**FOOD AND DRUG ADMINISTRATION**
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

MEMORANDUM

# Final CMC review

**To:** File (STN BL 125586/0) & Thomas Maruna

**From:** Mikhail V. Ovanesov, PhD, Research Biologist, Laboratory of Hemostasis (LH), Division of Hematology research and Review (DHRR)/OBRR

**Through:** Tim Lee, PhD, Acting Chief, LH/DHRR/OBRR

Basil Golding, MD, Director, DHRR/OBRR

**Subject:** Final CMC Review of Portola's BLA for Coagulation Factor Xa (Recombinant), Inactivated [ANDEXXA]

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**Contents**

# 1. Executive Summary

STN 125586/0 is an original biologics license application (BLA) submitted by Portola Pharmaceuticals Inc. (Portola) for Coagulation Factor Xa (Recombinant), Inactivated with the proprietary name ANDEXXA and International Nonproprietary Name (INN) *andexanet alfa*.

ANDEXXA is presented as a lyophilized powder for intravenous administration after reconstitution with sterile Water for Injection. The active ingredient of ANDEXXA is a genetically modified variant of human Coagulation Factor Xa (FXa) produced by recombinant DNA technology in a Chinese Hamster Ovary (CHO) cell line. ANDEXXA was designed to bind anticoagulant drugs that inhibit FXa (e.g., apixaban, edoxaban, rivaroxaban, enoxaparin). In the ANDEXXA molecule, the FXa proteolytic activity and its procoagulant lipid binding domain were genetically removed rendering it unable to activate blood coagulation but it can still bind to direct and indirect FXa inhibitors. ANDEXXA also binds to and inactivates Tissue Factor Pathway Inhibitor (TFPI), an endogenous inhibitor of blood coagulation, which may contribute to procoagulant activity of ANDEXXA *in vivo*.

ANDEXXA received *Breakthrough Therapy* designation on 22 November 2013 under the Investigational New Drug application (IND) 15089. 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) " on 23 February 2015. FDA has determined that an *Accelerated Approval* pathway is appropriate for a BLA for the reversal of anticoagulation with direct FXa inhibitors.

The BLA for ANDEXXA under STN 125586/0 was submitted as a rolling review. The initial modules received on 6 November 2015 included Nonclinical Module 2 (sections 2.4 and 2.6) and Module 4. The remaining modules, i.e., Modules 1, 2, 3 and 5, were received on 17 December 2015, starting the review clock. This application is reviewed under a *Priority Review* schedule and is subject to PDUFA-V requirements. The action date for this BLA is 17 August 2016.

The scope of this review covers all CMC product topics except 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), FDP release methods and development of associated reference standards (reviewed by a review team from OCBQ/DBSQC), justification of specifications and extractables and leachables studies (reviewed by Dr. Andrey Sarafanov).

Substantive CMC issues were identified during the review of the ANDEXXA BLA. The data on process development and validation are deficient, including those on the validation of commercial (b) (4) for the manufacture of (b) (4) Final Drug Product (FDP), in-process hold times, process control strategy, impurity evaluation and clearance, batch consistency, comparability between clinical ((b) (4) ) and commercial ((b) (4) ) batches, and stability.

CBER performed a Pre-License Inspection (PLI) of Portola's BDS contract manufacture (b) (4) in (b) (4) from (b) (4) covering the manufacturing of (b) (4) FDP. CBER issued a Form FDA 483

with the following four observations: (1) “process validation is incomplete”, (2) “deviations during production have not been thoroughly investigated”, (3) “preventative maintenance of critical equipment is not performed per written procedures”, and (4) “failure to follow standard operating procedure for deviation management”. Portola’s responses to observation 1, “process validation is incomplete”, are not acceptable and as a result, the inspection is not closed. For example, repeated out-of-specification (OOS) results for the (b) (4) of ANDEXXA observed for the (b) (4) batches at release, in stability studies and in-process intermediates were linked to the presence of (b) (4) activity in process intermediates, and possibly in the FDP. The identity of the (b) (4) impurities and the capability of the purification process in clearing these impurities are under intense investigation by Portola but no definitive conclusion has been reported to the FDA. In an attempt to partially mitigate the increase in the (b) (4) during purification process, (b) (4) will install new equipment to control (b) (4) at the point of use. The earliest date that this action can be completed is 15 November 2016 which is after the PDUFA goal date of 17 August 2016 and therefore is not acceptable. Validation of the FDP process at Portola’s contract manufacturer (b) (4) in (b) (4) was also found deficient in that the lyophilizers were not properly qualified and evidence of non-compliant practices of (b) (4) at the (b) (4) facility was identified.

The proposed shelf-life of 24 months for the FDP was not supported by available stability data - only 6 months of real-time data from the commercial (b) (4) batches are available at this time. Stability data from the batches using (b) (4) could not be used to support the proposed shelf-life because side-by-side comparison with (b) (4) demonstrated that these two preparations are not comparable. A trend towards (b) (4) in batches manufactured using the proposed commercial process was observed as evidenced by (b) (4) formation.

The release specifications of (b) (4) FDP for excipients, identity, and impurities are deficient and the analytical methods for release testing are not fully validated. ANDEXXA is a mutated coagulation factor product manufactured at large scale, formulated at high concentration (10 mg/mL) and administered at high doses. The inclusion of excipient specifications and enhanced identity tests ((b) (4) and characterization of (b) (4) modifications, e.g., (b) (4)) are required to provide assurance of consistent product quality to compensate for the limited manufacturing experience. In addition, the ANDEXXA potency standard is not properly qualified and the consistency of product potency in the event of future standard replacement is not assured. Portola proposed to develop new release assays and provide a properly qualified potency standard by 31 October 2016.

A comparability protocol (CP) was submitted in the BLA to support the introduction of a new manufacture suite at (b) (4) and scale-up of the manufacturing processes of (b) (4) FDP. 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 prior approval to CBE-30. However, the CP is found to be deficient and unacceptable. At this time, the FDA does not consider the CP to be suitable to support a downgrade of the supplement to add (b) (4) for the manufacture of (b) (4) FDP.

Multiple deficiencies were identified in the validations of the bioanalytical methods used in clinical studies. Portola failed to develop assays to measure anti-drug antibodies that inactivate endogenous FX and FXa or bind (b) (4). In addition, different versions of the critically important pharmacodynamics assays for TFPI activity and thrombin generation were used in Phase 1, 2 and 3 clinical trials without proper bridging studies. As a result, incorrect interpretation of the results obtained in the pivotal safety and efficacy Phase 3 trials was provided in the BLA, as evidenced from preliminary investigations of the retained clinical samples from the completed clinical trials. Additional validations of bioanalytical methods and re-analysis of the associated clinical trial data are in process and will not be completed until 31 October 2016.

**Recommendation:**
Many critical elements of the manufacturing process are not fully validated, which include (b) (4). In addition, the bioanalytical methods used to support and analyze samples from the pivotal safety and efficacy clinical trials were not properly validated and incorrectly analyzed. These deficiencies have been communicated to Portola, and Portola responded that additional process investigation and validation studies, analytical and bioanalytical method validations are under way. Portola estimated that these studies will be completed by 30 October 2016, which fails to meet the action due date for this BLA. Therefore, I recommend issuing a Complete Response letter.

# 2. Background

The active ingredient in ANDEXXA is a genetically modified variant of human FXa. The product is developed for the U.S. market under IND 15089 for the reversal of the anticoagulant effect of direct or indirect FXa inhibitors in patients who are experiencing a serious uncontrolled bleeding event (b) (4). ANDEXXA, if approved, will be the first recombinant FXa product and the first reversal agent for direct FXa inhibitors in the world.

ANDEXXA is intended to address an unmet medical need for the reversal of the activity of direct or indirect FXa inhibitors in bleeding patients. Indirect FXa inhibitors are low molecular weight heparins (LMWHs, e.g., enoxaparin) and heparin-related synthetic polysaccharides (e.g., (b) (4)) that inhibit the coagulation process through indirect interference on the reaction between antithrombin III, a serine protease inhibitor, and its target procoagulant enzymes. Antithrombin III is the primary plasma inhibitor of FXa and thrombin (FIIa), and binding to LMWH dramatically increases its innate ability to inactivate FXa and thrombin. Enoxaparin is indicated for prophylaxis and treatment of deep vein thrombosis (DVT), prophylaxis of ischemic complications of unstable angina and non-Q-wave myocardial infarction (MI) and treatment of acute ST-segment elevation myocardial infarction; (b) (4) is indicated for prophylaxis of DVT and treatment of DVT and pulmonary embolism (PE) when administered in conjunction with warfarin.

Direct FXa inhibitors bind and inhibit FXa activity directly, without the involvement of Antithrombin III. In the U.S., FXa inhibitors are approved for the prevention of stroke in patients with nonvalvular atrial fibrillation, prevention of DVT in hip or knee replacement surgery, and

treatment and secondary prevention of venous thromboembolism (VTE) including and/or DVT. 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 first product was approved 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].

Despite the clinical benefit of both direct and indirect FXa inhibitors, these drugs are associated with an increase in bleeding events, some of which are life-threatening or fatal. Protamine is indicated specifically for heparin overdose, with no specific mention of FXa inhibitors or LMWH (enoxaparin or (b) (4) ). Protamine sulfate has partial reversal effect on LMWH activity *in vitro* and the clinical evidence of protamine efficacy is lacking. An effective specific reversal agent for direct FXa inhibitors is not available, and alternative therapies, such as replacement of clotting factors, are considered non-specific, and the safety and effectiveness for this type of use have not been demonstrated in well-controlled clinical trials. Furthermore, infusions of coagulation factors (e.g., recombinant factor VIIa, prothrombin complex concentrate) have prothrombotic effects, and the long half-lives of some coagulation factors contribute to this risk. These facts underscore the urgency of the unmet medical need for an alternative strategy for the reversal of direct FXa inhibitory activities, which has a better benefit/risk profile.

Portola has designed andexanet alfa to serve as a reversal agent for both direct and indirect FXa inhibitors. FXa inhibitors reduce the ability of FXa to activate prothrombin to thrombin (Figure 1B). FXa inhibitors may interact with both free FXa and FXa in the prothrombinase complex with Coagulation Factor Va (FVa) on procoagulant lipid surfaces. Similar to FXa, andexanet alfa forms a 1:1 inactive complex with FXa inhibitors leading to their sequestration from plasma. Andexanet alfa 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. Andexanet alfa also lacks the γ-carboxyglutamic acid (Gla)-containing domain of FXa, thus preventing its incorporation into and inhibition of the prothrombinase complex. The lack of interference with prothrombinase is important for normal thrombin generation because prothrombinase is ~300,000 fold more active than FXa alone. Treatment with andexanet alfa should reduce the concentration of FXa inhibitors, which should result in restoration of normal thrombin generation needed to stop bleeding (Figure 1C).

The ability of andexanet alfa to bind TFPI may also contribute to its procoagulant activity. TFPI is the only known inhibitor of tissue factor (TF) which is a transmembrane glycoprotein responsible for the initiation of coagulation at the site of vascular lesions. Activation of coagulation starts with the formation of a complex between TF and activated coagulation Factor VII (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 tertiary complex of TF, FVIIa, FXa and TFPI, thereby inhibiting coagulation. Although andexanet alfa can bind TFPI, the absence of the Gla domain prevents the subsequent formation of the inactive tertiary complex[2].

[1] Barnes GD, Lucas E, Alexander GC, Goldberger ZD. National Trends in Ambulatory Oral Anticoagulant Use. Am J Med. 2015 Dec;128(12):1300-5.e2. http://www ncbi nlm nih.gov/pubmed/26144101

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

The end result is the acceleration of the generation of FXa and thrombin, as described in Portola's 2014 patent application[3] (Figure 1C).

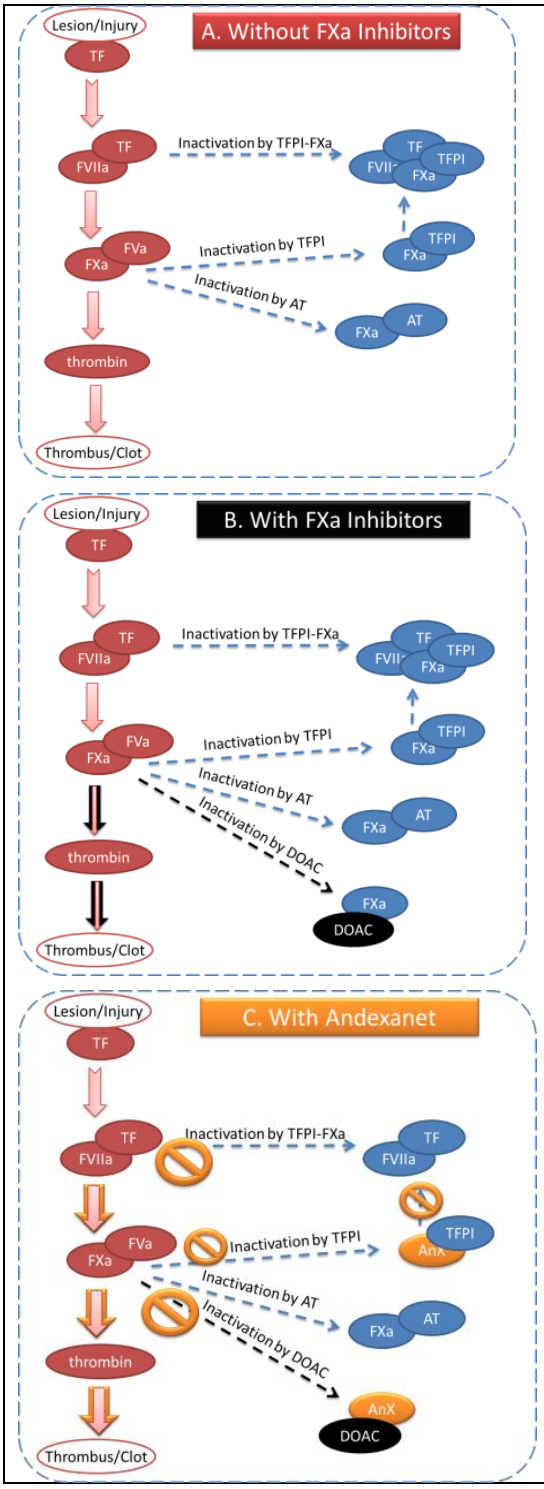


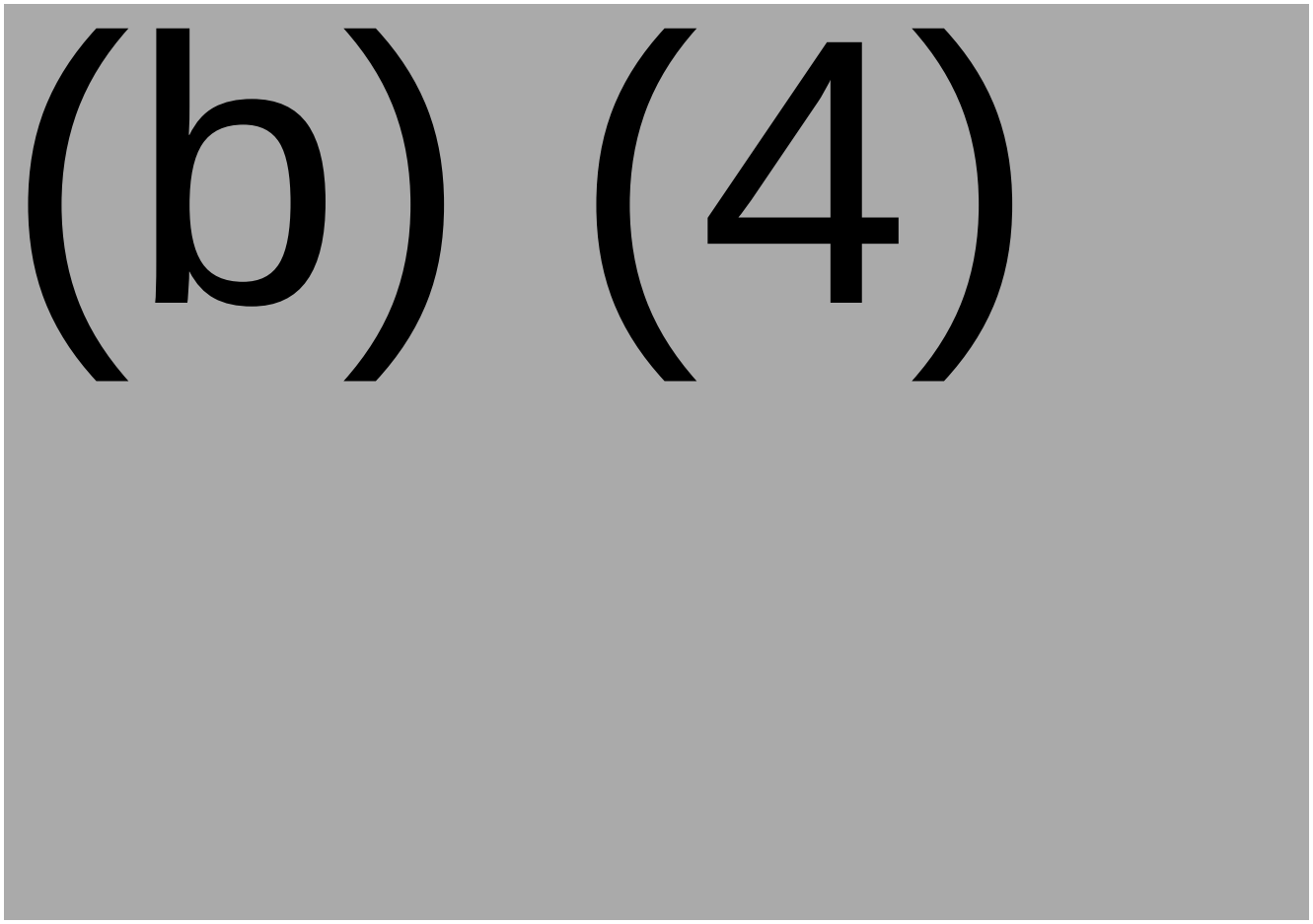

**Figure 1: Mechanisms of Action of direct FXa inhibitors and Andexanet.**

A. Coagulation process without FXa inhibitors and andexanet alfa. Coagulation is initiated by exposure of Tissue Factor (TF) at the site of vascular lesion followed by the formation of the TF-FVIIa complex (extrinsic tenase), 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 hemostatic plugo 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, a stable inactive complex of TFPI-FXa-FVIIa-TF is formed.

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

C. Andexanet alfa (AnX) blocks DOACs leading to restoration of thrombin generation. In addition, AnX inactivates TFPI preventing its inhibition of TF activity. This leads to a faster rate of FXa generation and an elevated rate of thrombin generation.

[3] 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




Since ANDEXXA is intended to address an unmet medical need, it is also qualified for the *Breakthrough Therapy* program. As a result, FDA has counseled Portola frequently to provide guidance on efficient drug development. 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 studies in healthy volunteers in which anti-FXa activity is used as a biomarker. An assay was used to measure the concentration of active FXa inhibitors in blood in these studies. Because the concentration of FXa inhibitor has been shown to be proportional to its anticoagulant action, Portola proposed that the reduction of anti-FXa activity by andexanet alfa is reasonably likely to predict clinical benefit, and could be used as a surrogate endpoint in clinical studies. As part of the accelerated approval pathway for ANDEXXA, Portola has initiated a Phase 3b/4 study (14-505) in patients on FXa inhibitors experiencing an acute major bleed to confirm the correlation between reversal of anti-FXa activity and clinical benefit. However, the determination of safety and efficacy under the *Accelerated Approval* pathway was based on review of data from healthy volunteer studies.

At the time of the writing of this memorandum, FDA is not prepared to give a class approval for the reversal of the activity of direct anti-FXa inhibitors given the differences in pharmacokinetics/ pharmacodynamics (PK/PD) characteristics between the anticoagulants, and will negotiate with Portola for a more limited indication, which may include specifying the particular direct FXa inhibitors. At this time, there are insufficient data to support an Accelerated Approval for the

reversal of anticoagulation with indirect FXa inhibitors, such as enoxaparin because adequate justification for use of a anti-FXa activity surrogate for this product has not been presented by Portola and because the data submitted to support the proposed dose of ANDEXXA is insufficient. In addition, there are also insufficient data to support an indication for perioperative/peri-procedural management of bleeding in patients receiving either direct or indirect FXa inhibitor anticoagulants.

# 3. Manufacturing Process

## *3.1. Manufacturers*

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).

(b) (4)

(b) (4)

### 3.2. Facility inspections

ORA conducted surveillance inspections of (b) (4) from (b) (4) (inspection was classified as VAI), (b) (4) . from (b) (4) (classified as VAI), and (b) (4) from (b) (4) (classified as NAI). *Reviewer's comment: On the advice of LCDR Donald Ertel, DMPQ reviewer, the CMC review team agreed to waive the pre-approval inspections of* (b) (4) *facilities, which have good compliance history and are responsible for the relatively standard downstream stages of the ANDEXXA manufacturing process.*

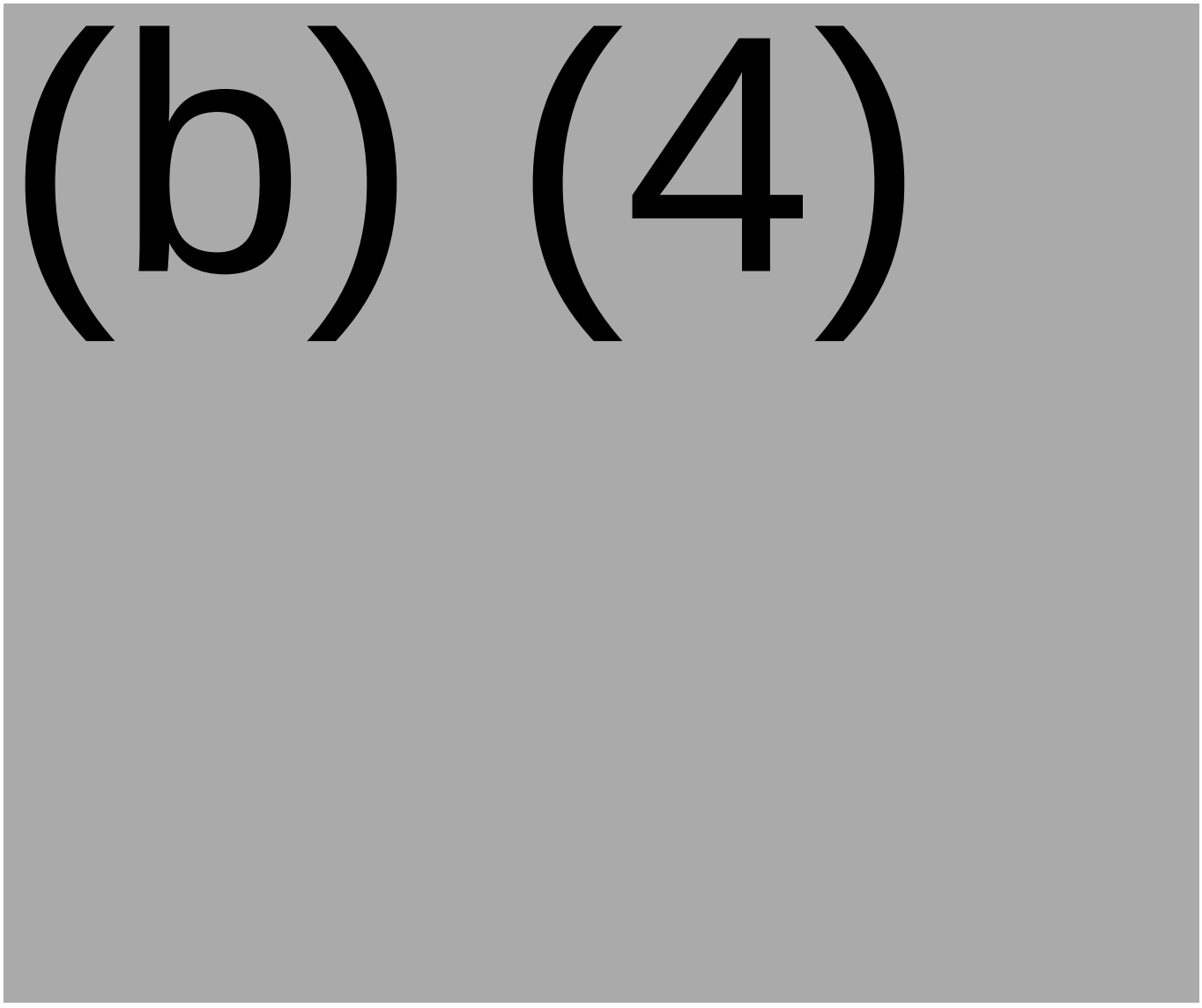
CBER performed a PLI of (b) (4) from (b) (4) covering the manufacturing of drug substance and drug product release testing. At the end of the inspection, CBER issued a Form FDA 483 with four observations. The firm responded to the observations on 16 May 2016 and again on 30 June 2016. The corrective actions were reviewed and found to be inadequate.

*Reviewer's comments: The inspection team consisted of two DMPQ inspectors, LCDR Donald Ertel and Ms. Joan Johnson, and two OBRR product reviewers, Drs. Mikhail Ovanesov and Yideng Liang. During the inspection, I evaluated process validation data, interviewed* (b) (4) *personnel responsible for ANDEXXA process development, implementation and control, and observed several manufacturing unit operations. I was able to observe the upstream manufacturing steps and preparations to downstream purification steps as well as laboratory testing. However, the critical downstream unit operations were not available for observation by FDA inspectors because* (b) (4) *has experienced a series of manufacturing problems which, according to Portola, led to the need for shifting the production schedule away from the previously planned window of FDA inspection. My overall impression was that the ANDEXXA process development was incomplete,* (b) (4) *process is not in a state of control, manufacturing deviations were not properly investigated and written procedures were not followed properly. Three of my objectionable observations were documented in Form FDA 483 issued to* (b) (4) *on* (b) (4) .

