performance and (b) (4) quality were consistent. (b) (4) Portola attempted, and failed, to conduct a new hold time validation study. The failures were attributed to errors in protocol execution, i.e., for reasons not directly related to product stability. In lieu of a validated hold time study, a review and assessment of historical intermediate hold times along with the process characterization studies evaluating both time and temperature impact on product quality were utilized to establish a conservative set of intermediate hold times. Revised in-process downstream intermediate hold times have been defined through an analysis of historical manufacturing batch data combined with process characterization studies. This approach defines more restricted (i.e., much shorter) hold time limits to ensure control of product-related variants, see Table 6. These limits are supported by the control of ANDEXXA variants monitored by the increase in product-related (b) (4) variants formation ((b) (4)) as measured by (b) (4).

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1d	***During the PLI, we observed that*** (b) (4) ***were associated with a decrease in yield at the*** (b) (4) ***step and loss of control over the content of the*** (b) (4) ***in the*** (b) (4)***. We acknowledge your 30 June 2016 commitment to implement and validate new equipment to control*** (b) (4) ***at the point of use no earlier than 15 November 2016, which is after the PDUFA V Action Date, and also does not include a "no later than" date. Please clarify your intent and timeline.***
	Review of Portola's Response to CRL Item No. 1d: The validated effective control over (b) (4), as described in response to Question 1c, is based on the manual control of the (b) (4) for the (b) (4) process step. To further enhance process control, and consistent with previous communications with the FDA, a (b) (4) has been implemented for batches starting manufacture in June 2017. Data to support the implementation of the (b) (4) will be submitted as a post approval supplement.
1e	***Complete the validation of hold times for process intermediates during the manufacture of the*** (b) (4) ***demonstrate the control over the*** (b) (4) ***and other quality attributes of the BDS.*** ***As you reported on 11 July 2016, the validation study performed per process hold time study protocol VAL-30234-01 failed due to an*** (b) (4) ***in the*** (b) (4) ***at the*** (b) (4) ***step. You had not identified the root cause for this deviation, and have initiated a new study per validation protocol VAL-30291-01 which will be completed by 31 October 2016, which is also after the PDUFA V Action Date.***
	Review of Portola's Response to CRL Item No. 1e: The root cause for the increased (b) (4) at the (b) (4) step in small scale study VAL-30234-01 was identified in study (b) (4)-CR-074 *Root Cause Analysis for* (b) (4) *per VAL-30234*, and determined to be a (b) (4) of the (b) (4) load material resulting in a (b) (4) variant. As described in response to Question 1c, an analysis of historical manufacturing hold times supplemented with characterization data for the rate of formation of product-related variants has been used to set conservative hold times sufficient to control the quality of the (b) (4). Portola states that these conservative hold times will only be extended with additional hold time validation data to support the extension. This response is acceptable.
1f	***Ensure that the FDP process performance qualification (PPQ) studies, and all manufacturing activities, are conducted in compliance with CGMP requirements. We note that these requirements were not followed when out of specification (OOS)*** (b) (4) ***Batch*** (b) (4) ***and Out of Limit (OOL)*** (b) (4) ***Batch*** (b) (4) ***were mixed with conforming*** (b) (4) ***batches to manufacture three PPQ FDP batches that met specifications as described below:***

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1f(i)	***According to the aforementioned deviation investigation, i.e., DEV-1632, (b) (4), Batch (b) (4) ((b) (4) number (b) (4)) was not released because the release testing for the (b) (4) was OOS ((b) (4)). Nevertheless, the final validation report for the ANDEXXA FDP process states that on 09 November 2015 Portola authorized the use of this batch for the production of PPQ FDP Batch (b) (4). As documented in the same report, Batch (b) (4) was (b) (4) with portions of (b) (4) Batches (b) (4) and (b) (4), which were well within specification for the (b) (4). As a result of this (b) (4), the content of the (b) (4) was (b) (4) to (b) (4) in FDP Batch (b) (4), which was within the release specification and this batch met the pre- determined acceptance criteria for the lyophilized vial finished product testing and was reported in 3.2.P.5.4 Batch Analyses. (b) (4) OOS and/or OOL batches with batches that are within specification is not considered to be acceptable CGMP.*** ***Please explain how these occurrences will be prevented in the future and report on the current disposition of these PPQ batches, which cannot be used to support the process validation.***
	Review of Portola's Response to CRL Item No. 1f & 1f(i): At the request of the FDA during the 17 October 2016 Type A CRL meeting, Portola provided a detailed explanation of the events that led to this CGMP excursion. Portola states that they understood the Agency's concerns, and have worked with the contract manufacturers to enhance controls. A complete description of the improvements made to the Quality System and the disposition of the referenced FDP lot was included in a prior submission [12]. The detailed description is also provided as an appendix to the FDP Process Consistency report, which has also been updated to include (b) (4) clinical lots and (b) (4) post-PPQ lots in addition to the (b) (4) PPQ lots, providing a total of (b) (4) lots detailing the consistency of the FDP process. This response is acceptable from the product perspective. I defer to the OCBQ to make the final determination from the compliance perspective. I also recommend that the implementation of corrective actions is followed up at the next biannual Team Bio inspection of ANDEXXA BDS and FDP facilities.
1f (ii)	***The amount of protein for (b) (4) process parameter "(b) (4) " exceeded the allowable range (which is reported in the BLA as (b) (4)). A total of (b) (4) of (b) (4) Batch (b) (4) was used in the manufacture of all (b) (4) FDP PPQ batches, which corresponds to (b) (4) of andexanet alfa in this (b) (4). PPQ Batches (b) (4) met the release acceptance criteria and were used in primary stability studies. PPQ Batch (b) (4) was also released for use in humans.***
	Review of Portola's Response to CRL Item No. 1f(ii): The meaning of the terminology "(b) (4) " has been clarified in the response to the CRL. The parameter "(b) (4) " with a (b) (4) allowable range specifically applied to the (b) (4) of (b) (4) solution used to (b) (4) the

[12] STN 125586 SN0075 Section 1.11.1, 29 December 2016

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	(b) (4) during the (b) (4) *Filtration and Filling* step. It did not refer to the allowable range for total content of ANDEXXA in the final (b) (4). The (b) (4) used in manufacturing of (b) (4) Batch (b) (4) was within the acceptance criteria of (b) (4). Accordingly, since total ANDEXXA in a (b) (4) is not a process parameter and therefore has no acceptance criteria, (b) (4) Batch (b) (4) with a content of approximately (b) (4) of ANDEXXA in the (b) (4) was not OOL and was therefore acceptable for use in FDP PPQ Batches (b) (4). In addition, the (b) (4) used in manufacturing of (b) (4) Batch (b) (4) was also within the acceptance criteria of (b) (4). To address the FDA concerns, in Section 3.2.S.2.2 *Description of Manufacturing Process and Process Controls* Table 3.2.S.2.2-25 *Filtration and Filling Process Key Parameters*, the wording has been refined to read "(b) (4)" to more clearly designate the intended meaning of this process parameter.
2	***The proposed release specifications for the (b) (4) FDP are incomplete and not representative of the experience with the proposed commercial process. We acknowledge your proposal to use (b) (4) release data to derive (b) (4) release specifications but do not find it acceptable because:*** • **The comparability of the (b) (4) and (b) (4) materials has yet to be established.** • **Empirical (b) (4) data are limited and insufficient to support the critical analytical methods used to monitor the identity, purity, and potency of the (b) (4) (these methods were replaced after the introduction of (b) (4), when only three (b) (4) batches were manufactured and with the simultaneous introduction of the proposed (b) (4) specifications).** • **Data obtained with the previous versions of methods for identity, purity, and potency was not trended quantitatively and therefore the comparability between the different versions of these methods, and different versions of processes, is not established.** • **To provide assurance of consistent product quality, please address the following deficiencies with release methods and specifications:**
2a	***Base all (b) (4) specifications on available (b) (4) manufacturing data, and FDP specifications on data from batch analyses of the FDP, not the (b) (4). The proposed specifications are deficient because they were developed prior to the execution of the (b) (4) PPQ campaign, when data from only (b) (4) out of (b) (4) currently manufactured (b) (4) batches were available. To develop meaningful specifications, use data from all (b) (4) FDP batches that were manufactured in compliance with the proposed control strategy and CGMP. Exclude the data for all batches that are not manufactured by the proposed commercial process, such as all (b) (4) batches and Batch (b) (4), which was manufactured at (b) (4).***
	Review of Portola's Response to CRL Item No. 2 and 2a: As noted in Response to Question 1b, new data have demonstrated comparability between (b) (4) and (b) (4). In addition, as part of the specification setting process, lot

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	release data for the (b) (4) processes were determined to be (b) (4), further demonstrating comparability between the processes. Therefore, specifications have been set using all available (b) (4) and (b) (4) lots manufactured to-date as well as all available FDP lots manufactured to-date. In certain cases, when appropriate, the specifications for (b) (4) FDP were aligned. Only lots that had been manufactured within the proposed Control Strategy were included in the statistical assessment for specifications. (b) (4) batches and those manufactured at (b) (4) (outside of control strategy) were excluded from specification setting. I agree with proposed specifications. Please also refer to Dr. Andrey Sarafanov's specifications review memorandum for details.
2b	***In reference to our Information Request (IR) dated 07 April 2016 and your 20 April, 08 July and 29 July 2016 responses, which are incomplete:***
2b(i)	***Validate the (b) (4) assay as an identity test for andexanet alfa based on protein structure, and validate the methods for determining the (b) (4) and (b) (4) content.***
	Review of Portola's Response to CRL Item No. 2b(i): The (b) (4) has been optimized and validated as an identity test. In addition, a specific assay for (b) (4) content has been developed and validated and a newly implemented (b) (4) assay is used for the quantitation of (b) (4).
2b (ii) 1	***Validate the analytical methods and establish release specifications for the excipients mannitol, sucrose, and Polysorbate 80.***
	Review of Portola's Response to CRL Item No. 2b(ii) [Part 1]: During the first review cycle, the development of release specifications for mannitol and sucrose was requested to mitigate the risk of acute kidney injury due to high level of these sugars[13]. The clinical reviewer, Dr. Lisa Faulcon, found Portola's previous explanation that "*[i]n the Phase 1-3 studies in healthy volunteers and in the > 100 bleeding patients treated in ANNEXA-4, there have been no sensitivity issues that have been specifically linked to the tolerability of sucrose or mannitol*" misleading because based on the data and current study protocol submitted, serum chemistries in the confirmatory study (ANNEXA-4) are only done at baseline, which is inadequate to assess sucrose-related renal toxicity[14]. In the CRL response, Portola provided evidence that analytical methods for the excipients have been validated, and FDP release specifications for each of these excipients have also been established.
2b (ii) 2	***Please also qualify all compendial analytical methods used for the release of raw materials intended for FDP formulation.***

[13] Dantal J. Intravenous immunoglobulins: in-depth review of excipients and acute kidney injury risk. Am J Nephrol. 2013;38(4):275-84.

[14] Dr. Lisa M. Faulcon's clinical review memorandum dated 12 August 2016

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	Review of Portola's Response to CRL Item No. 2b(ii) [Part 2]: This request was made during the first review cycle because neither the FDP release methods nor the in-process release methods for the same excipients were qualified at the time. The raw materials that are excipients in the FDP - *mannitol, sucrose, polysorbate 80, arginine, and Tris* - are all compendial, and the analytical methods used to release them for use in manufacturing have been verified now. The quality of the excipients is controlled as raw materials at the (b) (4) manufacturing stage since no further compounding occurs at the FDP manufacturing stage. This approach is acceptable.
2b (iii)	***Develop and validate potency units for ANDEXXA to replace the current unit of "percent of a reference standard." The existing percentage approach is not suitable for the evaluation of the stability of the product because the stability of the reference standard is not established. To address these deficiencies, the new potency units should be traceable to the international reference preparations distributed by the*** (b) (4) ***. Refer to the*** (b) (4) ***for examples. To illustrate a specific example of a possible method, the units can be defined as follows: "*** (b) (4) ***" and "*** (b) (4) ***"***
	Review of Portola's Response to CRL Item No. 2b(iii): The units for the direct and indirect potency assays have been established relative to international reference standards to allow for traceability of potency for the PRS (see Fig 4.), monitoring of stability of the ANDEXXA reference standards, and to correlate potencies of WRS over time (Table 7). The following definitions were introduced: • **Direct Potency Unit**: (b) (4) • **Indirect Potency Unit**: (b) (4) . • **Specific Activity**: *The number of activity units in 1 mg of andexanet alfa*. **Figure 4: Calibration of PRS and** (b) (4) **WRS in the Direct, Indirect, and TFPI Potency Assays**

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	(b) (4)
2c	*Develop quantitative acceptance criteria for the* (b) (4) *resolved by* (b) (4) *. ANDEXXA is a heterogeneous mutated protein product comprised of more than* (b) (4) *charged* (b) (4) *and additional variants with different* (b) (4) *modifications and* (b) (4) *content. Additional purity specifications are needed to demonstrate control over all* (b) (4) *forms that may arise during the purification process.* *These quantitative parameters may be used to investigate the comparability of the* (b) (4) *and* (b) (4) *materials, as well as the* (b) (4) *and lyophilized (FDP) formulations of* (b) (4) *materials.* *Please also explain why the product is treated with* (b) (4) *. The treatment* (b) (4) *, and in turn gives results that are not representative of the actual composition of the product.*

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	Review of Portola's Response to CRL Item No. 2c: New reporting and quantitative acceptance criteria for the (b) (4) resolved by (b) (4), and the recently introduced (b) (4) have been developed and validated for each of these analytical methods. These revised specifications were also applied for the demonstration of comparability between (b) (4) and (b) (4). The use of (b) (4) is needed to assure assay robustness and control of ANDEXXA variants, and explained in detail in *Elucidation of Structure* as well as *Justification of Specification*. The responses are acceptable.
2d	***Your justification for proposed specifications for Visual Appearance for (b) (4) reconstituted FDP ("Clear, colorless to slightly yellow solution, essentially free of visible particulates") is not acceptable. The presence of visible particles may indicate issues with protein solubility and stability. Revise the acceptance criteria to require "Clear, colorless to slightly yellow solution, free of visible particles."***
	Review of Portola's Response to CRL Item No. 2d: The specification for *Visual Appearance* for (b) (4) reconstituted FDP) has been revised and is expressed as "*Clear, colorless to slightly yellow solution, essentially free from visible particulates*" . The FDP specification is consistent with industry standards and regulatory expectations.
2e	***In reference to our IR dated 01 June 2016 and your 15 June and 19 July 2016 responses which are incomplete, develop a (b) (4) assay and associated release specifications to measure the inhibition of Tissue Factor Pathway Inhibitor (TFPI) activity by ANDEXXA FDP. Please base your assay for TFPI inhibition activity on the thrombin generation test (TGT) used as a biomarker in Phase 3 clinical studies.***
	Review of Portola's Response to CRL Item No. 2e: An additional (b) (4) assay was developed to measure the inhibition of TFPI activity by ANDEXXA FDP, and it has been instituted for FDP release. The specifications for TFPI inhibition activity were established using data from all available FDP lots.
2f	***In reference to our IR dated 22 June 2016 and your 08 July 2016 response which is incomplete, develop and validate a new method for the evaluation of endotoxins in FDP with a limit of detection comparable to that of the method used for (b) (4) release. Your specification for endotoxins in the FDP ((b) (4)) is very close to the compendial infusion limit for endotoxins and can be reduced as demonstrated by the capability of your manufacturing process.***
	Review of Portola's Response to CRL Item No. 2f: (b) (4) has validated the FDP endotoxin method to a limit of quantification (LOQ) of (b) (4) or (b) (4), which is equivalent to the LOQ of the (b) (4) method used for

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	(b) (4) release. The (b) (4) FDP endotoxin specifications have been lowered from (b) (4) .
2g	***We acknowledge your commitment to replace a commercially available assay for the measurement of Chinese Hamster Ovary (CHO) (b) (4) impurities with an ANDEXXA process-specific method. A new release method is required because (b) (4) impurities are suspected to originate from CHO cells. A process-specific (b) (4) preparation should be prepared from (b) (4) . Please refer to the ICH Guideline Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. This (b) (4) preparation should then be used to generate the antibodies used in the assay for (b) (4) impurities. Adequate coverage of the (b) (4) antibodies for CHO-derived impurities should be established.***
	Review of Portola's Response to CRL Item No. 2g: A process-specific (b) (4) assay has been implemented to replace the former commercially available assay for measurement of CHO (b) (4) impurities. Adequate coverage of the (b) (4) antibodies was demonstrated.
2h	***In reference to our IR dated 07 June 2016 and your 30 June and 13 July 2016 responses which are incomplete, develop new specifications for the (b) (4) to utilize the demonstrated sensitivity of this parameter to changes in critical process parameters and the purity of ANDEXXA. Support the specifications with a report on risk assessment of the (b) (4) and (b) (4) -producing impurities. This should include, but not be limited to, their impact on the purity, quality, potency, and stability of the product as they are related to its safety and effectiveness. In addition, please:***
	Review of Portola's Response to CRL Item No. 2h: The newly implemented (b) (4) method is used to specifically quantitate the (b) (4) variants and establish new specification limits. New CQA risk assessment and investigations of activity of the (b) (4) variants are provided in *Elucidation of Structure* (Section 3.2.S.3.1.4.0), and *Justification of Specifications* (3.2.S.4.5). The (b) (4) variants forming (b) (4) impurities are now discussed in *Manufacturing Process Development* Section 3.2.S.2.6 ((b) (4) clearance) and *Impurities* Section 3.2.S.3.2 (characterization of (b) (4)). The responses are acceptable.
2h (i)	***Provide complete reports for the investigations into the root causes behind the observed changes in product quality attributes after the introduction of (b) (4), which were evidenced by the (b) (4) in the levels of (b) (4) observed (i) at several unit operations (such as (b) (4)), (ii) in hold time studies, (iii) after the introduction of (b) (4), and (iv) over time in stability studies (under both accelerated and real-time conditions). These investigations should include, but not be limited to, evaluation of the effect of (b) (4), inconsistent impurity clearance and extended hold times on process performance.***
	Review of Portola's Response to CRL Item No. 2h(i):

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	Portola confirmed that the introduction of (b) (4) resulted in the (b) (4) levels. Comprehensive understanding of, and control over, the root causes behind the (b) (4) increases was obtained through the studies described above: (i) process characterization studies were performed to understand the impact of temperature and hold times on the formation of product-related substances and to define the appropriate Process Control Strategy for the (b) (4) process step; (ii) the root cause of the previously failed hold study that showed (b) (4) was determined to be due to sample contamination; (iii) additional stability data from the (b) (4) to (b) (4) comparability study demonstrated no (b) (4) in (b) (4) variants for the revised (b) (4), while lots manufactured outside of the defined Process Control Strategy for the (b) (4) process step showed elevated (b) (4) levels; and (iv) long-term stability studies demonstrated no increase in product-related substances under routine storage conditions.
2h (ii)	***Use (b) (4) methods for the measurement of the (b) (4) to compare the (b) (4) and (b) (4) batches, and to monitor the changes in the (b) (4) in stability studies for the (b) (4) FDP.***
	Review of Portola's Response to CRL Item No. 2h(ii): The (b) (4) method was developed as the principal (b) (4) assay to the (b) (4) for measurement and control of (b) (4) in (b) (4). When assessed with the (b) (4) method, no significant changes were seen in the (b) (4) content under routine storage conditions.
2h (iii)	***Explain how the available clinical data support the (b) (4) specifications. In your response, use (b) (4) methods to detect the ranges of levels for each (b) (4) form in all batches used in the completed clinical trials and address the possible effect of the (b) (4) on the ANDEXXA circulatory half-life. With reference to your proposal to increase the acceptance criterion of the (b) (4) by the existing (b) (4) method from (b) (4) to (b) (4), please note that the clinical batches contained less than half of the (b) (4) as defined by the increased upper specification limit, which does not support such an increase.***
	Review of Portola's Response to CRL Item No. 2h(iii): Portola explained how the clinical lots were taken into consideration by determining the tolerance intervals for the subset of FDP (b) (4) lots used in clinical trials to verify that the commercial specifications were appropriate, as a precursor step for the proposed specification-setting for the (b) (4) for (b) (4) FDP. This is described in the *Justification of Specifications.*
2h (iv)	***Use (b) (4) methods to compare the specific potencies of the (b) (4) with the other product-related molecular forms of ANDEXXA. In addition to validated potency methods, we suggest using a biomarker assay, e.g., TF-activated TGT.***
	Review of Portola's Response to CRL Item No. 2h(iv):

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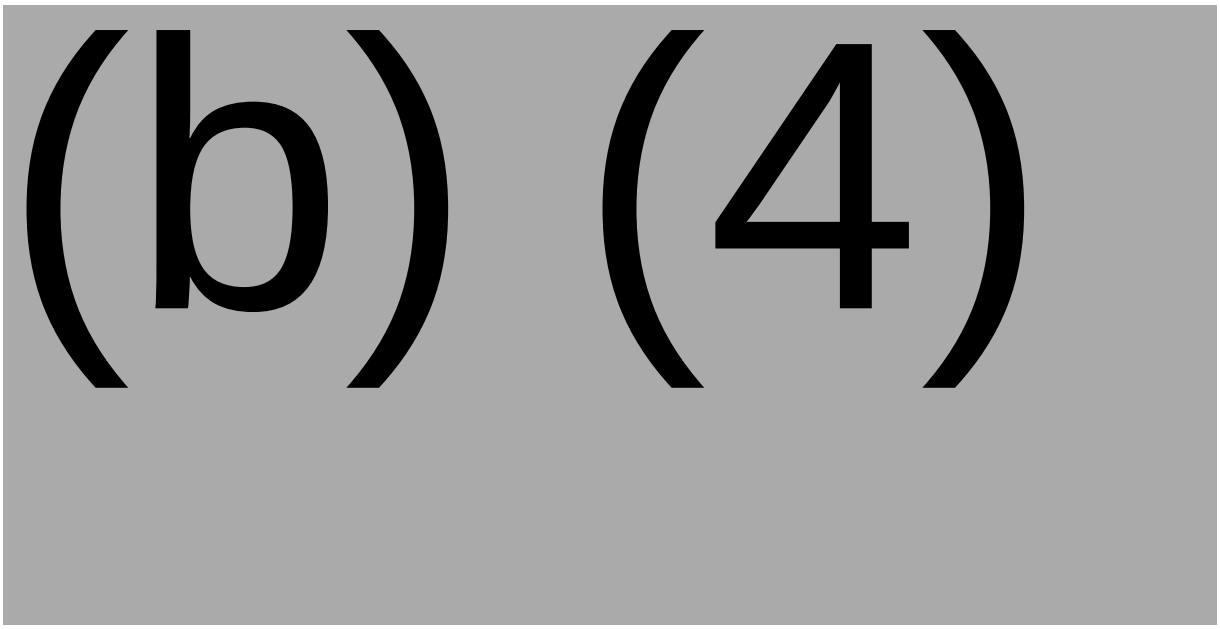
	The requested studies demonstrated that the clipped variants ((b) (4) forms) have full functional activity. To demonstrate this, the potencies of the (b) (4) ANDEXXA preparations were examined and compared to the parent, (b) (4) lot. Potencies were determined by direct, indirect, and TFPI assays, as well as by the (b) (4) and TF-initiated TG assay. Additionally, (b) (4) ANDEXXA was examined, and shown to have equivalent potency to the parent lot by direct, indirect and anti-TFPI activity methods.
2i	***Because the Phase 3 studies were conducted using materials manufactured by (b) (4) , please justify the proposed commercial release specifications for all release methods with the analytical studies of clinical batches. In these studies, the clinical batches and representative (b) (4) batches should be compared side by side using fully validated release methods and the pharmacodynamics methods used in the clinical trials to demonstrate the ANDEXXA effect, including the clinical assay TF-activated TGT and TFPI activity assays.***
	Review of Portola's Response to CRL Item No. 2i: Portola provided an update to justification for the commercial specifications, that takes clinical batches into account. An anti-TFPI activity release assay has been validated, that is based on TF-dependent activation of FX in the presence of TFPI. Data generated using a PD method (TF-initiated TG, with or without a FXa inhibitor) using (b) (4) lots of material, are included in the structure and function elucidation sections of the CRL amendment, *Elucidation of Structure and other Characteristics*, see Fig. 6.  The *in vitro* data presented in Fig. 6 demonstrate that ANDEXXA's dose needed for complete reversal of FXa inhibitor activity is approximately (b) (4) times higher than the ANDEXXA dose needed to induce a substantial procoagulant activity in a patient without FXa inhibitor. The data from TG assay experiments under the conditions that demonstrate the procoagulant

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	effect of TFPI inhibition by ANDEXXA, similar to the Fig. 6, were omitted from the original BLA submission, and were added as requested in the CRL.
2j	***Please note that your justification for specifications should explain how the finalized specification and validated release methods will demonstrate the consistent performance of your manufacturing process to produce drug product with the appropriate identity, quality, safety, purity, and potency attributes.***
	Review of Portola's Response to CRL Item No. 2j: An updated approach for setting specifications includes: (1) the CQAs, (2) inclusion of analytical methods that control for CQAs and/or process consistency, and (3) a robust statistical approach assessing all lots manufactured to date per the revised Process Control Strategy, which included assessment of method capability, stability data, and lots used in clinical studies to ensure the identity, quality, safety, purity and potency of ANDEXXA. I agree with Portola's conclusion that the revised release methods and specifications are now appropriate.
3	***In reference to our IR about ANDEXXA potency standards dated 12 February 2016 and your 22 February, 20 April, 18 May, 06 June, 21 June, 27 June, 06 July, 08 July, 13 July and 29 July 2016 responses which are incomplete, please note that a Primary Reference Standard (PRS) is required to control and preserve the existing and new unitages of the potency of ANDEXXA. A secondary standard is needed for routine control of the manufacturing process and QC of product quality. The PRS is critical in maintaining a consistent potency unit and allows "like vs like" comparisons when changes are made in assay reagents or methodologies, and manufacturing process. To demonstrate control over potency unitage, please:***
3a	***Provide your reference standard qualification protocol for review.***
	Review of Portola's Response to CRL Item No. 3a: The RS qualification protocol is sufficiently comprehensive and acceptable. The qualifications for RSs are also appropriate, see Table 7.
3b	***Qualify and establish one lot of andexanet alfa as the PRS and ensure that your Working Reference Standards are qualified against this PRS over the product life-cycle. You should perform an adequate number of replicate analyses to qualify the reference standards so that the potency can be assigned with sufficient statistical power.***
	Review of Portola's Response to CRL Item No. 3b: ANDEXXA PRS has been established and has subsequently been bridged to both the current and previously used WRSs (or the parent (b) (4) lots, when the respective WRSs were not available), see Table 7.
3c	***Qualify the reference standards independently for both the direct and the indirect potency assays.***

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	Review of Portola's Response to CRL Item No. 3c: The RSs were qualified independently for both the direct and the indirect potency assays, see Table 7.
3d	***Provide detailed information on the method and reagents used in the assignment of potency to the PRS and secondary standards, studies to monitor the stability of the reference standards, and protocol for the replacement or replenishment of these reference standards.***
	Review of Portola's Response to CRL Item No. 3d: Details on the method and reagents used in the assignment of potency are provided in Section 3.2.S.5 *Reference Standards or Materials*. They are appropriate.
3e	***List all reference standards used thus far for the release testing of*** (b) (4) ***FDP batches and in stability studies. In addition, apply new potency unitage to evaluate the potencies of all of your reference standards – primary, secondary, or working – in direct and indirect units in side-by- side comparative studies.***
	Review of Portola's Response to CRL Item No. 3e: A history of all RSs used during development for either release or stability testing for both (b) (4) FDP is provided in Section 3.2.S.5 *Reference Standard or Materials*. The new potency unitages were applied to these RSs.
3f	***Provide the reasons for the replacement of previous standards and the actions taken to ensure the linkage of products made as the manufacturing process was changed; as well as the preservation of the potency unit in stability studies.*** ***For example, reference standard Lot #*** (b) (4) ***was qualified on 10 November 2015 but was no longer available for use on 15 July 2016. Please provide the investigation report for its OOS pH result (pH*** (b) (4) ***was outside of the specification criterion of*** (b) (4) ***) which occurred on 16 March 2016 and explain the impact of this deviation on reference standard continuity.***
	Review of Portola's Response to CRL Item No. 3f: Due to the early development limitations of the manufacturing capacity, and the resulting supply challenges encountered in keeping large amounts of WRS available, once each RS was depleted, a new RS was created. Where available, Portola used either the retained WRS samples or samples of parent BDS lots to bridge the standards. The investigation for the OOS for pH in WRS Lot (b) (4) concluded that adsorption of (b) (4) from (b) (4) into loosely capped vials of standard was the likely root-cause for the OOS. During the normal use of the (b) (4) WRS, the pH was shown to gradually return to the specification range. Portola believes that the impact of non-conformant pH will be minimal for assays where use of (b) (4) standard is not required. Importantly, the establishment of a PRS now ensures continuity and traceability of future RSs.

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4	***The proposed shelf-lives of the commercial product are not supported with sufficient*** (b) (4) ***manufacturing experience. Your proposal to use*** (b) (4) ***stability data to support the stability of the*** (b) (4) ***product is not acceptable because of the following reasons:*** ***The comparability of the*** (b) (4) ***and*** (b) (4) ***materials has yet to be established.*** ***Empirical stability data on the batches for both processes are limited and insufficient because the critical analytical methods used to monitor the identity, purity and potency of ANDEXXA were introduced shortly after*** (b) (4) ***introduction. In addition, only the old methods continue to be used in many of the initiated stability studies.*** ***Stability data obtained with the previous versions of these methods were not trended quantitatively and therefore the linkage between the data from the old and new methods is not well established.***
4a	***To demonstrate product stability over time:*** ***Retest all available*** (b) (4) ***and*** (b) (4) ***batches using the new, validated release methods to demonstrate that the old batches meet all the stability specifications and possess comparable stability profiles.***
	Review of Portola's Response to CRL Item No. 4a: As part of the specification-setting process, all available (b) (4) lots were re-tested with the new, validated release methods (e.g., (b) (4)). Statistical analyses demonstrated that older lots could be pooled with more recently manufactured lots. Old batches met all stability specifications and are comparable to new ones. These findings also demonstrate product stability.
4b	***Investigate all adverse stability trends of all available data, which should include, but not be limited to, every*** (b) (4) ***and*** (b) (4) ***as resolved by your new and old methods. For example, please explain the steady*** (b) (4) ***in the*** (b) (4) ***by the*** (b) (4) ***which was observed in*** (b) (4) ***FDP Batch*** (b) (4) ***in real-time and accelerated stability studies. Please explain how this band is related to the*** (b) (4) ***detected by the new*** (b) (4) ***methods.***
	Review of Portola's Response to CRL Item No. 4b: As part of stability studies of (b) (4) FDP, data generated by fully validated quantitative stability-indicating methods are (b) (4) to identify any potential trends. The previous (b) (4) methods used throughout development were not optimized, nor fully validated. Portola asserts that stability data generated by these methods does not provide the most accurate assessment of ANDEXXA (b) (4) FDP stability. New and revised fully validated methods have been used for the stability analysis of all primary stability lots of (b) (4) FDP. The obtained stability data using the new methods are now monitored and trended on a regular basis. After 18 months in the stability programs, no adverse trend

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	has been observed at long term storage conditions. The data and trending of the future stability time points will be provided in the (b) (4) updates. In the CRL response, Portola confirmed that the (b) (4) by (b) (4) method, observed in the development FDP Batch (b) (4), is consistent with the (b) (4) for (b) (4) quantified by the new (b) (4) method. However, the steady (b) (4) in the (b) (4) was not explained. I therefore submitted the following information request dated 28 December 2017: 1. **Regarding the Complete Response Letter, question 3b, please explain the steady (b) (4) in the (b) (4) by the (b) (4) which was observed in (b) (4) FDP Batch (b) (4) in real-time and accelerated stability studies. Specifically, please discuss the impact of these findings on the overall conclusions from ANDEXXA stability studies.** In their 25 January 2018 response, Portola presented evidence that the apparent trends for (b) (4) increase in the (b) (4) stability study can be explained by assay artifacts. Specifically, the assay was drifting as evidenced from an identical trend seen for the (b) (4) in the RS in the same study. In addition, the major contributor to the value reported for the (b) (4) was found to be a (b) (4) identified as an (b) (4) impurity, (b) (4). Furthermore, the analysis of available stability data by all other assays collected in this study supported the conclusion that the various forms of ANDEXXA, including (b) (4), remained stable during storage of (b) (4) lot.
4c	***Describe all OOS results in completed and ongoing stability studies, including accelerated stability and stability of reference materials. For example, an OOS result for potency of (b) (4) batch # (b) (4) after (b) (4) of storage at (b) (4) occurred on 30 July 2015. The deviation investigation was closed on 14 October 2015 but this OOS was not reported in the BLA.***
	Review of Portola's Response to CRL Item No. 4c: Only one lot ((b) (4)) had an OOS result at the long-term storage condition. Other results that were observed that did not meet the long-term storage criteria were only seen under (b) (4) conditions designed to identify stability-indicating methods, with the most changes seen with either (b) (4) methods. There were no OOS results in the FDP stability studies.
4d	***Complete the in-use stability studies during which product compatibility with intravenous administration devices was also investigated. Please include assessment of parameters for microbiology, purity by (b) (4), and direct and indirect potency over the proposed (b) (4) period.***
	Review of Portola's Response to CRL Item No. 4d: The updated in-use stability study was conducted under the worst-case condition of the lowest ANDEXXA dose corresponding to 40 mL of reconstituted FDP in a 250 mL IV bag and the slower infusion dose rate of 4 mg/min (0.4 mL/min) maintained at room temperature and exposed to ambient light. No significant changes in the potency and purity of the reconstituted FDP, and no increase in microbial growth were observed under the conditions

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	studied. Storage condition limits of up to 16 hours at 2 to 8°C in primary container closure and up to 8 hours at room temperature in the IV bag are justified by the data.
5a	***Include*** (b) (4) ***testing as a critical process parameter for the*** (b) (4) ***step. We acknowledge that you are performing*** (b) (4) ***and*** (b) (4) ***testing as non-critical process parameters, however, the proposed surrogate critical control parameters, such as*** (b) (4) ***, by themselves are not sufficient to ensure the effectiveness of this viral clearance step.***
	Review of Portola's Response to CRL Item No. 5a: (b) (4) *Testing* has been established as a CPP for the (b) (4) step to ensure the effectiveness of this viral clearance step.
5b	***Explain the validation and criticality status for the process parameter*** (b) (4) ***.*** (b) (4) ***related parameters,*** (b) (4) ***targets, are listed in Table 35:*** (b) (4) ***and*** (b) (4) ***Andexanet*** (b) (4) ***Manufacturing Process Changes of the 21 June 2016 amendment to Comparability Protocol Andexanet Alfa (PRT064445)*** (b) (4) ***to*** (b) (4) ***and Resulting Drug Product. These parameters are not described in the BLA.***
	Review of Portola's Response to CRL Item No. 5b: In response to the FDA's request for information on the (b) (4) control strategy, and advice to seek approval of the (b) (4) process through a PAS rather than a CBE-30 supplement, Portola decided to no longer pursue the (b) (4) process at (b) (4) .
5c (i)-(v)	***[5c] List the validated*** (b) (4) ***FDP fill volume ranges for the commercial*** (b) (4) ***. The expected scaled-up*** (b) (4) ***(also known as*** (b) (4) ***) and the GEN2 Process at Lonza.*** ***Please provide a table with the following information:*** ***(i) BDS batch fill volume range (Formulated at*** (b) (4) ***)*** ***(ii) FDP batch fill volume range (Formulated at 10 mg/ml)*** ***(iii) Total BDS yield (*** (b) (4) ***)*** ***(iv) Number of BDS batches needed to produce*** (b) (4) ***FDP batch*** ***(v) Number of vials per FDP batch***
	Review of Portola's Response to CRL Item No. 5c (i)-(v): Portola is no longer pursuing the (b) (4) (formerly expected scaled-up) process at (b) (4) . As part of the lifecycle management of the ANDEXXA manufacturing process, as well as to address future commercial market demands, Portola has modified the process to increase product yield and scaled up to (b) (4) for the GEN 2 process at Lonza. **GEN 2 process is not included in the BLA**, but the preliminary GEN 2 process and GEN 2 product data were reviewed under the IND. It should be noted that FDA does not currently recognize GEN 2 as an improvement of the ANDEXXA process. Rather, FDA has concluded, repeatedly, that the GEN 2 process appears to be substantially different from the proposed ANDEXXA commercial process, and therefore FDA recommended that Portola should seek

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	approval for the GEN 2 product under a new BLA. A PK/PD comparability study was designed to understand the scope of clinically meaningful differences between the commercial ANDEXXA and GEN 2 materials. The results of the comparability study are yet to be communicated to the FDA.
6	***In reference to our IR dated 01 June 2016 and your 15 June 2016 response, which is incomplete, develop the (b) (4) assay for the characterization of the interactions between the (b) (4) and TFPI and perform the following studies:***
6a	***Use representative (b) (4) batches from (b) (4) ((b) (4) batches) and (b) (4) ((b) (4) batches) to study interactions between (b) (4) and TFPI. We are aware that the reported Kd values for Factor Xa and TFPI are near the limit of resolution of the (b) (4) assay and that the (b) (4) might be too (b) (4) to resolve the Kd accurately due to the (b) (4). However, the same experiments can provide an accurate assessment of n and ΔH - the former is an indicator of drug activity, and the latter of batch-to-batch variability and micro-heterogeneity within individual batches.***
	Review of Portola's Response to CRL Item No. 6a: The data shows that (b) (4) lots of ANDEXXA bind to TFPI with similar enthalpy and stoichiometry. Please refer to the memorandum of Dr. Wojciech Jankowski for details.
6b	***Use (b) (4) to investigate the interactions of the (b) (4) of andexanet alfa with TFPI.***
	Review of Portola's Response to CRL Item No. 6b: ANDEXXA and (b) (4) ANDEXXA appear to interact similarly with TFPI. Please refer to the memorandum of Dr. Wojciech Jankowski for details.
6c	***Investigate the sensitivity of the (b) (4) method to evaluate the degradation of ANDEXXA and consider including the (b) (4) assay in the (b) (4) release specifications. Establish acceptance criteria for its interactions with direct FXa inhibitors for these thermodynamics and stoichiometry parameters - Kd, ΔH, TΔS, ΔG and n.***
	Review of Portola's Response to CRL Item No. 6c: Portola conducted requested studies. Although (b) (4) was found to be informative of the ANDEXXA structural integrity, Portola believes that the currently proposed potency assays for release (including the recently established TFPI (b) (4) assay) are sufficient to capture the mechanisms of action of ANDEXXA. In addition, Portola has not been able to identify a contract lab to run the (b) (4) assay under GMP conditions. The response is acceptable.
7	***(b) (4) of your FDP (b) (4) samples, including (b) (4) batches of lyophilized drug product, (b) (4) lyophilized solution and the "reference standard", which we analyzed by (b) (4) using a (b) (4) all show (b) (4), in addition to (b) (4), when (b) (4) is replaced by (b) (4) in the (b) (4). Please identify the proteins in these (b) (4).***

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	Review of Portola's Response to CRL Item No. 7: The (b) (4) method using (b) (4) in the (b) (4) was investigated. Portola confirmed that this (b) (4) method variant shows partial resolution of product-related variants that (b) (4) as a (b) (4) by the release method. Similar partial resolution was confirmed by another (b) (4) method Portola developed using (b) (4). Good lot-to-lot consistency was demonstrated by this method for (b) (4) lots. The product variants in this (b) (4) were identified as (b) (4) variants. These variants are well resolved by at least (b) (4) of (b) (4) existing lot release methods, (b) (4). Because individual specification limits for these forms are already established, separation and reporting of the partially resolved (b) (4) species is not required. The response is acceptable.
8	***Line AB to C Comparability Protocol***
	Review of Portola's Response to CRL Item No. 8: (b) (4) at (b) (4) is not being considered for the BLA or for future use.
13a	***In reference to our IR on immunogenicity methods dated 17 February 2016 and your 03 March, 20 April, 08 July and 29 July 2016 responses, which are incomplete, we request that you develop and validate assays to measure the activity of the antibodies that bind (b) (4) or inhibit the activities of endogenous human Factors X and Xa. In your response, please address the following requests:*** ***a) Develop and validate the assay using clinically relevant methods (e.g., the (b) (4) assay for Factor X activity), and report the results in (b) (4) of Factor X inhibition activity.***
	Review of Portola's Response to CRL Item No. 13a: An assay to detect antibodies to (b) (4) has been developed using the (b) (4) preparations made from the (b) (4) CHO cells, in the same (b) (4) used for ANDEXXA expression. The (b) (4) antibody assay was qualified and validated to test clinical study samples. These assays did not identify any samples with confirmed (b) (4) antibodies in any of the 92 subjects tested. The development of assay to detect the antibodies that inhibit the activities of endogenous FX/FXa was requested by the FDA in 2010. Portola agreed to, but did not develop the assay, despite the claims in the submitted clinical study reports that no FX/FXa activity neutralizing antibodies were detected. During the review of original BLA submission, Portola repeatedly claimed that it is impossible to develop a (b) (4) assay for detection of such antibodies because FXa inhibitors will interfere with (b) (4). In the CRL response, Portola presented the validation of a modified (b) (4) assay for detection of the FX/FXa neutralizing antibodies, which is based on an (b) (4)-based (b) (4) assay for FX activity in FX-deficient plasma with the results reported as (b) (4). ANDEXXA, at very high concentrations of (b) (4), was shown to interfere with the

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	detection of neutralizing antibodies, indicating that this assay should not be used in patients within 2 days after administration. Apixaban ((b) (4)) did not interfere with the assay and had no significant impact on the signal. Rivaroxaban ((b) (4)) interfered with the intercept and antibody titers but had no significant influence on the linear slope of the assay, suggesting that the assay should not be used in patients within 2 days of rivaroxaban administration. Because Portola evaluated FX/FXa neutralizing antibodies before FXa inhibitor and ANDEXXA administration, and at days 14-20, 28-36, and 43-48 post treatment, the validation is acceptable and no interference with the study drugs is expected. Portola tested a representative subset of archival samples from the healthy volunteer studies, and identified no subjects positive for neutralizing antibodies in either placebo or ANDEXXA-treated study samples. Portola made a commitment to implement this assays in future clinical studies.[15]
13b	***Please note that the development of neutralizing antibodies against Factors X and Xa is an unwanted immune response to a therapeutic protein product as defined in the 2014 FDA Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products. To ensure protection of confirmatory study participants from exposure to a product with a non-redundant endogenous counterpart, you are required to have a means of testing for neutralizing antibodies against endogenous Factors X and Xa. FDA previously requested that you develop these assays during the pre-IND meeting on 16 June 2009 (CRMTS #7089, Ref. PS000698), and you included a commitment to develop these assays in the original IND submitted on 15 March 2012 and in your Clinical Study Protocol 15-507 dated 09 June 2015.***
	Review of Portola's Response to CRL Item No. 13b: An assay for detecting neutralizing antibodies was developed, see response to CRL Item No. 13a.
13c	***Develop an assay to assess the development of (b) (4) antibodies in subjects who have participated in the clinical studies. (b) (4) impurities are suspected to originate from CHO cells, which may be present in the FDP as evidenced from the formation of clipped (b) (4) in stability studies.***
	Review of Portola's Response to CRL Item No. 13c: An assay to assess the development of (b) (4) antibodies was developed, see response to CRL Item No. 13a.
13d (i)	***13d. Use validated immunogenicity methods to:*** ***(i.) Assess how the presence of anti-Factor X/FXa inhibitory antibodies may interfere with the assays used to evaluate the pharmacodynamics, pharmacokinetics, and immunogenicity in the clinical studies.***
	Review of Portola's Response to CRL Item No. 13d(i):

[15] STN 125586/0 - Sequence 0077 - Reviewer's Guide, page 15.