*In addition,* (b) (4) *was in complete shut down due to ongoing investigations into repeated bioburden excursions. The ANDEXXA process development was still ongoing as evidenced by the numerous deviations in the process parameters and release specifications. Several critical process deviations were under intense investigations at the time of the inspection. Furthermore, I found that* (b) (4) *has initiated multiple process evaluation studies to address the root causes of these deviations, including the impurity clearance studies and design of experiment investigations to understand the critical process parameters for* (b) (4) *. These deviations and*

*related process investigation studies were not reported in the BLA. Although the* (b) (4) *staff was well trained in general GMP procedures, the operators were struggling with the adoption of new process modifications, especially those related to increased operation burden related to the use of* (b) (4) *in the scaled-up* (b) (4) *(see below). Deviation management was not always handled properly, for example,* (b) (4) *had delayed the opening of official records of major deviations to give Portola time to review the data. Portola then guided* (b) (4) *in writing the initial description of the circumstances related to the deviations. Many of the deviation investigations were incomplete, and the conclusions were in direct contradiction to the available evidence.*

### *3.3. Bulk Drug Substance*



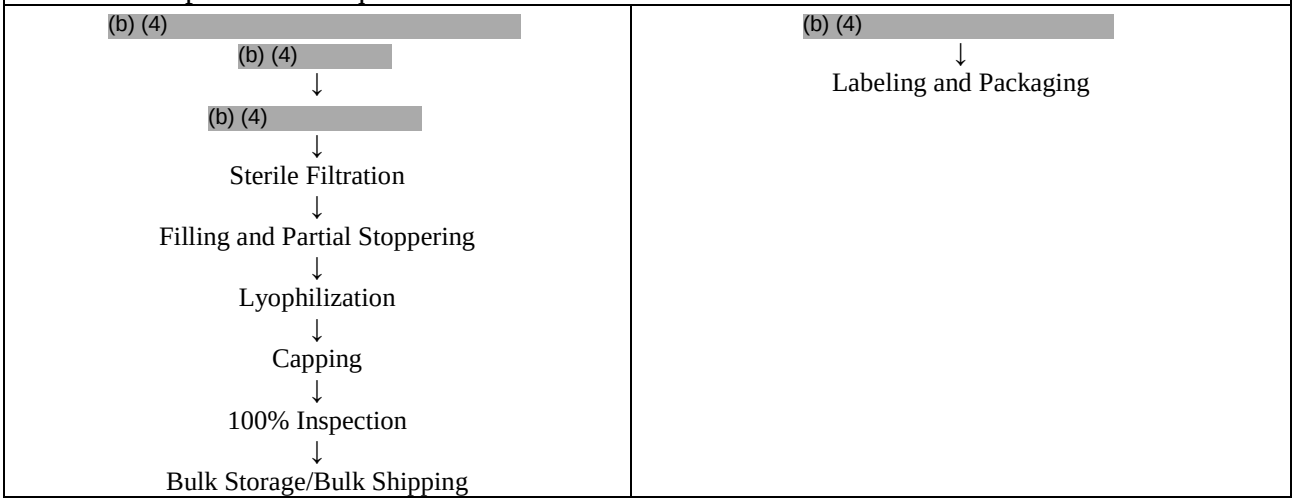

(b) (4)

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

## 3.4. Final Drug Product

A flow diagram for the manufacturing and packaging processes for andexanet alfa FDP is provided in Figure 4.

**Figure 4: ANDEXXA FDP manufacturing and labeling process.** (b) (4) is responsible for manufacturing the lyophilized ANDEXXA, bulk storage and bulk shipping to (b) (4) for primary labeling and packaging. (b) (4) engraves the aluminum caps with a unique batch number.




Between (b) (4) batches are used to manufacture one 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 or powder in single-use glass vials, each containing about 100 mg of andexanet alfa. 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) andexanet alfa, 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.

To date, (b) (4) FDP lots were manufactured comprising one clinical lot ((b) (4)) and (b) (4) PPQ lots ((b) (4)) all of which were released.

*Reviewer's comments:*

a) *Portola also reported* (b) (4) *100-mg vial lots,* (b) (4)*, which were allegedly manufactured using* (b) (4)*. However, the process used was not representative of the proposed* (b) (4) *because* (b) (4) *was an engineering batch and* (b) (4) *was manufactured using a different type of* (b) (4) *(see notes under Table 3).*

b) *I found that the PPQ series was manufactured using a* (b) (4) *scheme, i.e.,* (b) (4)

*Although* (b) (4) *of* ***compliant*** (b) (4) *batches is in principle acceptable,* (b) (4) *compliant* (b) (4) *batches with non-compliant ones is not.*

*A direct GMP violation occurred for PPQ3 FDP batch* (b) (4) *which was produced in November 2015 by* (b) (4) . (b) (4) *was OOS in the release test for the* (b) (4) *by* (b) (4) *and the FDP* (b) (4) *was tested within the* (b) (4) *specifications. Please see Review Memorandum Section* 4. Process Development, Validation and Qualification *for details.*

**Table 3: Andexanet Alfa FDP Process Batch History.** The batches produced by FDP (b) (4) were manufactured at (b) (4) (except for engineering small-scale batch (b) (4) , which was manufactured at (b) (4) ), and the batches produced by FDP (b) (4) were manufactured at (b) (4) .

| Portola Lot # | Process | vial strength (Fill volume) | Date of Manufacture or Release | Batch Designation/Use | BDS lots [e] | Out-of-specification in | Release method [a] | Stability methods [a] | Notes | Real-Time Stability |
|---|---|---|---|---|---|---|---|---|---|---|
| (b) (4) | | | | | | | | | | |

| Portola Lot # | Process | vial strength (Fill volume) | Date of Manufacture or Release | Batch Designation/Use | BDS lots [e] | Out-of-specification in | Release method [a] | Stability methods [a] | Notes | Real-Time Stability |
|---|---|---|---|---|---|---|---|---|---|---|
| (b) (4) | | | | | | | | | | |

*Reviewer's notes:*

** Endotoxin specification is* (b) (4) *. All subsequent batches were below* (b) (4) *.*

*** Particles* (b) (4) *are shown as an example. Other particle counts are also monitored. Note that all subsequent* (b) (4) *batches were below* (b) (4) *(specification* (b) (4) *). (b) (4) batches were within* (b) (4)

a) *The five new methods are:* (b) (4) .
b) *Stability samples were labeled as 10 mg/vial in the stability data sheet, which should be 100 mg/vial or 10 mg/mL.*
c) *Batch was produced using non-validated* (b) (4) *. All subsequent batches were manufactured using* (b) (4) *Glass.* (b) (4) *is classified as a key operating equipment because it is used to determine validated batch volume.*
d) (b) (4) *were submitted for CBER in-support lot release.*
e) *For FDP* (b) (4) *batch was used to manufacture a single FDP batch.*
f) *FDP batch* (b) (4) *was manufactured from* (b) (4) *batch* (b) (4) *. This* (b) (4) *batch was not reported in the BLA or during the PLI.*

**Container Closure System**

The drug product is filled into a 20-mL, clear (b) (4) glass vial having a 20-mm finish ((b) (4) ) and closed with a gray 20-mm (b) (4) and (b) (4) chlorobutyl rubber stopper ((b) (4) ). *Reviewer's comment: The Container Closure Integrity Testing data were reviewed by Dr. Christine Harman, DMPQ. Please refer to her review memorandum for details.*

**Compatibility with intravenous administration components**

Figure 5 illustrates Portola's recommendation of pooling multiple reconstituted vials into a single bag for intravenous (IV) administration of the proposed two dosing regimens for ANDEXXA. The lower dose totaling 880 mg is administered intravenously first in a bolus of 400 mg followed by a 2-hour infusion of 480 mg of the product. The higher dose totaling 1,760 mg is administered intravenously first in a bolus of 800 mg followed by a 2-hour infusion of 960 mg.

An exploratory compatibility study was performed to evaluate the stability and compatibility of the reconstituted ANDEXXA with commonly used IV administration components. The FDP ((b) (4) development batch (b) (4) ) was used to simulate low dose bolus, infusion and storage at (b) (4) exposed to ambient light. The following administration components were evaluated: (b) (4) . No changes in appearance, pH, concentration, (b) (4) and particulate matter were found in comparison with a control consisting of the FDP solution in a glass vial.

*Reviewer's comment: The study is deficient because microbiological attributes were not evaluated. In addition, the study did not utilize the validated versions of release test methods for stability-indicating parameters, e.g.,* (b) (4) *and Direct and Indirect Potency.*

**Figure 5: Portola's recommendation of pooling multiple reconstituted vials into a single bag.** Reference: promotional materials [5].

## 3.5. Definitions of Batch and Scale

[5] Amendment Sequence 61. File: Supplemental Speaker Bureau Slides PP-AnXa-US-0051 Clean Copy.pdf

Table 4 describes the history of BDS and FDP batch and scale definitions for (b) (4) (used in pivotal clinical Phase 3 studies), (b) (4) (proposed commercial process) and scaled-up of (b) (4) ((b) (4) process planned for introduction post-licensure). Only (b) (4) batch was manufactured. In the proposed commercial (b) (4) ) is used to produce (b) (4) leading to one finished BDS (b) (4) . An FDP batch is made of (b) (4) batches. FDP batch size varies typically between approximately (b) (4) vials, depending on the volume of available (b) (4). Portola did not indicate the validated ranges for the fill volumes of (b) (4) FDP. *Reviewer's comment: It is noteworthy that in* (b) (4) *batch was used to manufacture a single FDP batch. For FDP* (b) (4) *, Portola started* (b) (4) *batches without a clear operational need to* (b) (4) *batches. Evidence indicates that* (b) (4) *was introduced to achieve better consistency of* (b) (4) *batches during the PPQ campaign by* (b) (4) *batches of variable quality (please refer to Review Section* 4.4. Process Validation and Evaluation *for details).*

(b) (4)

*Reviewer's Notes:*

*a) Information not provided in the BLA*

*b) A total of* (b) (4) *of* (b) (4) *Batch* (b) (4) *were used for production of* (b) (4) *FDP PPQ batches* [6] *which corresponds to* (b) (4) *of andexanet alfa. This mass exceeds the range for the key* (b) (4) *process parameter* (b) (4) [7].

*c) Includes* (b) (4).

## 3.6. Control of Raw Materials

No materials of animal origin are used in the manufacture of ANDEXXA, except for the andexanet alfa master and working cell banks. Qualification of these banks is described below under the review of Section *4.1. Cell Substrate*.

Portola's BDS contract manufacturer, (b) (4), is responsible for the review and disposition of raw material lots (including excipients, media components, reagents, (b) (4), and filters) used to produce andexanet alfa BDS. Raw materials have been assessed for criticality to the process, and tested and qualified per the Raw Material Qualification Master Program. Prior to release, all raw materials used in the manufacturing process are tested according to (b) (4) in-house specifications and comply with the (b) (4) standards. *Reviewer's comment: During the facility inspection, I reviewed* (b) (4) *procedures for maintaining the list of approved suppliers as well as the plans for supplier audits, all of which were found acceptable.*

The excipients *tromethamine L-arginine HCl, sucrose, mannitol, polysorbate 80,* (b) (4), *sWFI,* and (b) (4) are tested per compendial requirements of the (b) (4) without customization, therefore Portola claims that no validation of the analytical methods is required.

*Reviewer's comment: FDA repeatedly requested development of release methods for excipients. Portola plans to develop these methods no earlier than 31 October 2016. Because excipients are not controlled at release and the qualification of compendial methods for raw materials was not provided, I conclude that excipients are not adequately controlled.*

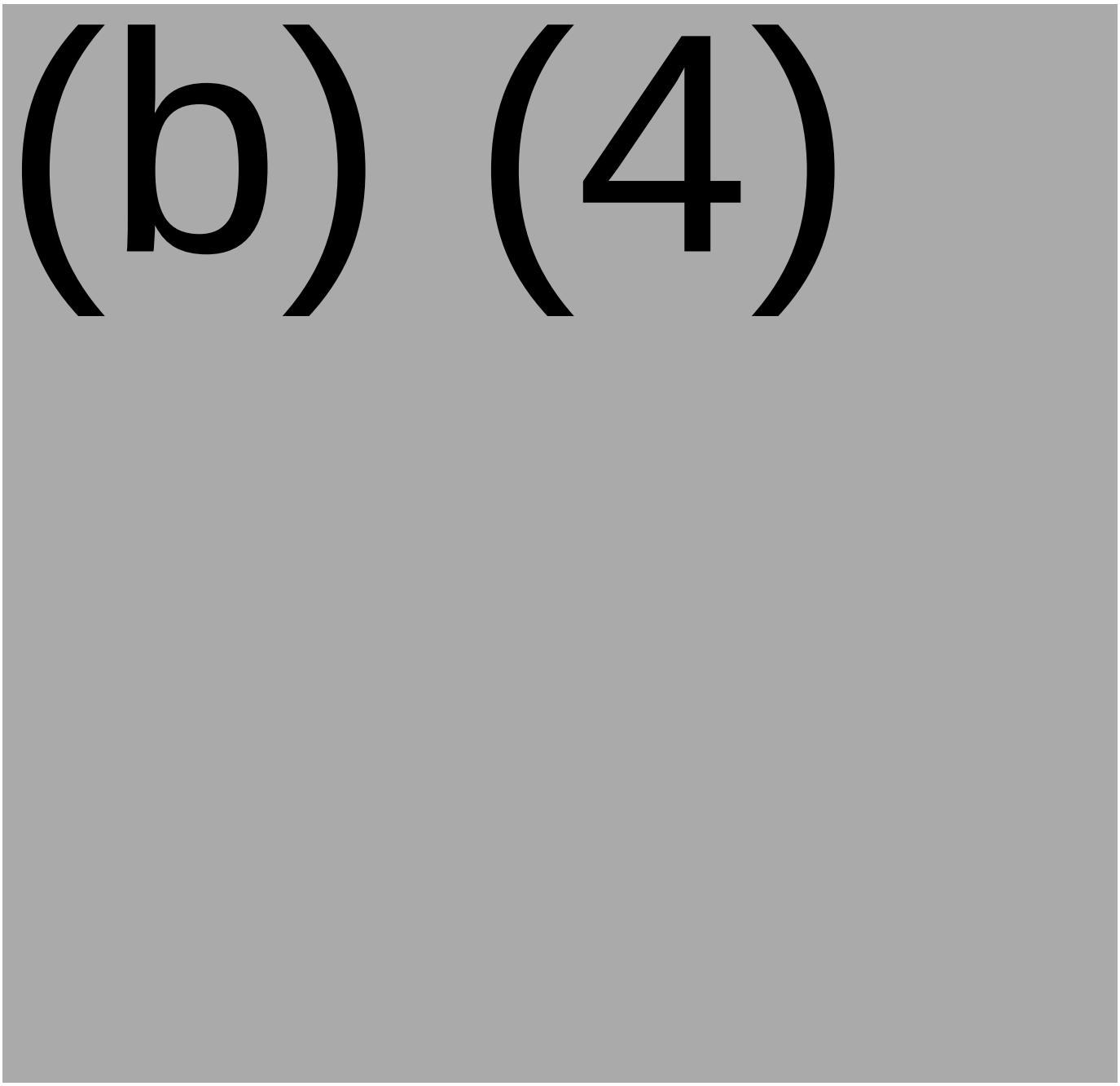
## 3.7. Controls of Critical Steps and Intermediates

There are no process intermediates identified for the manufacture of ANDEXXA BDS and FDP.

The critical process steps and the in-process controls used to monitor these steps in the production of BDS and FDP are listed in Tables 5 and 6, respectively. The manufacturing process is controlled using a Process Control Strategy (PCS). Process controls for each manufacturing step were developed using a risk-based approach to ensure the consistency of the manufacturing process and product quality. Portola used theoretical considerations to choose the in-process parameters that are more likely to affect the product quality attributes.

[6] See below Table : (b) (4) scheme for FDP Process Performance Qualification batches
[7] See below Table 5: ANDEXXA BDS Manufacturing In-Process Controls

The process parameters controlled during BDS manufacturing are classified as either a *Critical Process Parameter* (CPP, variability has an impact on a critical quality attribute [CQA]), a *se* (KOP, essential for process performance but does not affect CQAs) and a *Non Key Operating Parameter* (nKOP, easily controlled or has a wide acceptable limit). In addition, there are two types of performance attributes that cannot be directly controlled but monitored as indicators that the process performed as expected: *In-process Limit* (IPL, confirms consistency of the process at noncritical steps) and *In-process Specification* (IPS, confirms product quality or performance attributes are achieved).



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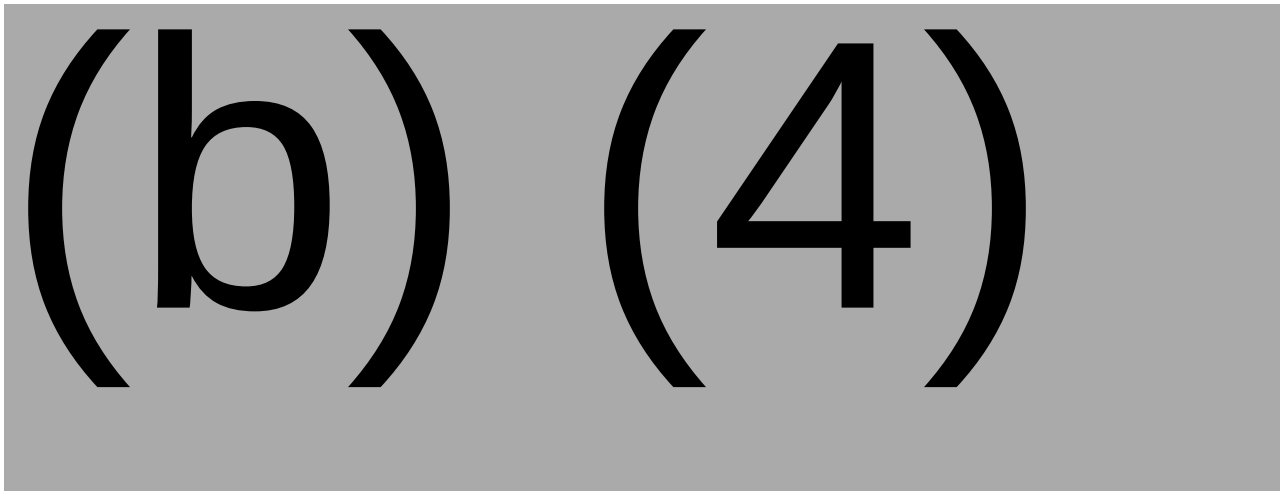


*Reviewer's comments:*

a) *It is noteworthy that many limits for the in-process parameters were based on the manufacturing experience or equipment capability (e.g., observed room temperature range was initially used to define temperature ranges for* (b) (4) *operation) rather than on the prospective process optimization studies which are normally done to define the acceptable boundaries for critical and non-critical in-process parameters. Moreover, because Portola has limited experience with the proposed commercial* (b) (4)*, the in-process control limits were developed using data collected from the investigational* (b) (4) *rather than* (b) (4)*. The use of data from* (b) (4) *was supported by the results of analytical studies that demonstrated general comparability of* (b) (4) *FDP batches manufactured by all* (b) (4) *processes (see review Section* 4. Process Development, Validation and Qualification*).*

b) *The deficiencies of Portola's approach were evidenced from several excursions in process parameters during manufacture following the completion of the BDS PPQ campaign. Portola initiated several small-scale studies to understand the relationship between process parameters and these deviations (summarized in Observation #1 of the FDA Form issued to* (b) (4) *during the PAI in* (b) (4) *). The studies are ongoing.*

*Portola claims*[8] *that at least two of the process parameters,* (b) (4) *and* (b) (4) *, should be upgraded from non-critical to critical because they were found to have a significant impact on the performance of* (b) (4) *steps as well as the quality and stability of the intermediates and finished* (b) (4)*. The scope of in-process control has been updated with the addition of new CPPs which should result in the modification of both the manufacturing process and facilities. For example,* (b) (4) *will implement control of* (b) (4) *at the point of use. The earliest date that this action can be completed is 15 November 2016.*

**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

[8] Revised Form 483 responses submitted in a 30 June 2016 BLA Amendment.

monitoring in the manufacturing process; (2) in-process controls, e.g., t(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 final product 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 contaminating adventitious viruses or transmissible spongiform encephalopathy (TSE) agents is minimized.

The potential of contamination by infectious viruses in cell culture is well controlled in the manufacture of ANDEXXA, which is produced in a genetically modified CHO cell line. (b) (4)

Additionally, the potential risk of viral contamination of ANDEXXA is further mitigated through two dedicated, (b) (4) viral clearance steps: (b) (4)

Portola has evaluated these viral clearance steps in relevant down-scale studies using model viruses. (b) (4)

*<u>Reviewer's comment:</u> Dr. Ze Peng finds these results supportive of the conclusion that the manufacturing process of ANDEXXA is effective in viral clearance. Please refer to his review memorandum for further details.*

## 3.8. Release Specifications, Analytical Methods and Reference Materials

*Reviewer's comment: Analytical method validations for FDP release methods and development of associated reference standards were reviewed by a review team from OCBQ/DBSQC. Release specifications were reviewed by Dr. Andrey Sarafanov. Multiple deficiencies were identified by these reviewers. As summarized below, deficiencies in method validations, reference standards and specifications remain unaddressed. Please refer to their review memoranda for details.*

(b) (4) manufacturer (b) (4) is responsible for all (b) (4) FDP release testing with the exception of FDP tests *Sterility* and *Endotoxin* which are performed by FDP manufacturer (b) (4) and FDP release test for *Particulate matter* and FDP stability test *Container closure integrity* which are performed by (b) (4) in (b) (4). Portola's Quality Assurance (QA) unit is responsible for the final release of (b) (4) FDP batches. The decision to release (b) (4) FDP batches is based on the final GMP documentation, including Certificate of Analysis, batch review reports and lot release reports, issued by (b) (4) and (b) (4), respectively.

*Reviewer's comment: At the PAI inspection of (b) (4) manufacturing facility, I found that (b) (4) conducts internal (b) (4) lot release testing and batch review process per internal GMP procedures which are expected to culminate in the issue of a Certificate of Analysis document which serves as a formal statement of GMP compliance. According to the Quality Agreements between Portola and its contractors (b) (4) and (b) (4), Portola is solely responsible for the final decision to release the (b) (4) FDP batches as well as the disposition of released lots, which includes coordination of (b) (4) shipment from (b) (4) to (b) (4) and shipment of FDP release samples from (b) (4) back to (b) (4). I found that (b) (4) internal release procedures include a provision that a (b) (4) batch should be placed on quarantine and should not be released if manufacturing deviations have occurred and related deviation investigations were not completed [9]. An issue of the GMP compliant Certificate of Analysis is only possible after the deviation investigations (e.g., critical deviations) are closed.*

**Potency**

ANDEXXA is dosed by mass. To ensure consistency of dosage, two potency assays were developed based on the anti-FXa activity assays for direct and indirect FXa inhibitors. The direct potency assay is designed to measure the identity and potency of ANDEXXA based on its ability to bind to direct FXa inhibitor (b) (4) (Portola's investigational product known as (b) (4)) and reverse the inhibition of human FXa in an assay mixture composed of ANDEXXA, human FXa and (b) (4). The indirect potency assay uses (b) (4), an indirect FXa inhibitor (b) (4), in place of (b) (4). In both assays, the restoration of human FXa activity is measured by an FXa-specific (b) (4) substrate, which releases a (b) (4) upon cleavage by FXa. The potency is determined by comparing the response of the test sample to that of the reference standard. The indirect potency assay uses a (b) (4) approach and the direct potency is based on an (b) (4).

[9] 30 June 2016 Amendment Sequence 48, file "3.2.R.2 DEV-1632 Rev 1_ Results for Lot (b) (4) Final.pdf"

*<u>Reviewer's comments:</u> I agree that the potency assays are suitable for characterization of anti-FXa activity reversal since they are relevant to the biomarker, anti-FXa activity, which was used as a surrogate endpoint for clinical benefit in Phase 1, 2 and 3 clinical studies. I noted the different sources of FXa protein in the potency assays for the product release and the clinical assays used to measure anti-FXa activity in patient plasma samples. The product potency assays use human FXa and the clinical assays use* (b) (4) *. The potency assay is acceptable because use of human FXa for potency method is more representative of the interactions of andexanet alfa with patient proteins. However, I identified the following deficiencies which were repeatedly communicated to Portola and have not been addressed to-date:*

1. *Potency assay is calibrated using a standard which was not properly qualified. The clinical assays for direct FXa inhibitors measure anti-FXa activity expressed as a concentration of the respective FXa inhibitor, i.e., in ng/mL of rivaroxaban, apixaban, or* (b) (4) *. This approach is standard for clinical laboratory methods because activity of direct inhibitor standards can be directly and easily traced to the mass concentration of the main pharmaceutical ingredient in the inhibitor drugs. The concentration is determined by the well-established and clinically validated physical and chemical analysis methods. In contrast, an anti-FXa assay for* (b) (4) *which is a biological drug, is calibrated in units of the* (b) (4) *.*

   *ANDEXXA is a biological product and therefore its potency should be defined by a bioassay calibrated with an andexanet alfa potency standard rather than by the mass of this standard. At this time, Portola did not develop a product-specific unit and instead proposed to report potency of the released material as a percent of the potency of the reference standard, which itself is calibrated in mass units. Although several product-specific standards were used since 2010, Portola did not bridge the potencies of these standards. Portola explained* [10] *that qualification of new standard is based on a single determination of new standard's potency by a routine potency assay. A new standard is released if it meets the current lot release specifications which are very wide at this time (*(b) (4) *% for Direct Potency assay and* (b) (4) *for Indirect Potency assay). I conclude that the continuity of the potency unit cannot be assured under these manufacturing practices.*

   *To ensure consistency of the potency, stability and integrity of the ANDEXXA primary product-specific standard, FDA asked Portola to develop a product-specific activity unit, and monitor the standard's stability using the* (b) (4) *as controls. Portola plans to develop product specific unit by 31 October 2016.*

2. *Since inhibition of TFPI is an important additional mechanism of action of ANDEXXA, FDA asked Portola to investigate this activity in* (b) (4) *batches and include an assay to assess it as part of control of product quality. Portola has not agreed to develop such assay.*

[10] See 27 June 2016 amendment, BLA sequence 45; Portola's response to 13 June 2016 FDA Question 1.