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	During the first review cycle, Portola stated that although an assay for detection of endogenous FX/FXa neutralizing antibodies was not developed, the presence of such antibodies would be obvious from the analysis of the existing PK/PD assays. Since Portola did not prospectively validate either of the PK/PD assays for the interference with neutralizing antibodies, this information was requested in a CRL. Assay interference by anti-FX/FXa antibodies was tested in human plasma in *in vitro* studies using a commercial anti-human FX polyclonal antibody as a surrogate neutralizing antibody. The following clinical PD assays were evaluated: 1) three versions of TG assay ((b) (4) , TF-CAT, and (b) (4) ; these assays are discussed in Response to CRL Item No. 14 below); 2) (b) (4) -based assays in plasma ((b) (4)) or whole blood (ACT); 3) anti-FXa activity assay, and 4) ADA-neutralizing activity assay. Portola found that the presence of FX/FXa neutralizing antibodies could interfere with and be detected by the TG assay. They do not interfere with the reversal activity of ANDEXXA toward FXa inhibitors. Likewise, addition of anti-human FX antibody caused a dose-dependent prolongation of the (b) (4) in all (b) (4) based assays ((b) (4)). ANDEXXA could neutralize the anticoagulant activity of each FXa inhibitor in the presence of the surrogate neutralizing antibody. As I was expecting, the minimal concentration of the neutralizing antibody to induce at least some inhibition ((b) (4) , for TG assay, (b) (4) , respectively) was (b) (4) times higher than the lowest end of the linear range ((b) (4)) for the validated assay for detection of the FX/FXa neutralizing antibodies. The result was expected because the validated (b) (4) assay is based on a more sensitive FX activity assay in FX-deficient plasma. The existing PD assays can be partially affected by very high titers of FX/FXa neutralizing antibodies (above (b) (4)).
13d (ii)	***Test retained clinical samples for anti-Factor X and anti-Factor Xa inhibitory antibodies and*** (b) (4) ***antibodies.***
	Review of Portola's Response to CRL Item No. 13d (ii): The retained samples were tested, see response to CRL Item No. 13a.
14	***In reference to our IR on pharmacodynamics methods dated 17 February 2016 and your 03 March, 20 April, 08 July and 29 July 2016 responses, which are incomplete, please provide the reports of bioanalytical studies which you have committed to perform to establish the comparability, or lack thereof, between the three versions of the TGT assay. The three versions are*** (b) (4) ***the commercially available TF-activated CAT (TF-CAT), and*** (b) (4) ***. The latter two assays were used in the phase 3 and 3b/4 trials. These studies should include side-by-side testing of samples spiked with ANDEXXA and FXa inhibitors and retrospective analyses of data from the clinical trials.***
	Review of Portola's Response to CRL Item No. 14:

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	As I was expecting, the side-by-side comparisons of the three versions of TG assay, i.e., the (b) (4) , commercial TF-CAT, and the (b) (4) demonstrated that these assays are inhibited similarly by the FXa inhibitors, but the reversal of the inhibitors by ANDEXXA is different, see Figs. 7 and 8.
	(b) (4)
14a	***Please also address the following examples of incorrect presentation and interpretation of TGT data in the BLA:***

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	On page 9 of the 27 July 2016 meeting materials, you claimed similarity between the correlation graphs of anti-fXa activity and TGT in the Phase 2 and Phase 3 clinical trials. However, you compared the mean TGT Phase 2 data from all (placebo and ANDEXXA-treated) subjects to the mean ETP Phase 3 data from the placebo arm only. Please revise these graphs to present data from the placebo and ANDEXXA arms separately.
	Review of Portola's Response to CRL Item No. 14a: During the first review cycle, FDA raised concerns regarding the proposed indication for edoxaban reversal, for which no clinical Phase 3 data were available. In support of the claim of edoxaban reversal, Portola argued that effects of ANDEXXA on edoxaban patients can be extrapolated from the Phase 3 study data on apixaban and rivaroxaban because (1) In Phase 2 studies, all three inhibitors demonstrated similar effects on the anti-FXa activity increase and TG assay decrease after administration of either apixaban, rivaroxaban, or edoxaban. (2) Per Portola, a good agreement between the Phase 2 and Phase 3 studies was shown for the anti-FXa and TG assay biomarkers. As discussed in Response to CRL Item No. 14, the claim of comparability of TG responses was incorrect because different versions of the TG assay were used. This deficiency was not apparent, because the graphs, provided by Portola in 2016, were based on the analysis of anti-FXa and TG assay parameters ***in the absence of ANDEXXA***. (b) (4) Although the Phase 2 and Phase 3 studies have demonstrated comparable effect of FXa inhibitors on the 2 biomarkers (e.g., Figs. 7a, 8a, and 9), the Phase 2 and Phase 3 studies showed that the effect of ANDEXXA on the TG assays was different (see Figs. 7b and 8b). Specifically, the effect of ANDEXXA on the TG was substantially higher in Phase 3 study (see Figs. 7b and 8b) because the Phase 3 TG assay (TF-CAT) was more sensitive to the anti-TFPI

[16] Addendum for data from more than one study (Section 5.3.5.3)

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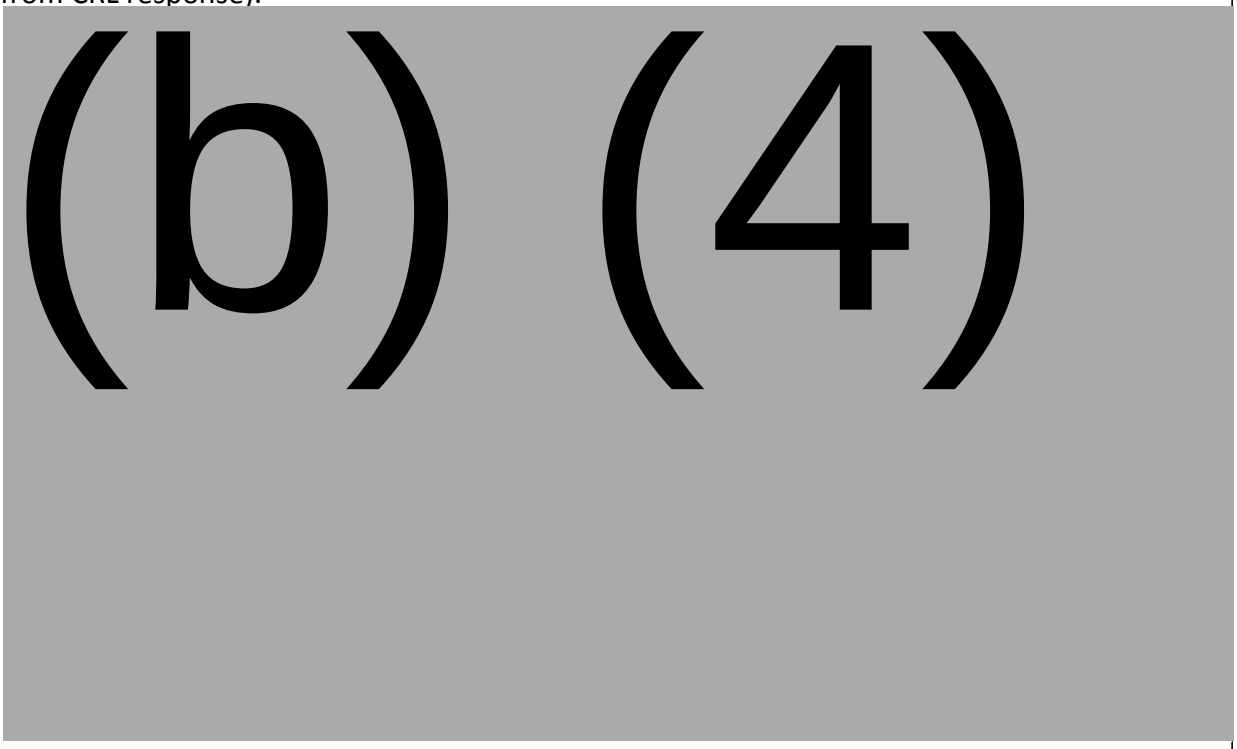
	action of ANDEXXA than the older (b) (4) method used in the Phase 2 studies (Fig. 10, data from CRL response).  Based on my previous analyses presented in the review memo dated 17 August 2016, which are now supported by the new data presented in the CRL responses, I conclude that the TG assay data from the Phase 2 and Phase 3 studies (in the presence of ANDEXXA) are not comparable, and the effect of ANDEXXA on edoxaban, which was not studied in the Phase 3, cannot be extrapolated from the Phase 2 results.
14b	***Your 03 March 2016 response states that the TG(b) (4) and CAT methods are similar. However, there appears to be a stronger effect of ANDEXXA on TF-activated TGT elevation (e.g., during the first 3 hours post-bolus) in the Phase 3 studies, as compared to the effect report in the Phase 2 study.*** ***For example, analysis of the clinical study data presented in Table A1-5 provided in your 03 March 2016 amendment demonstrates that in the apixaban studies, TF(b) (4) was elevated above the pre-apixaban baseline by 29% (Study 12-502, Module 1) and TF-CAT was elevated by 66% (Study 14-503 Part 1) and 40% (Study 14-503 Part 2). In the rivaroxaban studies, TF-(b) (4) was elevated by 15% (Study 12-502, Module 2) and TF-CAT was elevated by 30% (Study 14-504 Part 1) and 39% (Study 14-504 Part 2). In contrast to the differences in TGT elevation, TF-RFU and TF-CAT were inhibited to a similar degree by apixaban (50% inhibition in both methods) and rivaroxaban (80% in TF(b) (4) and 71% in TF-CAT). Please explain these findings and perform the anti-fXa activity versus TGT comparison separately for each of the FXa inhibitors.***
	Review of Portola's Response to CRL Item No. 14b:

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	Portola explained that the TF(b) (4) and TF-CAT assays use different TF and phospholipid concentrations. The TF-CAT may be more sensitive to the ANDEXXA-TFPI interaction due to the lower TF concentration used in the assay. This explanation is consistent with my expectations as described in the review memo dated 17 August 2016. A side-by-side comparison between the two assays was performed by *in vitro* spiking tests as discussed in the Response to CRL Item No. 14 above.
14c	***The preclinical report for study NC-15-0659-R0001 states that "andexanet alone had minimal effect in the absence of rivaroxaban." However, the raw data you submitted on 17 July 2016 to support this report show a 50% increase and 40% shortening in the commonly used TGT parameters, thrombin peak height and time to thrombin peak, respectively. These findings suggest that the effect of ANDEXXA is not represented by the presented parameter of the TGT method, ETP.***
	Review of Portola's Response to CRL Item No. 14c: Portola confirmed that in the TF-CAT assay, an endogenous thrombin potential (ETP) parameter was collected together with the other CAT parameters ((b) (4)). ETP was used in the original data analysis as it is analogous to the in-house TF-RFU endpoint readout. All (b) (4) CAT parameters are now provided in an addendum to the study report. Fig. 11 shows that the (b) (4) and TPH were the most sensitive, and ETP was the least sensitive, to the action of FXa inhibitors and ANDEXXA. This is in line with my analyses of the raw assay data which I requested during the first review cycle (see 17 August 2016 memo). (b) (4) In addition, in the CRL response, Portola included the following description of the TG parameter sensitivity to TFPI[17]: "*ETP (Endogenous Thrombin Potential, or area under the thrombin generation curve), <..> is less sensitive to TFPI effects than other CAT parameters. For the CAT parameter (peak thrombin), which is more sensitive to TFPI effects, there was a* (b) (4)

[17] 3.2.S.3.1 Elucidation of Structure & Other Characteristics. Page 96

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	(b) (4) *increase compared to control plasma".* This description confirms that in their prior publications and written communications with the FDA, Portola could plausibly claim that ANDEXXA has little procoagulant activity in the TG assay because the least sensitive parameter was used for the graphical presentation of TG data.
15	***In the 19 July 2016 re-analysis of the data from a subset of subjects in the Phase 3 clinical trial, you explained that the elevation of TGT over the pre-inhibitor treatment baseline was mediated by the inhibition of plasma TFPI activity, as evidenced by a reduced elevation in a contact- activated TGT assay. The finding that inhibition of TFPI was contributing to the procoagulant activity observed in the clinical studies implies a need to address this phenomenon in product labeling to assure that physicians will understand the effect of administration of ANDEXXA and the potential for enhanced thrombogenicity. To address this issue:***
15a	***Please propose language for the Package Insert that will inform physicians of this incompletely characterized phenomenon and the potential risk of enhanced and prolonged thrombogenicity that it may cause.***
	Review of Portola's Response to CRL Item No. 15a: The proposed language now acknowledges that ANDEXXA has the second procoagulant action mediated by inhibition of TFPI activity.
15b & 15b (i)	***Please perform additional analyses to delineate the magnitudes and durations of the respective contributions of anti-fXa reversal and TFPI inhibition on TGT elevation as a basis for relabeling of the product. The following approach is suggested to ensure that the relationship between the duration and magnitude of TGT elevation, and the reversal of anti-fXa activity is properly investigated:*** ***15b (i) Re-evaluate the conclusions regarding the contribution of anti-fXa activity reversal to the TGT elevation. Because the TF-activated TGT method you used was not specific to the effect of anti-fXa activity reversal, we conclude that a*** (b) (4) ***TGT (which you referred to as*** (b) (4) ***TGT) should be used instead of, or in addition to, the TF-activated TGT whenever you present the TGT results as evidence of the potentially hemostatic outcome of anti-fXa activity reversal by ANDEXXA;***
	Review of Portola's Response to CRL Item No. 15b(i): Portola performed comparisons between the TF-CAT and (b) (4) assays in both the *in vitro* spiking samples and in clinical samples from the Phase 3 studies (Part 2). These results were used to evaluate the overall conclusions regarding the contribution of the anti-FXa activity reversal to the TG assay elevation. The (b) (4) profile is more similar, nearly identical, to the anti-FXa activity reversal that was used as the primary PD marker to determine the clinical doses of ANDEXXA in the Phase 2 studies. These observations support the overall conclusions that:

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	• Sequestration of the FXa inhibitor by ANDEXXA plays a major role in the increase in TG during and immediately following the end of ANDEXXA administration, i.e., within 2-3 hours after bolus dose. • The inhibition of TFPI by ANDEXXA appears to contribute to the prolonged duration of the increase in TG in the TF-CAT assay, at least for 22 hours and possibly longer. This sustained elevation effect is not observed in the (b) (4) assay. • ANDEXXA has FXa inhibitor reversal capacity in both the TF-CAT assay (with the TFPI effect) and the (b) (4) assay (without the TFPI effect). • Substantial TG assay elevation above the pre-FXa inhibitor baseline was observed in TF-CAT, but a much smaller elevation was seen in the (b) (4) assay, suggesting that the procoagulant "overshoot" is mediated by the TFPI inhibition action of ANDEXXA.
15 b (ii)	***Re-analyze your TF-activated TGT assay data using the parameters suitable for evaluation of TFPI effect. For example, your data suggest that ETP is significantly less sensitive than the thrombin peak height to the procoagulant effect of TFPI inhibition by ANDEXXA. The use of a single parameter, e.g., ETP, could therefore be misleading;***
	Review of Portola's Response to CRL Item No. 15b(ii): The results of *in vitro* experiments are discussed above in Response to CRL Item No. 14c. (b) (4)

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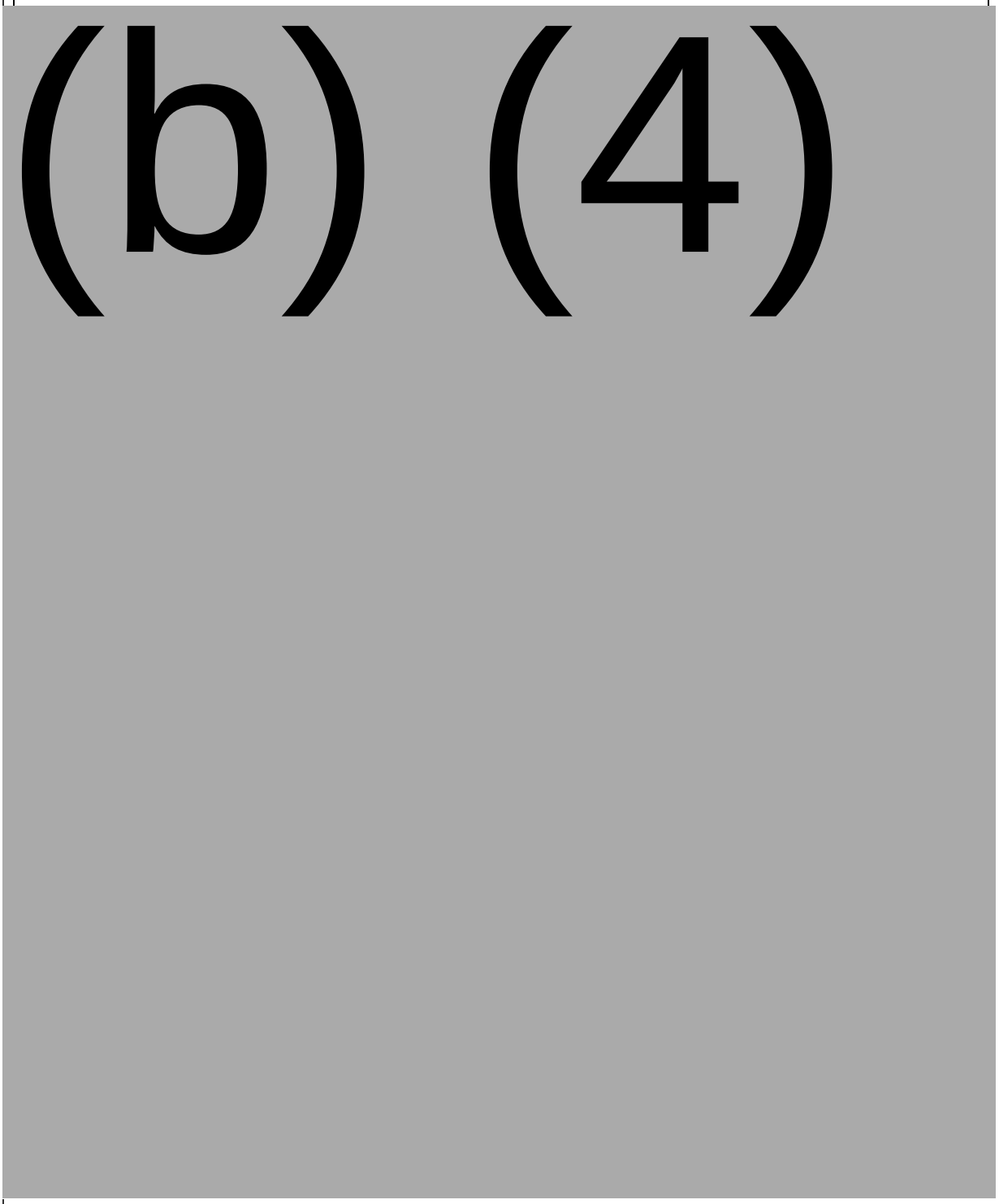
	(b) (4)  Portola reviewed the original analyses in the completed Phase 3 Studies 14-503 and 14-504, and performed additional analyses for the TF-CAT parameters. Portola re-graphed the TF-CAT time-course profiles for ETP, peak thrombin, and time-to-peak parameters for direct comparisons on the same time scale (an example is presented in Fig. 12).

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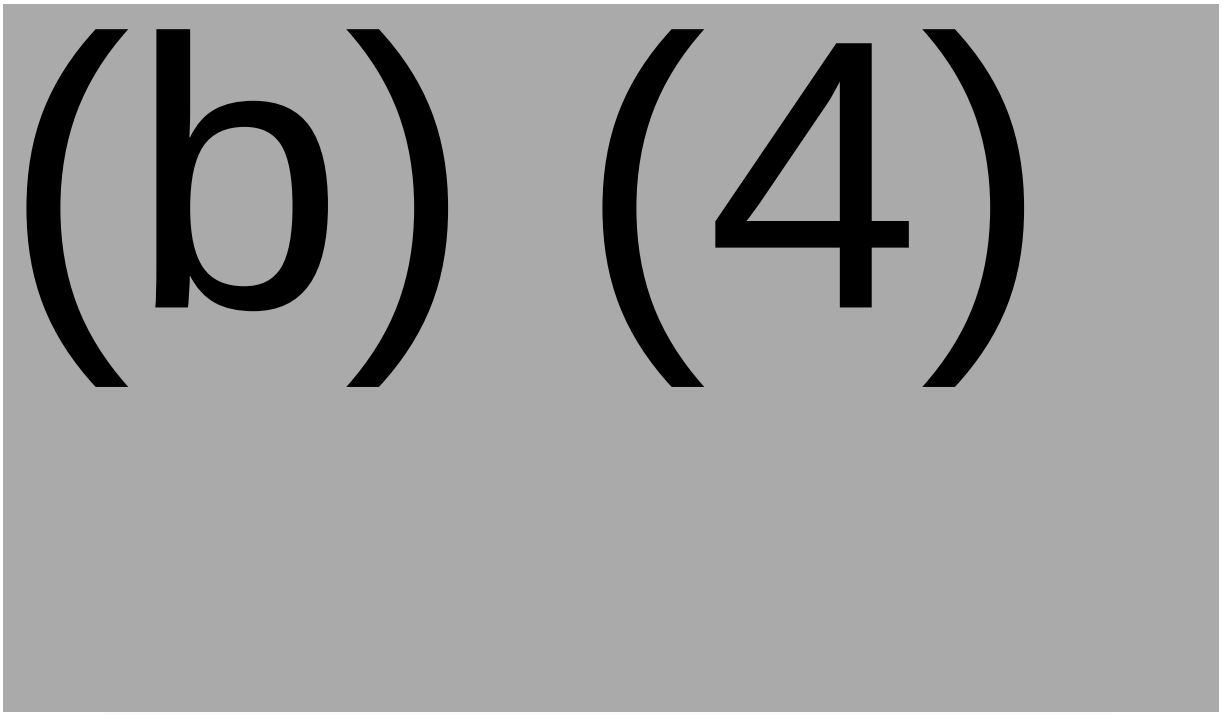
	I do not agree with Portola's conclusion that "*[t]he same conclusions as ETP can be reached based on each of the other CAT parameters*" because non-ETP parameters appear to show greater *overshoot over the normal range* after ANDEXXA administration. For example, for the graphs shown in Fig. 12, ANDEXXA increased the ETP by (b) (4) while the thrombin peak height was increased by (b) (4), and the time-to peak was shortened by (b) (4) (compared to the pre-apixaban level). In my view, these results explain the elevation of markers of thrombogenicity TAT and D-dimer, and confirm the potentially procoagulant effect of ANDEXXA on healthy volunteers treated with FXa inhibitors.
15b (iii)	***Compare the contributions of the anti-fXa reversal and TFPI inhibition actions of ANDEXXA to TGT elevation as you have already started doing in amendment dated 19 July 2016 by comparing the time courses of TF-activated TGT and contact-activated TGT methods;***
	Review of Portola's Response to CRL Item No. 15b(iii): A side-by-side comparison between the TF-CAT and (b) (4) is provided for the ETP parameter (Fig. 13, similar to the format in the NEJM 2013 paper), for direct comparisons to the previous results. As expected, ANDEXXA has a smaller and shorter effect on the non-TF assay, confirming the role of TFPI inhibition by ANDEXXA in the NEJM study. 
15b (iv)	***To demonstrate that the anti-fXa activity reversal, and not TFPI inhibition, was responsible for the successful normalization of the TGT, please apply the same statistical criteria you previously used in the Phase 3 study;***
	Review of Portola's Response to CRL Item No. 15b(iv):

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	The same statistical criteria previously used in the Phase 3 study for the TF-CAT assay have now been used for the analyses of the (b) (4) results for each of the (b) (4) CAT parameters. The results are summarized in the Addendum, Section 5.3.5.3, see Table 6 below. (b) (4)
15b (v)	*To facilitate the review of these data by the FDA, please re-plot all the graphs that show the time-courses of anti-fXa and TGT elevation using:*
15b (v) (1)	*The same time scales of no less than 24 hours after an ANDEXXA bolus. Your presentation of anti-fXa activity over 12 hours and TGT over 22 hours created a misleading appearance of good correlation between the duration of anti-fXa reversal (which is short) and that of elevation of TF-activated TGT (which is sustained).*
	Review of Portola's Response to CRL Item No. 15b(v)(1): All the graphs have been replotted, per the FDA specifications, and presented in the Addendum, Section 5.3.5.3. See discussion in Response to CRL Item No. 15b(i).
15b (v) (2)	*Error bars calculated as the standard deviation of the mean for all data points, which should include the pre-treatment (the so-called normal TGT range presented as a horizontal gray area on the TGT graphs) for the ANDEXXA and placebo arms of the study. Your proposal to compare two standard deviations of the pre-treatment levels of TGT with a standard error of the mean for the ANDEXXA arm creates an incorrect impression that the elevation of TGT after ANDEXXA administration remains within the "normal TGT range" while in fact a substantial elevation over the pre-treatment baseline was observed in the Phase 3 studies.*
	Review of Portola's Response to CRL Item No. 15b(v)(2): All the graphs have been replotted, per the FDA specifications, and presented in the Addendum, Section 5.3.5.3. See discussion in Response to CRL Item No. 15b(i).
15b (vi) Q 1.b. iv	*Please also reference the communication from FDA on 1 June 2016, which you have not yet addressed.* *Question 1.b.iv, from the 01 June 2016 RFI:* *Determine the TFPI activity in retained samples from Phase 1, 2 and 3 healthy volunteer studies. Please include enough data points to describe the effect of andexanet dose (bolus*

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	and bolus plus infusion) on the timing of the changes in TFPI activity in anticoagulated and non- anticoagulated subjects. Specifically, please determine the time of TFPI activity return to either the pre-andexanet treatment baseline or the normal range.
	Review of Portola's Response to CRL Item No. 15b (vi), Question 1.b.iv: **Portola did not provide the data needed to determine the time of TFPI activity return to either the pre-ANDEXXA treatment baseline or the normal range.** The available TFPI activity data from the Phase 1 study, as well as the total and free TFPI antigen data from the Phase 2 study in healthy subjects are provided in the Addendum to Studies 12-502, 14-503, and 14-504 [18]. From these data, it is impossible to discern the exact time when the TFPI activity returns to normal because no TFPI activity data are available to accurately cover the time-course of TFPI inhibition post 1 day after ANDEXXA bolus. Some information can be derived from the TFPI antigen data, as proposed by Portola, but, unfortunately, Portola did not establish a precise correlation between the TFPI antigen level and TFPI activity. This deficiency is important because Portola claimed that they were using TFPI antigen assays to estimate TFPI activity inhibition. This was done without validating the use of the TFPI antigen assays in lieu of the TFPI activity assay. The TFPI activity assay was not validated either. During the first review cycle, I noted that the TFPI inhibition effect was incorrectly presented as insignificant in the Phase 2 and 3 clinical studies [19]. In the clinical study reports, Portola noted that the TFPI antigen was partially inhibited, but discounted the finding as an artifact of ANDEXXA interference with the TFPI (b) (4) assay. Portola did not explain the antigen interference findings for what they were, i.e., the evidence that TFPI activity was inhibited. In contrast, in their public presentations that happened prior to BLA submission, Portola did the opposite by presenting their TFPI (b) (4) data in place of the TFPI activity data[20]. Although FDA requested that TFPI activity is investigated in all phases of clinical trials, and Portola agreed to do this, the TFPI activity assay was used only once, in one arm of the Phase 1 study. Portola initially claimed that the TFPI activity was always measured, but later clarified that "*[w]e have referred to this* (b) (4) *-based free TFPI results as TFPI activity in the Phase 2*

[18] Clinical Study Addendum, Section 5.3.5.3

[19] CMC review memo dated 17 August 2016

[20] In response to my 1 June 2016 request to "*[p]rovide the results of all relevant testing on plasma samples collected during the course of the Phase 1, 2 and 3 clinical studies, including but not be limited to the following data which either were not presented or appear to contradict the data presented in the BLA: <..> (ii) the Phase 2 TFPI activity testing which you acknowledged in the abstracts presented by Dr. Mark Crowther at the 2013 meetings of the American Society of Hematology and the International Society on Thrombosis and Haemostasis, and in Commission File Number 001-35935 (posted on the Securities and Exchange Commission's website)*", Portola explained that "*[i]n the Crowther abstract for the 2013 ASH meeting, the statement referring to TFPI "activity" was based on the TFPI* (b) (4) *using the* (b) (4) *, which detects "free" TFPI (see comments above regarding "free" TFPI, response to question 1c.viii). The same is true for any reference to TFPI activity in SEC filings regarding phase 2 TFPI activity.* Source: BLA 125586.0/ SN0039 dated 15 June 2016, 1.11.3 CLINICAL INFORMATION AMENDMENT.

#	*FDA CRL Comments* & My Review of Portola's Responses
	studies" because "*the effect of andexanet on TFPI activity could also be inferred from the change of the* (b) (4) *readout in the presence of andexanet.*" [21] Portola's position was that it would be impossible to measure TFPI activity in patients on FXa inhibitors[22]. The interference of FXa inhibitors with the TFPI activity assay was not demonstrated, and the use of TFPI antigen assays for estimation of TFPI activity was not qualified, and these studies were requested in the CRL. The validation of the TFPI activity assay is now presented in the CRL response, showing that the method is suitable for assessment of plasma samples with low levels of FXa inhibitors, i.e., as early as 18 hours after ANDEXXA bolus. I conclude that TFPI activity assay is suitable for the evaluation of the depth and duration of TFPI inhibition by ANDEXXA. The *in vitro* study to compare TFPI activity levels with TFPI antigen assay readouts is pending in the validation report (NC-17-0801-R0001). In lieu of *in vitro* analytical data, the correlation between the TFPI activity and free TFPI antigen level was investigated in ANDEXXA-treated subjects (Study 11-501, see Fig. 14). The clinical data set shows variability, likely due to individual variability in TFPI activity and antigen levels, but also due to variability between different measurements. Nevertheless, the data suggest that there is a linear relationship between plasma TFPI antigen levels and activity. **Based on the data presented in the CRL response, including indirect evidence, TFPI activity remains inhibited nearly fully for at least 24 hours post recommended bolus plus a 2-hour infusion. TFPI activity can return to normal sometime after 48 hours, or possibly later.** Regarding the reasons why the TFPI data were not provided, Portola stated that this is due to a delay in receiving the back-ordered commercial kits to measure free TFPI ((b) (4)) and TFPI activity ((b) (4)). The (b) (4) has been back-ordered since late November 2016 due to the failed QC releases for multiple lots. While Portola Quality group is working with (b) (4) to resolve the supply issue, there is a continued delay for release (next batch expected September 2017) and possible discontinuation of the product. Portola is in the process of finding a replacement for the free TFPI kit, and will perform additional assay validation should the (b) (4) kit be discontinued. B(b) (4) is now supplying the TFPI activity kit (previously from (b) (4)). Portola has been notified of multiple delays (since early June, 2017) due to failure in one of the in-process

[21] Source: BLA 125586.0/ SN0039 dated 15 June 2016. Portola's scientific rationale is explained as follows: "*Based on the fact that the* (b) (4) *binds to the same domain on TFPI that interacts with fXa (and andexanet), we reason that the levels of "free" TFPI determined using this assay are equivalent to TFPI "activity", as it is the full-length, TFPI-α form that provides the majority of activity, as defined by inhibition of fXa. This was confirmed in the Phase 1 study (11-501) in the 600 mg cohort where both "free" TFPI and TFPI activity were measured and found to be decreased in parallel.*"

[22] "*Because the TFPI activity assay is based on measuring fXa* (b) (4) *activity following fX activation by VIIa/TF, it is not possible to utilize this assay beyond the Phase 1 study because of interference due to the presence of fXa inhibitor in the clinical samples.*" Source: BLA 125586.0/ SN0039 dated 15 June 2016

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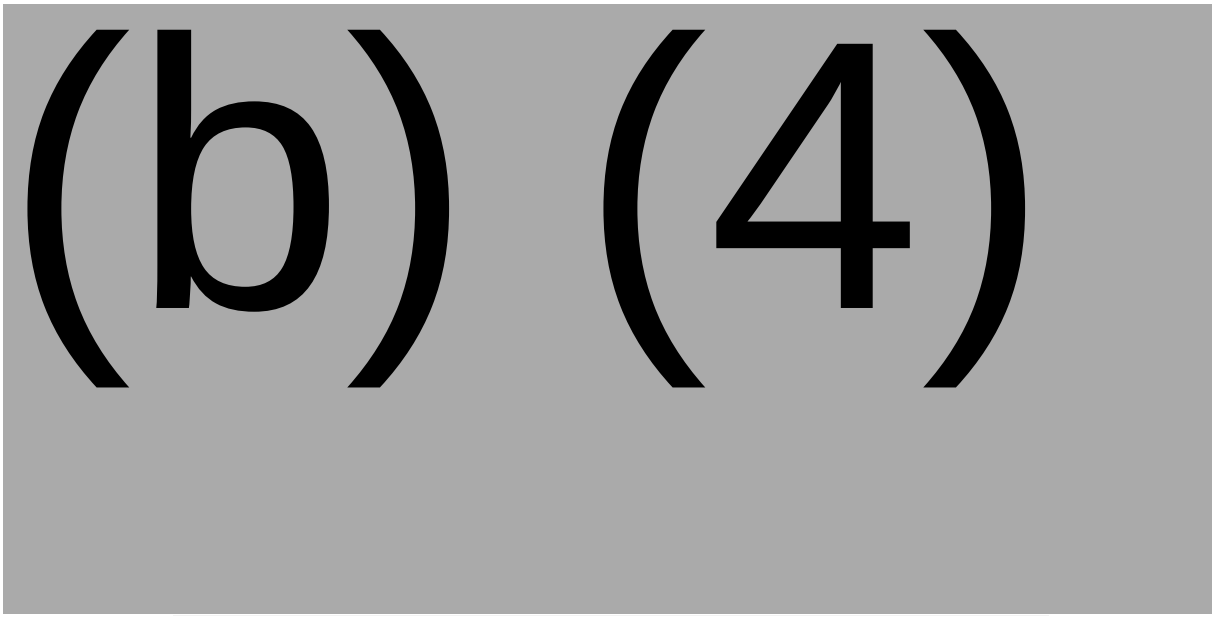
	tests for the kit release. Portola stated that they will need to resolve the supply issues for these specialty test kits should one or both kits be discontinued or further delayed, and they are currently researching potential alternatives.  I should note that Portola's inability to conduct TFPI activity testing is surprising and not consistent with their ability to develop and validate an anti-TFPI activity assay for the release of ANDEXXA product and in stability studies, reported in the CMC section of the CRL response. TFPI activity testing does not appear burdensome or unique. Use of TFPI activity assays for the analysis of clinical samples is reported widely in recent scientific literature, including for studies of anti-TFPI activity agents currently in clinical development for the prevention of bleeding in hemophilia[23,24,25,26]. Because Portola failed to test the TFPI activity in Phase 2 and 3 studies, and failed to properly qualify the use of TFPI antigen assays for estimation of TFPI activity, the question of the depth and duration of TFPI activity inhibition after recommended doses remains an issue that is insufficiently investigated. I therefore propose the following to be included in the labeling section 12.2. "*Inhibition of TFPI activity in plasma has been sustained for at least 22 hours following ANDEXXA administration. The time from decrease following ANDEXXA treatment to increase to pre-ANDEXXA levels of TFPI activity was not determined*."

[23] Peterson et al. Targeting TFPI for hemophilia treatment. *Thromb Res*. 2016 May;141 Suppl 2:S28-30.
[24] Gu et al. Mechanistic Modeling of the Pharmacodynamic and Pharmacokinetic Relationship of Tissue Factor Pathway Inhibitor-Neutralizing Antibody (BAY 1093884) in Cynomolgus Monkeys. *AAPS J*. 2017 Jul;19(4):1186-1195.
[25] Waters et al. Concizumab, an anti-tissue factor pathway inhibitor antibody, induces increased thrombin generation in plasma from haemophilia patients and healthy subjects measured by the thrombin generation assay. *Haemophilia*. 2017 Sep;23(5):769-776.
[26] Parng et al. Translational Pharmacokinetic/Pharmacodynamic Characterization and Target-Mediated Drug Disposition Modeling of an Anti-Tissue Factor Pathway Inhibitor Antibody, PF-06741086. *J Pharm Sci*. 2018 Mar 20. pii: S0022-3549(18)30150-3.

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	Portola made another commitment to use the TFPI activity assays in all studies going forward. Importantly. Portola committed to investigate the time-course of TFPI activity inhibition in the healthy volunteer PK/PD study, which was designed to compare the commercial ANDEXXA product with the GEN 2 material. When these new TFPI activity data become available, Portola should update the product labeling to more accurately explain the effect of ANDEXXA on TFPI activity.
15b (vi) Q 1.c. xi	***Question 1.c.xi, from the 01 June 2016 RFI:*** ***With reference to preclinical Study # NC-12-0439-R0001, please explain your conclusion that the absence of increase in the TAT and F1.2 levels in andexanet-treated whole blood samples demonstrates a lack of andexanet thrombogenicity. Since TF had no effect on coagulation in whole blood in the absence of andexanet, this suggest that whole blood was activated by the contact pathway, possibly by red blood cells surfaces, making the assay unsuitable to study the anti-TFPI action of andexanet. Please study andexanet procoagulant activity using TF-dependent blood coagulation which may be obtained by using*** (b) (4) ***, which inhibits contact activation, and the appropriate amount of TF.***
	Review of Portola's Response to CRL Item No. 15b (vi), Question 1.c.xi: Portola re-did the TAT and F1.2 experiments in whole blood and plasma using the TF-dependent blood coagulation in the presence of (b) (4) . As I was expecting, their previous result, that ANDEXXA does not increase TAT and F1.2, was confirmed to be an experimental artifact. However, I do not agree with the following Portola's new conclusion because it misleadingly downplays the potentially thrombogenic action of ANDEXXA: "*These results indicate that AnXa has no procoagulant activity on its own, as expected, due to the active site mutation. However, AnXa-TFPI interaction may contribute to increased thrombin formation, as reflected by increased F1+2 and TAT levels observed in the current study, when the reactions are initiated with TF either in whole blood or plasma*". The first part of this conclusion, that ANDEXXA "*has no procoagulant activity on its own*", is in contradiction with the revised results of the study described in the second part of the same conclusion, that ANDEXXA activity is "*reflected by increased F1+2 and TAT levels*". I conclude that the new results of the correctly performed *in vitro* study are in excellent agreement with the elevation of thrombogenicity markers in monkeys and in healthy volunteer studies. These elevations were observed both in the presence and absence of FXa inhibitors, confirming that ANDEXXA has procoagulant activity "*on its own*".
15b (vi) Q 1.c. xii	***Question 1.c.xii, from the 01 June 2016 RFI:*** ***With reference to the preclinical investigation of TFPI inhibition on endothelial cells presented in Study #NC-15-0662-R0001, please explain your conclusion that rivaroxaban blocks the interaction of TFPI and andexanet. Figure 8 demonstrates that in the presented purified system in the absence of plasma proteins,*** (b) (4) ***rivaroxaban contributes to less than a*** (b) (4) ***decrease in andexanet binding to TFPI on endothelial cells, suggesting that***

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	rivaroxaban may provide no protection from TFPI inhibition 6-12 hours after rivaroxaban dose or in the presence of plasma proteins. ***Please investigate the effect of anticoagulant concentration for each of the inhibitors (***(b) (4)***, rivaroxaban, edoxaban, and apixaban) on andexanet binding to TFPI expressed on endothelial cells in the presence and absence of plasma proteins, and submit the results to the BLA.***
	Review of Portola's Response to CRL Item No. 15b (vi), Question 1.c.xii: This request was made because Portola repeatedly used the results from the endothelial cell study to suggest that the TFPI inhibition by ANDEXXA is negligible. Two points were made, incorrectly: 1. Regarding the ANDEXXA binding to TFPI on endothelial cells in a system of purified proteins, Portola claimed that FXa inhibitors "block" this effect, and therefore TFPI inhibition will be negligible in the target population of FXa inhibitor-treated patients. I disagreed with this conclusion because complete "blocking" was only seen at an unreasonably high level of rivaroxaban. 2. Regarding the TFPI inhibition found in systems of purified proteins in general, Portola claimed that such effect is artificially high compared to experiments in plasma or whole blood (see, e.g., [27]). This statement was made without the evidence from direct side-by-side studies. (b) (4) Per the FDA request, the endothelial cell experiments were repeated in the absence and presence of human plasma proteins with the direct FXa inhibitors (b) (4), rivaroxaban,

[27] NC-12-0450-R0001: Interaction of PRT064445 with TFPI Page 15 of 23: "*In summary, PRT064445 has the ability to bind TFPI in purified systems. However, the activity is substantially reduced when tested in systems which are more complex and require multi-component enzyme cofactor assembly. Moreover, we have not detected measurable effect of the interaction of PRT064445 with TFPI in global assays of the coagulation cascade or in animal models.*"

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	edoxaban, and apixaban. The inhibitors differed in their ability to interact with the ANDEXXA binding to endothelial cells. However, the effect of anticoagulants on ANDEXXA binding was similar in the presence of plasma protein compared to buffer control. Therefore, the systems of purified proteins appear suitable for the evaluation of TFPI inhibition by ANDEXXA. Portola also repeated the studies with FXa inhibitors to confirm that they neutralize ANDEXXA binding to endothelial cell surface (Fig. 15). The IC50 for rivaroxaban was (b) (4), or about (b) (4), confirming my previous conclusion that FXa inhibitors are not able to block TFPI inhibition for 12 hours post ANDEXXA dose, and later.

6. Outstanding non-CMC substantive regulatory issues

Several substantive regulatory issues, that are not directly related to CMC, were covered by my review and are described in this section. These regulatory issues are related to ANDEXXA's mechanisms of action, bioanalytical assays, and comparability studies; and they are related to the safe and effective use of the product.

As an expert in hemostasis, a product reviewer, and the chairperson of the review committee, I have reviewed the studies that are needed to ensure the safe and effective use of ANDEXXA, which are discussed below. The scientific issues that are not directly related to product safety and efficacy are not included in this section.

6.1. Anti-FXa activity reversal may not be reasonably likely to predict clinical benefit

During the review of the ANDEXXA IND and BLA, FDA has repeatedly questioned the evidence used to support the notion that reversal of anti-FXa activity is predictive of clinical benefit. The history of the issues related to the anti-FXa assay is described in the 12 August 2016 Clinical Review memo of Dr. Lisa Faulcon[28], and my Final CMC review memo dated 17 August 2016 [29].

To address these questions during the BLA review, it was determined that Portola would submit clinical data derived from the subjects experiencing acute major bleed who received ANDEXXA in the ongoing Phase 3b/4 study entitled "Prospective, Open-Label Study of Andexanet Alfa In Patients Receiving A Factor Xa Inhibitor Who Have Acute Major Bleeding" (14-505, ANNEXA 4). The ANNEXA 4 study was initiated to meet a requirement for the *Accelerated Approval* pathway,

[28] Pages 23-28 of Clinical Review memorandum dated 12 August 2016 by Dr. Lisa M. Faulcon.
[29] CMC review memo dated 17 August 2016. Section *7. Suitability of the proposed biomarker as a surrogate endpoint for clinical benefit*

to confirm that reversal of anti-FXa activity by ANDEXXA is associated with clinical benefit [30]. Upon review of these data, the clinical reviewers concluded that anti-FXa reversal may not be predictive of clinical benefit:

- During the first BLA review cycle, Dr. Lisa Faulcon evaluated the data from 35 subjects in the ongoing ANNEXA 4 study, and noted that "*Adjudication of hemostatic efficacy as successful (i.e. rating of excellent or good) despite nadir anti-FXa activity that remained within the therapeutic (anticoagulated) range following andexanet administration questions the adequacy of anti-FXa activity as a surrogate marker likely to predict clinical outcomes. Preliminary data show that the depth of reversal is not as robust in patients presenting with supratherapeutic anti-FXa levels, which could result in continued bleeding or evidence of re-bleeding*."

  Dr. Faulcon had since left CBER, and Dr. Bindu George replaced her as the clinical reviewer for this file.

- Additional data from the confirmatory study were submitted in Portola's response to the CRL. In her clinical review [31], Dr. Bindu George noted "*The lack of correlation between change from baseline anti-fXa activity (surrogate endpoint) and hemostatic response in the 185 subjects from the ANNEXA 4 study.*"

The poor correlation between Portola's surrogate endpoint and clinical outcomes observed in patients who are treated with FXa inhibitors, in the ongoing confirmatory trial confirms the FDA long-standing doubts about the contribution of ANDEXXA's sequestration of FXa inhibitors to controlling bleeding in this patient population. **Although it is logical to assume that a *full and sustained reversal* can be beneficial in the control of bleeding, the reversal provided by ANDEXXA is short, inconsistent, and may not be deep enough to explain the hemostasis observed in the confirmatory study.** To this end, the discrepancy between the surrogate endpoint and hemostatic efficacy in the ANNEXA 4 study should not be discounted as outliers that in some bleeding cases hemostasis cannot be restored even when the anti-FXa activity is reversed by ANDEXXA, nor should this be viewed as an artifact of analytical variability. Rather, we should question the relevance of the MOA that is represented by the surrogate endpoint assay because

[30] ANNEXA 4 **Primary Objectives**: 1. The percent change from baseline in anti-FXa activity to the nadir from the evaluation period (where the evaluation period starts 5 minutes following the end of the ANDEXXA bolus and ends just prior to the end of the ANDEXXA infusion) 2. The achievement of hemostatic efficacy of stopping an ongoing major bleed at 12 hours from the end of the ANDEXXA infusion. **Secondary Efficacy Objective:** To assess the relationship between decrease in anti-FXa activity and the achievement of hemostatic efficacy in patients receiving FXa inhibitors who have acute major bleeding and reduced FXa activity. **Exploratory Objectives:** • For patients receiving apixaban or rivaroxaban, to evaluate the decrease in the free fraction of the FXa inhibitor following ANDEXXA treatment. • To evaluate the use of red blood cells transfusions. • To evaluate the use of other blood products and hemostatic agents. **Safety Objectives:** • To evaluate the overall safety of ANDEXXA, including adjudicated TEs and antibodies to FX, FXa, and ANDEXXA. • To evaluate the 30-day all-cause mortality. *Source: Dr. Lisa Faulcon's 12 August 2016 memorandum.*

[31] BLA 125586/0 Clinical Review Memo dated 22 April 2018 by Dr. Bindu George, MD.

we see that hemostasis can be achieved without ANDEXXA, or even *despite* ANDEXXA if a rebound of inhibitor activity is taken into consideration.

According to the 2014 Guidance on Expedited Programs for Serious Conditions[32], FDA must review the evidence provided in the BLA that a proposed surrogate endpoint is reasonably likely to predict the intended clinical benefit of a drug. I, therefore, conclude that the *Accelerated Approval* pathway should not be used here because the proposed surrogate endpoint is not reasonably likely to predict clinical benefit when ANDEXXA is used in the intended patient population.

6.2. Unknown duration of potentially thrombogenic inhibition of TFPI

As stated in the 2014 guidance[33] for industry for *Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products*, "*[t]he reasons for the absence of an expected correlation between pharmacologic and clinical effects are diverse and can include an incompletely understood relationship between the pharmacologic effect and the clinical benefit and the presence of other pharmacologic effects attributable to a drug in addition to the effect being measured and thought to be beneficial.*" In the case of ANDEXXA, the second mechanism of action, inhibition of TFPI, was contributing to the pharmacologic procoagulant activity observed in the clinical studies. However, Portola did not measure TFPI activity in patients treated with FXa inhibitor. The data presented during the first review cycle was obtained with TFPI assays that were not properly validated. Furthermore, ANDEXXA's effect on TFPI activity was not adequately discussed in Portola's clinical study reports and published manuscripts.

The data submitted in Portola's CRL response provide conclusive pharmacologic evidence that the sustained elevation of thrombin generation in healthy volunteers was mediated by TFPI inhibition, not by sequestration of FXa inhibitor[34]. However, the contribution of TFPI inhibition to the *clinical efficacy* of ANDEXXA in patients taking a FXa inhibitor has yet to be investigated. Portola did not develop or investigate the pharmacologic endpoint of TFPI inhibition in any of the studies. Of note, Portola cited animal studies as evidence of potential negligible contribution of TFPI inhibition. However, the proposed animal models are not relevant to the human system because

[32] 2014 Guidance on Expedited Programs for Serious Conditions. https://www.fda.gov/downloads/Drugs/Guidances/UCM358301.pdf

[33] Quote from the FDA Guidance for Industry Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products May 1998: "*Note, however, that plausible beneficial pharmacologic effects have often not correlated with clinical benefit, and, therefore, caution must be observed in relying on a pharmacologic effect as contributing to evidence of effectiveness. For example, pharmacologic effects such as arrhythmia suppression by Type 1 antiarrhythmics and increased cardiac output by phosphodiesterase inhibitors or beta adrenergic inotropes resulted in increased mortality, rather than, as was expected, decreased sudden death and improved outcome in heart failure. The reasons for the absence of an expected correlation between pharmacologic and clinical effects are diverse and can include an incompletely understood relationship between the pharmacologic effect and the clinical benefit and the presence of other pharmacologic effects attributable to a drug in addition to the effect being measured and thought to be beneficial. Generally, the utility of pharmacologic outcomes in providing independent substantiation will be greatest where there is prior experience with the pharmacologic class. Even in this case, however, it is difficult to be certain that a pharmacologic effect that correlates with a clinical benefit accounts for all the clinical benefit or that other effects are not present and relevant.*"

[34] Portola's Responses to CRL Items No. 14 & 15

ANDEXXA, which is based on a human FXa sequence, interacts differently with animal TFPI, TF, and FVIIa in plasma and endothelial cells, as evidenced from the monkey studies in which TAT and D-dimer elevation were lower than that in the human studies, although the doses in monkeys were higher than those in human.