**Release Specifications**

Table 7 lists 5 new quantitative release methods, (b) (4) , and the (b) (4) methods (direct inhibitor potency, indirect inhibitor potency) which were introduced less than one year ago, for the (b) (4) FDP PPQ campaigns. The specifications of (b) (4) FDP are summarized in Tables 8, 9 and 10 below.

The methods and specifications are established based on limited manufacturing experience and theoretical safety considerations. Specifications have not been revised since the PPQ campaign with the exception of the (b) (4) specification for the (b) (4) method (discussed in detail below).

Portola developed specifications for FDP using (b) (4) data, and (b) (4) specifications were based on only (b) (4) batches with additional supportive data from (b) (4) batches (up to (b) (4) and (b) (4) FDP batches). However, no (b) (4) batch release experience was available for the 5 quantitative potency and purity release methods listed in Table 5 because these methods were not available during release of all (b) (4) batches and the pre-PPQ (b) (4) batches. Specifications for these 5 methods were derived from the retrospective analyses of older batches as described in the method bridging studies in which new methods were compared side-by-side with the previous non-quantitative versions of the same methods.

**Table 7: New Quantitative Andexanet Alfa-Specific Methods for (b) (4) FDP Release and Stability Testing**

| New Methods for Commercial DS | Rationale for Change |
|---|---|
| (b) (4) | Existing method optimized to simplify quantitation of andexanet alfa variant (b) (4) |
| (b) (4) | Existing method modified to validate (b) (4) for quantitative results |
| (b) (4) | Existing method modified to validate (b) (4) for quantitative results |
| Direct Inhibitor Potency Assay | More robust than the existing (b) (4) method. Supplemental identity test per FDA request |
| Indirect Inhibitor Potency Assay | New (b) (4) assay to indirect FXa inhibitors per FDA request |

(b) (4)

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*f)* (b) (4)

**Table 9**: **FDP Specifications**

| **Test Method** | **Parameter monitored** | **Acceptance Criteria** |
|---|---|---|
| Visual Appearance | Characteristics | White to off-white lyophilized cake |
| Reconstitution Time | Characteristics | (b) (4) |
| Moisture Content per (b) (4) | Purity | (b) (4) |
| Sterility 1 per (b) (4) | Purity | Sterile |
| Endotoxin per (b) (4) | Purity | (b) (4) |
| Appearance after Reconstitution | Characteristics | Clear, colorless to slightly yellow solution, essentially [f] free of visible particulates. |
| pH per (b) (4) | Characteristics | 7.8 (b) (4) |
| (b) (4) | Characteristics | (b) (4) |
| Direct Potency | Identity and Potency | |
| Indirect Potency | Potency | |
| Protein Concentration by (b) (4) | Potency [c] | |
| Purity by (b) (4) | Purity | |
| (b) (4) | Purity | |
| (b) (4) | Purity | |
| (b) (4) | Purity | |
| Particulate Matter per (b) (4) | Purity | |

(b) (4)

*Reviewer's notes:*

a) *Specification for endotoxin is not supported by manufacturing capability but derived using the allowable pharmacopeial limit per a 2,000-mg dose (current dose is 1,700 mg) which, according to Portola, translates into a 1,300 mg/hr for a 70-kg individual. This approach is not acceptable because higher doses are to be explored in clinical trials. Portola responded that tighter specification cannot be introduced because the method is not sensitive enough. Portola stated that they will develop a better method by 31 October 2016.*
b) *Specifications are too wide. In addition, FDA requested Portola to develop product-specific potency units to replace the "*(b) (4)*" unit. This will ensure continuity of unitage of the potency standards.*
c) *"Strength" will be more appropriate because ANDEXXA is dosed by mass.*
d) *Portola indicated that insufficient data exist to develop numerical specifications at the time of BLA submission. FDA will request numerical specifications to be based on the analysis of batches which were manufactured after the BLA submission.*
e) *Tighter specification limits (*(b) (4)*) were used during the PPQ campaign. During the PLI, FDA found that PPQ specifications are still used by the* (b) (4) *facility. Several OOS results were experienced during the release of* (b) (4) *lots and in stability studies. The investigations into the root causes for the increased* (b) (4) *are incomplete at this time. FDA disagrees with Portola's proposal to widen the* (b) (4) *specifications until the investigations are completed.*
f) *"Essentially free of visible particles" is not acceptable. The specifications should read "free of visible particles" because lots with visible particles may indicate issues with protein solubility.*

**Table 10**: **Additional** (b) (4) **FDP Specifications Requested by the FDA**

| **Test Method** | **Parameter monitored** | **Acceptance Criteria** |
|---|---|---|
| Identity by (b) (4) | Identity ((b) (4)) | To be developed by Portola[a] by 31 October 2016 |
| (b) (4) | Purity ((b) (4)) | To be developed by Portola[a] by 31 October 2016 |
| Sucrose | Purity (FDP) | To be developed by Portola[a] by 31 October 2016 |
| Mannitol | Purity (FDP) | To be developed by Portola [a] by 31 October 2016 |
| Polysorbate 80 | Purity (FDP) | To be developed by Portola [b] by 31 October 2016 |
| Anti-TFPI activity by thrombin generation assay | Potency (FDP) | Portola does not agree [c] |

*Reviewer's notes:*

a) *An IR was sent to Portola on 6 April 2016 and again on 22 June 2016. Portola agreed to develop specifications using their existing partially validated assays by 1 August 2016 and committed to complete the validation of the methods by 31 October 2016.*
b) *An IR was sent to Portola on 6 April 2016 and again on 27 June 2016. Portola agreed to develop this method by 31 October 2016.*

c) *Portola claims that TFPI inhibition is a minor mechanism of ANDEXXA action. This claim contradicts with the data obtained in the Phase 3 trial which demonstrated that sustained procoagulant effect of ANDEXXA as measured by the thrombin generation assay (TGA) was mediated by the anti-TFPI action of the drug. Therefore, FDA will propose Portola to use a new potency assay which is based on the TGA method.*

*Reviewer's comments:* *The proposed specifications are not acceptable for the following reasons:*

1. *The proposed* (b) (4) *specifications are too wide and should be revised with the analysis of the available* (b) (4) *data which now include at least* (b) (4) *batches and* (b) (4) *FDP batches since the introduction of the proposed release methods and were reported to the FDA during the PLI in April of 2016. Furthermore, as it is customary in the review of original BLAs, Portola should make a commitment to re-evaluate the acceptance limits of release parameters after Portola has obtained data from* (b) (4) *batches of FDP or* (b) (4) *post licensure, whichever comes first.*

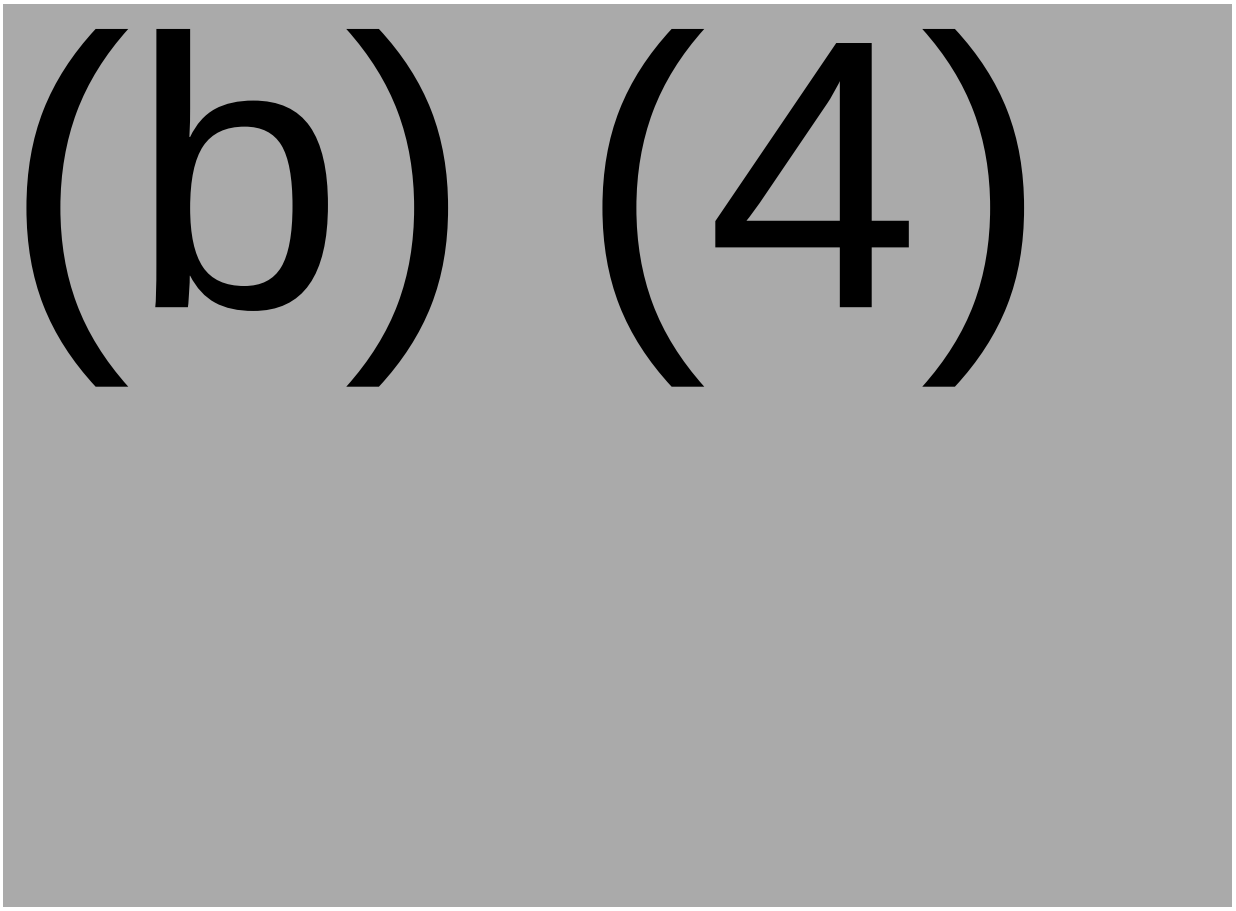
2. *The use of* (b) (4) *data to support* (b) (4) *specifications requires additional justification because the* (b) (4) *were found at higher levels in routine* (b) (4) *batches in comparison with the* (b) (4) *batches used in the pivotal safety and efficacy clinical trials, see* (b) (4) *trending on Figure 6. The root cause for the differences in* (b) (4) *between* (b) (4) *should be identified and the potential impact on the other parameters of the release specifications should be evaluated.*

3. *I disagree with Portola's proposal to revise the* (b) (4) *specification for the* (b) (4) *from* (b) (4) *to* (b) (4) *. This is the only specification revised after the PPQ campaign. Portola claims that the revision of* (b) (4) *specification was needed to align it with new process data: "*As additional lots have been manufactured, the specifications for (b) (4) methods have been revised, applying statistical analysis to include the additional manufacturing release data with the expectation that 95% of the population will fall within the interval with 99% confidence*." However, during the inspection, I found that* (b) (4) *batch* (b) (4) *(*(b) (4) *number* (b) (4) *) was OOS for the* (b) (4) *on 09 November 2015, and the investigation on this deviation was ongoing. Portola claims that the investigation was closed on 19 April 2016. However, although* (b) (4) *was identified as a contributing factor for the differences in* (b) (4) *content at different stages of product development, the real root cause for the formation of the* (b) (4) *was not identified, please refer to review section* 5.2. Characterization of Process-related Impurities *for details.*

   *In addition, insufficient evidence from clinical trials is available to support the proposed specifications for the* (b) (4) *which are* (b) (4) *wider than those batches used in the clinical trials. Portola used* (b) (4) *batches for the pivotal safety and efficacy Phase 2 and 3 studies (for detailed discussion, please refer to section Process Development).* (b) (4) *was introduced for use in the Phase 4 studies in the spring of 2016 and limited safety and efficacy data are available at this time.*

*Finally, analysis of Figure 6 indicates that the new* (b) (4) *specification was based on the analysis of batches manufactured using the non-validated* (b) (4) *process.*

4. *Numerical specifications should be developed for all* (b) (4) *reported by methods* (b) (4) *. Similar to the analyses of the* (b) (4) *in Figure 6, the new quantitative methods should be used to re-analyze all* (b) (4) *batches and the data should be analyzed for consistency with* (b) (4) *and presented in the BLA.*




**Analytical Methods**

Portola informed FDA that the validation of (b) (4) *content, sucrose, mannitol* and *Polysorbate 80* methods is ongoing and will be completed by 31 October 2016. The non-compendial release methods were validated for their suitability for the intended use. The compendial release test methods were qualified for their intended use and comply with their respective (b) (4) . Portola's contract (b) (4) manufacturer (b) (4) is responsible for the following compendial (b) (4) FDP methods: *Visual appearance* (FDP only), *Moisture content* (FDP only), *pH*, (b) (4) , *bioburden* and *endotoxin* (FDP only). (b) (4) performs the

[11] 30 June 2016 Amendment Sequence 48, file "3.2.R.2 DEV-1632 Rev 1_ Results for Lot (b) (4) Final.pdf"

FDP release methods *Sterility* and *Endotoxin* and (b) (4) is responsible for the FDP release methods *Particulate Matter* and *Container closure integrity testing.*

A CHO (b) (4) kit from (b) (4) is used to measure (b) (4) in andexanet alfa (b) (4).

*Reviewer's comment: Portola proposed to develop a process-specific assay as a post-marketing commitment but did not provide a time line, which they should. In addition, Portola should perform bridging studies between the existing and new* (b) (4) *methods using samples from all available* (b) (4) *FDP batches.*

**Reference Standards and Materials**

The key reference standards are the product reference standards (stored at (b) (4) ) and the (b) (4) antibody standard.

*Reviewer's comment: A commercial* (b) (4) *standard is not representative of the* (b) (4) *found in ANDEXXA because the cells have different genetic make-ups, for example, the ANDEXXA cell line is prepared from cells* (b) (4).

The initial andexanet alfa reference standard Lot # (b) (4) was prepared from a developmental lot of andexanet alfa (b) (4), Lot (b) (4) , in May 2011. This reference standard was manufactured at a concentration of (b) (4) formulated in (b) (4) Tris, (b) (4) L-arginine HCl, (b) (4) (w/w) sucrose, 0.01% (w/w) polysorbate 80, pH 7.8. Following the change in the manufacturing process to (b) (4) , a new reference standard Lot # (b) (4) ((b) (4) number (b) (4) ) was qualified in November 2015. It was based on (b) (4) Lot (b) (4) manufactured in December 2014 and formulated at 10 mg/mL in 10 mM Tris, 45 mM L-arginine HCl, 2% (w/w) sucrose, 5% (w/w) mannitol, 0.01% (w/w) polysorbate 80, pH 7.8.

*Reviewer's note: During the PLI, I found that reference standard Lot #* (b) (4) *was OOS for pH (pH* (b) (4) *outside of the specification criterion of* (b) (4) *) on 16 March 2016. This OOS was not reported in the BLA. The investigation is ongoing.*

Portola did not bridge the first and the second potency standards. The following release data for the current reference standard Lot # (b) (4) : (b) (4) by the old direct potency (kinetic) assay, (b) (4) by the direct potency assay (endpoint) gave and (b) (4) by indirect potency assay. The accuracy of potency assignment was not established.

*Reviewer's comments: The following deficiencies with the respective reference standards for purity and potency were identified and remain unresolved:*

a) *Because Portola was presenting* (b) (4) *in* (b) (4) *of the assay standard and because the potency assay was changed less than a year ago, no assessment of the linkage and comparability of product potency between* (b) (4) *or during stability studies was provided.*

b) *At this time, it is not clear which reference standard Portola uses for product release. On 15 July 2016, Portola informed the FDA that the current reference standard will not be provided for use by the FDA Lot Release branch because this standard is not available. Below is a direct quote from Ms. Janice Castillo, Portola's Senior Vice President, Regulatory Affairs and Quality Assurance, in an email on 15 July 2016 to Lt Thomas J. Maruna, FDA's Senior Regulatory Management Officer:*

> PTLA can provide GMP10 (PTLA lot (b) (4) ) bulk material (parent (b) (4) of vialed ref std lot (b) (4) ). PTLA does not vialed ref std. If (b) (4) is acceptable, we will make arrangements to send this material accompanied by GMP10 (b) (4) CofA and not the RS CofA the beginning of next week

*On 22 July 2016, CBER received Portola's standard* (b) (4) *which was manufactured on 02 May 2016, only 13 months after the introduction of the previous standard. No bridging studies were reported to demonstrate the comparability of all predecessor reference standards.*

**In-support testing**

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 (Figure 7) when the (b) (4) is used instead of (b) (4) is used in the (b) (4) . The significance of this issue is uncertain at this time.

**Recommendation for 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.

However, ANDEXXA is developed under the *Expedited Programs for Serious Conditions* and Portola has limited experience with the product and manufacturing process. In addition, major manufacturing changes are being developed to be introduced within months after the ADD. Therefore, FDA is proposing to place ANDEXXA on either CBER Lot Release or surveillance to ensure product quality and process consistency.

*Reviewer's comment: I am supporting placing this product on CBER Lot Release/Surveillance. CBER Lot Release/Surveillance will provide additional assurance of product quality and will be helpful to Portola. We need this additional assurance because:*

a) *The specification limits are only partially supported with the manufacturing experience. ANDEXXA product was under an accelerated development which means that Portola has limited knowledge with the product and the manufacturing process. The FDA lot release data can be helpful in bridging the knowledge gaps.*

b) *Major manufacturing changes are going to be introduced within months after the ADD. Portola is planning to submit several CMC Supplements to increase the manufacturing*

*scale. The FDA lot release data can provide an independent analytical assurance of product consistency.*

c) *There is a precedent of placing recombinant product on the lot release program: STN BL 125248/0 for Thrombin topical (Recombinant) RECOTHROMT, see quote from the approval letter:* ***"Thrombin topical (Recombinant) is exempt from the lot release requirement of 21 CFR 610.2.*** (b) (4) ***"***

(b) (4)

# 4. Process Development, Validation and Qualification

## *4.1. Cell Substrate*

(b) (4)

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(b) (4)

To address an anticipated product shortage if the BLA is approved in August 2016, Portola intends to introduce a scale-up version of (b) (4) denoted as (b) (4) . In the (b) (4) process, a new suite at (b) (4) in (b) (4) will be added and the (b) (4) will be replaced with (b) (4) . The scale of all unit operations will be (b) (4) of the current (b) (4) process. The corresponding FDP process will be scaled-up as well and represent an approximately (b) (4) of the current FDP process, which will include additional lyophilizers at the (b) (4) facility in (b) (4) .

Portola had planned to include (b) (4) in the original BLA but was dissuaded by FDA. Instead, Portola submitted in the BLA a Comparability Protocol (CP) which outlines its plan to demonstrate the comparability of the existing (b) (4) and the scaled-up (b) (4) . Along with the CP, Portola requests a downgrade of reporting category for the supplement for the introduction of (b) (4) .

*Reviewer's comment: At this time, FDA has identified several deficiencies in the CP, e.g., absence of well-defined criteria of comparability, and does not consider the CP to be suitable to support a downgrade of the supplement to add* (b) (4) *for the manufacture of ANDEXXA* (b) (4) *FDP. Please refer to review section* 4.3. Process comparability studies *below.*

## *4.3. Process comparability studies*

(b) (4) **to** (b) (4) **Comparability**

The main process changes with the potential for an adverse impact on product quality were introduction of (b) (4) . To evaluate the impact of these changes on product quality, purity and potency, a comparison of (b) (4) FDP was performed using the release test data, (b) (4) ((b) (4), to compare interactions of andexanet alfa with FXa inhibitors), (b) (4) for the reversal of the (b) (4) (a non-validated version of the Indirect Potency assay), reversal of inhibition of TF-initiated thrombin generation in human plasma by direct and indirect FXa inhibitors (a pharmacodynamics method used in Phase 1 and 2 clinical trials), reversal of inhibition of non-TF-dependent thrombin generation in human plasma induced by direct and indirect FXa inhibitors (this method is not sensitive to anti-TFPI action of andexanet), interaction with TFPI, and a pharmacokinetic study in (b) (4) monkeys. The results of these studies provided evidence that the dosage form change to a lyophilized formulation (used in the pivotal Phase 3 clinical trials) resulted in a product that is comparable to the (b) (4) FDP that was used in toxicology and in the Phase 1 and 2 clinical trials. According to Portola, the lyophilized FDP appeared comparable in its identity, purity, potency, and impurity profile to (b) (4) formulation.

*Reviewer's comment: Although Portola's investigations demonstrated analytical comparability of the* (b) (4) *and lyophilized* ((b) (4) *) FDP materials, Portola later found that the lyophilized formulation was more immunogenic in humans. On 11 November 2016, Portola stated that*

"The initial (b) (4) formulation had a very low rate of confirmed low titer non-neutralizing antibodies against andexanet (2%) while the rate observed for the lyophilized formulation was higher (20%). The overall rate of confirmed anti-andexanet antibodies was 12.1%" *Reference: Document entitled "Immunogenicity Assessment of Andexanet Alfa", see amendment Sequence 98 to IND 15089* [12].

*At that time, there were 30 confirmed ADAs observed against andexanet out of 247 andexanet alfa treated subjects (plus one confirmed antibody that was a placebo-treated subject). For* (b) (4) *, confirmed ADAs were observed in 2 out of 102 treated subjects; for lyophilized formulation: 28 out of 146 subjected.*

*Although the cause for the increased immunogenicity was not definitively identified, I conclude that* (b) (4) *material is reasonably likely non-comparable to* (b) (4) *material. Because the proposed commercial product is lyophilized, in the description of product safety in the product labeling, I propose to exclude immunogenicity data obtained with the* (b) (4) *formulation (*(b) (4) *material) in clinical studies 11-501, 12-502 Module 1 (apixaban), 12-502 Module 2 (rivaroxaban), and 12-502 Module 3 Cohort 1 & 2 (enoxaparin).*

*Portola claims that "*these ADAs do not appear to pose a risk to patients who will receive andexanet administration to stop excessive bleeding due to anticoagulation*." I defer to clinical reviewers to assess the clinical implications of this finding*

**Comparability study between** (b) (4) **and** (b) (4)

A comparability study was conducted to demonstrate that (b) (4) batches manufactured on (b) (4) and (b) (4) are comparable. All (b) (4) release specifications were met for (b) (4) produced using both (b) (4) and (b) (4) and Portola concluded that these two lines are considered interchangeable. These conclusions are supported by the comparability of the equipment utilized by these two lines. Process parameters are identical for both lines.

(b) (4) **to** (b) (4) **Comparability**

Comparability of (b) (4) and (b) (4) was assessed in FDP (b) (4) studies. FDP study evaluated data from release testing, supplemental side-by-side testing using quantitative lot release methods described in Table 7 above, side-by-side stability testing of (b) (4) lots and (b) (4) batch ((b) (4) ) at the storage temperature (2-8°C) and under (b) (4) conditions ((b) (4) ), and formal stability testing (not side-by-side) for (b) (4) batch (b) (4) at the storage temperature (2-8°C) and under (b) (4) conditions ((b) (4) ) with time-points aligned with the side-by-side stability testing using the same methods. The results of the analytical testing met all acceptance criteria and indicated that (b) (4) FDP is comparable to (b) (4) FDP.

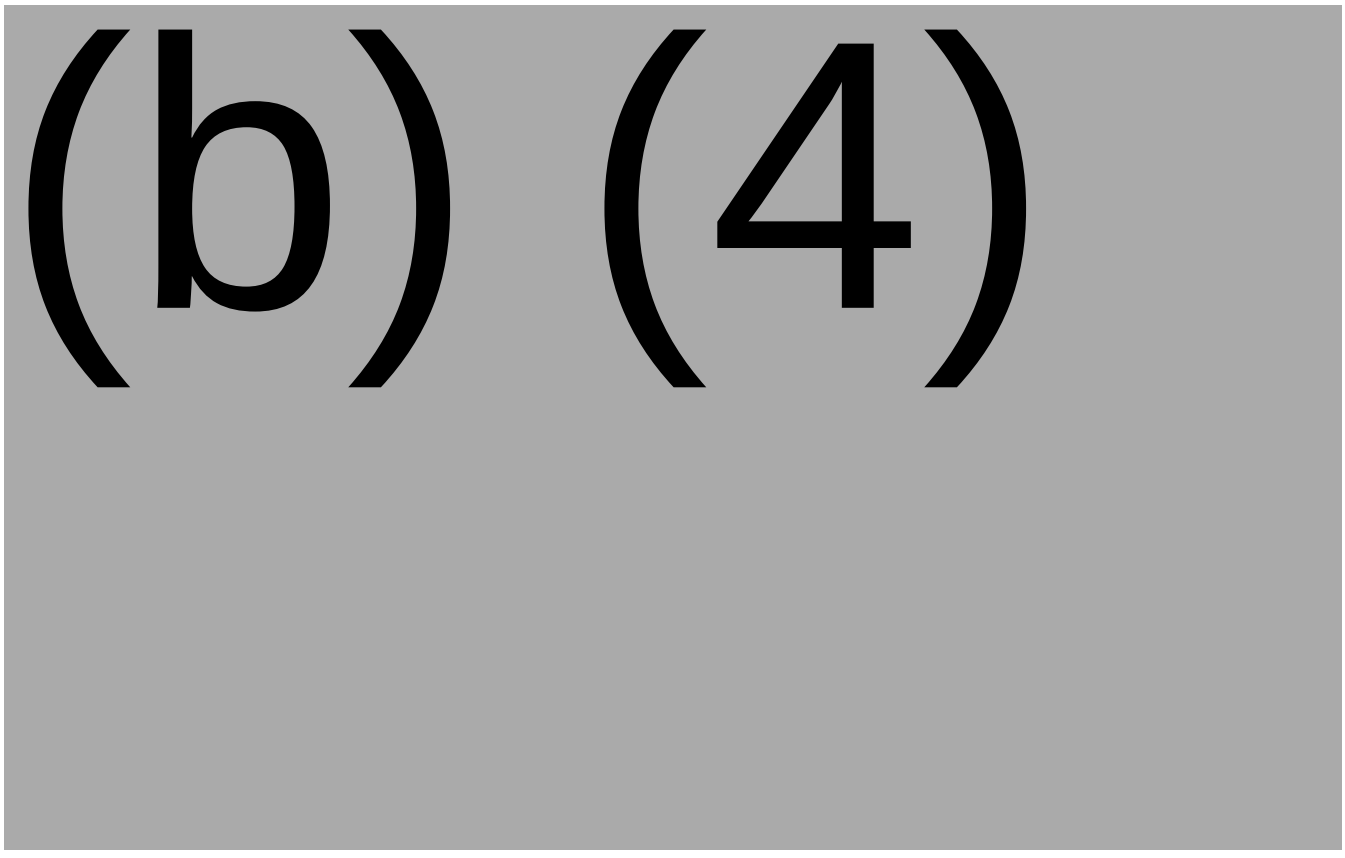
(b) (4) comparability was assessed in a formal *Comparability Report NC-15-0614-R0001* using (b) (4) batches ((b) (4) ) and (b) (4) batches from (b) (4) . The comparability evaluation was based on assessments from three categories of testing: (1) Comparison of results from release testing of lots from each process and retrospective tolerance

[12] Document entitled "Immunogenicity Assessment of Andexanet Alfa", see 11 November 2016 amendment Sequence 98 to IND 15089.

interval criteria-based evaluation of historical batch data from (b) (4), (2) comparison of additional select physicochemical characteristics in side-by-side analysis of lots from each process and Reference Material as comparator, and (3) side-by-side stability comparison of (b) (4) profiles using results obtained from (b) (4) from a side-by-side stability study.

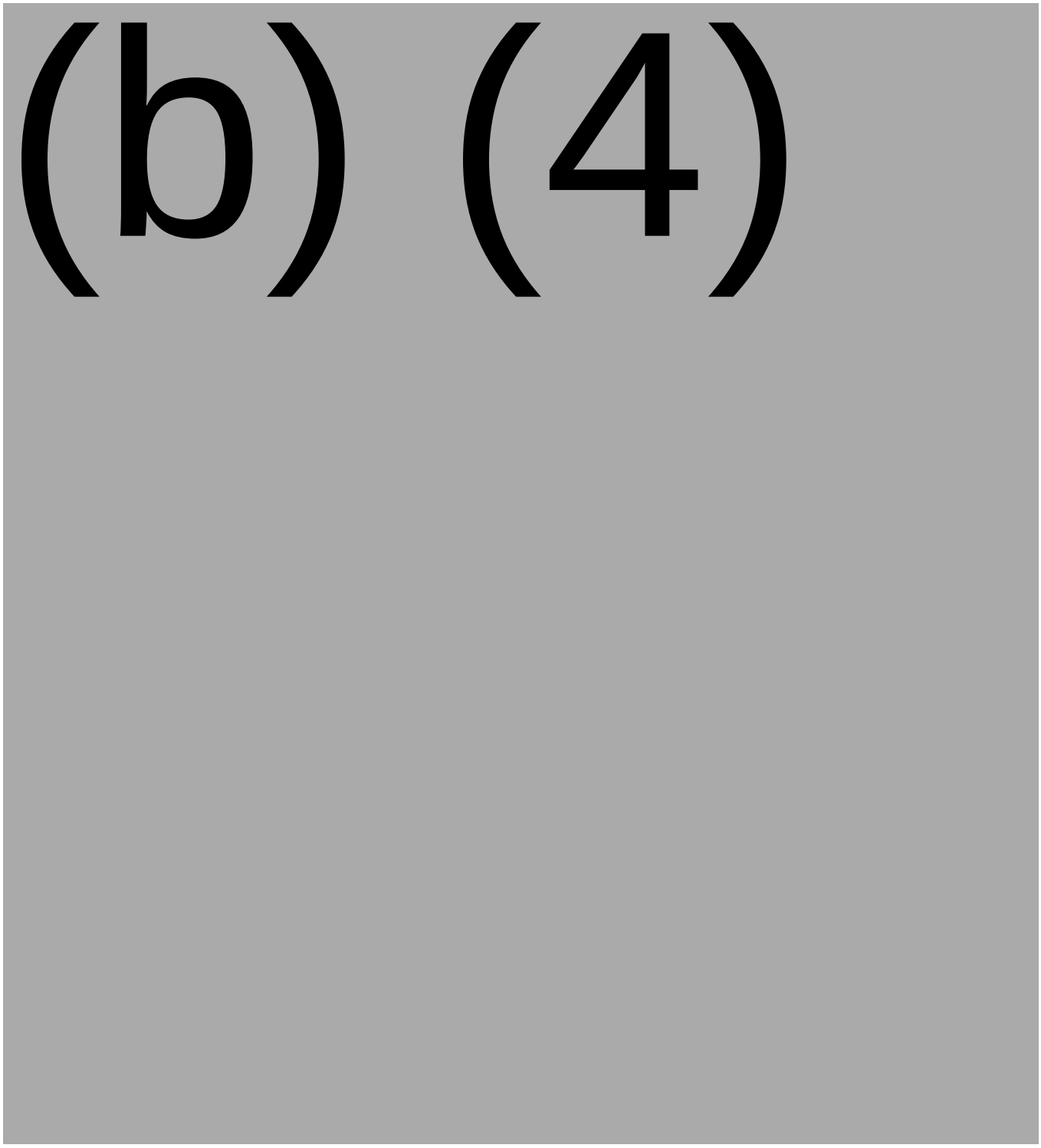
All (b) (4) investigations suggested comparability of (b) (4) from (b) (4) with the exception of (b) (4) at release and (b) (4) rate of (b) (4) studies in (b) (4) vs. (b) (4) batches. The (b) (4) levels measured by the (b) (4) assay exceeded the shelf-life specifications of (b) (4) at the (b) (4) stability time-point of (b) (4) for (b) (4) lots from (b) (4) (Figure 8). This is most likely due to the (b) (4) in the lots produced by (b) (4).

The increase in the (b) (4) was seen in batches from both processes, but the (b) (4) were (b) (4) in the (b) (4) batches (Figure 9A). The (b) (4) in (b) (4) was confirmed by two (b) (4) methods, (b) (4), and was accompanied by a (b) (4) in the (b) (4) identified by both methods (Figures 9B and 10B). Portola did not investigate the accelerated stability beyond a time-point of (b) (4). Preliminary results from the real-time stability studies did not demonstrate an obvious beta trend by the (b) (4) time-point but these stability studies are ongoing.




[13] NC-15-0614-R0001_ Andexanet Alfa DS Comparability Report.pdf

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




*<u>Reviewer's comments</u>: Higher levels of the* (b) (4) *in* (b) (4) *vs.* (b) (4) *batches is consistent with the batch analyses presented in Figure 6 above. Portola failed to identify the root cause for the* (b) (4) *difference in* (b) (4) *batches and claimed that the investigations are not needed because the* (b) (4) *are expected to be fully functional, please refer to review section* 5. Elucidation of Structure, Function and Impurities *for details.*

*I conclude that the comparability of* (b) (4) *and* (b) (4) *batches has not been established. The implications for process consistency, safety and stability should be investigated. Specifically,*

a) *I disagree with Portola's conclusion that the* (b) (4) *in the* (b) (4) *has no impact on product safety, purity and efficacy. Of particular concern is a possibility that an unknown* (b) (4) *impurity is present at higher levels in the* (b) (4) *batches, please refer to review section* 5.2 Characterization of Process-related Impurities *for details.*
b) *I recommend commencing additional process evaluation and development studies to reveal the sources of the increase in the* (b) (4) *during manufacturing and under accelerated storage conditions.*
c) *I also recommend performing some nonclinical, and possibly clinical, studies to look at a high dose toxicology and PK study in animals to compare materials from* (b) (4) *and* (b) (4) .

**Comparability Protocol "Andexanet Alfa (PRT064445)** (b) (4) **to** (b) (4) **Resulting Drug Product".**

To support post-licensure introduction of a modified scaled-up version of (b) (4) termed "(b) (4) ", Portola submitted in the BLA a *Comparability Protocol: Andexanet Alfa (PRT064445)* (b) (4) *to* (b) (4) *Resulting Drug Product*. The CP describes major changes in the manufacturing processes of the (b) (4) FDP, specifically the introduction of (b) (4) , the use of (b) (4) lyophilizers, and (b) (4) lyophilizers.

*Reviewer's comment: The protocol is deficient and does not follow the recommendations provided in the FDA Guidance on Comparability Protocols [16]. Therefore, the CP will not support a downgrade of the submission for* (b) (4) *from a Prior Approval Supplement to a CBE-30 Supplement. I found that*

a. *The CP does not describe nor takes into consideration the totality of data gathered in process and product development. This includes* (b) (4) *failed* (b) (4) *Process PPQ campaigns and repeated excursions which had resulted in the termination of* (b) (4) *out of* (b) (4) *of initiated* (b) (4) *lots.* (b) (4) *was out of operation during the PLI on* (b) (4) *. FDA inspectors had reviewed the investigations of several* (b) (4) *deviations and informed Portola and* (b) (4) *that* (b) (4) *was not in a state of control as was evidenced from* (b) (4) *inability to consistently manufacture* (b) (4) *lots in accordance with established process parameters.*

b. *The CP does not provide sufficient information on the substantive differences in equipment used in* (b) (4) *. For example, during the PLI in* (b) (4) *provided evidence that* (b) (4) *deviations were caused by deficiencies in the cleaning procedures of the new equipment in* (b) (4) *. Therefore, the revised CP should address the need for new validation studies or abbreviated bridging studies performed on the* (b) (4) *equipment, including* (b) (4) *and cleaning validation,* (b) (4) *, and* (b) (4) *studies.*

[16] Draft Guidance "Comparability Protocols for Human Drugs and Biologics: Chemistry, Manufacturing and Controls Information, April 2016" for additional information in regards to the expectations for Comparability Protocols.

c. *Since the* (b) (4) *upstream process may include variable numbers of* (b) (4)*, the PPQ study should use a bracketing approach in which the minimally acceptable number of* (b) (4) *and all* (b) (4) *are used to manufacture successful* (b) (4) *lots. In addition, a successful PPQ lot should be defined as a lot with no failed* (b) (4)*. At this time, the cumulative failure rate was 16.6% for all completed* (b) (4) *campaigns or* (b) (4) *for the* (b) (4) *PPQ series. A similar bracketing approach should be used in the manufacture of FDP lots produced from* (b) (4) *lots.*

d. *To demonstrate process consistency, Portola should provide data from* (b) (4) *consecutive BDS lots. The* (b) (4) *consecutive lots may include the* (b) (4) *PPQ lots.*

e. *Portola should include product activity and antigen levels in the assessment of the performance for most of the unit operations. These parameters should be used to calculate process yield and recovery, and added as performance attributes for comparison between* (b) (4) *and* (b) (4) *.*

f. *The acceptance criteria in the CP should be expressed as quantitative values or ranges and the following methods should be added:*
   - *potency of the product to be described in absolute values, instead of percentages, referencing publicly available standards;*
   - *identity by a* (b) (4) *method;*
   - (b) (4) *and* (b) (4) *content; and*
   - *identity and quantity of excipients - sucrose, mannitol and Polysorbate 80.*

g. *The comparability exercise should include the analysis of results from all* (b) (4) *and* (b) (4) *lots, including the pre-PPQ campaign* (b) (4) *lots, manufactured using the proposed commercial procedure in* (b) (4) *, in addition to those from the* (b) (4) *PPQ* (b) (4) *lots.*

h. *The FDP lots manufactured using the* (b) (4) *lots should be enrolled in stability studies, and compared with the stability trends of* (b) (4) *lots.*

## *4.4. Process Validation and Evaluation*

Process Performance Qualification (PPQ) covers the two major stages of production - (b) (4) ((b) (4) PPQ batches, see Table 2 above) and FDP ((b) (4) PPQ batches, see Table 3 above). The provided PPQ reports claim that the PPQ studies were successful.

*<u>Reviewer's comment</u>: I found the following deficiencies with the PPQ studies performed both at* (b) (4) *and* (b) (4) *:*

a) *During the* (b) (4) *PLI of the* (b) (4) *facility, several post-PPQ manufacturing deviations were reported to the FDA. The deviation investigations revealed the need to adjust the existing in-process control limits. Two additional CPPs were introduced,* (b) (4) *and pH. The implementation of the improved Process Control Strategy for these two parameters is ongoing. Because these CPPs were not*

*investigated during the completed process qualification studies, the* (b) (4) *PPQ investigation was deficient.*

(b) (4)

b) *I found that the FDP PPQ series was manufactured using a* (b) (4) *scheme that is not in compliance with the GMP regulations (Table 11). Specifically, FDP was produced by* (b) (4) *batches such that a* (b) (4) *batch was used for up to* (b) (4) *FDP batches, each FDP batch consisting of* (b) (4) *from* (b) (4) *batches. A direct GMP violation is evident for the PPQ* (b) (4) *batch* (b) (4) *which was produced in November 2015 by* (b) (4) . (b) (4) *had a release OOS for* (b) (4) *by* (b) (4) *and its status was reported to the FDA during the PLI in* (b) (4) *as "not released for GMP use". The lot was not considered released because* (b) (4) *does not release batches with ongoing deviation investigations* [17]. *Despite the non-approved status of* (b) (4) *, Portola authorized* (b) (4) *to use* (b) (4) *lots* (b) (4) *in a letter dated 09 November 2015 which is documented in the FDP PPQ report* [18]. *As a result of* (b) (4) . *Note that at the time of manufacturing of these* (b) (4) *FDP batches, the pre-defined PPQ specification for* (b) (4) *FDP was consistent with the release and stability specification for the* (b) (4) *of* (b) (4) *for both the* (b) (4) *FDP (e.g., as evidenced by the 20 January 2016 CoA for batch* (b) (4) *).*

[17] 30 June 2016 Amendment Sequence 48, file "3.2.R.2 DEV-1632 Rev 1_ Results for Lot (b) (4) Final.pdf"
[18] Process Performance Qualification Protocol for Andexanet Alfa, PRT064445 Lyophilized Drug Product, file "32r2-20160318-(b) (4) -2500l-ppq-report-final.pdf"
[19] BLA document "CoA for Andexanet Alfa DP (cGMP) Lot #(b) (4) (16Sep2015).pdf" dated 20 January 2016

c) (b) (4) *Batch* (b) (4) *which was used as source material for all* (b) (4) *FDP PPQ batches was OOL. A total of* (b) (4) *of* (b) (4) *Batch* (b) (4) *were reported in the FDP PPQ report* [20] *(Table 11) which corresponds to* (b) (4) *of andexanet alfa. This amount of protein exceeds the range* (b) (4) *) for the key* (b) (4) *process parameter* (b) (4) *[* [21]*].*

d) *Contrary to the obvious OOS and OLL statuses of* (b) (4) *batches* (b) (4) *, FDP PPQ protocol states that all source materials were conformant and "all materials are approved prior to use". The FDP PPQ investigation is deficient because it did not document and investigate these* (b) (4) *OOS results.*

In addition to the PPQ studies, several ancillary validation studies were performed to support the consistency of the manufacture of ANDEXXA BDS (Table 12). The studies included *Impurity Clearance Validation, In-Process Hold Time Validation,* (b) (4) *Validation,* (b) (4) *Validation* and *Shipping Qualification*.

*Reviewer's comment: Deviations were encountered in two of the studies,* In-Process Hold Time Validation *and* Resin Lifetime Validation. *The assessment of the impact of these deviations is incomplete. On 11 July 2016, Portola informed the FDA that new intermediate hold time validation protocol VAL-30291-01 was initiated* [22]*. Per this protocol,* (b) (4) *. According to Portola, the new study is more robust as it is not dependent on small-scale processing. In addition, the new validation protocol (VAL-30291-01) will challenge hold times at maximum process step hold time limits as opposed to the previous validation protocol VAL-30234-01 where cumulative maximum hold times in many cases were used well over maximum hold conditions for each process step. Portola proposes to submit the validation final report (VAL-30291-02) by the end of October 2016.*

**Table 12: Process validation and evaluation studies reported in the BLA**

| Process | Study |
|---|---|
| (b) (4) validation studies | • Comparability Study Between (b) (4) and (b) (4)<br>• Process Performance Qualification<br>• Process Performance Qualification Deviations<br>• Hold Time Studies<br>• Product (b) (4) Validation Study<br>• (b) (4) Validation Studies<br>• Filter Validation Studies<br>• (b) (4) and (b) (4) Studies<br>• Extractable and Leachable Studies<br>• Shipping Validation Studies<br>• Cleaning Validation Study<br>• Validation of Viral Inactivation and Viral Clearance |

[20] 3.2.R.2 414-21-04-001-SR2_ Process Performance Qualification Summary Report for Andexanet Alfa, PRT064445 Lyophilized Drug Product, 20 mL_ 20 mm Vial, (b) (4) mL Fill, 100 mg_Vial (b) (4), Fill Line (b) (4) ((b) (4) ).pdf
[21] See below Table 5: ANDEXXA BDS Manufacturing In-Process Controls
[22] 11 July 2016 Amendment; Sequence 56. File: 1111-m1-ax-00123rd.pdf

| | |
|---|---|
| FDP validation studies * | • Performance Qualification Study for andexanet alfa DP, (b) (4) Fill Line (b) (4) (b) (4))<br>• Andexanet alfa DP Consistency Lots Manufacturing Summary<br>• Component Compatibility Operational Qualification of 20 mL/20 mm Components Using the (b) (4) Filling Machine ((b) (4)) and Capper Model Number (b) (4)<br>• Summary of the Operational Qualification of 20 mL/20 mm Components<br>• Container Closure Integrity Test<br>• Media Fill Performance Qualification and Confirmation<br>• Formulation Equipment Sterilization Validation<br>• Filling Equipment Sterilization Validation<br>• Stopper Sterilization Validation<br>• Vial Washer Performance Qualification<br>• (b) (4) Performance Qualification<br>• Lyophilizer Validation<br>• Microbial Retention Validation<br>• Membrane Compatibility Screening<br>• Product (b) (4) Determination<br>• Extractables Test Validation |

** Reviewed by DMPQ reviewer Dr. Christine Harman*

Portola developed Continued Process Verification (CPV) plans for both (b) (4) and (b) (4) facilities to ensure that the ANDEXXA manufacturing process is in a state of control throughout the product lifecycle. The CPV program is designed to collect process data and perform statistical evaluation of the datasets in order to routinely confirm the process to be in a state of control, and to identify and evaluate planned and unplanned changes in the manufacturing process.
*Reviewer's comment: The proposed CPV plan did not address the deficiencies in PPQ investigations and the existing PCS at* (b) (4) *. Therefore, the CPV plan is not acceptable at this time.*

**Reviewer's overall assessment of the status of ANDEXXA process validation**

According to the FDA guidance on Process Validation [23], *quality cannot be adequately assured merely by in-process and finished-product inspection or testing. Each step of a manufacturing process is controlled to assure that the finished product meets all quality attributes including specifications. The* guidance defines process validation as *the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product*. Process validation involves a series of activities taking place over the lifecycle of the product and process. My review of the data provided in the BLA and during the PLI of (b) (4) facility in (b) (4) indicated serious deficiencies in the first two stages of process validation activities described in the FDA guidance:

[23] FDA Guidance for Industry. Process Validation: General Principles and Practices. January 2011. Current Good Manufacturing Practices (CGMP) Revision 1

- Stage 1 – Process Design: During this stage, Portola was expected to define the commercial manufacturing process based on knowledge gained through development and scale-up activities. However, two of the CPPs, (b) (4) and (b) (4) were not identified at this stage. These CPPs were identified later through small-scale process development studies which were initiated in response to manufacturing deviations that occurred after the completion of the PPQ investigation. In addition, the source of (b) (4) activity responsible for product (b) (4) in process intermediates and during storage of finished (b) (4) FDP were not investigated and are under intense investigation at this time, please refer to review section *5.2 Characterization of Process-related Impurities* for details.

- Stage 2 – Process Qualification: During this stage, the process design should have been evaluated to determine if the process is capable of reproducible commercial manufacturing. Although Portola reported their PPQ as successful, the need to introduce new CPPs to control the (b) (4) process and an apparent compliance violation during execution of the FDP PPQ series indicate that the process qualification is incomplete.

- Stage 3 – Continued Process Verification: At this stage, routine production is expected to provide assurance the process remains in a state of control. However, Portola's post-PPQ production cannot be considered as routine production activity because manufacturing is occurring concurrently with the additional process design studies and changes to the process control strategy. The process control modifications have not been fully implemented at this time as is the case of the control of temperature at the point of use at the (b) (4) facility.

According to the FDA guidance, *before any batch from the process is commercially distributed for use by consumers, a manufacturer should have gained a high degree of assurance in the performance of the manufacturing process such that it will consistently produce APIs and drug products meeting those attributes relating to identity, strength, quality, purity, and potency.* It is apparent that Portola is unable to meet this requirement because insufficient manufacturing experience is available and the analytical methods and related reference standards need to be developed for Portola to define the attributes for identity, strength, quality, purity, and potency.

During the CMC review, I have considered that the *process validation for drugs (finished pharmaceuticals and components) is a legally enforceable requirement under section 501(a)(2)(B) of the Act (21 U.S.C. 351(a)(2)(B)). FDA regulations describing current good manufacturing practice (CGMP) for finished pharmaceuticals are provided in 21 CFR parts 210 and 211. The CGMP regulations require that manufacturing processes be designed and controlled to assure that in-process materials and the finished product meet predetermined quality requirements and do so consistently and reliably. Process validation is required, in both general and specific terms, by the CGMP regulations in parts 210 and 211. The foundation for process validation is provided in § 211.100(a). This regulation requires manufacturers to design a process, including operations and controls, which results in a product meeting these attributes.*

If approved, ANDEXXA will be a single-source product that involves complicated manufacturing process. Homogeneity within a batch and consistency between batches are goals of process validation activities. Because ANDEXXA is intended to address an unmet medical need and is

orphan designated, validation was expected to offer *assurance that a process is reasonably protected against sources of variability that could affect production output* and cause supply problems, and thus negatively affect public health.

The lack of ANDEXXA process protection from variability is well illustrated by the lack of understanding of the nature of (b) (4) impurity responsible for the increased (b) (4) content in the (b) (4) material and loss of the (b) (4) in accelerated stability studies. With regard to impurities, the guidance states that *although often performed at small-scale laboratories, most viral inactivation and impurity clearance studies cannot be considered early process design experiments. Viral and impurity clearance studies intended to evaluate and estimate product quality at commercial scale should have a level of quality unit oversight that will ensure that the studies follow sound scientific methods and principles and the conclusions are supported by the data.*

Because (b) (4) has not yet implemented the equipment that will permit control of (b) (4) at the point of use, which as a new critical process parameter, Portola's process controls are not able *to address variability to assure quality of the product.* Furthermore, *FDA expects controls to include both examination of material quality and equipment monitoring.* During the PLI, FDA inspectors found that (b) (4) does not properly qualify and maintain critical equipment used in production of ANDEXXA (please refer to Observation # 3 on the Form FDA 483). Similarly, the use of OOS (b) (4) for the manufacture of FDP PPQ series demonstrated the deficiency in material quality examination at Portola and (b) (4) .

I noted that although the degree and types of documentation required by CGMP vary during the validation lifecycle, the overall deficiency in Portola documentation was obvious from the lack of any description in the BLA of the initiated process investigations related to the excursions in (b) (4) specifications. The guidance instructs that *documentation requirements are greatest during Stage 2, process qualification, and Stage 3, continued process verification. Studies during these stages must conform to CGMPs and must be approved by the quality unit in accordance with the regulations (see §§ 211.22 and 211.100).* It is surprising that no mention of (b) (4) impurity investigations can be found in any of the validation reports or process description summaries provided by Portola in the BLA.

Deficient documentation and OOS lot and material release practices were also demonstrated by the use of OOS (b) (4) in the production of FDP PPQ series. During the PLI, (b) (4) acknowledged that at least one batch, (b) (4) was not released per written release procedures because it failed to meet the manufacturer's requirements for GMP release. This batch should not have been used for the FDP PPQ batch (b) (4) at (b) (4) because *the PPQ combines the actual facility, utilities, equipment (each now qualified), and the trained personnel with the commercial manufacturing process, control procedures, and components to produce commercial batches.*

# 5. Elucidation of Structure, Function and Impurities

## *5.1. Structure and Function Studies*

Elucidation of Structure and Product-related Impurities and Substances

Andexanet alfa is a recombinant variant of human FXa that lacks proteolytic activity due to a substitution of the active site serine residue to alanine at position (b) (4), and the deletion of the γ-carboxyglutamic acid (Gla)-containing domain (b) (4) of native FX. Andexanet alfa is expressed in CHO cells as a functional protein, i.e., it does not require either *in vitro* or *in vivo* cleavage of the AP, which is necessary for converting native FX to its activated form FXa. This is accomplished by (b) (4) of andexanet. (b) (4). Andexanet alfa has (b) (4) amino acid (AA) residues and an approximate molecular weight of 41 kDa based on the cDNA sequence. Table 13 describes the analytical methods utilized to characterize the primary, secondary, and higher order structure of andexanet alfa.