More importantly, the evidence that TFPI inhibition by ANDEXXA can be associated with an increased risk of thrombosis should not be ignored. Because TFPI inhibition by ANDEXXA elevates the markers of thrombogenicity, and this effect is not as transient as anti-FXa activity reversal, it is necessary for the treaters to be aware of the duration and magnitude of both mechanisms of action of ANDEXXA. Despite their commitment to do so, Portola did not provide the response to CRL Item 15b (vi) Q 1.b. iv, i.e.,

> *Determine the TFPI activity in retained samples from Phase 1, 2 and 3 healthy volunteer studies. Please include enough data points to describe the effect of andexanet dose (bolus and bolus plus infusion) on the timing of the changes in TFPI activity in anticoagulated and non-anticoagulated subjects. Specifically, please determine the time of TFPI activity return to either the pre-andexanet treatment baseline or the normal range.*

Portola explained that the testing was not done because the three TFPI kits were back-ordered by the manufacturers.

The questions of what the depth and duration of TFPI activity inhibition can be after the recommended dose of ANDEXXA remains unanswered, nor is the effect of TFPI inhibition on patient safety addressed. In the absence of data and based on our current understanding of the role of these proteins in hemostasis, I recommend that the potential risks associated with a sustained inhibition of TFPI activity be included in the highlight and full prescribing information sections of the product label.

6.3. Unexplained failure of Thrombin Generation biomarker in bleeding patients

Anti-FXa activity is not a marker of hemostasis, but a measure of the concentration of the FXa inhibitor in blood[35], therefore it is important to show the effect of ANDEXXA on blood coagulation by at least one hemostasis assay. Although Portola used several hemostasis assays in the studies[36], it chose only one hemostasis assay, TG, to illustrate the correlation between the improvement of hemostasis markers and anti-FXa reversal by ANDEXXA. The TG data from the healthy volunteer studies are presented in the BLA, draft product labeling, public presentations and published manuscripts. For example, Portola's proposal for draft labeling section 12.2 Pharmacodynamics states "*The effects of ANDEXXA can be measured through pharmacodynamic*

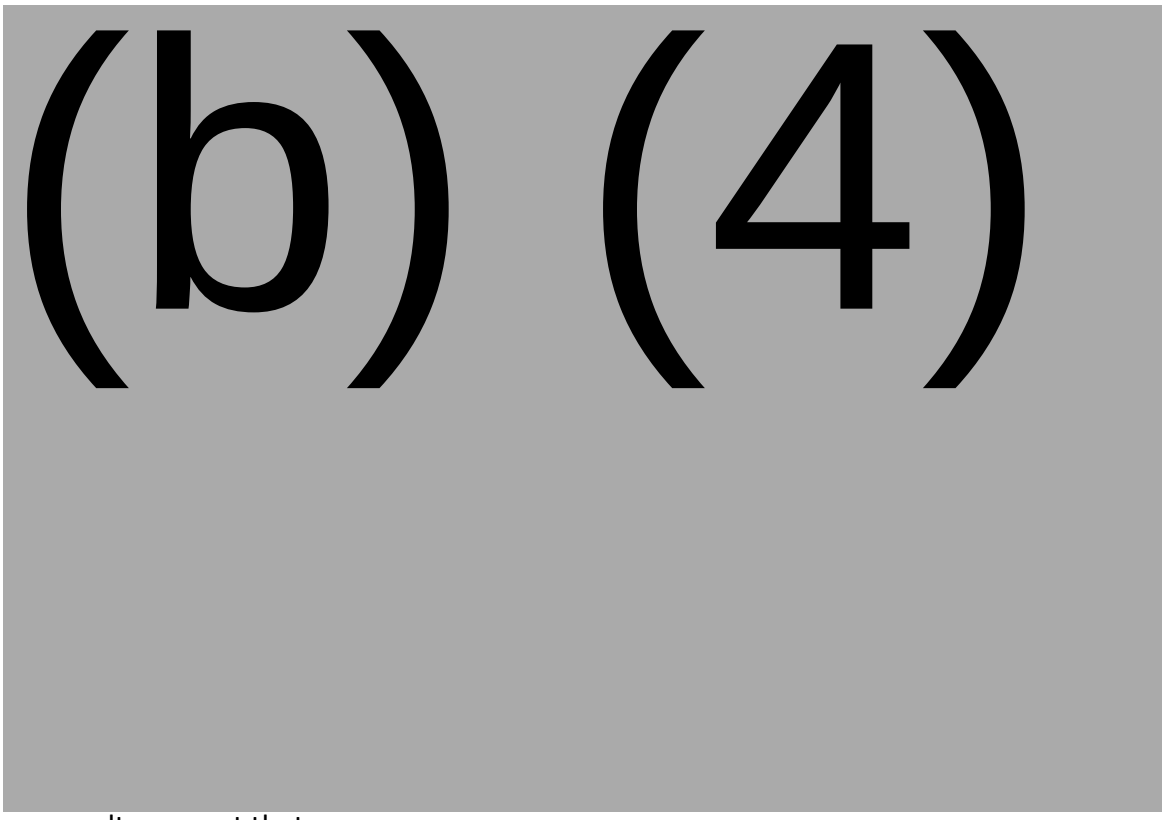
[35] See my Final CMC Memo dated 17 August 2016 Section 7.3. The relevance of anti-FXa activity to pharmacodynamics of ANDEXXA

[36] E.g., see my Final CMC Memo dated 17 August 2016, Figure 26: Effect of ANDEXXA administration on PD assays used in the clinical trials.

markers, including anti-FXa activity, free fraction of available FXa inhibitor, and through recovery of thrombin generation."

However, **all** TG results presented by Portola so far have been derived from the healthy volunteer studies. Portola submitted, but did not analyze, the TG data from the ANNEXA 4 confirmatory trial with patients who are treated with FXa inhibitors, see Figure 16 and Appendix D below. My preliminary review of these data indicated significant differences in the TG results between healthy volunteers and bleeding patients who are taking FXa inhibitors, i.e., the intended patient population. Specifically, while there appears to be a strong correlation between anti-FXa reversal by ANDEXXA and TG elevation in healthy volunteers, it does not appear so in bleeding patients who are taking FXa inhibitors. In these bleeding patients,

1. The pre-ANDEXXA TG level is rarely below the normal range[37], and is often above the normal range.
2. 7 to 16 hours post-ANDEXXA, the TG level is often not elevated[38], or elevated insignificantly,
3. Similar TG level is seen several days after ANDEXXA administration.



These results suggest that:

1. Either the TG assay used in the study is not suitable for the evaluation of hemostasis in bleeding patients, or

[37] For example, of the first 37 patients for whom the graphical TG data were presented in the BLA (presented in Appendix D), 76% of patients had normal or above normal TG before ANDEXXA.
[38] Of these 37 patients, TG was elevated above 10% in 59% of patients.

2. Hemostasis inhibition by FXa inhibitors is *not* significant in the patients who bleed, and ANDEXXA has no detectable procoagulant effect on these patients.

Based on these observations, I conclude that the use of TG assay to monitor ANDEXXA response should not be recommended in the label. Likewise, the presentation of TG data in the label and promotional materials should be carefully reviewed, and revised to instruct the treaters of the limitations of using a hemostasis assay, such as TG assay, to assess the pharmacologic effect of ANDEXXA in bleeding patients. Finally, I recommend that Portola commits to publishing the TG data from the ANNEXA 4 study to better inform the stakeholders on the risks and benefits of ANDEXXA in the intended patient population.

6.4. Use of non-comparable GEN 2 material may compromise ongoing confirmatory trials

In March 2018, Portola stated that approximately 80 patients in the ANNEXA 4 study have been treated with the GEN 2 product. FDA had previously advised Portola against using the GEN 2 material in the ANNEXA 4 confirmatory study unless and until an agreed-upon PK/PD study can demonstrate comparability between the commercial ANDEXXA product and the GEN 2 product. FDA had stated that results of the human PK/PD study are needed to establish comparability before the FDA could agree on accepting the data from the ANNEXA 4 study generated using the GEN 2 material to support the *Accelerated Approval* pathway. As of 23 April 2018, the PK/PD study results have yet to be presented to the FDA for review while this study should have been completed in October 2017.

FDA's concerns about the use of the GEN 2 material are based on the assessment that the proposed changes in the manufacture process for the (b) (4) FDP are major, and constitute a substantially modified process that produces a material that is not comparable to the commercial ANDEXXA product in analytical studies. As such, the safety of the GEN 2 material is unknown. Based on the available evidence, FDA had recommended Portola to consider the GEN 2 material as a new product.

The following manufacturing and analytical evidence demonstrate the differences between the commercial ANDEXXA and GEN 2 products:

1. The manufacturing changes introduced in the GEN 2 process can be classified as major because they are likely to have an impact on product quality, safety and efficacy. The following GEN 2 changes are the most significant:
    a. (b) (4)

(b) (4)

2. Results of characterization studies demonstrated differences in ANDEXXA purity and quality. For example, while no new product-related substance was found in the GEN 2 material, the distribution of the existing protein (b) (4) has changed substantially. The changes in GEN 2 vs. commercial ANDEXXA materials are evidenced from the following analytical studies:

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Regarding the ongoing ANNEXA 4 study proposed by Portola as a confirmatory study to meet the requirements under the *Accelerated Approval* pathway, I see two types of risks associated with the use of the GEN 2 material in it:

1. Depending on the outcomes of the human PK/PD comparability study of the existing commercial ANDEXXA and GEN 2 products, the sample size for the ANNEXA 4 study may need to be adjusted. Indeed, FDA has already explained that the review of the PK/PD study results is needed before FDA could determine the acceptability of the GEN 2-based ANNEXA 4 study data as a confirmatory study under the *Accelerated Approval* pathway. As of 23 April 2018, Portola has not provided the requested PK/PD study results to address the issue of acceptability of the use of the GEN 2 product in ANNEXA 4 as the confirmatory study to support *Accelerated Approval*.

2. The PK/PD profile of the GEN 2 material remains unknown, exposing the patients to additional risk. Although Portola is obligated to promptly report all serious adverse events, we are relying on Portola's due diligence in reporting and investigating them. However, past experience indicates that Portola does not always follow FDA recommendations, and could disregard the agreements reached with the FDA.

6.5. Inaccurate promotion and non-proprietary name may result in unnecessary and unsafe use

An accurate description of the *Mechanisms of Action* (MOA) is needed to ensure that ANDEXXA, if approved, will be marketed truthfully, and used safely and effectively. According to the promotional materials submitted in the amendment dated 18 July 2016, Portola is planning to promote ANDEXXA as a therapy that has **coagulation activity** (as evidenced by an increase in thrombin generation), but has **no thrombogenic activity** of its own (because elevation of thrombin generation was within the normal range for this assay; the statement about the lack of inherent procoagulant activity was included in the promotional slides in 2016[41]). These statements are not representative of the MOA of this product as described above in this section.

My concerns about inaccurate advertisement for ANDEXXA are substantiated by the Portola letter we received on 2 April 2018 [42]. In this letter, Portola states that *"The MOA for andexanet is to bind and sequester FXa inhibitors, period. It has no pro or anticoagulant activity on its own, like a coagulation factor would have."* This statement is incorrect because it ignores the evidence that

[41] See my review dated 17 August 2016. Figure 25: Comparison of experimental data with promotional claims submitted in the amendment dated 18 July 2016

[42] 125586 / SN0118 Response to 15 February 2018 BLA Resubmission Information Request dated 30 March 2018

ANDEXXA has a direct procoagulant MOA, inhibition of TFPI. This MOA was not fully acknowledged in the original BLA, as documented in several CRL items. In Portola's CRL response, Portola provided the missing data, and correct interpretations, and at the request of FDA, Portola acknowledged the existence of a procoagulant MOA of ANDEXXA in the revised clinical study reports and draft labeling. However, Portola appears to be stepping back from their acknowledgement of the involvement of TFPI inhibition in ANDEXXA action in the 2 April 2018 letter, probably because it is incongruent with its intent to promote ANDEXXA as simply a safe and effective antidote to FXa inhibitors.

Portola's intention to advertise ANDEXXA as a non-procoagulant antidote is further illustrated by its disagreement with the FDA on the FDA-assigned proper name, *coagulation factor Xa (recombinant), inactivated.* Instead, it wants to use the adopted USAN name, *andexanet alfa*, as the proper name of its product. The FDA-assigned proper name clearly describes ANDEXXA as a molecule derived from a coagulation factor.

FDA determined that it is scientifically valid and clinically meaningful to put ANDEXXA in the established pharmacologic class of "coagulation factors". The "coagulation factor" assignment is supported by documented and submitted empiric evidence that connects ANDEXXA's structure, function, and MOAs to its proposed indication. The *coagulation factor Xa (recombinant), inactivated* pharmacologic classification is clinically meaningful because it enhances the ability of clinicians to understand the physiologic effects of the product, and to anticipate undesirable effects associated with this product, and others in this pharmacologic class. These effects can either be a lack of efficacy due to under-dosing, or thrombogenicity due to over-dosing.

Our advice is in accordance with the following recommendation from the *FDA Guidance for Industry and Review Staff: Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information*[43]:

> **"A *clinically meaningful* pharmacologic class term or phrase enhances the ability of professionals to understand physiologic effects related to the indication or to anticipate undesirable effects that may be associated with the drug or pharmacologic class"**

In my view, it is important for stakeholders to appreciate the risk of thrombosis. Putting ANDEXXA in a **pharmacologic class** as "antidote" or "reversal agent" will not highlight the thrombotic risk because these terms imply that ANDEXXA has no procoagulant effect on the coagulation cascade (apart from short-term removal of the FXa inhibitor, as would be expected of a FXa inhibitor antidote), and that overdosing carries no additional risk of thrombosis. The risks of thrombosis arise from the action of coagulation FXa on the coagulation cascade via sustained inhibition of TFPI. Overdosing will increase the risk. In the real-world setting, the urge to administer repeat or higher doses, thus overdoing the patient, can be anticipated because the reversal action of FXa

[43] FDA Guidance for Industry and Review Staff: Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information Good Review Practice October 2009
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM186607.pdf

inhibitors by ANDEXXA can appear too short to be effective per some standards of life-threatening bleed treatment. However, higher, longer and repeat doses had not been investigated by Portola in part because of the risk arisen from the procoagulant action Portola had discovered in the Phase 1 dose escalation studies.

For additional discussion, please refer to Appendix A. *Review of the risks associated with the promotion of ANDEXXA as an "antidote"*, Appendix B. *Review of Portola's request to use the INN for the non-proprietary name of ANDEXXA*, and Appendix C. *Review of the risks associated with promotional materials*.

6.6. An RCT is needed to confirm favorable risk/benefit

A well-designed randomized control trial (RCT), proposed by the clinical reviewer, will be able to address the issues brought up by the new findings from the ANNEXA 4 study. I should note that the recommendation for an RCT is not a new demand of the FDA but a re-affirmation of a long-standing FDA position that such study is needed. The recommendation is supported by the new concerns arisen from the lack of correlation between anti-FXa reversal and hemostasis observed in the intended patient population in the ANNEXA 4 study, and increased risk of thrombosis. With all that is known now, I request our management to seriously consider the consequences and ramifications of the regulatory action of this BLA. It should also be noted that the prospect of Portola agreeing to conducting an RCT will be greatly diminished if the FDA approves the BLA now.

6.7. Outstanding regulatory issues: Conclusion

My most serious concerns regarding an ANDEXXA approval are the safety risks to patients who may receive ANDEXXA despite the lack of evidence that ANDEXXA is more effective than the available usual care or standard of care. I agree with the clinical reviewer that a pre-licensure RCT is needed to establish the clinical benefit of ANDEXXA. Even if FDA approves ANDEXXA with limited indications, i.e., for reversal of rivaroxaban and apixaban in ICH, we can expect wide-spread off-label uses of the product. In an effort to control bleeding, the product may be used in patients having non-life-threatening bleeding episodes, patients with low starting levels of anti-FXa activity (in the ANNEXA 4 study, 20% of the treated patients had FXa inhibitor levels below 75 ng/mL[44,45]), or patients on FXa inhibitors other than rivaroxaban and apixaban. In these patients, the use of ANDEXXA could put them in undue risk of thrombosis.

[44] Clinical review memorandum dated 12 August 2016: "*… Furthermore, in the absence of a companion diagnostic to obtain anti-FXa activity in real time it is very likely that if Andexxa is approved patients with anti-FXa activity levels <75 ng/mL will be treated. In fact, 8/35 (23%) of the subjects treated in the confirmatory study had anti-FXa activity levels <75 ng/mL. Therefore the efficacy of the product need to be established for the whole clinical trial population in order to allow for generalizability of these data to the target population.*"

[45] Clinical review memorandum dated April 2018: "*In theory, for every 200 patients who receive the product based on these clinical parameters, 46 are likely to have received the product even though the anti-fXa levels were less than 75ng/mL.*"

At the very least, the concerns about the potential risks should be adequately and prominently communicated in the ANDEXXA labeling and promotional materials.

7. Chemistry, Manufacturing and Controls - Conclusion

The responses to CMC deficiencies in the CRL are acceptable because Portola has addressed the observed (b) (4) degradation of the product, which turned out to be the root cause behind the issues related to manufacturing robustness and product quality:

- Portola confirmed that the stability of process intermediates was indeed affected by (b) (4) degradation by (b) (4) found in the (b) (4) impurities. The increased degradation in the commercial (b) (4) was observed due to faster (b) (4) at (b) (4) at the (b) (4) step and longer hold times.

- In the CRL response, Portola identified the (b) (4) in these (b) (4) impurities, demonstrated the clearance of these impurities in the existing manufacturing process, revised the process control strategy to address the (b) (4) degradation, and demonstrated that the revised process is validated and robust.

- Finally, Portola demonstrated that partially cleaved ANDEXXA variants are fully functional and well controlled by the revised lot release assays and release specification limits.

It should be noted that Portola's original approach to the design of ANDEXXA process and its control strategy were generally consistent with ICH and FDA guidance documents. As it was expected of a product developed under accelerated clinical programs for unmet medical needs, and in prior agreement with the FDA, many of the process development activities were not finalized at the time of BLA submission because the manufacturing process development was lagging behind the clinical program. Moreover, Portola had not always followed GMP procedures when dealing with these issues. Specifically, a (b) (4) batch showing the (b) (4) (b) (4) was not released by the (b) (4) contract manufacturer, but Portola shipped this batch to the FDP contract manufacturer where this batch was (b) (4) with compliant (b) (4) batches to make the FDP PPQ batch. **A combination of an insufficiently validated process that had produced a potentially unstable product, and lapses in GMP compliance were the reasons why Portola was unable to demonstrate its ability to manufacture a safe and effective product. This was the basis for the issuance of the CRL at the first review cycle.**

In the CRL responses, Portola presented evidence that the ANDEXXA process is well controlled, and the ANDEXXA FDP and its intermediates are sufficiently stable. The investigations were thorough and adequate. In addition, Portola acknowledged that deviations from GMP requirements had occurred, and they had responded by introducing the necessary corrective and protective actions.

I, therefore, found the CMC information adequate to support the claims of quality, identity, purity, and potency of ANDEXXA.

The deficiencies in bioanalytical method validations and mechanism of action studies were also addressed in a series of new validation and bridging studies. As a result, the product immunogenicity was re-investigated by appropriate assays, and the procoagulant mechanism of ANDEXXA action, i.e., inhibition of TFPI activity, was correctly described.

My remaining concerns are related to the potentially unsafe use of the product, if it is granted *Accelerated Approval*:

- *Accelerated Approval* would be based on the surrogate marker, anti-FXa activity reversal, which may not be suitable to reasonably likely predict the clinical benefit of ANDEXXA. It is therefore not certain that the target population can benefit from replacing the current usual standards of care for the ANDEXXA administered at the proposed dosage. I agree with the clinical reviewer that an appropriately designed RCT may be needed.

- Also concerning to me are the safety risks that may arise from Portola's proposal to promote the ANDEXXA as a universal and specific antidote to FXa inhibitors. The sustained TFPI inhibition and elevation of thrombogenicity markers, and insufficient reversal of FXa inhibitors other than rivaroxaban and apixaban, indicate that ANDEXXA is neither specific nor universal, nor is it an antidote that the treaters are waiting for. ANDEXXA efficacy and safety may be unfavorable if it is used off label, especially at higher, longer and repeat doses in patients with minor bleeds.

To the extent possible, these safety concerns should be addressed in labeling and promotional materials. I defer the resolution of these issues to the clinical reviewers and my management.

Conclusion:

There are no outstanding CMC issues associated with this BLA. However, I have concerns about the potentially unsafe use of this product. I defer to our management to develop a strategy that can mitigate these risks, if the product is to be approved.

Appendix A. Review of risks associated with promotion of ANDEXXA as "antidote"

A *pharmacologic class* is a group of drugs that share scientifically documented properties [46]. FDA guidance defines pharmacologic class on the basis of any one of the following three attributes of the drug:

1. Mechanism of action (MOA)— Pharmacologic action at the receptor, membrane, or tissue level
2. Physiologic effect (PE) — Pharmacologic effect at the organ, system, or whole body level
3. Chemical structure (CS)

An *established pharmacologic class* is represented by a term or phrase that is scientifically valid and clinically meaningful according to the following definitions:

- A scientifically valid pharmacologic class is supported by documented and submitted empiric evidence showing that the drug's pharmacologic class is known, not theoretical, and relevant and specific to the indication.
- A clinically meaningful pharmacologic class term or phrase **enhances the ability of professionals to understand physiologic effects related to the indication or to anticipate undesirable effects that may be associated with the drug or pharmacologic class**.

Based on the MOAs, ANDEXXA can be classified as ***either*** a "coagulation factor concentrate" **or** an "anticoagulant reversal agent". However, I strongly recommend that ANDEXXA be classified as "coagulation factor concentrate" to educate the stakeholders about the risk of thrombotic adverse events associated with ANDEXXA's MOA that is unrelated to its transient sequestration of FXa inhibitors.

My rationale:

1. **Precedent exists to place ANDEXXA in 2 classes, "anticoagulant reversal agent" & "coagulation factor concentrate".**

    - ANDEXXA's intended use, anticoagulant reversal, places it in the same category as PRAXBIND and KCENTRA. Note, however, that although KCENTRA and PRAXBIND are both used for anticoagulant reversal, only PRAXBIND is classified as "anticoagulant reversal agent" while KCENTRA is classified as "coagulation factor concentrate". ANDEXXA is:
        i. a coagulation factor concentrate, like KCENTRA;
        ii. similar to PRAXBIND's action against dabigatran in its binding and sequestration of FXa inhibitors. Note, however, that PRAXBIND binds dabigatran with higher affinity than the target of dabigatran's inhibition,

[46] Guidance for Industry and Review Staff: Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information Good Review Practice. October 2009

thrombin. ANDEXXA binds the FXa inhibitors with similar affinity as endogenous FXa, because ANDEXXA *is* FXa.

2. **Like all coagulation factor concentrates, and unlike PRAXBIND, ANDEXXA's hemostatic effect is mediated by the facilitation of coagulation cascade reactions.**

    a. Reversal of anti-FXa activity results in faster and higher rates of thrombin generation because FXa inhibitors inhibit FXa-dependent prothrombinase complex leading to reduced generation of thrombin. In contrast, dabigatran inhibits thrombin and has little effect on thrombin *generation*. Reversal of dabigatran activity has limited effect on thrombin *generation*.

    b. TFPI inhibition removes the breaks on the coagulation cascade activation, because TFPI is the only known plasma inhibitor of tissue factor (TF), the molecule responsible for initiation of blood coagulation at the site of vascular lesion.

3. **Like NOVOSEVEN and FEIBA (but not like KCENTRA), ANDEXXA has a procoagulant action that is mediated by inhibitor by-passing activity.**

    - NOVOSEVEN and FEIBA increase thrombin generation through facilitation of coagulation reactions that by-pass the action of anti-FVIII/FIX inhibitors, similar to acceleration of thrombin generation in plasma of patients with FXa inhibitors via the anti-TFPI action of ANDEXXA.
    - ANDEXXA/NOVOSEVEN/FEIBA actions are different from the action of KCENTRA and other factor concentrates which act by supplementing the deficiency of certain coagulation factors.