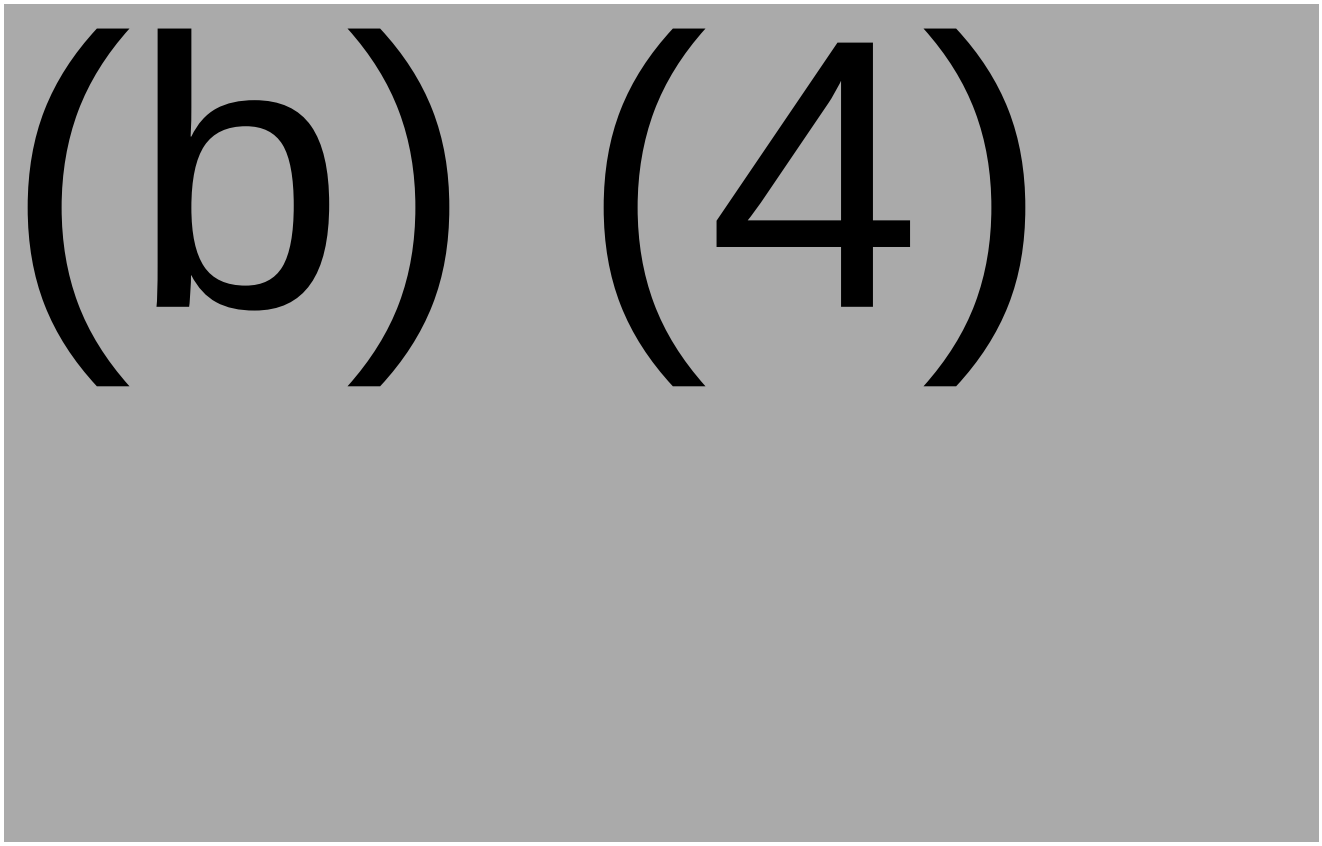
**Table 13: Summary of Methods and Attributes for Andexanet Alfa Characterization**

| Attribute | Characterization Method |
|---|---|
| Primary Sequence | (b) (4) |
| Analysis of (b) (4) | |
| (b) (4) Modifications | |
| Higher Order Structure | |
| Aggregates | |
| Purity | |
| Protein Identity | |
| (b) (4) | |
| (b) (4) | |

At least (b) (4) variants have been identified in andexanet alfa (see Figures 11, 12 and 13). They result from (b) (4)

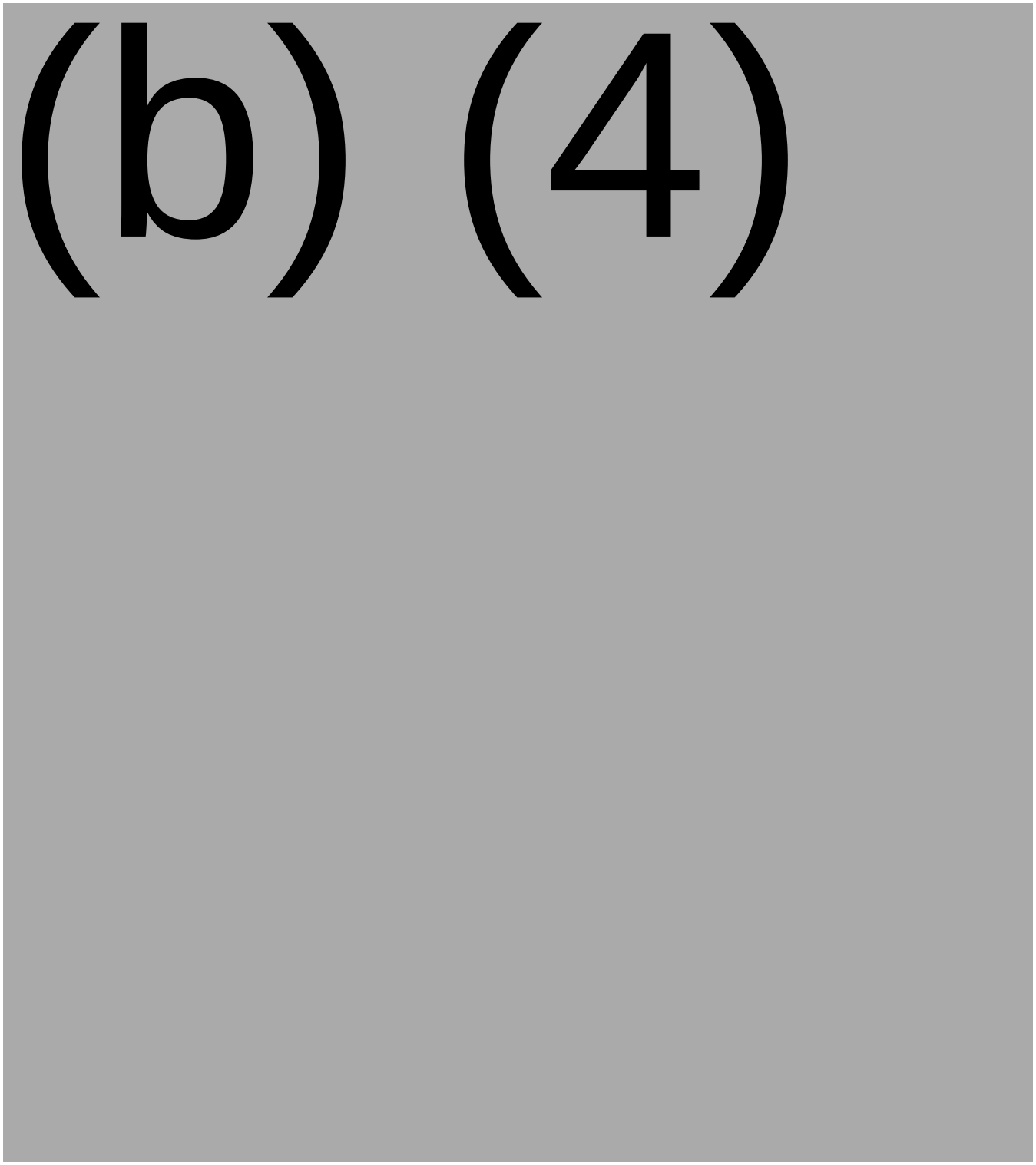
(b) (4) These modifications can occur individually, but more typically occur as a combination of the different modifications to generate a complex mixture of andexanet alfa variants.

The majority of these modifications also occur in the native FXa protein. Portola claims that variants of the molecule that are formed during the manufacturing process, (b) (4), but have properties comparable to the desired product are considered product-related substances, not impurities. 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.




Product-related impurities originate from modifications to the andexanet alfa primary structure during the manufacturing process, (b) (4), and do not have properties comparable to the desired product.

In-process and release testing of andexanet alfa are controlled by (b) (4). (b) (4) products of andexanet alfa are controlled during release and stability testing by (b) (4). Aggregates of andexanet alfa are controlled during release and stability testing by (b) (4).




Portola claims that formation of the (b) (4) is not expected to affect product potency. The (b) (4) of native human FXa to produce the (b) (4) has been reported. From the

[24] 3.2.S.3.1 Elucidation of Structure and Other Characteristics.pdf
[25] 3.2.S.3.1 Elucidation of Structure and Other Characteristics.pdf

crystal structure of human FXa, the primary binding pocket of the protein to the small molecule direct FXa inhibitors is at the HC region equivalent to andexanet alfa (b) (4) which is distant from the (b) (4). The (b) (4) of human FXa was found to have equal coagulant activity as the intact FXa. Additionally, the (b) (4) of (b) (4) FXa was found identical to the intact FXa for interaction with the active site-directed inhibitor, reaction with ATIII in the presence or absence of heparin as well as assemble of prothrombinase and activation of prothrombin.

Furthermore, Portola claims that human FXa-(b) (4) has the same activity as the full-length FXa for peptidyl substrate cleavage, which interacts mainly with the (b) (4) at the FXa active site, the same binding site for FXa inhibitors. Portola concludes that since andexanet alfa is a modified FXa molecule, the (b) (4) and full-length andexanet alfa molecules would have the same binding affinity for FXa inhibitors because they have the same (b) (4). Using two different functional activity assays (direct potency assay and (b) (4)) on several lots of (b) (4) of the product is essentially 100%, irrespective of the percent (b) (4) present in the individual (b) (4) lots.

*<u>Reviewer's comments</u>: Portola believes that the variants that can bind FXa inhibitors will be fully active. This conclusion is indirectly supported by the relative similarity in potencies of (b) (4) and (b) (4) batches despite their slightly variable distributions of product variants. At the request of the FDA, Portola performed purification of the major (b) (4) and full-length andexanet alfa species which were found to be (b) (4) by the direct and indirect potency assays. However, data on the (b) (4) were not provided in the BLA.*

*Although I agree that theoretical considerations may support the hypothesis that the (b) (4) of andexanet alfa may be functional in the ANDEXXA potency assays, I think that additional investigation of the (b) (4) is needed:*

a) *The sources of (b) (4) responsible for the formation of the andexanet (b) (4) should be identified. It is noteworthy that formation of human FXa (b) (4) is a result of (b) (4) by FXa-alfa. Because andexanet alfa is designed to have (b) (4), Portola should investigate the possibility of (b) (4) activity in the andexanet alfa protein.*

b) *The similarity of specific activities in different (b) (4) batches cannot be used as a direct proof of (b) (4) activity because the (b) (4) represent a relatively small portion of andexanet alfa by mass and the (b) (4) content is not sufficiently variable (between (b) (4)) to have an effect on the existing potency assays. Portola should investigate potencies of preparations consisting of (b) (4) (similar to those presented in Figure 13) and (b) (4) andexanet alfa samples which can be produced by (b) (4) or (b) (4). Furthermore, one may expect the potency assay to be not sensitive to the activity of the (b) (4) because (b) (4) may be generated in situ during the potency assay procedure which uses high amounts of human FXa as a reagent.*

c) *Although human FXa-(b) (4) in many respects is functionally similar to FXa, evidence also exists that FXa (b) (4) has unique functions, for example (b) (4) found that (b) (4), and not FXa, is necessary for (b) (4). Additional investigation of functional interactions between the (b) (4) of andexanet alfa on (b) (4), are warranted.*

d) *Interaction of (b) (4) with TFPI have not been investigated despite repeated requests by the FDA. Because inhibition of TFPI activity by andexanet alfa is dependent on interactions with TF and FVIIa proteins, and because andexanet alfa has been demonstrated to bind human FVII (reported in Portola's patent application[27] WO 2014116275 A1), the ability of (b) (4) to bind and inhibit TFPI activity should be investigated in the presence of TF and FVIIa.*

## 5.2. Characterization of Process-related Impurities

The levels of impurities in the product were evaluated in clinical studies and have not been directly associated with adverse events. Process-related impurities that have been assessed for clearance include (b) (4), and process additives, such as (b) (4). Risk assessment considered the number of purification steps, capacity of the purification steps, amount per one 2-g dose (a borderline estimate above the maximum dose of 1,760 mg that would be administered to a patient), toxicological risk of the potential impurities, and evaluation of the literature. The impurities identified in the risk analysis as requiring demonstration of impurities clearance were evaluated in the (b) (4) consecutive PPQ (b) (4) lots.

However, in the Final Investigation Report[28] for deviation DEV-1632 submitted on 30 June 2016, Portola reported that a (b) (4) impurity may exist and be responsible for the formation of the (b) (4) throughout the manufacturing process and during storage of the (b) (4) lyophilized FDP. Furthermore, in the 17 July 2016 amendment to the BLA[29], Portola also acknowledged that "*As of yet, we have not identified the source of the (b) (4) in the upstream process*".

*Reviewer's comments: I found that Portola's impurity investigations were incomplete because no information on the sources of the (b) (4) that is responsible for the formation of the (b) (4) was provided in the BLA. In the 17 July 2016 response[30] to FDA's 08 July 2016 Information Request, Portola provided a brief summary of the ongoing investigations into the identity of the*

[26] Bhattacharjee G, Ahamed J, Pawlinski R, Liu C, Mackman N, Ruf W, Edgington TS. Factor Xa binding to annexin 2 mediates signal transduction via protease-activated receptor 1. Circ Res. 2008 Feb 29;102(4):457-64.
[27] Patent WO 2014116275 A1 "INHIBITION OF TISSUE FACTOR PATHWAY INHIBITOR WITH FACTOR Xa DERIVATIVES". Publicly available sources: http://google.com/patents/WO2014116275A1?cl=en and https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2014116275
[28] 30 June 2016 Amendment Sequence 48, file "3.2.R.2 DEV-1632 Rev 1_ Results for Lot (b) (4) Final.pdf"
[29] 17 July 2016 amendment; Sequence 59. File: 1.11.1 Quality Information Amendment.pdf
[30] 17 July 2016 amendment; Sequence 59. File: 1.11.1 Quality Information Amendment.pdf

(b) (4) *but no data were provided to substantiate any of the conclusions provided. The existence of these studies were not reported to the FDA to-date.*

# 6. Stability

*Reviewer's comment: The stability data were reviewed by Dr. Yideng Liang. Please refer to her review memorandum for details.*

(b) (4) **Drug Substance stability**

(b) (4)

(b) (4)

(b) (4)

(b) (4)

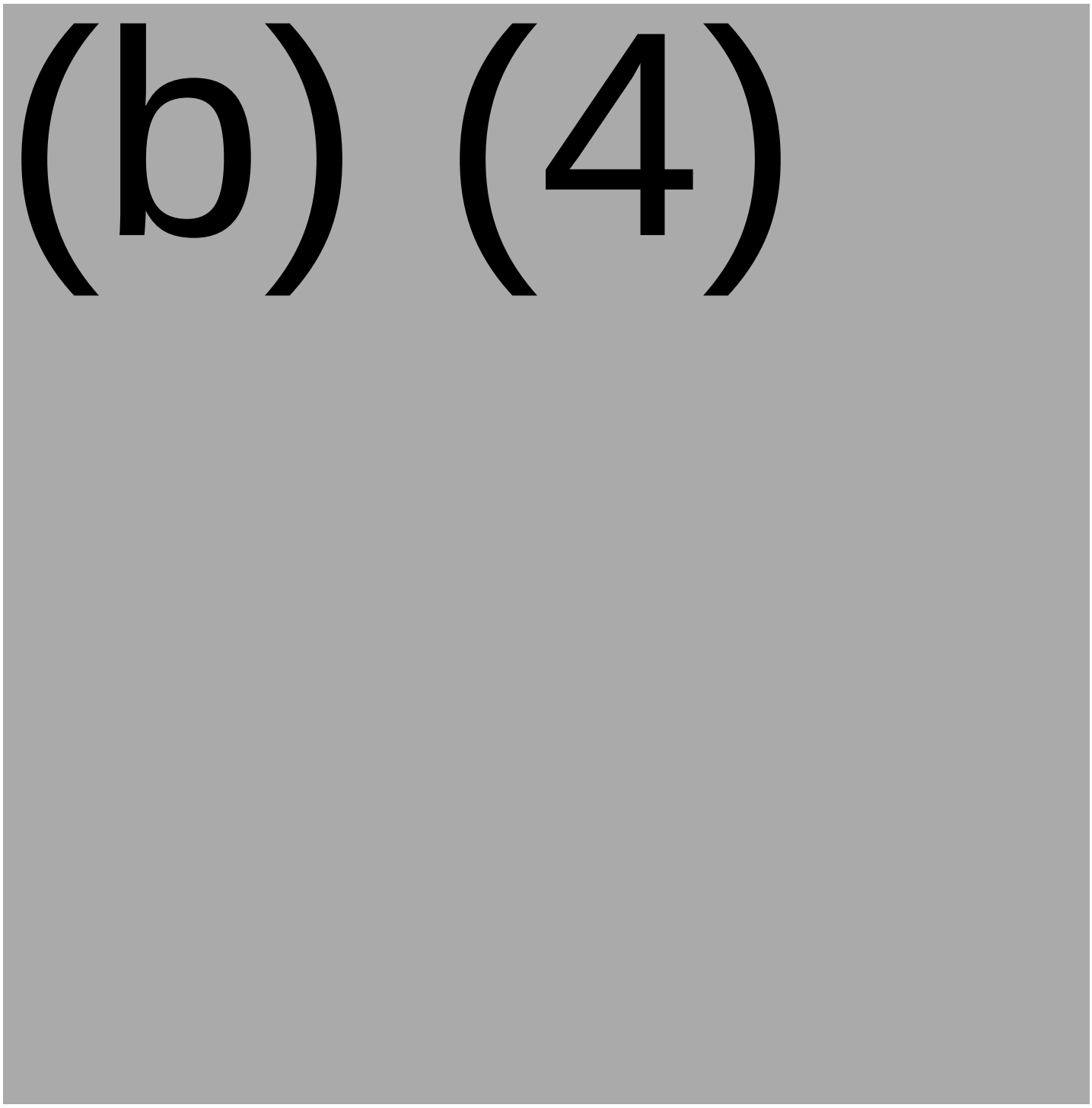
(b) (4)

(b) (4)

(b) (4)

Analysis of FDP stability studies indicated an (b) (4) associated with a (b) (4) in the (b) (4) and the (b) (4) for at least (b) (4) batches manufactured at (b) (4) facility, see Figure 15.

*<u>Reviewer's comment</u>: (b) (4) batch (b) (4) was OOS for the (b) (4) at (b) (4) of storage at - (b) (4). This batch was manufactured into the FDP batch (b) (4) which demonstrated (b) (4) in real-time stability studies, see Figure 15.*




## 7. Suitability of the proposed biomarker as a surrogate endpoint for clinical benefit

*<u>Reviewer's comment</u>: The analysis below summarizes my review of the evidence from the clinical and preclinical studies based on my scientific understanding of the mechanisms of action (MOA) of andexanet alfa. My review was focused on the data derived from the clinical trials, which were used to support the Accelerated Approval of ANDEXXA. In the process of review, I assessed the suitability of the potency and bioanalytical assays used in these studies and the validity of Portola's interpretation of the results derived from these assays. My conclusions are meant to be complementary to the primary discipline review performed by the clinical, clinical pharmacology, and preclinical reviewers of their respective disciplines. The clinical data were reviewed by Dr. Lisa Faulcon, clinical pharmacology by Dr. Iftekhar Mahmood, and preclinical data by Drs. Yolanda Branch and Ann Pilaro. Please refer to their review memoranda for details.*

## *7.1. Anti-FXa activity as a surrogate endpoint to support Accelerated Approval*

The ANDEXXA 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*. Under *Accelerated Approval*, FDA can rely on a particular kind of evidence, such as a drug's effect on a surrogate endpoint, as a basis for approval (reference is made to *2014 FDA Guidance on Expedited Programs for Serious Conditions*). The data used to support *Accelerated Approval* of ANDEXXA came from studies in healthy volunteers in which **a biomarker**, anti-FXa activity, is used as **a surrogate endpoint**. For purposes of accelerated approval, a surrogate endpoint is a marker, such as a laboratory measurement, that is thought to predict clinical benefit, but is not itself a measure of clinical benefit. In rare cases, a PD biomarker may be considered a clinically significant endpoint if it strongly suggests the potential for a clinically meaningful effect on the underlying disease.

According to the 2014 guidance[31], 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. The following specific questions should be addressed during review:

1. The evidence supporting the use of the PD biomarker.

2. The strength of the evidence supporting the ability of the marker to predict clinical benefit.

As FXa inhibitors target the coagulation enzyme FXa, a PD assay that measures activity of these inhibitors in blood may appear relevant for it to serve as a surrogate PD marker. Specifically, reduction of inhibitor activity below the pharmacologically active level can be viewed as reasonably likely to predict the clinical outcome related to reversal of anticoagulation.

Portola proposed to use the reversal of anti-FXa activity as the PD biomarker to support ANDEXXA approval. In this assay method, (b) (4) Portola provided strong evidence of ANDEXXA's effect

[31] FDA Guidance for Industry Expedited Programs for Serious Conditions – Drugs and Biologics. May 2014

on this biomarker. In the preclinical and clinical studies, andexanet alfa reversed the anti-FXa inhibitory activity of all four FXa inhibitors, rivaroxaban, apixaban, (b) (4) and edoxaban, in a dose dependent manner.

However, the anti-FXa activity assay is yet to be validated as a surrogate PD marker that can predict bleeding risk in recipients of FXa inhibitors. That is to say, the action of ANDEXXA on anti-FXa activity has not been validated in clinical studies to predict a hemostatic effect in bleeding patients. Therefore, Portola claims that the reversal of anti-FXa activity is a surrogate endpoint which is *reasonably likely* to predict ANDEXXA's intended clinical benefit in bleeding patients. If FDA will accept Portola's proposal to use reversal of anti-FXa activity to support the accelerated approval of ANDEXXA, a post-marketing confirmatory trial will be required to verify and describe the anticipated clinical benefit for the indications proposed. These trials must be completed with due diligence.[32]

*Reviewer's comment: I conclude that it is imperative to describe all unresolved scientific issues related to the use of the anti-FXa activity assay because the confirmatory trial should be designed to address any remaining concerns.*

Determining whether an endpoint is reasonably likely to predict clinical benefit is a matter of judgment that depends on the biological plausibility of the relationship between the bleeding conditions, reversal of anti-FXa activity, the desired clinical effect and the empirical evidence to support that relationship. Evidence of pharmacologic activity, i.e., reversal of anti-FXa activity, alone is not sufficient. Some clinical data should be provided to support the surrogate endpoint or intermediate clinical endpoint to be reasonably likely to predict the clinical outcome.[33]

*Reviewer's comment: Below is my review of the use of the reversal of anti-FXa activity and its associated assay as a PD biomarker and surrogate endpoint to support the accelerated approval of ANDEXXA, and a summary of the potential issues I identified. My review was based on the analysis of data across multiple disciplines as presented in the BLA.*

## 7.2. Concerns about the magnitude of the reversal of anti-FXa activity

In communications during the IND, Portola often presented the results of the anti-FXa activity assay as % of pre-ANDEXXA treatment, see Figure 16. This approach is suitable for comparing the ANDEXXA and placebo arms of the clinical trials. For example, Figure 16 demonstrates that anti-FXa activity returns to placebo levels within 2 hours after ANDEXXA administration. However, the percent presentation does not allow the comparison of the magnitude of the reversal of anti-FXa activity with the numerical values of the pharmacological range of FXa inhibitor concentrations in blood. Specifically, for the reversal of anti-FXa activity to be reasonably likely to predict clinical benefit, the remaining level of anti-FXa activity should be below the clinically effective

[32] Section 506(c)(3)(A) of the FD&C Act and §§ 314.510 and 601.41. Where confirmatory trials verify clinical benefit, FDA generally will terminate the requirement (21 CFR 312.560 and 601.46).
[33] Guidance for Industry Expedited Programs for Serious Conditions – Drugs and Biologics. May 2014

concentration of FXa inhibitor in blood. Furthermore, because FXa inhibitors differ in their established (or not established) pharmacological ranges, the presentation of anti-FXa activity will highlight the importance of analyzing the effect of ANDEXXA on each of the FXa inhibitors independently. Therefore, FDA requested the analysis of anti-FXa activity assay data to be presented in units of inhibitor concentration. Portola had these data because the commercially available anti-FXa inhibitor assays were already calibrated with the relevant standards of rivaroxaban, apixaban, (b) (4) and edoxaban.

*Reviewer's note: The issue of presentation of anti-FXa activity as a % of the pre-treatment level was raised during the pre-BLA discussions with Portola. At my advice, the following IR was submitted to Portola on 18 September 2015:*

"1. With regard to the data on the relationship between (b) (4) plasma concentration and the risk of bleeding submitted by Portola by email on September 15th, 2015, please provide additional information on the anti-factor Xa activity assay employed in Portola's clinical investigations. Specifically,

a) Please provide analytical data used to establish the quantitation ranges for apixaban, rivaroxaban, and edoxaban by the anti-factor Xa activity assay (in the absence of added andexanet alfa) and comment on their relation to the peak, trough and no-effect plasma levels for each inhibitor.