4. **Like NOVOSEVEN, FEIBA and KCENTRA, but unlike PRAXBIND, overdosing of ANDEXXA carries potential risks of thrombosis because higher doses increase the hemostasis potential above normal.**

5. **Classification of ANDEXXA as "anticoagulant reversal agent" can create an impression that ANDEXXA is "a specific antidote to FXa, period". Calling ANDEXXA an antidote can lead erroneously to disregarding the existence of the second, non-antidote action of ANDEXXA.**

    - Wikipedia states: "*Antidotes for anticoagulants are often referred to as reversal agents*" (https://en.wikipedia.org/wiki/Antidote). It is therefore logical to assume that **all** reversal agents are antidotes.

    - Antidote is commonly defined as "*a medicine taken or given to counteract a particular poison*". While ANDEXXA can counteract anti-FXa activity, it can also promote coagulation through the inhibition of TFPI. These two actions are independent of each other, i.e., TFPI inhibition cannot be implied from the ability of

ANDEXXA to reverse anti-FXa activity, and anti-FXa activity reversal is independent of TFPI inhibition.

6. **Underestimating the procoagulant (non-antidote) action of ANDEXXA can result in the unsafe use of the product because TFPI inhibition continues for several days after ANDEXXA administration and can be associated with thrombotic risks.**

- Underestimating the anti-TFPI action leads to underestimation of thrombotic risks associated with overdosing.
    - It is obvious that FXa inhibitor reversal by a *specific antidote* can be thrombogenic because these patients take FXa inhibitors to reduce the risk of thrombosis. However, if the user considers ANDEXXA a true antidote, then the user would think that reversal of anti-FXa activity would not push the risk above the pre-FXa inhibitor level, and even overdosing of the antidote should not increase the thrombotic risk further. But, this is not the case with ANDEXXA.
    - ANDEXXA is not specific, and its non-FXa inhibitory effect is TFPI inhibition. In the Phase 1 dose escalation study, this off-target MOA resulted in the increase of coagulation potential above normal even in healthy subjects, and higher doses increased the *duration* of this effect. The procoagulant potential of TFPI inhibition is clearly understood because TFPI is the only known plasma inhibitor of TF, the molecule responsible for the initiation of blood coagulation at the site of vascular lesion. Indeed, several investigational anti-TFPI agents have progressed into late-stage clinical trials for the prevention of spontaneous bleeding in hemophilia [47].

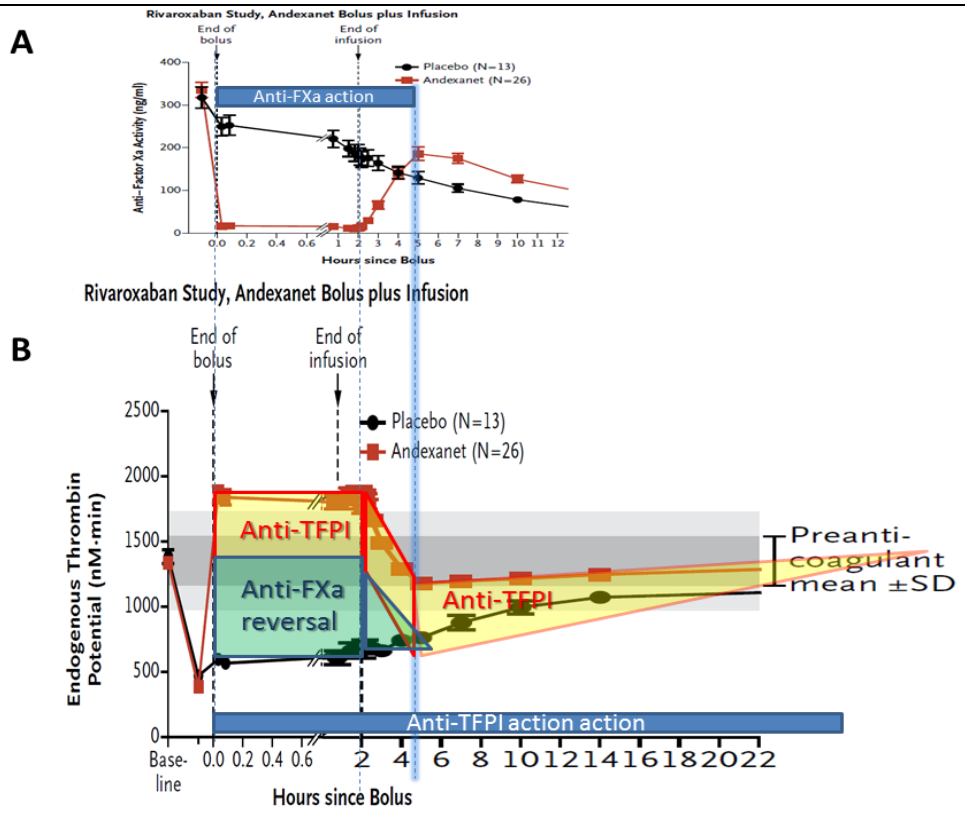
- Underestimating the anti-TFPI action leads to underestimation of the duration of the ANDEXXA action, and thrombotic risks. The anti-FXa reversal action is short, about 2 hours after the end of infusion because it requires very high doses of ANDEXXA, and is limited by the short ANDEXXA half-life. However, anti-TFPI action requires 100-1000-fold lower doses of ANDEXXA, and it continues well over several terminal half-lives. In healthy volunteers, ANDEXXA was detected out to 24-hour at low levels (i.e., <0.1 µg/mL) due to a prolonged terminal elimination phase[48]. Complete inhibition of TFPI is seen for *at least* 22 hours after ANDEXXA administration at recommended doses, and partial inhibition of TFPI continues for about 2 days or possibly longer.

- Underestimating the anti-TFPI action of the drug can facilitate off-label dosing of ANDEXXA.

[47] Peterson et al. Targeting TFPI for hemophilia treatment. *Thromb Res*. 2016 May;141 Suppl 2:S28-30.
[48] CDER's 15 July 2016 Consult Review from the Office of Clinical Pharmacology. Drs. Lars Johannesen, Jeffry Florian, Rajnikanth Madabushi, and Mehul Mehta.

- Because anti-FXa activity reversal is short, some doctors may want to go off-label to extend the anti-FXa inhibitor action of ANDEXXA by overdosing the patients, extending the duration of infusion beyond 2 hours, or administering the second dose. This off-label use may appear justified when a specific antidote is being administered because overdosing with a specific antidote would not increase the antidote action-dependent thrombogenic risks.
- ANDEXXA is not specific. An off-label use (higher dose, longer infusion and second dose) will extend the anti-TFPI action beyond those studied in clinical trials, leading to an increased risk of thrombosis.

Supplemental Figure S1 [49]: Estimates of the relative contributions of anti-FXa reversal and TFPI inhibition actions of ANDEXXA to the observed elevation of TG assay in the Phase 3 studies (FXa inhibitor: rivaroxaban, ANDEXXA: bolus plus infusion). Time-course of anti-FXa activity reversal (Panel A) and TG elevation (Panel B) are reproduced with modifications from the NEJM paper[50]. The difference between the TG assay values in ANDEXXA and placebo-treated volunteers should be a result of two actions: anti-FXa activity reversal which can bring the TG assay to the pre-treatment baseline and the TG assay which elevates the TG assay over this baseline.



[49] Source: Final CMC review memo dated 17 August 2016. Section 13.1. Appendix A: Supplemental Figures
[50] Siegal DM et al. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. *N Engl J Med*. 2015 Dec 17;373(25):2413-24

- Underestimating the anti-TFPI action may lead to unnecessary use of ANDEXXA in patients who are not likely to benefit from anti-FXa reversal. A belief in short antidote action of ANDEXXA can result in a desire to use ANDEXXA as the first line treatment to control bleeding in all patients on FXa inhibitors, regardless of bleed severity or anti-FXa level. Indeed, reversal of FXa inhibitors by a *true* antidote would add no harm, even if the benefit would be small in bleeding patients who received FXa inhibitor even long time ago. ANDEXXA is not specific. Its anti-TFPI inhibition will be observed in all patients, even those who have no anti-FXa activity in blood.

7. There are similarities between indications and mechanisms of action of ANDEXXA, PRAXBIND, NOVOSEVEN, KCENTRA, and FEIBA

Supplemental Table S1 provides comparative analysis of indications, mechanisms of action and pharmacodynamics sections in ANDEXXA, PRAXBIND, NOVOSEVEN, KCENTRA, and FEIBA labeling.

Supplemental Table S1: ANDEXXA, PRAXBIND, NOVOSEVEN, KCENTRA, and FEIBA labeling sections. Source: https://dailymed.nlm.nih.gov (April 17, 2018)

Indications
ANDEXXA[51] is coagulation factor Xa (recombinant), inactivated is a recombinant modified human Factor Xa (FXa) protein indicated for patients treated with rivaroxaban and apixaban, when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding. (1) This indication is approved under accelerated approval based on the change from baseline in anti-FXa activity in healthy volunteers. Continued approval for this indication may be contingent upon the results of ongoing patient studies to demonstrate an improvement in hemostasis. (1,14) Limitation of Use ANDEXXA is not indicated for the treatment of life-threatening or uncontrolled bleeding related to edoxaban, enoxaparin or other FXa inhibitors. (1)
PRAXBIND is indicated in patients treated with Pradaxa when reversal of the anticoagulant effects of dabigatran is needed: For emergency surgery/urgent procedures In life-threatening or uncontrolled bleeding This indication is approved under accelerated approval based on a reduction in unbound dabigatran and normalization of coagulation parameters in healthy volunteers [see Clinical Studies (14)]. Continued approval for this indication may be contingent upon the results of an ongoing cohort case series study.
NovoSeven RT, Coagulation Factor VIIa (Recombinant), is indicated for[52]: • Treatment of bleeding episodes and peri-operative management in adults and children with hemophilia A or B with inhibitors[53], congenital Factor VII (FVII) deficiency, and Glanzmann's thrombasthenia with refractoriness to platelet transfusions, with or without antibodies to platelets.

[51] Reviewer's comment: based on labeling draft dated 13 April 2018

[52] Reviewer's comment: Like ANDEXXA, NOVOSEVEN RT is *not* a replacement product

[53] Reviewer's comment: This indication resembles patients treated with rivaroxaban and apixaban

• Treatment of bleeding episodes and peri-operative management in adults with acquired hemophilia[54].

Kcentra, (Prothrombin Complex Concentrate (Human)), is a blood coagulation factor replacement product[55] indicated for the urgent reversal of acquired coagulation factor deficiency induced by Vitamin K antagonist (VKA, e.g., warfarin) therapy in adult patients with:
acute major bleeding or
need for an urgent surgery/invasive procedure.

FEIBA is an Anti-Inhibitor Coagulant Complex indicated for use in hemophilia A and B patients with inhibitors for[56]:

- Control and prevention of bleeding episodes
- Perioperative management
- Routine prophylaxis to prevent or reduce the frequency of bleeding episodes.

FEIBA is not indicated for the treatment of bleeding episodes resulting from coagulation factor deficiencies in the absence of inhibitors to coagulation factor VIII or coagulation factor IX.

12.1 Mechanism of action

Coagulation factor Xa (recombinant), inactivated is a reversal agent[57] for direct FXa inhibitors, rivaroxaban and apixaban. Coagulation factor Xa (recombinant), inactivated binds and sequesters the FXa inhibitors, reducing their anticoagulant effects. Coagulation factor Xa (recombinant), inactivated was also shown to inhibit the activity of tissue factor pathway inhibitor (TFPI) through binding of coagulation factor Xa (recombinant), inactivated to TFPI. TFPI, which inhibits TF-initiated thrombin generation, is present on the endothelium and in plasma.
Suggested addition: "TFPI inhibition can increase tissue factor-activated thrombin generation."

Idarucizumab is a specific[58] reversal agent for dabigatran. It is a humanized monoclonal antibody fragment (Fab) that binds to dabigatran and its acylglucuronide metabolites with higher affinity[59] than the binding affinity of dabigatran to thrombin, neutralizing their anticoagulant effect.

NovoSeven RT is recombinant Factor VIIa and, when complexed with tissue factor can activate coagulation Factor X to Factor Xa, as well as coagulation Factor IX to Factor IXa. Factor Xa, in complex with other factors, then converts prothrombin to thrombin, which leads to the formation of a hemostatic plug by converting fibrinogen to fibrin and thereby inducing local hemostasis[60]. This process may also occur on the surface of activated platelets.

Kcentra contains the Vitamin K-dependent coagulation Factors II (FII), VII (FVII), IX (FIX), and X (FX), together known as the Prothrombin Complex, and the antithrombotic Protein C and Protein S.
A dose-dependent acquired deficiency of the Vitamin K-dependent coagulation factors occurs during Vitamin K antagonist treatment. Vitamin K antagonists exert anticoagulant effects by blocking carboxylation of glutamic acid residues of the Vitamin K-dependent coagulation factors during hepatic synthesis, lowering both factor synthesis and function. The administration of Kcentra rapidly increases plasma levels of the Vitamin K-dependent coagulation Factors II, VII, IX, and X as well as the antithrombotic Proteins C and S.
Coagulation Factor II
Factor II (prothrombin) is converted to thrombin by activated FX (FXa) in the presence of Ca2+, FV, and phospholipids.
Coagulation Factor VII

[54] Reviewer's comment: This indication resembles patients treated with rivaroxaban and apixaban
[55] Reviewer's comment: Unlike ANDEXXA, KCENTRA is a replacement product
[56] Reviewer's comment: Like ANDEXXA, FEIBA is *not* a replacement product
[57] Reviewer's comment: I suggest replacing "reversal agent" with "modified coagulation factor for reversal"
[58] Reviewer's comment: In contrast, ANDEXXA is not specific
[59] Reviewer's comment: In contrast, ANDEXXA's affinity is similar to that of FXa
[60] Reviewer's comment: An identical description can be used when describing ANDEXXA

Factor VII (proconvertin) is converted to the activated form (FVIIa) by splitting of an internal peptide link. The FVIIa-TF complex activates Factor IX and initiates the primary coagulation pathway by activating FX in the presence of phospholipids and calcium ions.

Coagulation Factor IX

Factor IX (antihemophilic globulin B, or Christmas factor) is activated by the FVIIa-TF complex and by FXIa. Factor IXa in the presence of FVIIIa activates FX to FXa.

Coagulation Factor X

Factor X (Stuart-Prower factor) activation involves the cleavage of a peptide bond by the FVIIIa-Factor IXa complex or the TF-FVIIa complex. Factor Xa forms a complex with activated FV (FVa) that converts prothrombin to thrombin in the presence of phospholipids and calcium ions.

Protein C

Protein C, when activated by thrombin, exerts an antithrombotic effect by inhibiting FVa and FVIIIa leading to a decrease in thrombin formation, and has indirect profibrinolytic activity by inhibiting plasminogen activator inhibitor-1.

Protein S

Protein S exists in a free form (40%) and in a complex with C4b-binding protein (60%). Protein S (free form) functions as a cofactor for activated Protein C in the inactivation of FVa and FVIIIa, leading to antithrombotic activity.

FEIBA

Multiple interactions of the components in FEIBA restore the impaired thrombin generation of hemophilia patients with inhibitors. In vitro, FEIBA shortens the activated partial thromboplastin time (aPTT) of plasma containing factor VIII inhibitor.4,5

12.2 Pharmacodynamics

ANDEXXA

The effects of ANDEXXA can be measured through pharmacodynamic markers, including anti-FXa activity, free fraction of available FXa inhibitor, (b) (4) . In addition to binding and sequestering direct and indirect FXa inhibitors, ANDEXXA has also been shown to bind the endogenous anticoagulant, tissue factor pathway inhibitor (TFPI). (b) (4)

In prospective, phase 2 and phase 3, randomized, placebo-controlled, dose-ranging studies in healthy subjects, the dose and dose regimen of ANDEXXA required to reverse anti-FXa activity and restore thrombin generation were determined.

The reversal of anti-FXa activity was achieved within two minutes after completing the bolus administration. Administration of ANDEXXA as a bolus followed by continuous infusion resulted in a rapid and sustained decrease in anti-FXa activity *[see Clinical Studies (14)]*. The anti-FXa activity returned to the placebo levels approximately 2 hours after completion of a bolus or infusion.

The effect of ANDEXXA on plasma unbound FXa inhibitors occurred within 2 minutes following completion of ANDEXXA administration. When ANDEXXA was administered as a bolus followed by a continuous infusion, the decrease in unbound FXa inhibitors occurred within 2 minutes of the end of the bolus, was sustained over the course of the infusion, then gradually increased for approximately 2 hours following the end of infusion, and then decreased at a rate similar to placebo. Plasma TFPI activity has been shown to be inhibited for at least 22 hours following ANDEXXA administration.

Elevation of Tissue Factor (TF)-initiated thrombin generation above the baseline range (prior to anticoagulation) was achieved within two minutes following a bolus administration of ANDEXXA. The TF-initiated thrombin generation was elevated above placebo for up to 22 hours after completion of the

continuous infusion. In a contact-activated thrombin generation assay (which is not affected by ANDEXXA-TFPI interaction), the magnitude in thrombin generation elevation was smaller and its duration was shorter.

PRAXBIND

In healthy subjects aged 45 to 64 years, the plasma concentrations of unbound dabigatran were reduced to below the lower limit of quantification immediately after the administration of 5 g idarucizumab. Subjects' diluted thrombin time (dTT), ECT, aPTT, thrombin time (TT), and activated clotting time (ACT) parameters returned to baseline levels (see Figure 4 and Figure 5). This reduction of dabigatran plasma concentration was observed over the entire observation period of at least 24 hours. Similar findings were also observed in elderly subjects (aged 65 to 80 years) as well as subjects with mild and moderate renal impairment *[see Clinical Pharmacology (12.3)].*

In a limited number of patients, re-distribution of dabigatran from the periphery to plasma led to re-elevation of dTT, ECT, aPTT, and TT *[see Warnings and Precautions (5.2)].*

Re-dosing with 2.5 g idarucizumab in 6 healthy subjects aged 45-64 years at 2 months after first infusion revealed no differences in safety and no indication of allergic reactions *[see Clinical Pharmacology (12.3)]*.

No changes in the pharmacokinetics or pharmacodynamics of dabigatran were noted upon re-initiation 24 hours after the administration of idarucizumab [see Dosage and Administration (2.4)].

Figure 4 Plasma-Levels of Unbound Dabigatran in the Representative Group of Healthy Subjects (Administration of Idarucizumab or Placebo at 0 h)

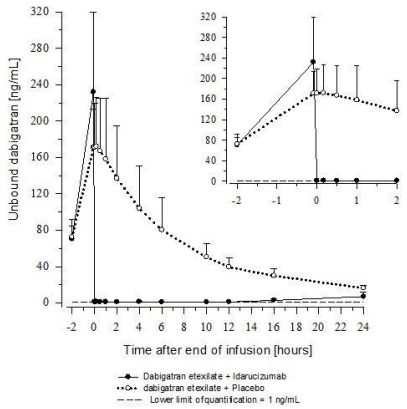
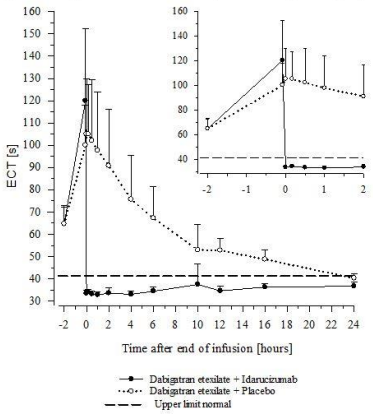


Figure 5 Change of ECT from Baseline in the Representative Group of Healthy Subjects (Administration of Idarucizumab or Placebo at 0 h)



Thrombin Generation Parameters
Idarucizumab alone has shown no procoagulant effect measured as endogenous thrombin potential (ETP).

Cardiac Electrophysiology
Clinical trials with idarucizumab in healthy subjects measured heart rate and electrocardiogram (ECG) parameters (waveform morphology, P wave duration, and PR, QRS, QT, and QTc intervals). There were no clinically relevant abnormal findings related to ECG.

Drug Interactions
In vitro Assessment of Drug Interactions
In vitro data suggest that the inhibition of dabigatran by idarucizumab is not affected by coagulation factor concentrates [3- or 4-factor prothrombin complex concentrates (PCCs), activated PCC, or recombinant Factor VIIa].

Assessment of Drug Interactions in Animal Studies
The potential effect of the binding of idarucizumab to dabigatran in the presence of volume replacement agents (e.g., crystalloids, colloids, and retransfusion of washed red blood cells) was investigated in swine.

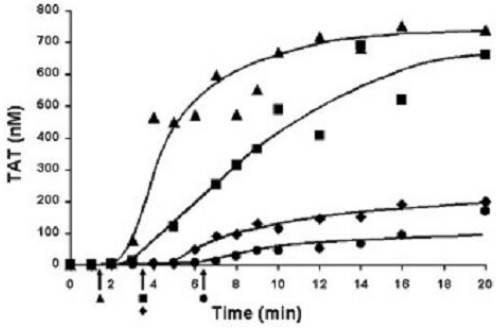
The results of this study suggest that neutralization of dabigatran anticoagulant activity is not influenced by 50% hemodilution with routinely used volume replacement strategies.

NOVOSEVEN

The effect of NovoSeven RT upon coagulation in patients with or without hemophilia has been assessed in different model systems. In an in vitro model of tissue-factor-initiated blood coagulation (Figure A),3 the addition of rFVIIa increased both the rate and level of thrombin generation in normal and hemophilia A blood, with an effect shown at rFVIIa concentrations as low as 10 nM. In this model, fresh human blood was treated with corn trypsin inhibitor (CTI) to block the contact pathway of blood coagulation. Tissue factor (TF) was added to initiate clotting in the presence and absence of rFVIIa for both types of blood.

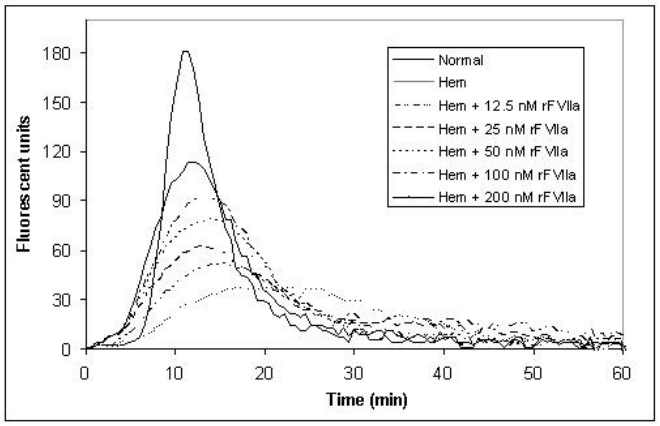
In a separate model, and in line with previous reports,4 escalating doses of rFVIIa in hemophilia plasma demonstrate a dose-dependent increase in thrombin generation (Figure B). In this model, platelet rich normal and hemophilia plasma was adjusted with autologous plasma to 200,000 platelets/microliter. Coagulation was initiated by addition of tissue factor and CaCl2. Thrombin generation was measured in the presence of a thrombin substrate and various added concentrations of rFVIIa.

Figure A



TF-initiated clotting of normal blood and congenital hemophilia A blood in the presence of factor VIIa. Clotting of CTI-inhibited (0.1 mg/mL) normal blood initiated with 12.5 pM TF (■) and addition of 10 nM factor VIIa (▲) and of hemophilia A blood with (♦) and without (●) addition of 10 nM factor VIIa. Figure A shows Thrombin Anti-Thrombin generation over time. Arrows indicate clotting times.

Figure B



TF-initiated clotting of normal and hemophilia A platelet rich plasma in the presence of rFVIIa.

KCENTRA

International Normalized Ratio (INR)

In the plasma-controlled RCT in acute major bleeding, the INR was determined at varying time points after the start or end of infusion, depending upon study design. The median INR was above 3.0 prior to the infusion and dropped to a median value of 1.20 by the 30 minute time point after start of Kcentra infusion. By contrast, the median value for plasma was 2.4 at 30 minutes after the start of infusion. The INR differences between Kcentra and plasma were statistically significant in randomized plasma-controlled trial in bleeding up to 12 hours after start of infusion [see Table 9].

The relationship between these or other INR values and clinical hemostasis in patients has not been established [see Clinical Studies (14)].

Table 9: Median INR (Min-Max) after Start of Infusion in RCTs

Study	Treatment	Baseline	30 min	1 hr	2-3 hr	6-8 hr	12 hr	24 hr
Acute Major Bleeding Study	Kcentra (N = 98)	3.90 (1.8–20.0)	1.20* (0.9–6.7)	1.30* (0.9–5.4)	1.30* (0.9–2.5)	1.30* (0.9–2.1)	1.20* (0.9–2.2)	1.20 (0.9–3.8)
	Plasma (N = 104)	3.60 (1.9–38.9)	2.4 (1.4–11.4)	2.1 (1.0–11.4)	1.7 (1.1–4.1)	1.5 (1.0–3.0)	1.4 (1.0–3.0)	1.3 (1.0–2.9)

(b) (4)

INR = international normalized ratio; NC = not collected.

* Statistically significant difference compared to plasma by 2-sided Wilcoxon test

FEIBA – no section 12.2

Appendix B. Review of Portola's request to use INN as a non-proprietary name

On 30 March 2018, Portola submitted an amendment[61] entitled "*Response to 15 February 2018 BLA Resubmission Information Request (Proposed suffixes for non-proprietary name and designation of non-proprietary name)*". In this amendment, Portola expressed concerns with the Agency proposing to use a chemical name, *coagulation factor Xa (recombinant), inactivated*, instead of the adopted USAN name, *andexanet alfa* [62], for the non-proprietary name of ANDEXXA.

Portola stated: *"Based on the product's designed Mechanism of Action (MOA) and intended use as an antidote (it is not a replacement factor, nor does it promote hemostatic activity on the coagulation cascade), we believe that categorizing it in the same class with other "Coagulation Factor" products is misleading and may result in healthcare providers assuming it acts in the same way as others in this class (e.g., FVIII, FVII, PCCs). Hence, andexanet is in a class by itself. Please note the following:*

1. *Andexanet alfa has been designed to lack all the critical functions of FX or FXa, except for the ability to bind and sequester direct and indirect FXa inhibitors with high affinity.*
   a. *The enzymatic activity of FXa has been eliminated, by mutating the active site serine to alanine. Thus, andexanet does not have the ability to generate thrombin from prothrombin, unlike native FXa.*
   b. *In addition, removal of the Gla domain from native FX/FXa was critical to the design of the andexanet molecule, in that andexanet does not compete with native FXa for assembly into the prothrombinase complex (with FVa). Therefore, andexanet cannot act as a "dominant negative" and lacks anticoagulant activity (since it does not compete with native FXa for assembly into the prothrombinase complex). Thus, andexanet is functionally different from an inactivated native FXa, such as FXa inactivated by using a* (b) (4) *or by active site serine to alanine mutation (e.g.,* (b) (4) *), which is a potent anticoagulant.*
2. *Andexanet alfa was designed as a reversal agent, first and foremost. The MOA for andexanet is to bind and sequester FXa inhibitors, period. It has no pro or anticoagulant activity on its own, like a coagulation factor would have. The only activity of native FXa that is retained in andexanet is the ability to bind to FXa inhibitors, which confers its reversal activity and allows it to act as a decoy molecule.""*

I do not agree with Portola's rationale presented above and I recommend that FDA denies Portola's request to use the INN (andexanet alfa), and continues to use "*coagulation factor Xa (recombinant), inactivated*".

The rationale presented by Portola is misleading because it disregards the second MOA, inhibition of TFPI, which is a procoagulant action of ANDEXXA, and therefore underestimates the potential

[61] STN 125586\0\117 eCTD Sequence Number: 0118 CBER Receipt Date: 02-Apr-2018, BLA 125586 / SN0118
[62] Reviewer's note: andexanet alfa is also an INN.

risks of thrombosis that can be associated with ANDEXXA use, especially when ANDEXXA is used off-label at higher doses. Specifically,

1. ANDEXXA has demonstrated at least two MOAs, (1) sequestration of FXa inhibitors, and (2) inactivation of plasma inhibitor TFPI. The second mechanism is a potentially thrombogenic procoagulant activity, which is not related to the first action (interaction of ANDEXXA with FXa inhibitors). Therefore, it would be misleading to refer to this product as only an "antidote" to FXa inhibitors.

2. Both mechanisms of ANDEXXA action rely on ANDEXXA being a coagulation FXa-like molecule. ANDEXXA competes with human plasma FXa for FXa inhibitors, and TFPI because ANDEXXA's binding sites for FXa inhibitors and TFPI are identical to human FXa in terms of DNA sequence and secondary protein structure.

3. I agree that ANDEXXA is in a class of itself, very much like FEIBA is in a class of itself, and FVIIa is in a class of itself. Of note, it is misleading to say that FVIII, FVIIa and PCCs are in the same class. These three products have different indications and mechanisms of action. The mechanisms of action of FVIII, FVIIa, PCCs and ANDEXXA are different from each other but at the same time they are similar in a sense that all 4 products interact with the coagulation cascade to improve hemostasis.

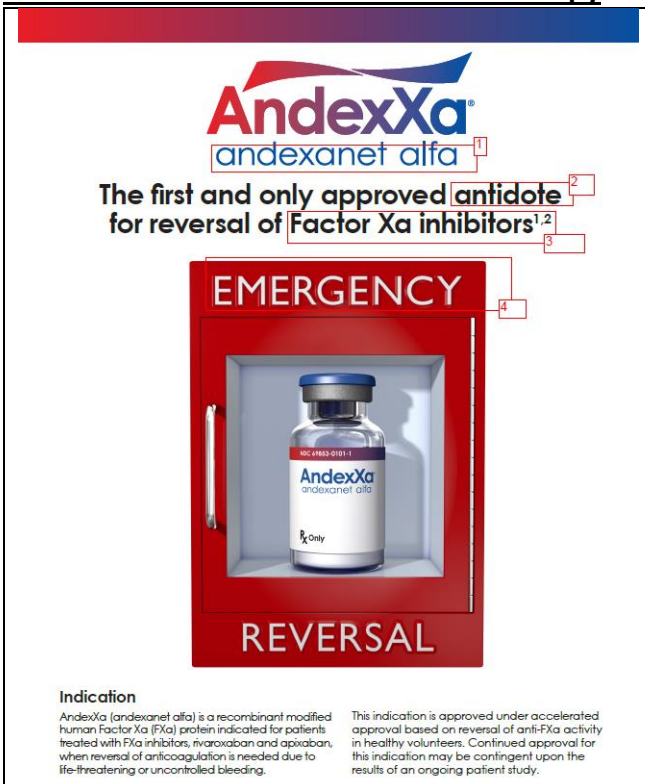
4. The adverse events associated with this product (bleeding because of the lack of action and thrombosis because of too much action) are very like other coagulation factor products, and therefore it would be appropriate for healthcare providers to assume the same risks as for other coagulation factor products

5. Finally, the statements in this amendment indicate that Portola is hoping to advertise this product in a misleading manner. Their proposed arguments misleadingly present ANDEXXA as a simple antidote for FXa inhibitors with no other targets or activity in the coagulation cascade. This representation ignores available preclinical and clinical evidence that ANDEXXA is a powerful procoagulant molecule because it interacts and completely obliterates TFPI activity for at least 22 hours and elevates markers of thrombin generation *ex vivo* (TG assay) and *in vivo* (D-dimer, TAT, and PF1.2). We do not know at this time whether this procoagulant action of ANDEXXA is clinically relevant, but we cannot ignore the evidence, and disregard the associated risks that it may.

Appendix C. Review of risks associated with promotional materials

This appendix describes my review of promotional materials submitted in the CRL response.

Core Visual Aid PP-AnXa-US-0012 Clean Copy

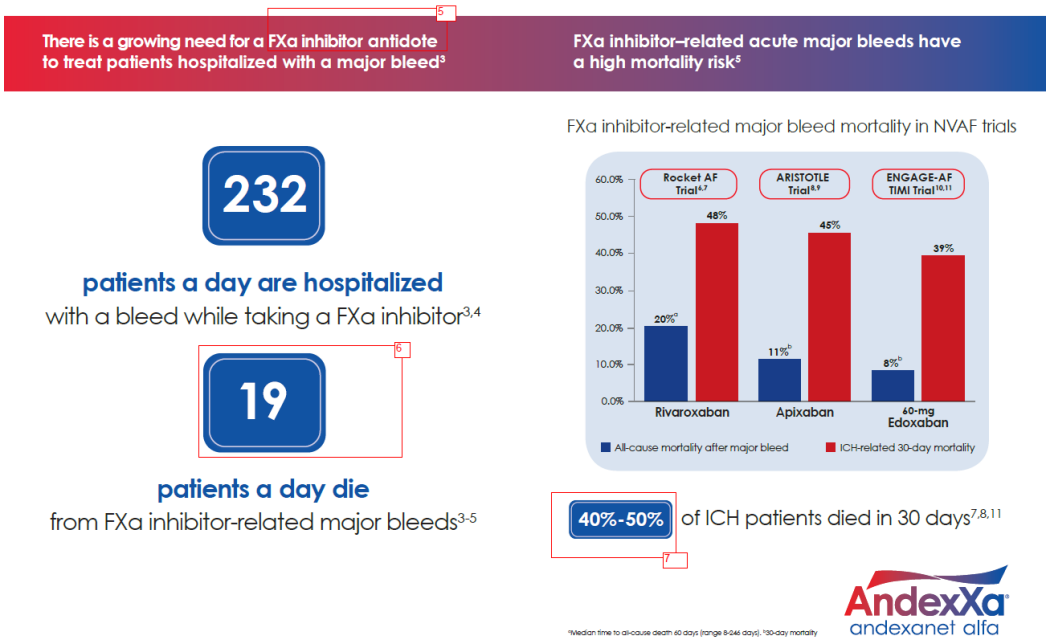


1. **Andexanet alfa**
   "andexanet alfa" is an INN. "*coagulation factor Xa (recombinant), inactivated*" should be used.

2. **Antidote**
   Misleading. Implies that ANDEXXA's biological action is limited to neutralization of FXa inhibitor, disregarding the potential thrombotic risks associated with the second action, mediated by neutralization of plasma inhibitor TFPI.

3. **Factor Xa inhibitors**
   Reference to a class of FXa inhibitors is misleading because ANDEXXA is indicated for reversal of only two FXa inhibitors, rivaroxaban and enoxaparin. Reversal of the remaining licensed FXa inhibitors is not demonstrated in clinical trials and may be ineffective because incomplete reversal of at least one anti-FXa, apixaban, activity was observed.

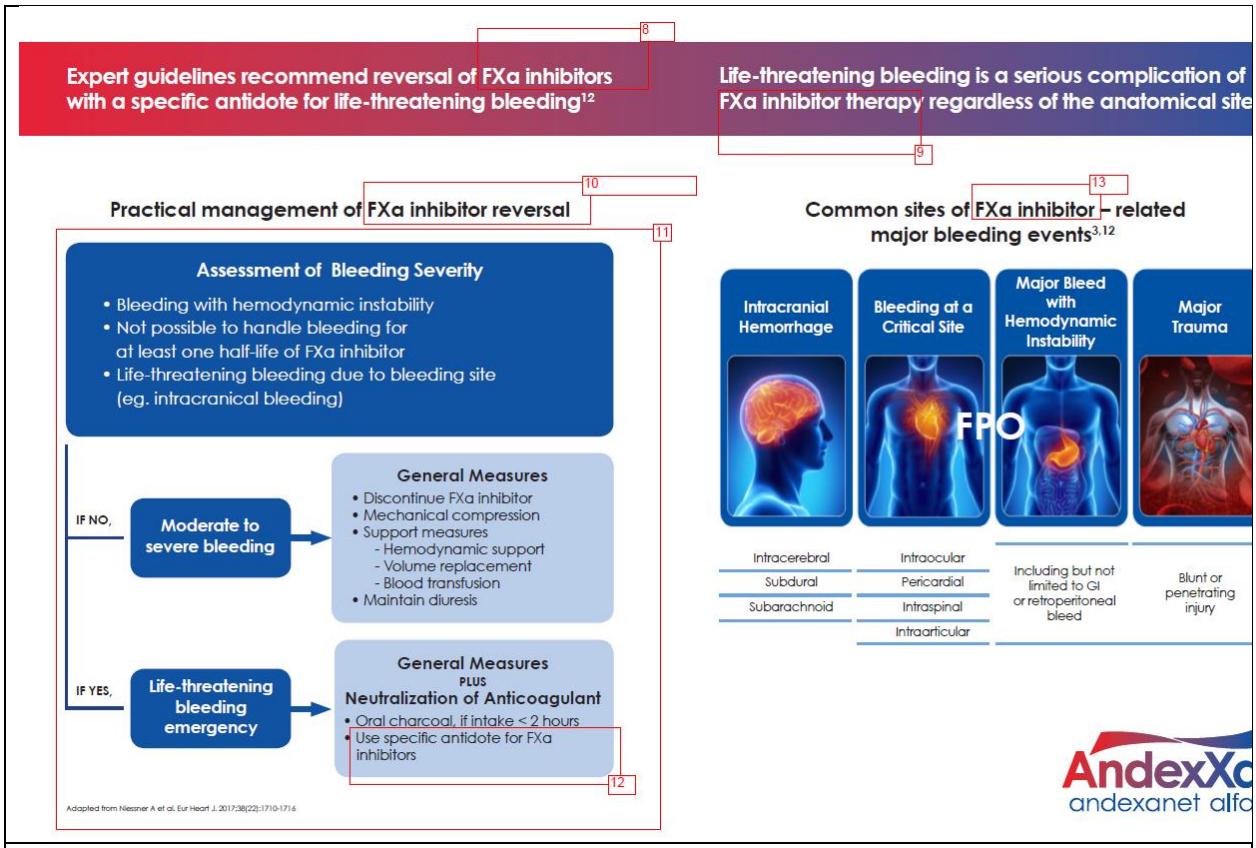
4. **Emergency**
   Misleading. Implies that ANDEXXA is available for a wide range of conditions. ANDEXXA is indicated for patients treated with rivaroxaban and apixaban, when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding.



5. **FXa inhibitor antidote**
   See Comments #2 & #3

6. **19 patients a day die**
   7 patients per reference 3. Range should be indicated, e.g., 7-19.

7. **40%-50% of ICH patients died in 30 days**
   Reference 3 provides 14% mortality in ICH. Should be 14%-50%



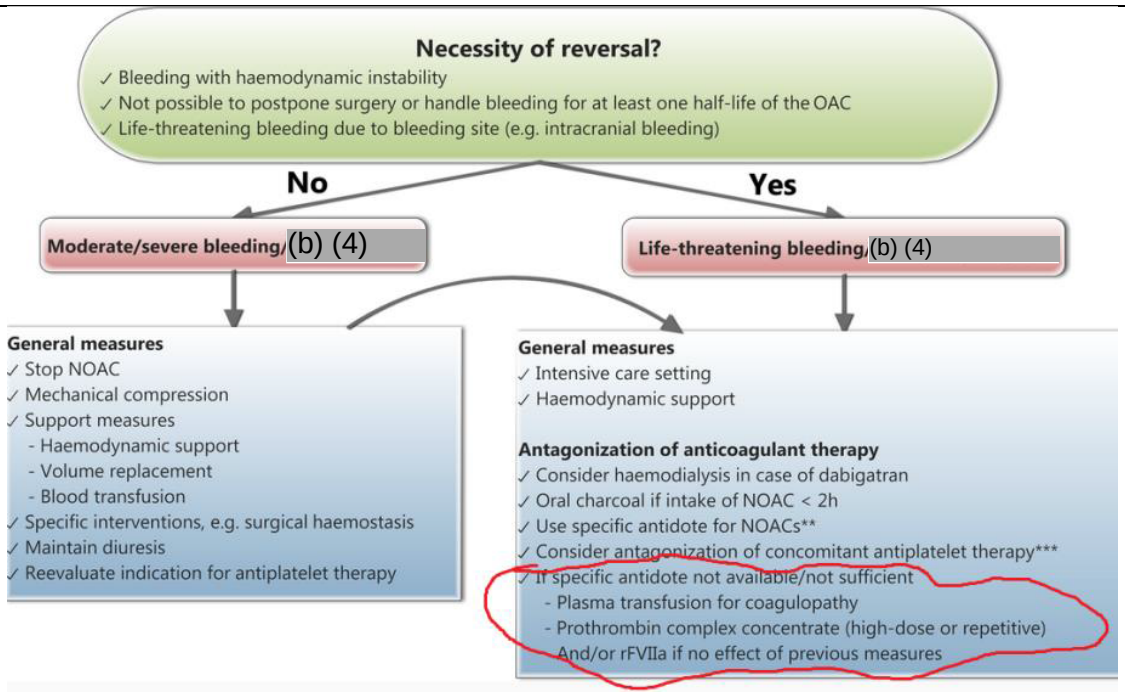
8. **FXa inhibitors**
   See Comment #3.

9. **FXa inhibitor therapy**
   See Comment #3.

10. **FXa inhibitor reversal**
    See Comment #3.

11. **Assessment of bleeding severity (adapted from Niessner A et al. *Eur Heart J*)**
    The table was misleadingly modified to remove recommended use of PPCs and create an appearance that only specific antidote is recommended. See original figure reproduced from Niessner A et al.[63]

[63] Niessner A et al. Reversal strategies for non-vitamin K antagonist oral anticoagulants: a critical appraisal of available evidence and recommendations for clinical management-a joint position paper of the European Society of Cardiology Working Group on Cardiovascular Pharmacotherapy and European Society of Cardiology Working Group on Thrombosis. *Eur Heart J*. 2017 Jun 7;38(22):1710-1716. https://academic.oup.com/eurheartj/article/38/22/1710/3056894

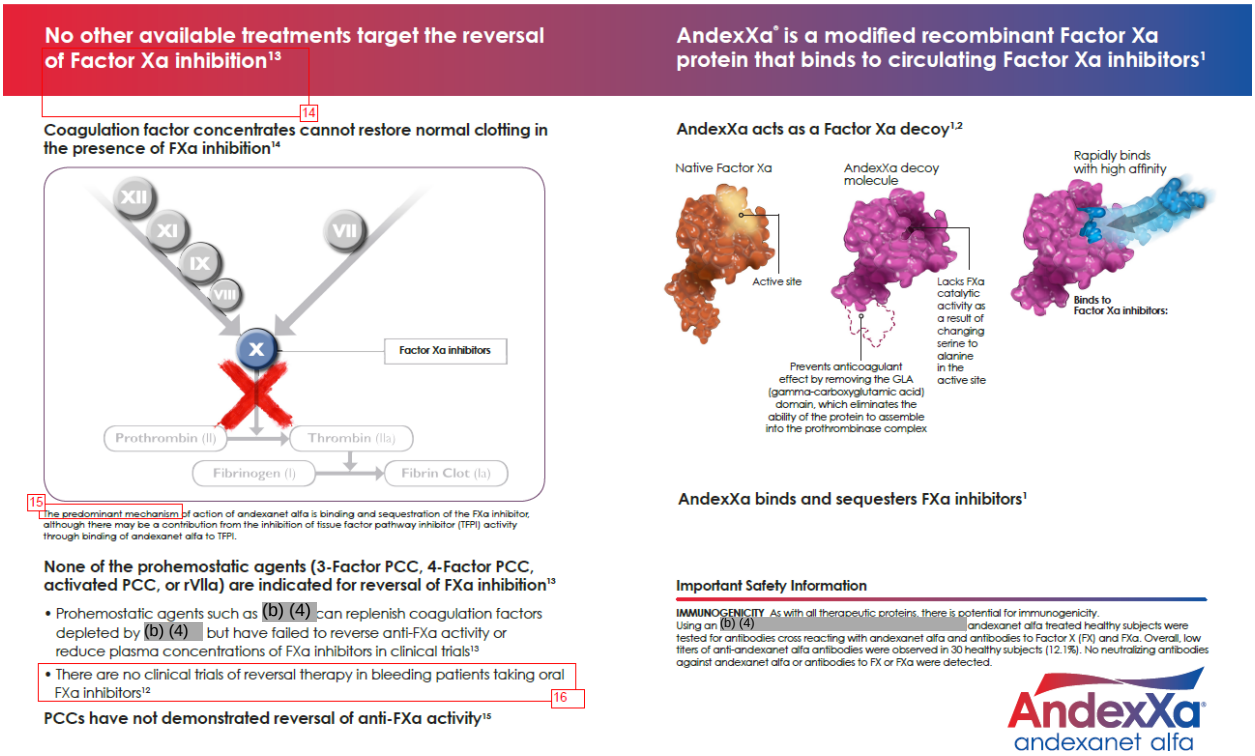


12. Use of specific antidote for FXa inhibitors

See Comments #2 & #3

13. FXa inhibitor

See Comment #3



14. Reversal of Factor Xa inhibition

See Comment #3

15. The predominant mechanism of action

"Predominant" is misleading, creating an appearance that TFPI inhibition is minor mechanism that can be disregarded. Clinical studies were not designed to provide evidence on the relative importance of the two mechanisms of action.

Underestimating the non-antidote action of ANDEXXA can result in unsafe use of the drug because TFPI inhibition continues for several days after ANDEXXA administration and can be associated with thrombotic risks.

- Underestimating the anti-TFPI action leads to underestimation of thrombotic risks associated with overdosing.
    - FXa inhibitor reversal can be thrombogenic because patients receive FXa inhibitors to reduce the risk of thrombosis. However, reversal of anti-FXa activity does not push risk above the pre-FXa inhibitor level, and ANDEXXA overdosing will not increase the thrombotic risk further.
    - TFPI inhibition increases coagulation potential above normal even in healthy subjects, and overdosing increases the duration of this effect. TFPI inhibition is potentially thrombogenic because TFPI is the only known plasma inhibitor of tissue factor, the molecule responsible for

initiation of blood coagulation at the sites of vascular lesions. Indeed, several investigational anti-TFPI agents have progressed into the late stage clinical trials for prevention of spontaneous bleeding in hemophilia.