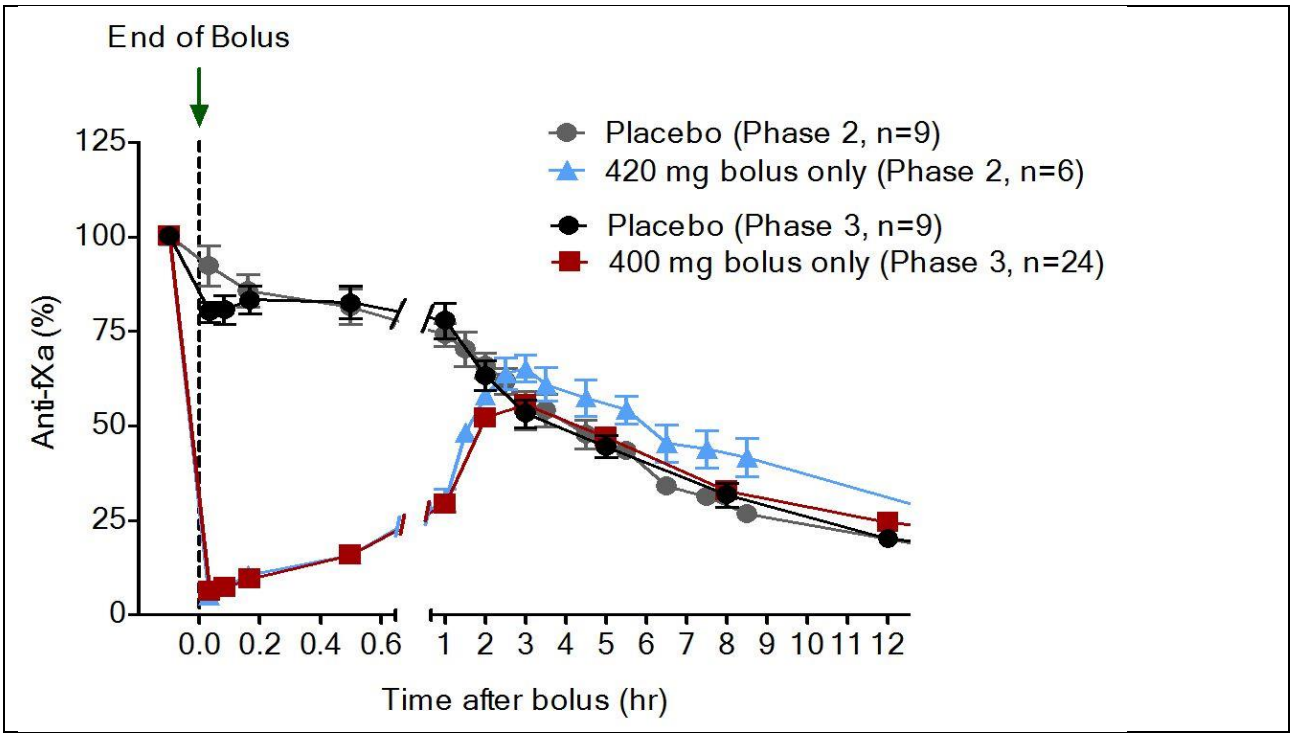
b) Please describe the relationship between the anti-factor Xa activity (in %, as reported by the assay) and plasma concentrations of these inhibitors (in (b) (4)). Specifically, please provide tables to assist with the conversion of anti-factor Xa activities into plasma concentrations of the inhibitors.

c) For a representative anti-factor Xa activity time course in a typical patient or a healthy volunteer, please re-plot the anti-factor Xa activity in inhibitor units, i.e., an estimate of the apparent inhibitor concentration versus time.

d) Please comment on the robustness of the anti-factor Xa activity assay. Please provide the raw data for the anti-factor Xa system suitability control included in each assay for the (b) (4) consecutive anti-factor Xa determinations. In addition, please explain how the assay's system suitability control is representative of the activity of apixaban, rivaroxaban, and edoxaban."

**Fig. 16: Example of anti-FXa activity data presentation as the % of pre-treatment.** This figure is taken from Portola's publication which is publicly available on the SEC website[34]. Portola's description: *The following diagram depicts the data from the first part of our Phase 3 ANNEXA-A study of Andexanet alfa in subjects taking apixaban*

[34] ANNUAL REPORT PURSUANT TO SECTION 13 OR 15(d) OF THE SECURITIES EXCHANGE ACT OF 1934 For the Fiscal Year Ended December 31, 2014. https://www.sec.gov/Archives/edgar/data/1269021/000156459015001190/ptla-10k_20141231.htm




## *7.3. The relevance of anti-FXa activity to pharmacodynamics of ANDEXXA*

Pharmacodynamics refers to the relationship between drug concentration at the site of action and the resulting effect, including the time course and intensity of therapeutic and adverse effects.[35] Although the anti-FXa activity assay is often presented as a PD method for FXa inhibitors, there are concerns about its physiological relevance, and therefore its predictive value in clinical outcome. Anti-FXa activity does not measure the response of patient plasma to the effect of FXa inhibitors. Instead, it measures the interaction between the exogenous human or (b) (4) FXa with the FXa inhibitor from a diluted sample of patient plasma. The assay is optimized such that the contribution of endogenous FXa and endogenous FXa inhibitors is minimized, i.e., the amount of added FXa is very high and the plasma is substantially diluted compared to their physiological levels. As a result, the anti-FXa activity assay becomes a very robust measure of FXa inhibitor concentration, and it has been demonstrated to agree 1 to 1 with the tandem chromatography-mass spectrometry methods which are considered the gold standard for the quantification of FXa inhibitor concentration in plasma[36, 37].

[35] Concepts in Clinical Pharmacokinetics 6th edition. American Society of Health-System Pharmacists, 2014

[36] Schmitz EM *et al.* Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulantsJ Thromb Haemost. 2014 Oct;12(10):1636-46

[37] Rathbun S *et al.* Comparison of methods to determine rivaroxaban anti-factor Xa activity. Thromb Res. 2015 Feb;135(2):394-7.

Finally, the anti-FXa assay is used to measure anti-coagulation. Because ANDEXXA is developed to control bleeding in urgent situations, a predictive PD method would be expected to correlate with the pro-coagulant effect of ANDEXXA.

*Reviewer's comment: Portola claims that any reduction of anti-coagulant activity of FXa inhibitors by ANDEXXA should improve hemostasis in a bleeding patient.*

To address the potential shortcomings of the anti-FXa activity assay, Portola proposed to use a Thrombin Generation Test (TGT) as a secondary PD measure which validates the use of anti-FXa activity assay.

*Reviewer's comment: In other words, Portola is proposing to use the TGT as a surrogate marker of the hemostatic effect of ANDEXXA in patients. Portola, however, does not claim the TGT to be a surrogate endpoint for the purpose of supporting Accelerated Approval, but will instead use the TGT data to validate the chosen biomarker, anti-FXa activity.*

## 7.4. Use of thrombin generation as a surrogate for the reversal of anti-FXa activity

Portola explained that because thrombin is formed from activation of prothrombin by FXa in the prothrombinase complex, and is the last protease in the coagulation pathway leading to fibrin (clot) formation, the thrombin generation assay is a more physiologically relevant measurement of both anticoagulation and restoration of hemostasis distal to FXa inhibition. Portola claims that FXa inhibitor-induced inhibition of thrombin generation correlates with anti-FXa activity and its reversal.

To support this statement, Portola presented the time courses for both methods in the BLA and the NEJM paper[38] which described the results from the pivotal Phase 3 clinical trials, see Figure 17 A. In addition, Portola presented correlation graphs obtained with the anti-FXa activity and TGT assays in the Phase 2 and 3 clinical studies, see Figure 18 A.

*Reviewer's comments: I disagree with Portola's statements about good correlation between anti-FXa activity assay and TGT as evidence of ANDEXXA effect.*

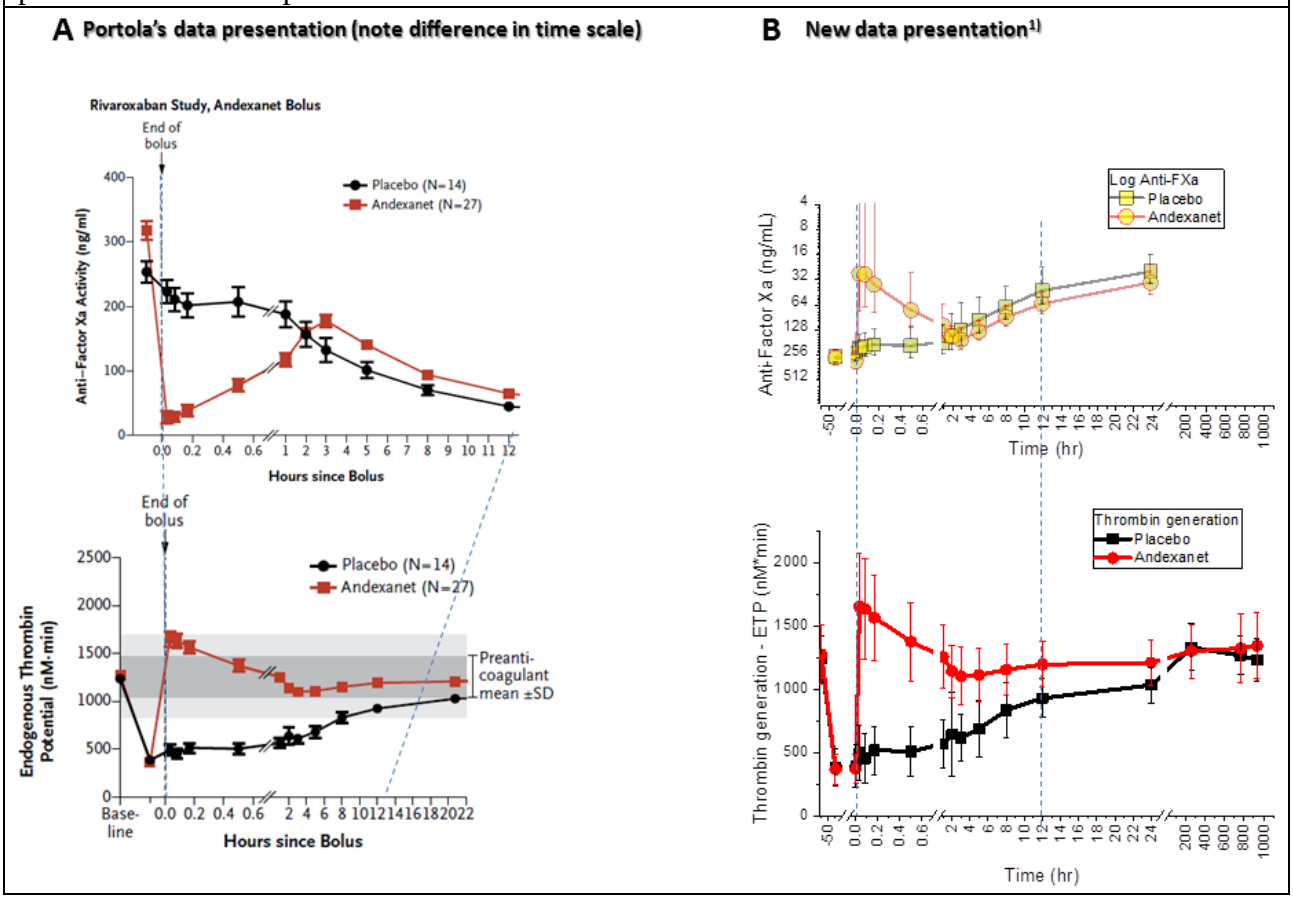
1. *The data in the NEJM paper presented in Figure 17 below demonstrate that the reversal of anti-FXa activity was very brief (anti-FXa activity levels in the ANDEXXA treatment group returned to those in the placebo group within 2 hours) while the elevation of TGT is much longer (TGT was elevated for at least 22 hours).*

   *Please note that Portola's presentation of the time courses of anti-FXa activity over 12 hours and TGT over 22 hours creates a misleading appearance of good correlation between the duration of the reversal of anti-FXa activity (which is short) and that of elevation of TGT*

[38] 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

*(which is sustained). Therefore, I re-plotted these graphs using the same time scales, see Fig. 17B.*

**Fig. 17: Time courses of anti-FXa activity and thrombin generation before and after the administration of andexanet.** Adapted from Figures 1B and 2B of Siegal DM *et al.* N Engl J Med. 373(25):2413-24 [Reference: [39]]. Note the difference in error bar presentation. Portola presented S.E. and I presented S.D.



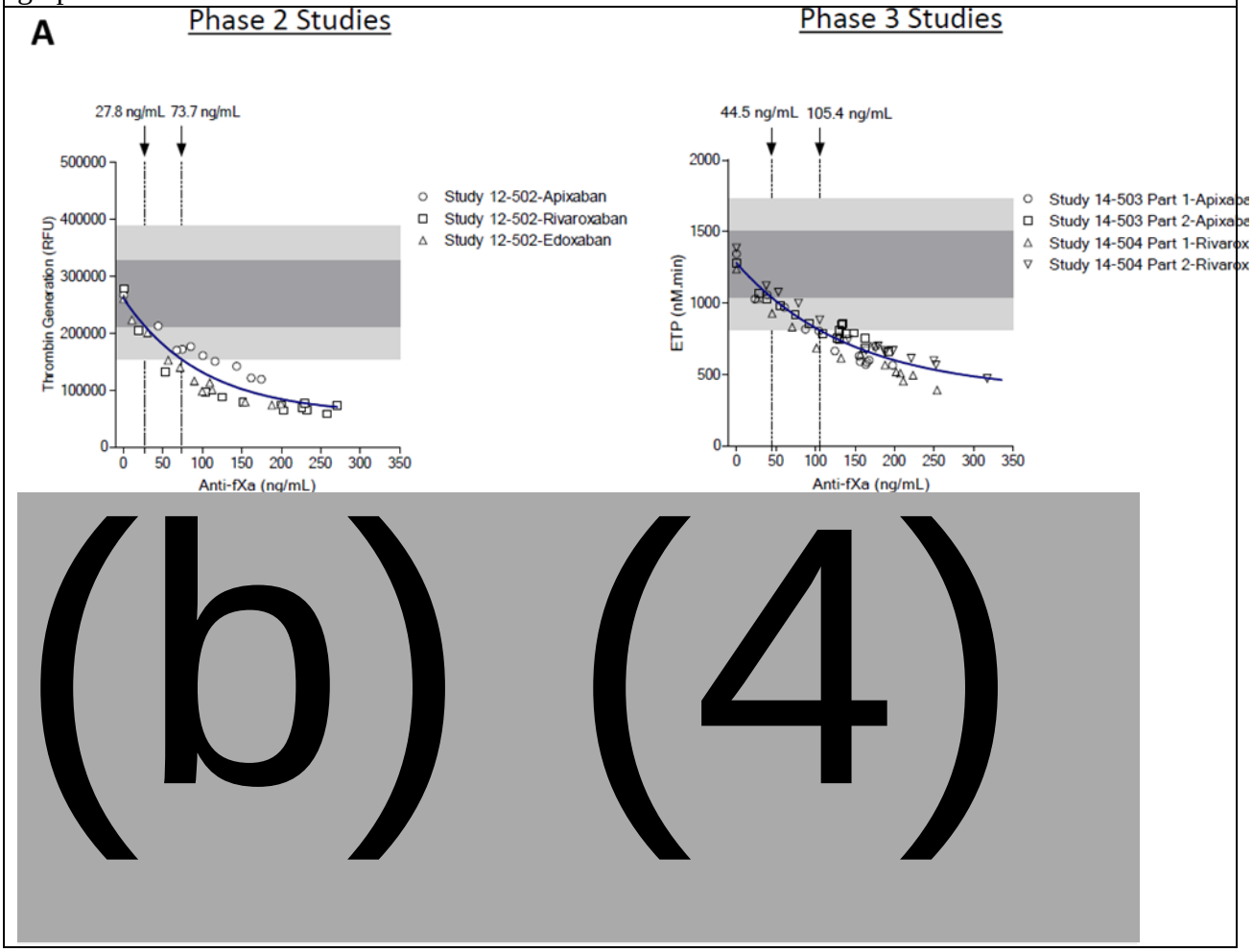

*Furthermore, to assist with the visual analysis of the pro-coagulant effects of ANDEXXA, on Fig. 17B, I changed the direction of the anti-FXa activity axis such that an increase in anti-FXa activity will be directed downwards. The revised Fig 17B illustrates that ANDEXXA has a much stronger and longer effect on the TGT than on the anti-FXa activity assay.*

2. *I found that ANDEXXA effect on TGT was incorrectly presented on the correlation between the anti-FXa activity and TGT. Portola compared the Phase 2 data presented as an average of the data from the placebo and ANDEXXA-treated subjects with the Phase 3 data from the placebo arm only (Fig. 18A, left). I re-analyzed a subset of the data from these studies and*

[39] 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

*found the stronger effect of ANDEXXA on TGT elevation during the first 3 hours post-bolus in the Phase 3 vs. the Phase 2 studies (see Fig. 18B).*

**Figure 18: Correlation between anti-FXa activity and TGT in Phase 2 and 3 studies.** (A) Data presentation from Portola's Pre-BLA and BLA meeting packages[40]. (B) My analysis of raw data presented in the BLA. Note that ANDEXXA has a profound effect on the correlation graphs.




The likely explanation for the prolonged elevation of TGT in clinical trials is the action of ANDEXXA on the endogenous inhibitor of blood coagulation. In the BLA, Portola stated that ANDEXXA has no significant interaction with other major plasma coagulation proteins, except for TFPI. Portola claims that ANDEXXA-TFPI interaction may enhance reversal of FXa inhibitor-induced inhibition of thrombin generation while it has minimal effect on thrombin generation in the absence of a FXa inhibitor.

[40] BLA amendment Seq. 63 dated 22 July 2016.

*Reviewer's comment: I found that Portola's assertion of ANDEXXA's "minimal effect on thrombin generation in the absence of a FXa inhibitor" contradicts with Portola's own data. See section "7.6. Incorrect interpretation of thrombin generation data" for further discussion.*

*Reviewer's comment: It should be noted that FDA had repeatedly cautioned Portola about the potential sensitivity of their TGT assay to the TFPI inhibition action. At my advice, the following IR was submitted to Portola on 18 September 2015:*

> *"*2. Portola suggests that the thrombin generation assay may be used to quantitate the antidote's activity, which is directed against anti-factor Xa inhibitors. In this regard, please provide data from testing andexanet alfa's activity against the tissue factor pathway inhibitor measured by Portola's tissue factor-activated thrombin generation assay. Specifically,
>
> a. Please provide an updated response to, "*Additional FDA Question 1*," referenced in the FDA October 16, 2012 Meeting Response Memorandum, CRMTS #8618, which stated, "*Regarding the PD assay used to detect the activity of the antidote against small FXa inhibitors, the tissue factor-mediated thrombin generation assay (TF-TGA) should be replaced with and/or validated against a TF-independent assay. Anti-TFPI activity of the antidote may interfere with the detection of anti-FXa activity in any TF-dependent assay."*
>
> b. Please provide data on the effect of increasing andexanet alfa concentrations in Portola's tissue factor-activated thrombin generation assay in healthy donor plasma.
>
> Please compare the activity of andexanet alfa in two versions of the thrombin generation assay, based on tissue factor or intrinsic pathway activators, respectively. In the intrinsic pathway-activated thrombin generation assay, the following coagulation triggers may be used: (b) (4) or any other suitable reagent derived from the (b) (4) test. In this experiment, please test a pharmacological range of andexanet alfa concentrations spiked into pooled human plasma, alone or in the presence of pharmacologically relevant concentrations of each anti-factor Xa inhibitor."

*In their response to this IND request, Portola reiterated their belief that TFPI inhibition is a minor action of ANDEXXA which has minimal effect on the TF-activated TGT and therefore the use of contact-activated TGT is not needed.*

## *7.5. Inhibition of TFPI by andexanet alfa – An inconvenient mechanism of action*

Throughout its communications with the FDA all the way to the BLA, Portola has claimed that ANDEXXA has no pro- or anti-coagulant activity. The following evidence was provided:

1. Change of the serine residue to alanine in the active site of FXa eliminated the catalytic capability of andexanet alfa to cleave the FXa (b) (4) -FXa, and its physiological substrates, prothrombin and FVII

2. Removal of the Gla domain eliminated the anticoagulant activity of ANDEXXA. For example, ANDEXXA does not inhibit TF-initiated thrombin generation, as compared to FXa-(b) (4), a human FXa containing an intact Gla-domain but with the active site inhibited by a (b) (4) .

3. ANDEXXA does not activate human platelets, leukocytes, or endothelial cells in cell-based assays.

4. Binding experiments indicate that ANDEXXA has no significant interaction with major plasma coagulation proteins, except for TFPI

*Reviewer's note: The above studies were requested at my recommendation at a pre-IND Meeting Response Memorandum dated 12 June 2009* [41].

Portola stated that ANDEXXA and TFPI interaction was expected because andexanet alfa is a modified human FXa molecule, and FXa has a high affinity for TFPI. The ANDEXXA-TFPI interaction has been characterized in detail in a (b) (4) system using purified proteins, cell-based assays with endothelial cells ((b) (4) ) and thrombin generation in human plasma. ANDEXXA was found to bind with high affinity TFPI that was added in the (b) (4) -system or endogenous TFPI that is expressed on (b) (4) surfaces. This interaction does not induce activation of (b) (4) .

However, Portola asserted that the binding of ANDEXXA to TFPI has minimal biological consequences. This conclusion was repeated in multiple sections of the BLA.

The following arguments were provided:

1. A FXa inhibitor, such as rivaroxaban, is able to dose-dependently block the andexanet-TFPI interaction on (b) (4) because FXa inhibitors and TFPI bind to the same site on andexanet.

2. ANDEXXA-TFPI interaction had minimal effect on thrombin generation in human plasma in the absence of a FXa inhibitor.

3. Binding of ANDEXXA to TFPI enhances restoration of thrombin generation in the presence of a FXa inhibitor, rivaroxaban, when the anticoagulant level is higher than andexanet in the plasma, i.e., under anti-coagulation state.

*Reviewer's comment: After analysis of the data presented in the BLA, I concluded that each of these three statements is either not accurate or wrong and should be corrected as follows.*

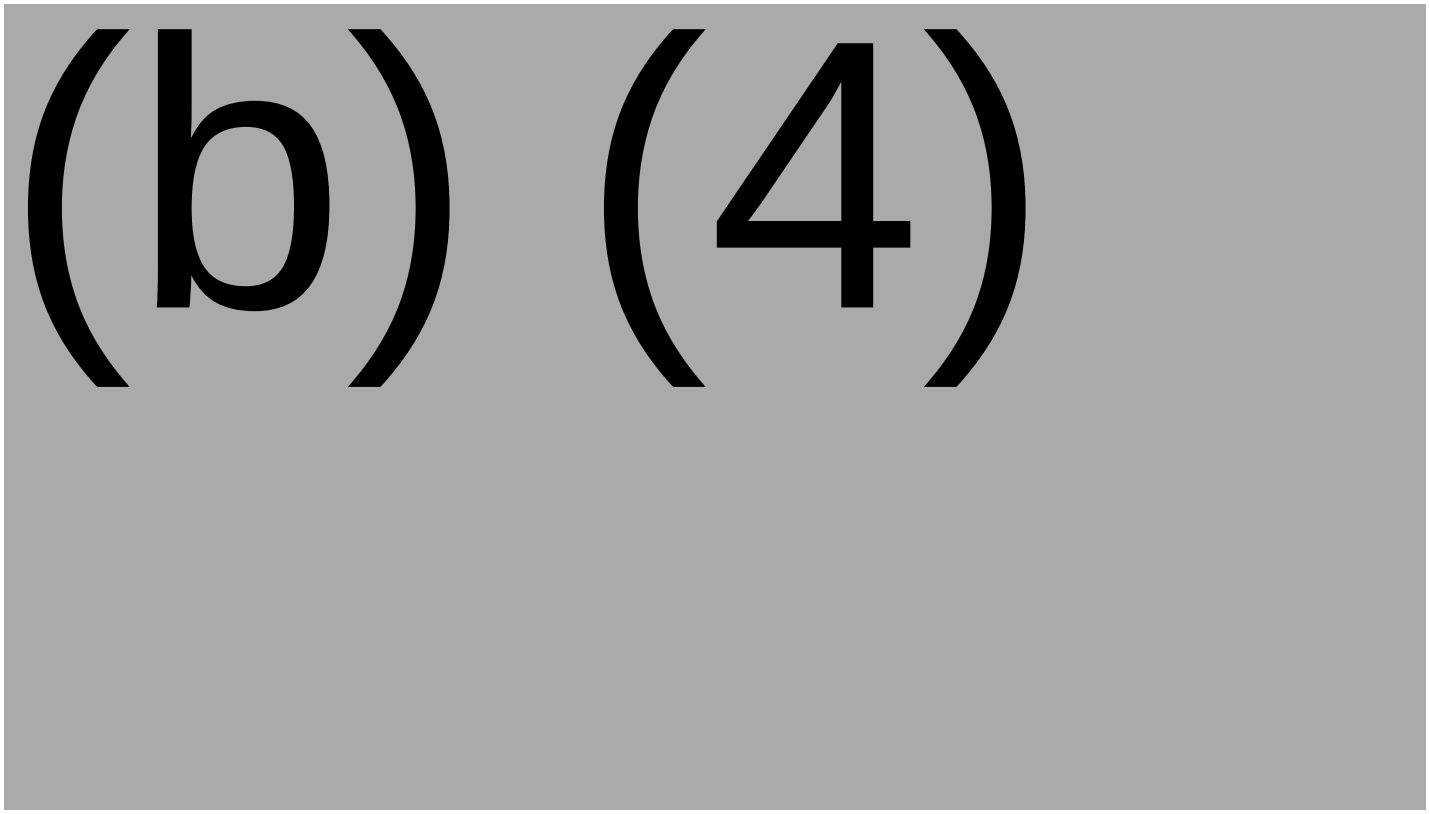
[41] Meeting Response Memorandum CRMTS #7089 Ref # PS000698 dated 12 June 2009

1. *Rivaroxaban **does not block** the andexanet-TFPI interaction on HUVECs.*

2. *ANDEXXA-TFPI interaction **has a profound effect** on the TGT*

3. *ANDEXXA-TFPI interaction **not only restores but also elevates** the TGT to the pre-FXa inhibitor treatment baseline.*

*These conclusions are supported by the following observations:*



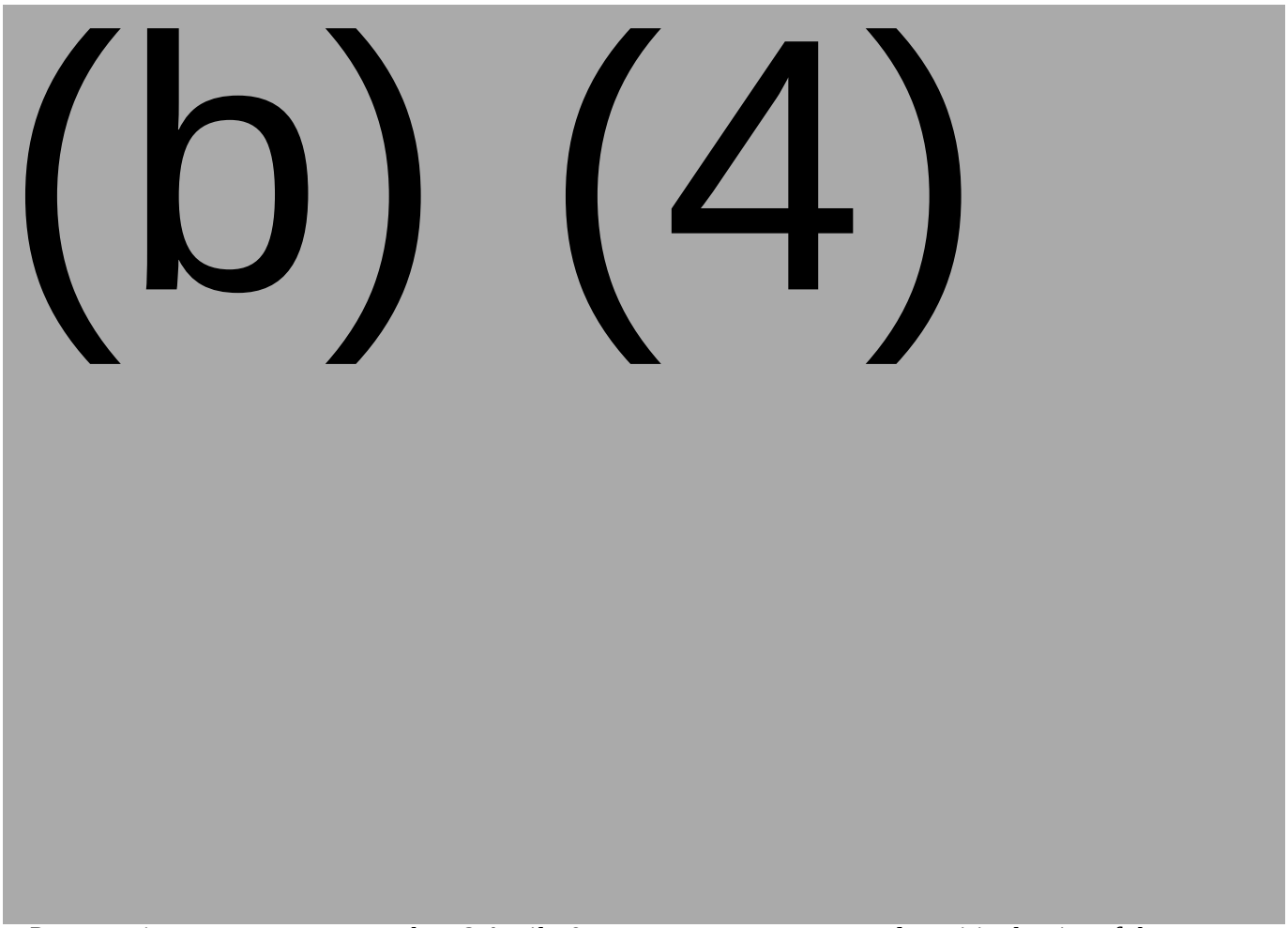

2. *I requested the raw data for the preclinical report NC-15-0659-R0001and found that ANDEXXA had strong dose-dependent effect on the thrombin generation curve, see Figure 20. The presented parameter of the TGT method, ETP, did not reflect the effect of ANDEXXA but this effect is clearly detected by a 50% increase and 40% shortening in the commonly used TGT parameters thrombin* (b) (4) *and time to thrombin* (b) (4)*, respectively. Therefore, the data contradict the conclusions in report NC-15-0659 which stated that "andexanet alone had minimal effect in the absence of rivaroxaban".*

3. *Analysis of the time courses of TGT in clinical trials presented in the NEJM paper[42] indicates that ANDEXXA administration does not simply restore TGT to the pre-FXa inhibitor treatment baseline but results in the elevation of TGT over this baseline, see Figure*

[42] 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

*17 as one example. Furthermore, analysis of the correlation graphs for anti-FXa activity vs. TGT demonstrates that reduction of anti-FXa activity by ANDEXXA is associated with elevation of TGT over the values which are observed in a placebo arm at low or in the absence of anti-FXa activity, see Figure 18 above.*

During the review of the BLA, clinical reviewers expressed concerns about the potential risk of thrombogenicity arising from the elevated thrombin generation in patients. In response to these concerns, Portola submitted an email dated 18 April 2016 in which Portola acknowledged that the increased TGT can be explained by the effect of ANDEXXA on TFPI activity, as evidenced from the contact-activated TGT assay which was used in the clinical trials as a control. However, Portola also reiterated that TFPI inhibition is a minor effect of ANDEXXA which does not translate into any pro-coagulant effect that may lead to thrombogenicity of the product.




*<u>Reviewer's comment</u>: Because the 18 April 2016 statements represented a critical point of the review cycle, but the email itself was not submitted as an official amendment to the BLA, I am reproducing the 18 April 2016 email as Appendix B of my memo.*

In the 22 April 2016 email, Portola stated "*Since andexanet also binds to TFPI and therefore removes this "Tissue Factor Pathway Inhibitor" from the patient plasma in the TG assay, the Tissue Factor reagent added to the assay is no longer inhibited by TFPI. As a result, a small*

(b) (4) *of extra TG is observed in the assay. This is entirely due to the sequestration of TFPI by andexanet in the assay mixture. This* (b) (4) *is not seen in the* (b) (4) *TG assay. If TFPI is removed from human plasma before it is added to the Tissue factor TG assay, the* (b) (4) *is also not seen. Both of these assays – the* (b) (4) *TG and TFPI-depleted plasma Tissue Factor TG assay – demonstrate that andexanet on its own has no prothrombotic activity as measured by enhanced thrombin generation."*

*Reviewer's comments: I disagree with Portola's conclusion that TFPI inhibition has no prothrombotic potential.*

1. *There is non-clinical evidence that TFPI inhibition has procoagulant effect in bleeding conditions such as hemophilia. It should be noted that Portola's assertion of the insignificant procoagulant effect of ANDEXXA on TFPI contradict with Portola's 2014 patent application WO 2014116275 A1 entitled "Inhibition of tissue factor pathway inhibitor with factor Xa derivatives" in which Portola reviewed the theoretical evidence for the procoagulant effect of TFPI inhibition which can be used for treatment of bleeding. For example, Portola cited multiple publications in which TFPI inhibition by various pharmacological agents was studied as a potential therapy for the treatment of hemophilia, including such agents as BAX499 (Gorczyca et al., J Thromb Haemost. 15 10(8):1581-90, 2012), ARC19499 (Waters et al., Blood, 117(20):5514-22, 2011), mAb2021 (Hilden et al., Blood, 119(24):5871-8, 2012), NASP (Liu et al., Thromb Haemost. 95:68-76, 2006).*

2. *Procoagulant action of ANDEXXA was documented in clinical trials. In Phase 1 clinical trials, reduction of TFPI activity was demonstrated to coincide with the elevation of all markers of in vivo thrombogenicity (TAT, PF1.2, D-dimer and so on). Elevated D-dimer is especially convincing because it indicates that thrombin generation did result in the fibrin clot formation which has then undergone fibrinolysis. The marker elevation continued for up to several weeks*

3. *Thrombin elevation over the pre-FXa inhibitor baseline may correlate with thrombogenicity because TGT is elevated in most conditions which are associated with thrombotic event development in humans. Although the TGT method is not validated as a predictive tool for clinical thrombogenicity, it is reasonably likely that elevated thrombin generation increases the risk of thrombosis.*

4. *Furthermore, Portola's reference to the results of comparison of the contact- and TF-activated TGT experiments demonstrates that ANDEXXA's effect on TGT is mediated by a mechanism via the inactivation of TFPI . Therefore, TFPI-dependent elevation of thrombin generation in vitro is in perfect agreement with the ANDEXXA-dependent thrombin generation in vivo as seen in the very first clinical studies in healthy volunteers.*

## *7.6. Relative contributions of reversal of anti-FXa activity and inhibition of TFPI action as assessed by thrombin generation in clinical trials*

*I should note that prior to the 22 April 2016 email, Portola did not report that a contact-based TGT assay had been used in the clinical studies as a control. Although the contact activated TGT was indeed reported for the characterization of ANDEXXA batches in vitro, no evidence on the use of contact-activated TGT in the clinical trials was provided for FDA review until July of 2016 (see below). Portola's conclusion that the elevation of TGT* ***in clinical trials*** *over the pre-inhibitor treatment baseline was mediated by the inhibition of TFPI activity, was new and contradicted their statements about the insignificance of TFPI inhibition in the presence of FXa inhibitors. This new information on the procoagulant effect of TFPI inhibition prompted me to examine more closely the results in the clinical studies regarding the duration of the procoagulant effect and the risk of thrombogenicity.*

Portola studied TFPI activity inhibition in the Phase 1 study. TFPI inhibition was observed for as long it was monitored, i.e., at least 22 hours, see Figure 20. Portola explained that the decrease in TFPI antigen was related to the interaction of ANDEXXA with TFPI because ANDEXXA was blocking the binding of TFPI to antibodies used to capture TFPI in the (b) (4) assays. In theory, inhibition of TFPI activity is correlated with a decrease in TFPI antigen, but a quantitative relationship between activity and antigen could not be established without additional experiments which Portola has failed to provide.

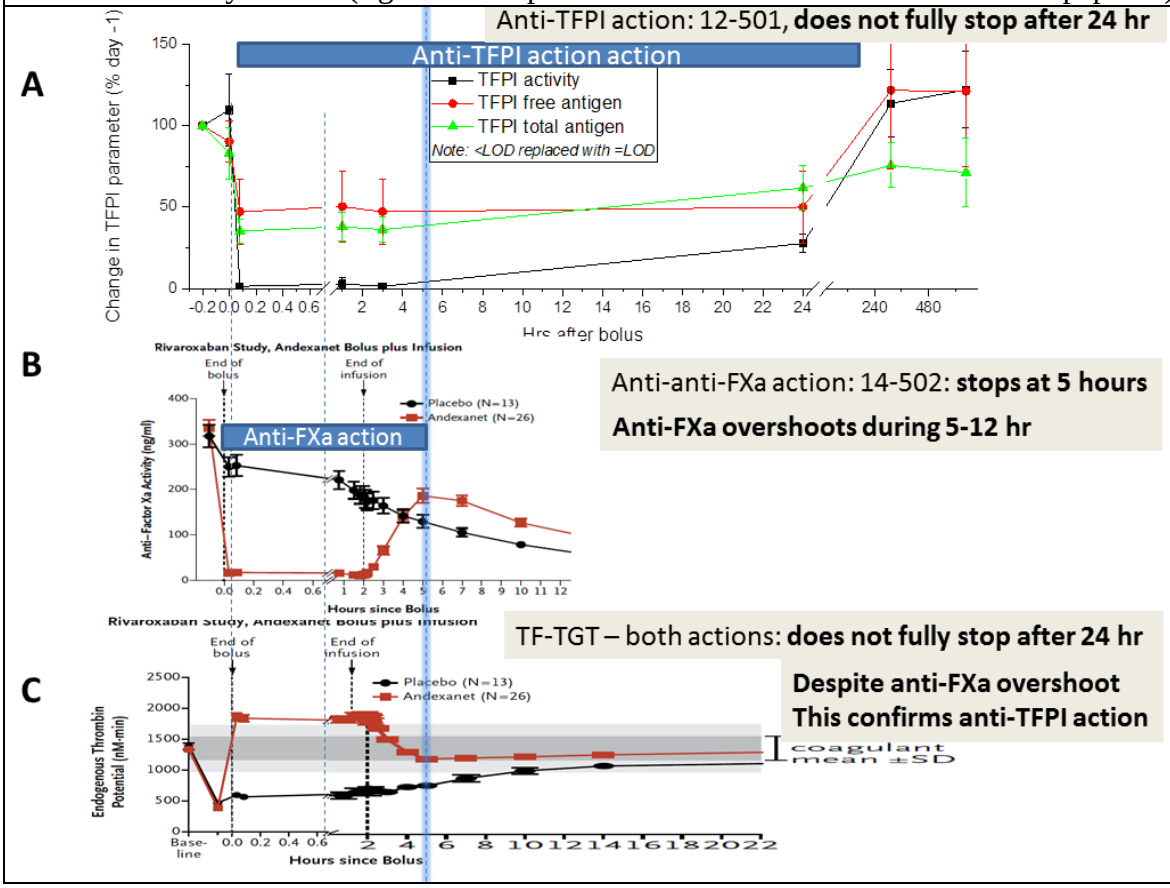
Contrary to the previously communicated commitments, Portola did not measure TFPI activity in the Phase 2 and Phase 3 clinical trials. However, Portola used two (b) (4) methods to measure TFPI antigen in the Phase 2 studies. The degree of decrease in TFPI antigen in the Phase 2 study was similar to that of the Phase 1 study (data not shown).

Side-by-side comparison of the time courses of the inhibition of TFPI with reversal of anti-FXa activity and elevation of TGT obtained in Phase 1 and Phase 3 studies, respectively is provided in Figure 21.

*Reviewer's comment: I conclude that anti-FXa reversal is very short (about 2 hours) and TFPI inhibition is prolonged (at least 24 hours but possibly much longer) and therefore all of the sustained elevation of TGT is probably a result of TFPI inhibition by ANDEXXA.*

**Figure 21: Duration of TFPI inhibition and anti-FXa activity reversal actions of ANDEXXA.** A. Data on TFPI activity and TFPI antigen inhibition in healthy volunteers without

FXa inhibitor. Data from Phase 1 study 12-501. B and C. Anti-FXa activity and TGT data from the Phase 3 study 14-502 (figures are reproduced with modification from NEIJM paper [43])




Because in the absence of ANDEXXA (a placebo arm), the time-courses of anti-FXa activity correlate with the time-courses of TGT extremely well (see Figure 22A), a placebo arm may be used to estimate the relative contributions of the anti-FXa reversal and TFPI inhibition actions of ANDEXXA, see Figure 22B and supplemental Figures 1, 2 and 3.

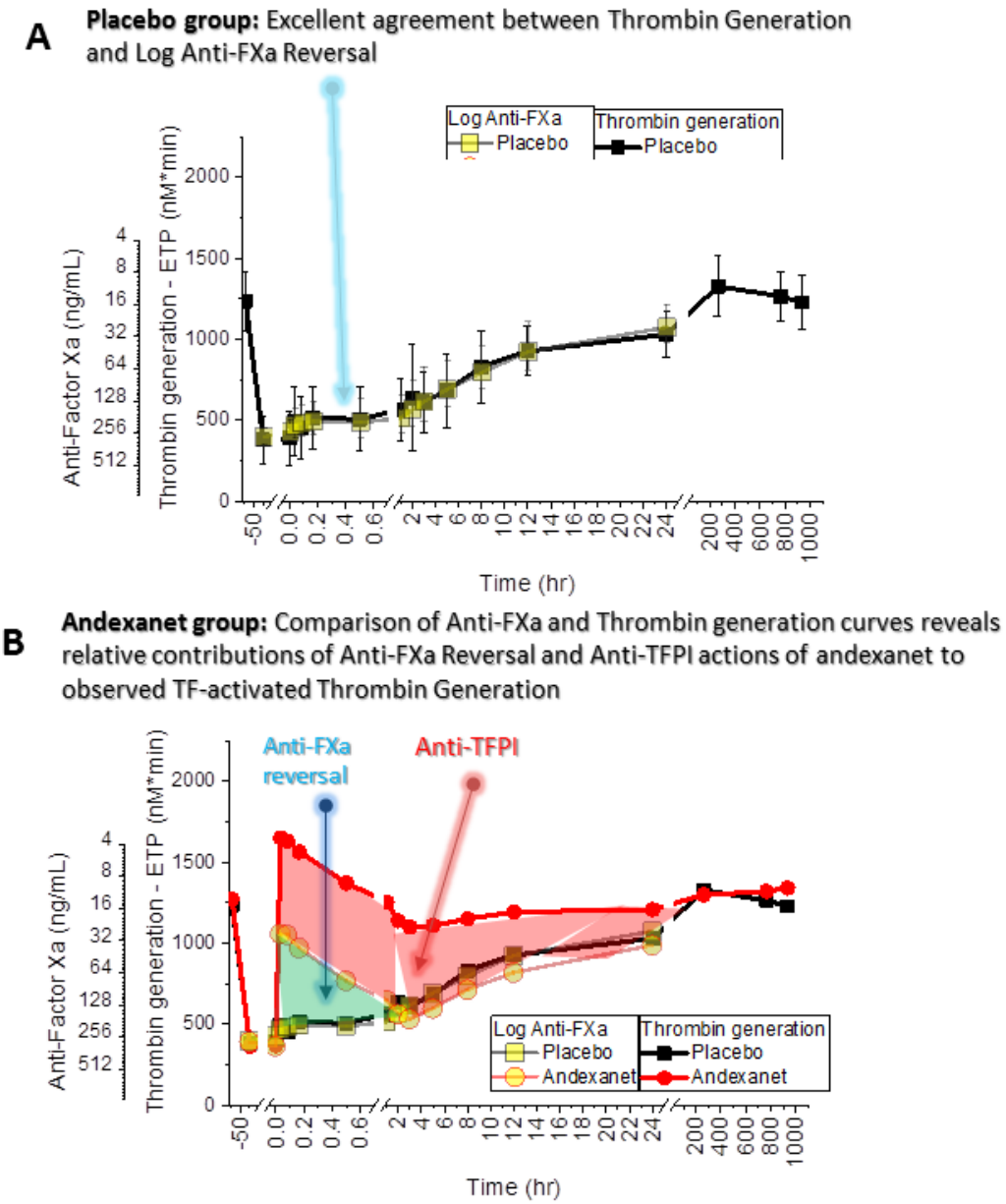
*Reviewer's comment: I conclude that TFPI inhibition may be responsible for ~50% of the observed TGT elevation during the first hour after ANDEXXA bolus and 100% of elevation for the remaining 24 hours or longer, see Figure 22.*

In a 19 July 2016 response to the FDA IR dated 1 June 2016, Portola submitted their preliminary data obtained with the contact-activated TGT. These data demonstrated a substantially reduced and shortened effect of ANDEXXA on TGT in the absence of TF, see Fig. 23.

**Figure 22: Estimation of the relative contributions of anti-FXa reversal and TFPI inhibition actions of ANDEXXA to the observed elevation of TGT in Phase 3 studies published in**

[43] 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

**NEJM paper**[44]**.** A. Overlay of the time courses of anti-Xa activity reversal and TGT elevation demonstrates excellent agreement of these two parameters in placebo treated subjects. B. The difference between the TGT values in ANDEXXA- and placebo-treated volunteers should be a result of two actions: anti-FXa activity reversal which can bring the TGT to the pre-treatment baseline and the inhibition of TFPI which elevates the TGT over this baseline.




*<u>Reviewer's comment</u>: The preliminary data seem to support my conclusion that TFPI inhibition plays a substantial role in sustaining the elevated TGT. It is interesting that TGT was elevated over the pre-treatment baseline even in the absence of TF. This may be explained by the following:*

[44] 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

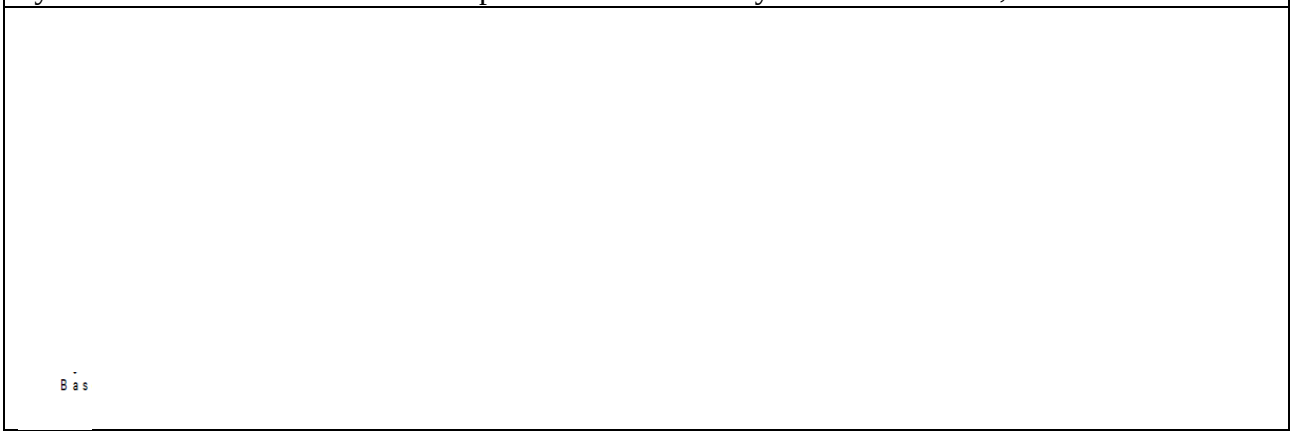
*(1) Artifacts of incomplete dataset analysis. Only about 30% of samples were studied by the contact-activated TGT. Raw data were not provided to the FDA for review.*

*(2) It is known that TFPI inhibits FXa even in the absence of TF. This effect may contribute to the elevation of TGT after TFPI inhibition.*

*(3) ANDEXXA may have an additional procoagulant effect, e.g., ANDEXXA may partially inhibit* (b) (4) *. Note that* (b) (4) *activity has not been investigated in clinical trials.*

*I recommend that Portola complete the re-testing of all retained samples from the clinical trials and submit the results for our review.*

**Figure 23. Preliminary results of TGT time courses by two assays, TF-activated TGT[45] (left) and contact-activated TGT (right).** Note that only a small subset of subjects (30%) was studied by the contact-activated method. Reproduced from 19 July 2016 amendment, see [46]



It is noteworthy that the preclinical studies, including studies using human plasma spiked with ANDEXXA, have not predicted the strong elevation of TGT observed in the Phase 3 clinical trials. *Reviewer's comment: For example, substantially stronger effect of ANDEXXA in the clinical trials is evident from the comparison of correlation graphs for anti-FXa activity and TGT, see figure 23.*

It is possible that TFPI effect on TGT is sensitive to the analytical conditions of the TGT assay. For example, Portola's 2014 patent application WO 2014116275 A1 contains an explanation that ANDEXXA's procoagulant effect on TGT in normal and hemophilia plasma is inversely proportional to the concentration of TF, e.g., this TGT elevation was lower in the presence of high amounts of TF and the effect is higher at low ((b) (4) ) concentrations of TF.