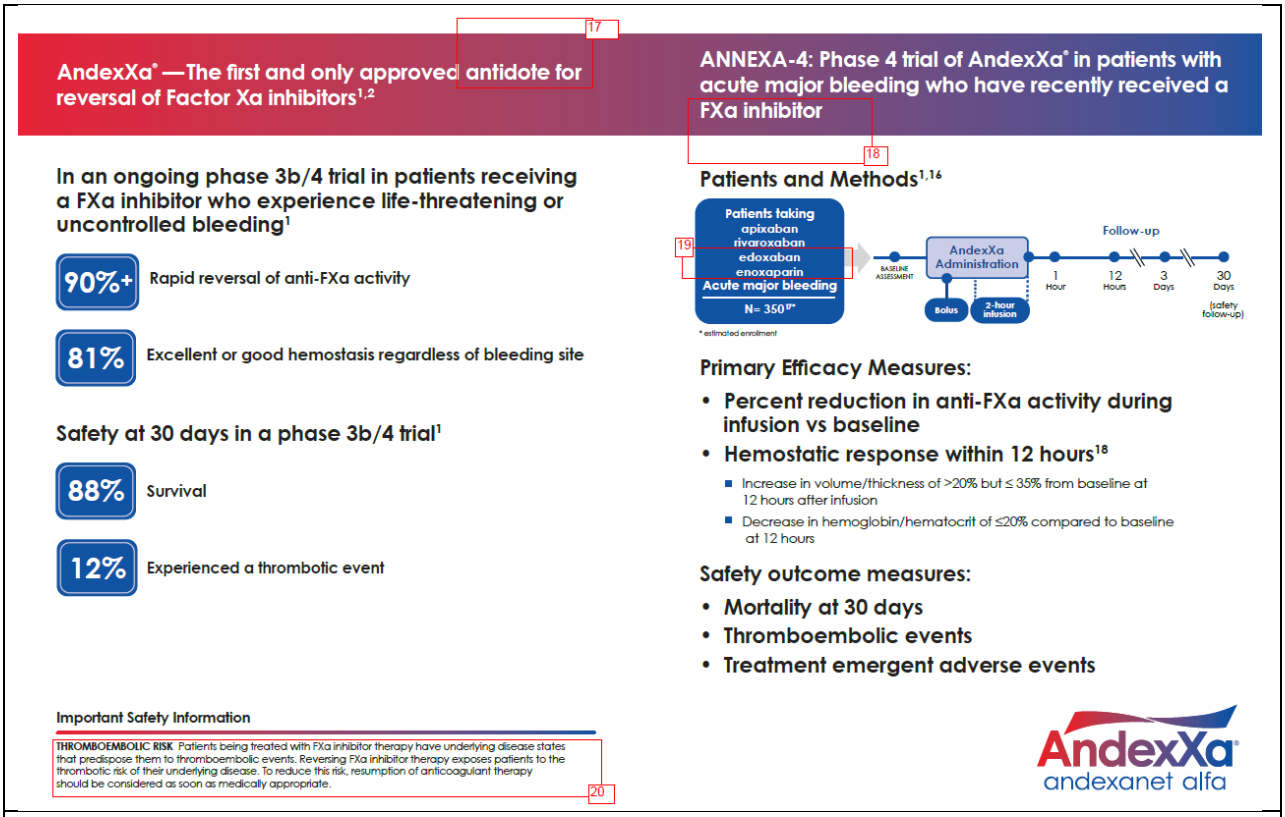
- Underestimating the anti-TFPI action leads to underestimation of the duration of the ANDEXXA action, leading to underestimation of thrombotic risks. Anti-FXa reversal action is short, about 2-4 hours after the end of infusion, because it requires very high doses of ANDEXXA and therefore is limited by the ANDEXXA half-life. However, anti-TFPI action requires 100-1000-fold lower doses of ANDEXXA, and it continues well over several terminal half-lives. Complete inhibition of TFPI is seen for 24 hours after ANDEXXA administration at recommended doses, and partial inhibition of TFPI continues for about 2 days or possibly longer.

- Underestimating the anti-TFPI action of the drug can facilitate off-label dosing of ANDEXXA.
    - Because anti-FXa activity reversal is short, and overdosing does not increase the antidote action-dependent thrombogenic risks, some doctors will go off-label to extend the anti-FXa inhibitor action of ANDEXXA by overdosing the patients, extending the duration of infusion beyond 2 hours, or administering the second dose.
    - An off-label use (higher dose, longer infusion and second dose) will extend the anti-TFPI action beyond those studied in clinical trials, leading to increased risk of thrombosis.

Underestimating the anti-TFPI action may lead to unnecessary use of ANDEXXA in patients who are not likely to benefit from anti-FXa reversal. Belief in short antidote action of ANDEXXA can result in a desire to use ANDEXXA in all patients on FXa inhibitors, regardless of the bleed severity or anti-FXa level. Indeed, reversal of FXa inhibitors by ANDEXXA will add no harm, but may be of limited benefit in bleeding patients who received anti-FXa inhibitor long time ago. The anti-TFPI inhibition will be observed in all patients, even those who have no anti-TFPI activity.

16. There are no clinical trials of reversal therapy

Not true. E.g., this study: Ammar Majeed, Anna Ågren, Margareta Holmström, Maria Bruzelius, Roza Chaireti, Jacob Odeberg, Eva-Lotta Hempel, Maria Magnusson, Tony Frisk and Sam Schulman. Management of rivaroxaban- or apixaban-associated major bleeding with prothrombin complex concentrates: a cohort study. Blood 2017 130:1706-1712; doi: https://doi.org/10.1182/blood-2017-05-782060 Study conclusion: "*The administration of PCCs for the management of MBEs associated with rivaroxaban or apixaban is effective in most cases and is associated with a low risk of thromboembolism. Our findings are limited by the absence of a control group in the study*."



17. Antidote for reversal
See Comments #2 & #3

18. Received FXa inhibitor
See Comment #3

19. Edoxaban, enoxaparin
ANDEXXA is not indicated for reversal of edoxaban and enoxaparin. See Comment #3.

20. THROMBOEMBOLIC RISK Patients being treated with FXa inhibitor therapy have underlying disease states that predispose them to thromboembolic events. Reversing FXa inhibitor therapy exposes patients to the thrombotic risk of their underlying disease. To reduce this risk, resumption of anticoagulant therapy should be considered as soon as medically appropriate.
Disregards potential thrombotic risks associated with inhibition of TFPI by ANDEXXA. See Comment #15.

AndexXa® safety profile[1]

In the pooled safety analysis for clinical trials of AndexXa with FXa inhibitors in older healthy volunteers (N=223)

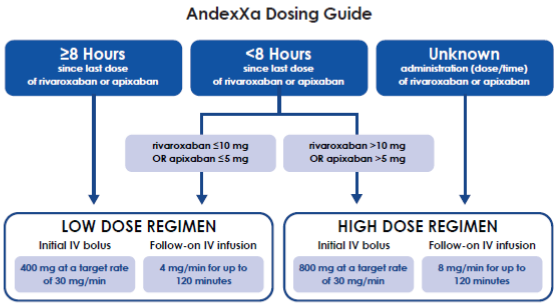
- The overall frequency of adverse events was similar between AndexXa-treated subjects and placebo-treated subjects
- No serious or severe adverse events were reported
- No thromboembolic events were reported during the older healthy volunteer studies
- In healthy volunteers, the most frequently reported adverse reactions (≥3%) in healthy subjects treated with andexanet alfa were infusion-related reactions

Safety results from an ongoing phase 4 study in patients with an acute major bleed who are on apixaban, rivaroxaban, edoxaban, or enoxaparin (N=185)

- 88% (162/185) survival at 30 days
  - 74% experienced an event ≥4 or more days after AndexXa administration
  - 78% were not on antithrombotic therapy at the time of the event
- No patients have reported an infusion reaction
- One patient discontinued study drug prematurely

AndexXa® dosing should be individualized[1]

AndexXa has 2 regimens specific to FXa inhibitors used and time of last dose[1]

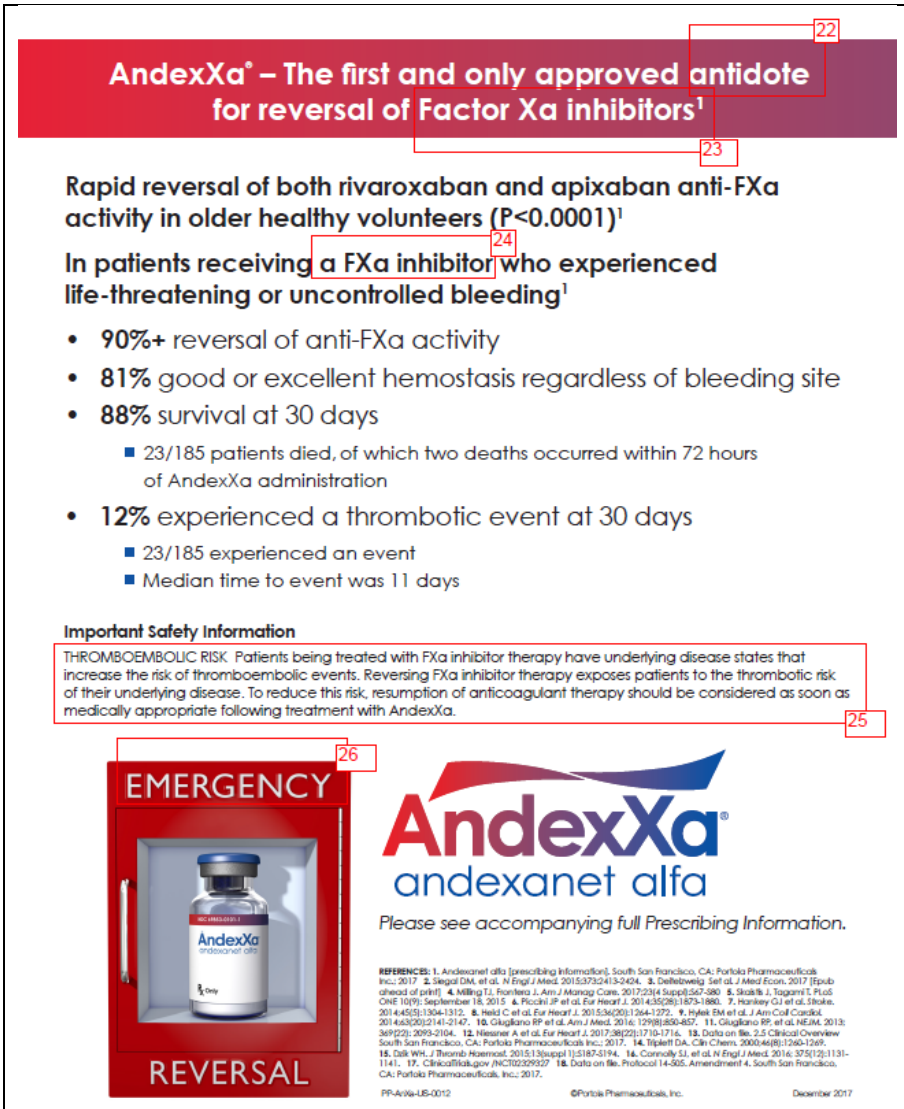


AndexXa is available in 100 mg vials (10 mg/mL)

AndexXa® andexanet alfa

21. Edoxaban and enoxaparin

See Comment #19.



22. Antidote
See Comments #2 & #3

23. Factor Xa inhibitors
See Comment #3

24. FXa inhibitor
See Comment #3

25. THROMBOEMBOLIC RISK
See Comment #20.

26. EMERGENCY
See Comment #4

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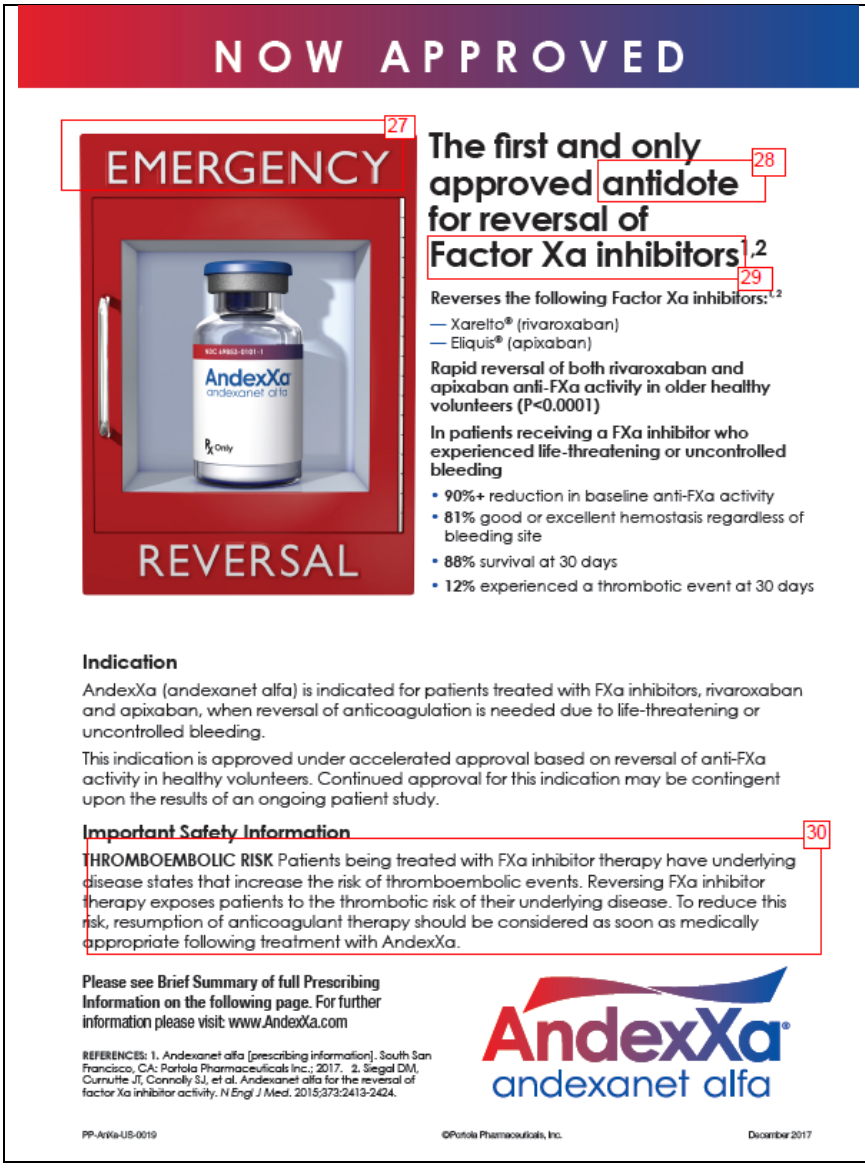


27. EMERGENCY
See Comment #4

28. Antidote
See Comments #2 & #3

29. Factor Xa inhibitors
See Comment #3

30. THROMBOEMBOLIC RISK
See Comment #20.

Appendix D. Review of thrombin generation data in poor responders in ANNEXA 4

To better understand the scientific reasons for patients who responded poorly to ANDEXXA treatment in the ANNEXA 4 study, I reviewed the TG assay data (measured by the ETP parameters) and listed the signs of vital/hemodynamic presented in the BLA files, see Table S2. The intent of this analysis was limited to the study of TG assay. The clinical signs were no adjudicated.

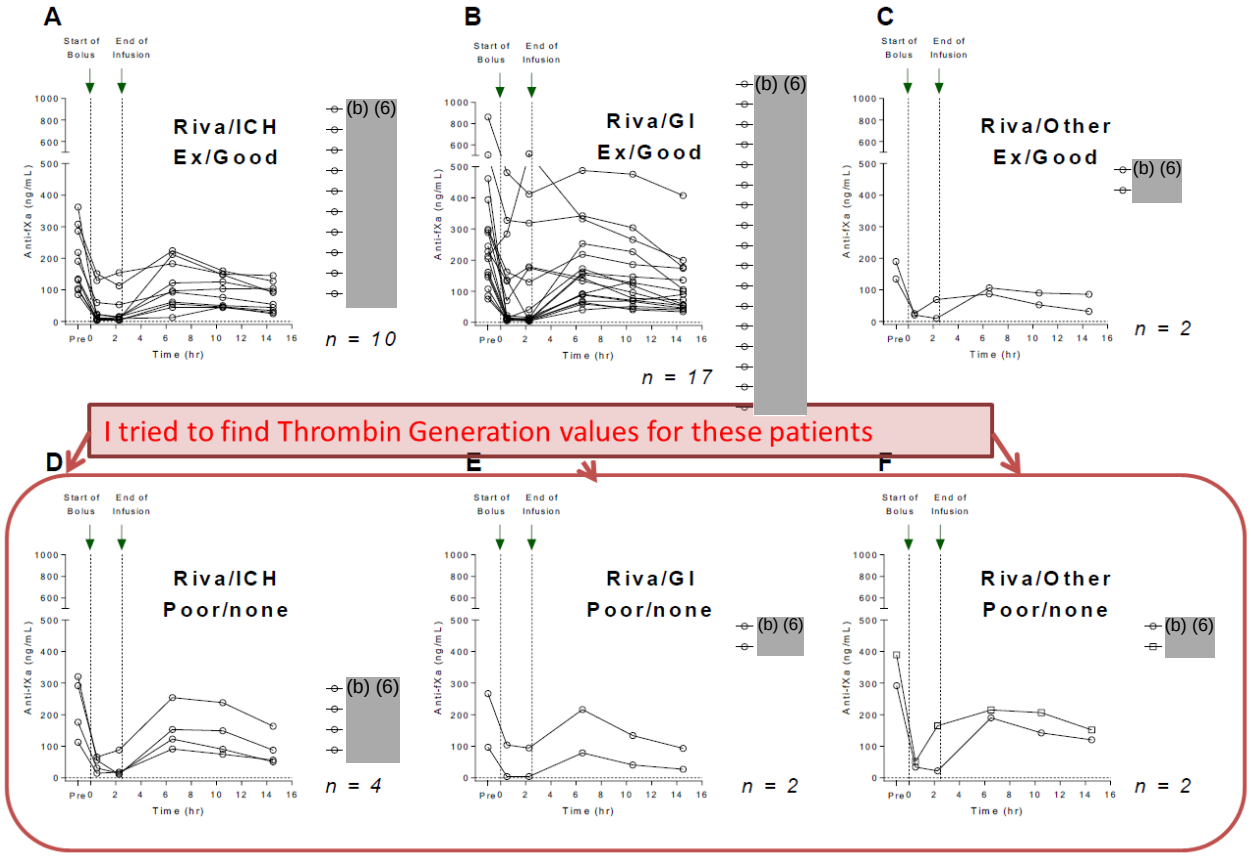
The time-courses of anti-FXa activity in excellent/good responders and poor/non-responders are shown in Tables S3-S4 below. Raw TG data and copies of original reports on vital/hemodynamic compromise signs are shown in Figs. S2-S8 below. For comparison, Fig. S7 presents available time course data for anti-FXa and TG assay parameters in excellent/good responders in the ANNEXA 4 study. Preliminary visual comparison of TG assay time course data indicates no obvious differences between the responder groups.

Supplemental Table S2: Overview of TG assay results in poor responders/non-responders from Tables S3-S4.

Group	Ptnt #	Vital/hemodynamic compromise signs		ETP at screening		ETP at 3 days	
		3 hr before bolus	15 min before bolus	Value	Relative to normal	Value	Relative to normal
Apix/ICH Poor/none	(b) (6)	*No data*	*No data*		*No data*		*No data*
		No	No	681	Low normal	947	Mid normal
		No	No	1100	Upper normal	1550	**Very high**
		No	No	900	Mid normal	1000	Upper normal
		Yes (ICH)	No	467	**Below normal**	790	Low normal
Apix/GI Poor/none		No	No	1050	Upper normal	1000	Upper normal
		No	No	1200	Upper normal	1400	**Above normal**
Riva/ICH Poor/none		*No data*	*No data*	336	Very low	died	1280@12hr UpNorm
		Right side weakness, slurr	Right side weakness	750	Low normal	1650	**Very high**
		Yes (poor skin perfusion)		1180	Upper normal	1100	Upper normal
		Yes (unconscious)	No	850	Mid normal	1150	Upper normal
Riva/GI Poor/none		No	No	900	Mid normal	1200	Upper normal
		Yes (dyspnea, pale tongue)	Yes (dyspnea)	1272	Upper normal	2142	**Super high**
Riva/Other Poor/none		Yes **(Hypotension)**	No	1160	Upper normal	1243	Above normal
		No	No	750	Low normal	1000	Upper normal

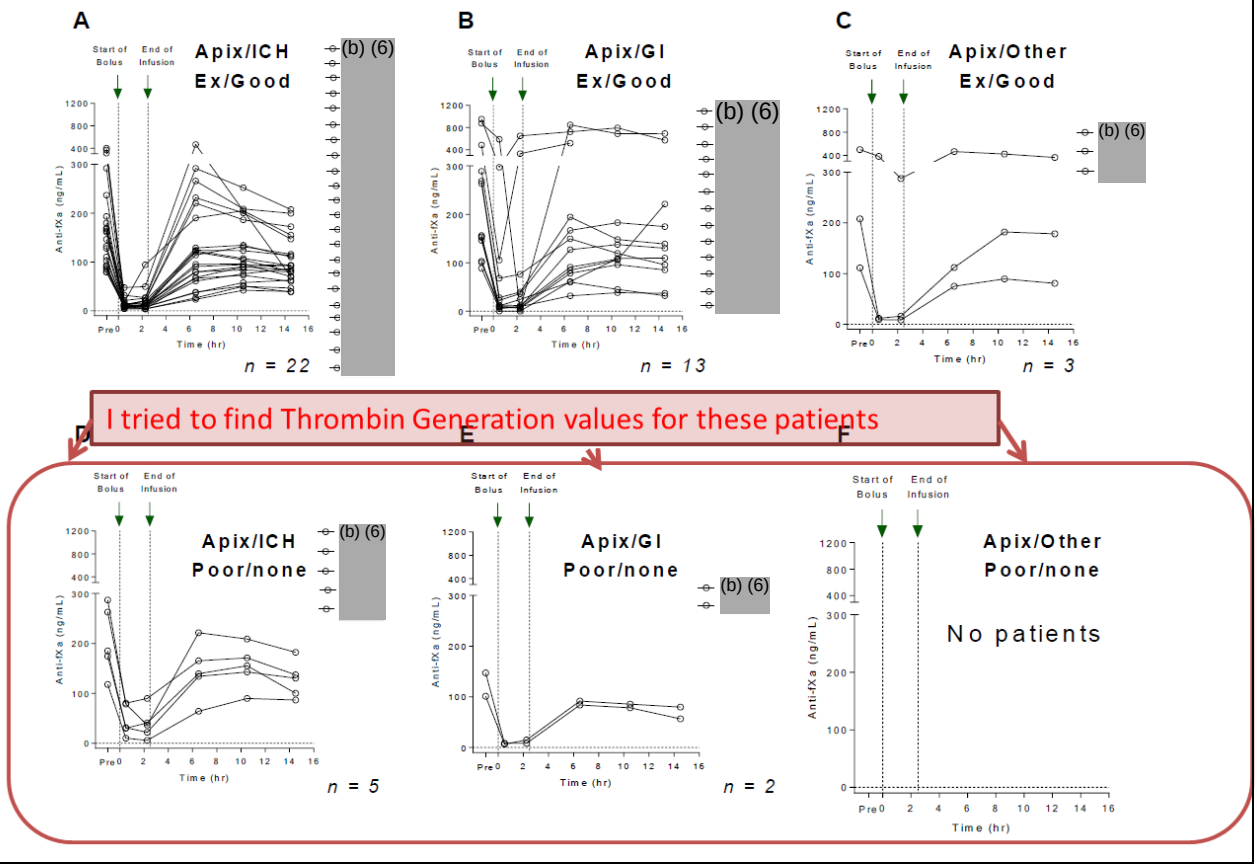
Supplemental Table S3: The time-courses of anti-FXa activity in excellent/good responders and poor/non-responders in *rivaroxaban* patients.

Figure 1.11.3-4: ANNEXA-4 Study: Change in Anti-fXa Activity over Time in Efficacy Evaluable Patients Tak Rivaroxaban, Stratified by Hemostatic Efficacy (20 April 2017 Data Cut)



Supplemental Table S4: The time-courses of anti-FXa activity in excellent/good responders and poor/non-responders in *apixaban* patients.

Figure 1.11.3-3: ANNEXA-4 Study: Change in Anti-fXa Activity over Time in Efficacy Evaluable Patie Taking Apixaban, Stratified by Hemostatic Efficacy (20 April 2017 Data Cut)



12 pages have been determined to be not releasable: (b)(4)