[45] 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

[46] 19 July 2016 Amendment Sequence 60: File 1.11.3 Clinical Information Amendment - TFPI and Additional Items.pdf

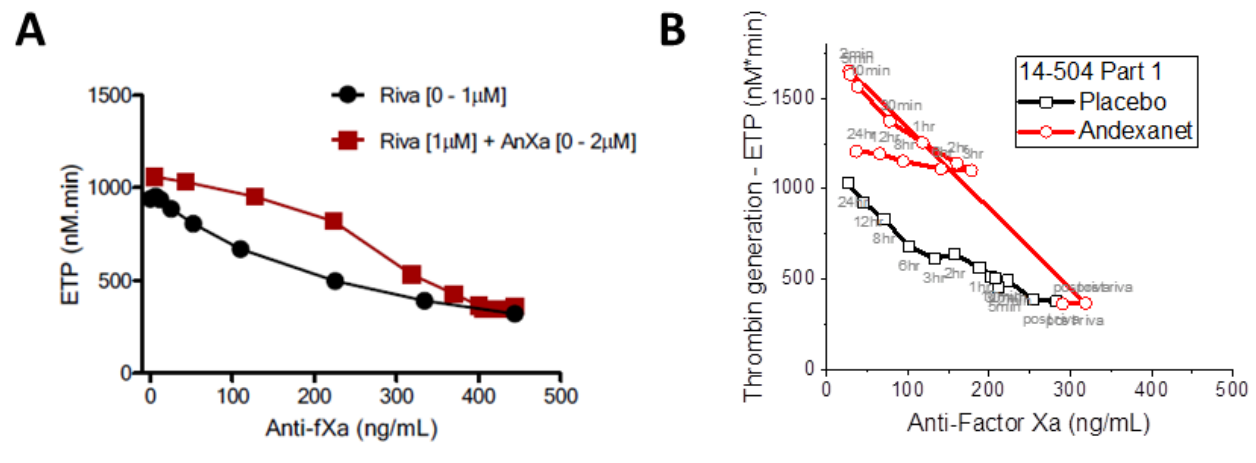
*Reviewer's comment: These conclusions from* WO 2014116275 A1 *and some data were not presented in the BLA. On 1 June 2016, I submitted the following IR to which Portola has not responded at the time of the writing of this memorandum:*

a. *To address the apparent deficiency in your prior conclusions from the analytical method qualification and preclinical studies that the effect of TFPI inhibition may be insignificant,*

   i. *Please explain why the preclinical studies using human plasma spiked with andexanet in vitro were not able to predict the TFPI-inhibition-dependent TG elevation seen in plasma samples from individuals receiving andexanet in vivo. Although on average a* (b) (4) *elevation in TG above the baseline was documented in the Phase 3 clinical studies, the spiking studies reported only a* (b) (4) *increase in TG above the pre-treatment baseline in plasma samples with or without a fully reversed anti-FXa activity (Figures 3-3 and 3-4 in preclinical report NC-15-0659-R0001, Figures 1 and 2 in report NC-12-0451-R0001, and Figure 1 in report NC-12-0452-R0001).*

   *Please consider the possibility of laboratory artifacts (including matrix effects such as inhibition of thrombin generation by excipients), the impact of plasma levels of TFPI, FXa inhibitor and andexanet which may have been different in the clinical versus spiked preclinical studies (e.g., use CAT to measure TG in normal plasma spiked with* (b) (4) *of andexanet in the presence of* (b) (4) *of rivaroxaban, in the presence and absence of inhibitory anti-TFPI antibody), and the impact of assay conditions, including but not be limited to assay temperature, plasma dilution factor, stability of plasma samples before and after andexanet spiking, and concentration of TF.*

**Figure 24: Correlation between anti-FXa activity and TGT in the presence of rivaroxaban *in vitro* (left) and ex vivo (right).** A. *In vitro* experiments in normal plasma, reproduced from Portola's study NC-15-0659-R0001. B. Data from Phase 3 studies. Note that TGT is elevated during the first two hours after ANDEXXA bolus.



## 7.7. Potential thrombogenic consequences of TFPI inhibition

The evidence of a sustained and nearly complete loss of TFPI activity up to 24 hours after ANDEXXA administration raises a concern that the safety of ANDEXXA as related to its ability to inhibit TFPI has not been adequately investigated in certain clinical situations, for example in bleeding patients with sub-therapeutic rivaroxaban levels. Such patients are less likely to benefit from the brief anti-FXa reversal but will be exposed to prolonged thrombogenic effect of TFPI inhibition. It should also be noted that FXa inhibitors are cleared from the circulation relatively quickly (half-lives around 15-18 hours), therefore the sustained procoagulant effect of ANDEXXA will not be modulated by the presence of FXa inhibitors. The following evidence is available on the thrombogenic roles of TF and TFPI:

- **TF is involved in atherothrombosis and cancer-associated thrombosis:** TF is expressed in the heart, lungs and throughout subendothelium where it is required for hemostasis. High levels of TF are also expressed in atherosclerotic plaques and likely contribute to atherothrombosis after plaque rupture[47]. Inhibition of the TF/Factor VIIa complex is unlikely to be an effective strategy to reduce atherothrombosis due the essential role of the complex in hemostasis. However, selective blockade of pathologic TF without affecting protective TF may be effective in reducing atherothrombosis.[48] In cancer, the principal mechanisms of thrombosis include the expression of TF by tumor cells. TF, constitutively expressed on malignant cell surface, plays a fundamental role in thrombin generation in cancer, but also contributes to tumor progression by directly inducing VEGF expression by both malignant and host vascular cells.[49]

- **TFPI inhibition is needed to control activation of coagulation**[50]**:** TFPI impacts a broad range of bleeding and thrombotic disorders. A number of mouse models have demonstrated physiological synergies between TFPI deficiency and procoagulant proteins such as TF, FVIIa, FV Leiden, and thrombomodulin. Total TFPI deficiency has not been observed clinically, suggesting that it is required for human embryonic development, as it is in mice. While low plasma TFPI is associated with venous and arterial thrombotic disease, in most studies, an increased risk is only observed in individuals with plasma TFPIα levels at or below the 10$^{th}$ percentile of the normal reference range. Of particular interest is that oral estrogen therapies decrease the total plasma TFPI concentration and activity by about 25%. The severe perinatal thrombosis observed in mice with heterozygous TFPI deficiency and FV Leiden suggests that these two procoagulant risk factors synergize to produce severe thrombotic disease. Women with FV Leiden increase their risk for thrombotic disease five-fold when they use oral contraceptives. It is reasonable to speculate that when the oral

[47] Bode MF, Mackman N. Protective and pathological roles of tissue factor in the heart. Hamostaseologie. 2015;35(1):37-46. http://www.ncbi nlm nih.gov/pubmed/25434707

[48] Tatsumi K, Mackman N. Tissue Factor and Atherothrombosis. J Atheroscler Thromb. 2015;22(6):543-9. http://www.ncbi.nlm.nih.gov/pubmed/26016513

[49] Falanga A, Marchetti M, Russo L. The mechanisms of cancer-associated thrombosis. Thromb Res. 2015 Feb;135 Suppl 1:S8-S11. http://www.ncbi.nlm.nih.gov/pubmed/25903541

[50] Maroney SA, Mast AE. New insights into the biology of tissue factor pathway inhibitor. J Thromb Haemost. 2015 Jun;13 Suppl 1:S200-7 http://onlinelibrary.wiley.com/doi/10.1111/jth.12897/full

contraceptive decreases TFPI levels, which then synergize with FV Leiden to greatly reduce endogenous anticoagulant activity and cause thrombosis.

In addressing FDA concerns, Portola argued that no evidence of thrombotic effect was observed in their animal preclinical studies.

*Reviewer's comment: I disagree with Portola's use of animal experiments to claim the lack of thrombogenicity in humans. Such risks should be investigated in human trials.*

*I propose the following to be included in the CR letter as an unresolved review issue:*

1. *Please note that animal experiments are generally not suitable for demonstration of relative contributions of anti-FXa activity reversal and TFPI inhibition effects in humans. You should base your conclusions on the results obtained in studies in humans. If you continue to want to use animal studies as supportive, please revise your reports to include accurate and scientifically valid interpretation of available evidence. Please address the following deficiencies:*

    a. *The existing animal models have not been validated to describe the pathophysiology of bleeding in humans. For example, the following differences between animal and human studies were not addressed:*
        1. *Contribution of TFPI inhibitor to bleeding and hemostasis may be different in different animal species, different injuries and different degrees of injury;*
        2. *Sensitivity of your animal models to TFPI inhibition was not established, e.g., in a positive control experiment with inhibitory anti-TFPI antibody.*
        3. *Similarity between human and animal studies was not established in the absence and presence of anti-FXa inhibitor by relevant pharmacodynamics markers of anti-TFPI action, e.g., TGT test and elevation of PF1.2, D-dimer and TAT.*
        4. *Your studies of animal TFPI activity should be performed at least with the relevant animal FVIIa, TF and FXa reagents because ANDEXXA may interact differently with each of the animal and human proteins TFPI, FVIIa, TF and FXa.*
        5. *Your conclusion that sequence homology can indicate similarity of function of animal and human TFPI molecules is deficient and should be supported with biochemical experiments. Minor differences in the sequence of animal and human proteins may result in drastic changes of the mechanisms of action as illustrated by the following FVIIa-dependent reactions which may be relevant to the anti-TFPI action of ANDEXXA in animals: (i) mouse TF does not bind human FVIIa but binds mouse FVIIa while human TF binds both mouse and human FVIIa, and (ii) rabbit TF does not support auto-activation of FVII while human TF does.*

    b. *Your animal models did not address the following mechanisms of thrombogenicity:*
        1. *The TFPI-dependent restoration of thrombosis observed in a rabbit model of recurrent arterial thrombosis under the control of anticoagulant therapy*

*(Ragni et al., Circulation 2000;102(1):113-7) and rabbit model of venous rethrombosis after lysis (Kaiser and Fareed. Thromb Haemost. 1996;76(4):615-20)*

2. *The loss of TFPI control over the initiation of thrombotic events at the sites of TF exposure. For example, because ANDEXXA reduces TFPI activity in plasma, the activation of coagulation will no longer be inhibited at TF-expressing, atherosclerotic plaques and cancer cells and vascular injuries. Specifically, please consider vascular injuries in trauma patients, during surgery, and in catheter-related events.*

## 7.8. Review of the draft labeling section 12. 1 Mechanism of Action section

Because ANDEXXA has been found to inhibit TFPI and express procoagulant activity in clinical trials, I disagree with the following section *12.1 Mechanism of Action* proposed by Portola in the draft prescribing information submitted on 30 June 2016:

> *"Andexanet alfa is a specific reversal agent for both direct and indirect FXa inhibitors. The predominant mechanism of action of andexanet alfa is binding and sequestration of the FXa inhibitor, although there may be a minor contribution from the inhibition of tissue factor pathway inhibitor (TFPI) activity through binding of andexanet alfa to TFPI. Andexanet alfa binds direct FXa inhibitors with high affinity, and also binds to indirect FXa inhibitors complexed with ATIII, making them unavailable to exert their anticoagulant effects. In addition to reversal of FXa activity, andexanet alfa has been shown to reverse the anti-IIa activity of a low molecular weight heparin, enoxaparin, in vitro.*
>
> *In animal studies in two different species using three different FXa inhibitors in both prophylactic and treatment models, andexanet alfa administration (either bolus alone or bolus-plus-infusion) reversed the anticoagulant activity of FXa inhibitors, restored hemostasis and reduced bleeding."*

Specifically, I found that the following statements to be inconsistent with the available evidence:

- ANDEXXA's reversal of FXa inhibitors is **not specific** because ANDEXXA also inhibits endogenous TFPI.
- TFPI inhibition is **not a minor contribution**. In the Phase 3 trials, TFPI inhibition was responsible for 30% to 100% of ANDEXXA effect on TGT at different time points after administration. In any case, there is insufficient evidence to compare the mechanisms of action to the effect of the drug in humans.
- This product is licensed on the basis of clinical trials in humans. **Animal models were not** demonstrated to correlate with the effect in humans.

I propose the following description in section *12.1 Mechanism of Action*:

> *"Andexanet alfa inhibits the action of FXa inhibitors apixaban, rivaroxaban, and edoxaban and endogenous tissue factor pathway inhibitor (TFPI). Andexanet alfa binds and sequesters FXa inhibitors and TFPI, making them unavailable to exert their anticoagulant effects. By inhibiting FXa inhibitors and TFPI, andexanet alfa increases FXa production at the sites of vascular injury. FXa, 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."*

This description of the mechanism of action is based on the data provided in the BLA and is consistent with the labeling of FXa inhibitors, activated factor VIIa and Idarucizumab, see Table 14.

**Table 14: Description of the Mechanism of Action for anti-FXa inhibitors, activated factor VII, and idarucizumab**

| **Drug** | **Prescribing Information section 12.1 *Mechanism of Action*** |
|---|---|
| XARELTO, direct FXa inhibitor | XARELTO is a selective inhibitor of FXa. It does not require a cofactor (such as Anti-thrombin III) for activity. Rivaroxaban inhibits free FXa and prothrombinase activity. Rivaroxaban has no direct effect on platelet aggregation, but indirectly inhibits platelet aggregation induced by thrombin. By inhibiting FXa, rivaroxaban decreases thrombin generation. |
| ELIQUIS, direct FXa inhibitor | Apixaban is a selective inhibitor of FXa. It does not require antithrombin III for antithrombotic activity. Apixaban inhibits free and clot-bound FXa, and prothrombinase activity. Apixaban has no direct effect on platelet aggregation, but indirectly inhibits platelet aggregation induced by thrombin. By inhibiting FXa, apixaban decreases thrombin generation and thrombus development. |
| SAVAYSA, direct FXa inhibitor | Edoxaban is a selective inhibitor of FXa. It does not require antithrombin III for antithrombotic activity. Edoxaban inhibits free FXa, and prothrombinase activity and inhibits thrombin-induced platelet aggregation. Inhibition of FXa in the coagulation cascade reduces thrombin generation and reduces thrombus formation. |
| LOVENOX, indirect FXa inhibitor | Enoxaparin is a low molecular weight heparin which has antithrombotic properties. |
| HEPARIN SODIUM, (b) (4) | Heparin inhibits reactions that lead to the clotting of blood and the formation of fibrin clots both in vitro and in vivo. Heparin acts at multiple sites in the normal coagulation system. Small amounts of heparin in combination with antithrombin III (heparin cofactor) can inhibit thrombosis by inactivating activated Factor X and inhibiting the conversion of prothrombin to thrombin. Once active thrombosis has developed, larger amounts of heparin can inhibit further coagulation by inactivating thrombin and preventing the conversion of fibrinogen to fibrin. Heparin also prevents the formation of a stable fibrin clot by inhibiting the activation of Factor XIII, the fibrin stabilizing factor. Heparin does not have fibrinolytic activity. |

| Drug | Prescribing Information section 12.1 *Mechanism of Action* |
|---|---|
| NOVOSEVEN, Activated factor VII | 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. This process may also occur on the surface of activated platelets. |
| PRAXBIND Idarucizumab | Idarucizumab is a specific reversal agent for dabigatran. It is a humanized monoclonal antibody fragment (Fab) that binds to dabigatran and its acylglucuronide metabolites with higher affinity than the binding affinity of dabigatran to thrombin, neutralizing their anticoagulant effect. |

An accurate description of the *Mechanism of Action* is needed to ensure that ANDEXXA, if approved, will be marketed truthfully. According to the promotional materials submitted in amendment dated 18 July 2016, Portola is planning to promote ANDEXXA as a therapy that has **coagulation activity** (as evidenced from 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 is included on one of the promotional slides), see Figure 25B for an example of promotional material.

I recommend modifying the promotional materials (and all related figures in the BLA) such that the time courses of TGT are plotted using *standard deviation* of the mean for all data points, which should include the pre-treatment (the so-called normal TGT range) for the ANDEXXA and placebo arms of the study. As evidenced from Figure 25B, Portola's 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.

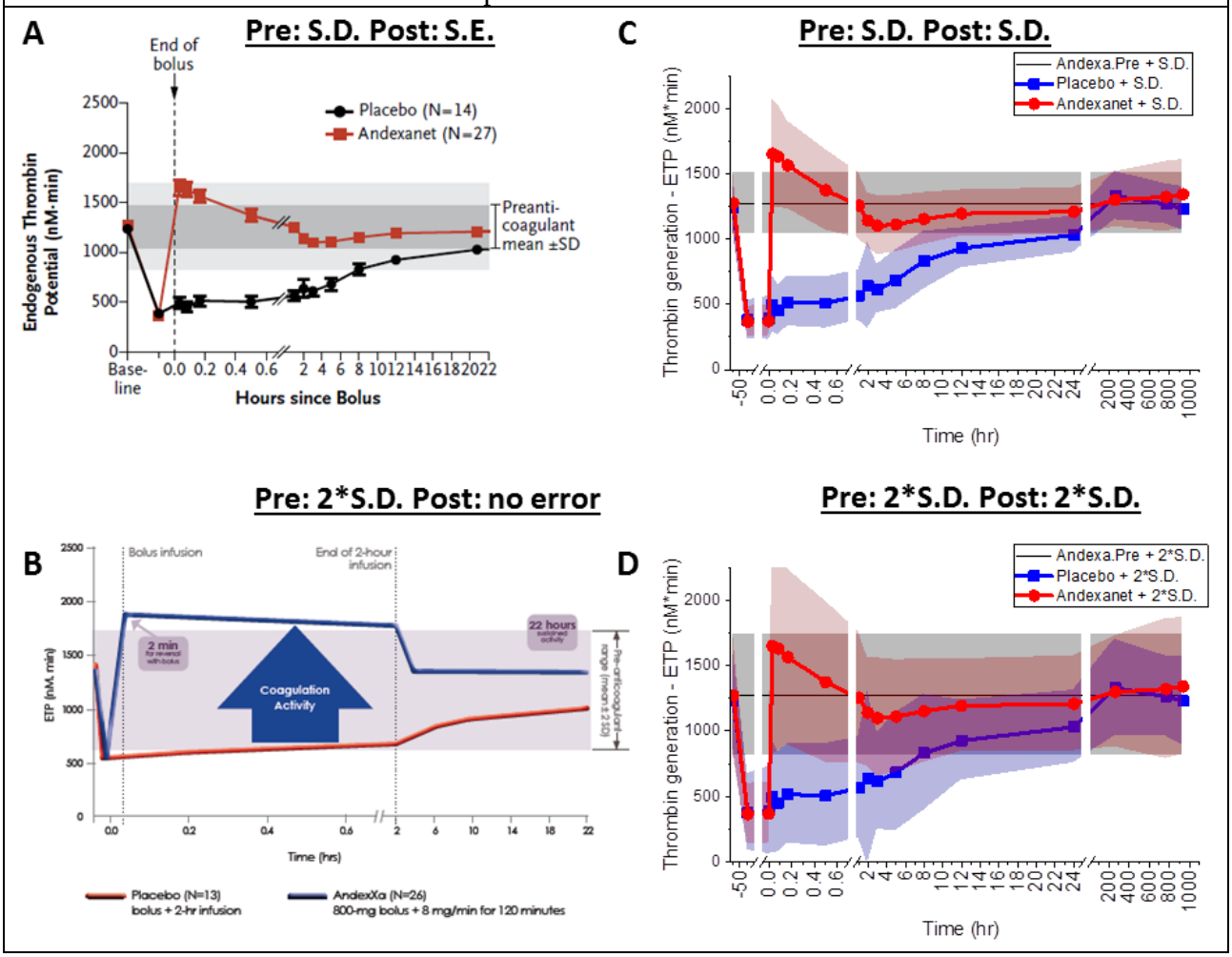
## *7.9. Conclusion*

I conclude that the documents in the BLA were substantially deficient in the description of one of the major mechanisms of action of ANDEXXA, i.e., its inhibition of the activity of TFPI which was correlated with the increase in thrombin generation and clot formation both *in vitro* and *in vivo*. These observations of TFPI inhibition was either not presented in the BLA or incorrectly described as having minimal impact on ANDEXXA effects in the clinical trials. As a result, the available experimental evidence was exaggerated to support the use of the anti-FXa activity as a surrogate endpoint to support the *Accelerated Approval* of ANDEXXA. Consequently, the evidence of the potentially thrombogenic effect of ANDEXXA was inadequately presented and addressed in the BLA and in the proposed labeling.

Because TFPI inhibition is potentially thrombogenic and is not as transient as the reversal of anti-FXa activity, the *Prescribing Information* should include accurate descriptions of the duration and magnitude of both outcomes of the effect of ANDEXXA in the clinical trials. I recommend requesting revisions to every sections in the BLA in which the mechanism of action of ANDEXXA

was described incorrectly, which includes all the *Clinical Study Reports* and summaries of Clinical and Preclinical disciplines.

**Figure 25: Comparison of experimental data with promotional claims submitted in the amendment dated 18 July 2016 [51].** *Please note that panels A, C and D and panel B show results of two different clinical trials, one with bolus and another with bolus plus infusion of ANDEXXA. The two experiments are presented together with the goal to show the effect of error bars on the interpretation of study results. The time courses should not be compared.* Panel A shows data from the NEIJM paper[52]. Error bars for the time courses of TGT in the placebo and ANDEXXA arms of the study are shown as S.E. They are compared with the one and two S.D.s of the mean for the pre-treatment time-point (gray area). Panel B. Error bars for the time courses are removed but means for the pre-treatment time-point are replaced with double S.D.'s (gray area). Panel C. Same as panel A but single S.D. is used to compare the time courses with pre-treatment baseline. Panel D. Same as panel C but error bars are shown as double S.D.




[51] Amendment Sequence 61 dated 18 July 2016. File: Efficacy Booth Panel PP-AnXa-US-0038 Clean Copy.pdf
[52] 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

With reference to the use of anti-FXa activity as a surrogate endpoint to support the accelerated approval of ANDEXXA, I conclude that Portola is yet to provide convincing evidence that the reversal of anti-FXa activity is reasonably likely to correlate with clinical benefits. I agree that it is plausible to assume that the reduction of anti-FXa activity by ANDEXXA may be associated with an improved hemostasis in a bleeding patient. However, an assay to measure anti-FXa activity alone does not reflect all of the actions of ANDEXXA, e.g., this assay does not measure the interactions of ANDEXXA with endogenous coagulation proteins, including TFPI. As a result, anti-FXa activity may not be found to correlate with the clinical benefit if this benefit is due to ANDEXXA's effect on TFPI.

According to the FDA Guidance on Expedited Programs [53], the post-marketing trial intended to verify the clinical benefit must be conducted promptly to facilitate determination, as soon as possible, of whether clinical benefit has been verified. I conclude that a meaningful confirmatory trial should also investigate the hypothesis that other ANDEXXA activities not reflected by the chosen surrogate may be responsible for the clinical outcomes observed. To achieve this goal, the effect of ANDEXXA on the magnitude and duration of the reversal of anti-FXa activity, inhibition of TFPI activity, and elevation of TGT activated by TF and contact pathways, should be analyzed.

*Reviewer's comment: I defer to the clinical reviewers to make final decisions on the appropriateness of the chosen surrogate marker and the need to modify the design of the confirmatory trials.*

# 8. Validation of Methods Used in the Clinical Trials

Pharmacokinetics (PK), pharmacodynamics (PD) and immunogenicity assays used in the clinical trials are summarized in Table 15 below. Figure 26 shows the effect of ANDEXXA on the PD assays.

**Table 15: Summary PK, PD and immunogenicity assays used in the clinical trials**

| Method Name | Principle | Intended Use of Method |
|---|---|---|
| **PK assays** | | |
| Andexanet Plasma Quantitation | (b) (4) | Quantify andexanet alfa concentrations in human plasma |
| Andexanet Urine Quantitation | (b) (4) | Quantify andexanet alfa concentrations in human urine |
| Apixaban Quantitation | (b) (4) | Quantify apixaban concentrations in human plasma matrix |

[53] FDA Guidance for Industry Expedited Programs for Serious Conditions – Drugs and Biologics. May 2014

| Method Name | Principle | Intended Use of Method |
|---|---|---|
| Apixaban Unbound Quantitation | (b) (4) | Quantify unbound apixaban concentrations in human plasma and mixtures containing human plasma and phosphate-buffered saline |
| Apixaban Urine Concentration | | Quantify apixaban concentrations in human urine matrix |
| Rivaroxaban Quantitation | | Quantify rivaroxaban concentrations in human plasma matrix |
| Rivaroxaban Unbound Quantitation | | Quantify unbound rivaroxaban concentrations in human plasma and mixtures containing human plasma and phosphate-buffered saline |
| Rivaroxaban Urine Concentration | | Quantify rivaroxaban concentrations in human urine matrix |
| Edoxaban and D21-2393 Quantitation | | Quantify edoxaban concentrations in human plasma matrix |
| Edoxaban and D21-2393 Quantitation | | Quantify edoxaban concentrations in human plasma matrix |
| Edoxaban and D21-2393 Unbound Quantitation | | Quantify unbound edoxaban concentrations in human plasma and mixtures containing human plasma and phosphate-buffered saline |
| Edoxaban Urine Concentration | | Quantify edoxaban concentrations in human urine matrix |
| Enoxaparin Quantitation | | Quantify amount of enoxaparin present in human plasma sample |
| **PD assays** | | |
| Anti-FXa Activity (Portola) | | Measuring anti- FXa activity of FXa inhibitors |
| Anti-FXa Activity (b) (4) | | Measuring anti- FXa activity of FXa inhibitors |
| Tissue Factor (TF)-Initiated Thrombin Generation ((b) (4)) | | Measuring thrombin formation in the presence and absence of FXa inhibitors |

| Method Name | Principle | Intended Use of Method |
|---|---|---|
| | (b) (4) | |
| Calibrated Automated Thrombogram (CAT) | | Measuring thrombin formation in the presence and absence of FXa inhibitors |
| **PD - Coagulation Markers** | | |
| D-dimer | | Measuring D-dimer in human plasma |
| dRVVT | | Assess potential presence of antibodies against endogenous factor X or Xa in human plasma |
| (b) (4) and FX Antigens | | Quantify ATIII and factor levels in human plasma |
| F1+2, TAT and total TFPI | | Quantify F1+2, TAT and total TFPI levels in human plasma |
| Free TFPI | | Quantify free TFPI in human plasma |
| (b) (4) and total TFPI Antigens | | ATIII and total TFPI antigens |
| F1+2, TAT, D-dimer | | F1+2, TAT and D- dimer levels |
| **Immunogenicity assays** | | |
| Anti-Andexanet Antibodies | | Detect anti- andexanet antibody |

| Method Name | Principle | Intended Use of Method |
|---|---|---|
| Anti-FXa Antibodies | (b) (4) | Detect antibody against FXa protein |
| Anti-fX Antibodies | (b) (4) | Detect antibody against fX protein |
| Anti-FXa Activity (for neutralizing antibody activity) | (b) (4) | Assessing neutralizing antibody activity in samples positive for anti-andexanet antibodies. |

*<u>Reviewer's comments</u>: The following methods are relatively standard and their validation is acceptable:*

1. *The PK methods used for detection of FXa inhibitors are described in the literature. These methods were validated to determine the limits of detection and interference with ANDEXXA.*

2. *The methods for markers of coagulation factor and inhibitor activity and coagulation were based on commercially available kits used by clinical laboratories. These methods were validated to determine the limits of detection and interference with ANDEXXA.*

3. *The anti-FXa activity assay which was used as surrogate endpoint of clinical response was based on the commercially available anti-FXa activity kits comprised of* (b) (4) *FXa and relevant FXa inhibitor standards for rivaroxaban, apixaban,* (b) (4) *, edoxaban and enoxaparin.*

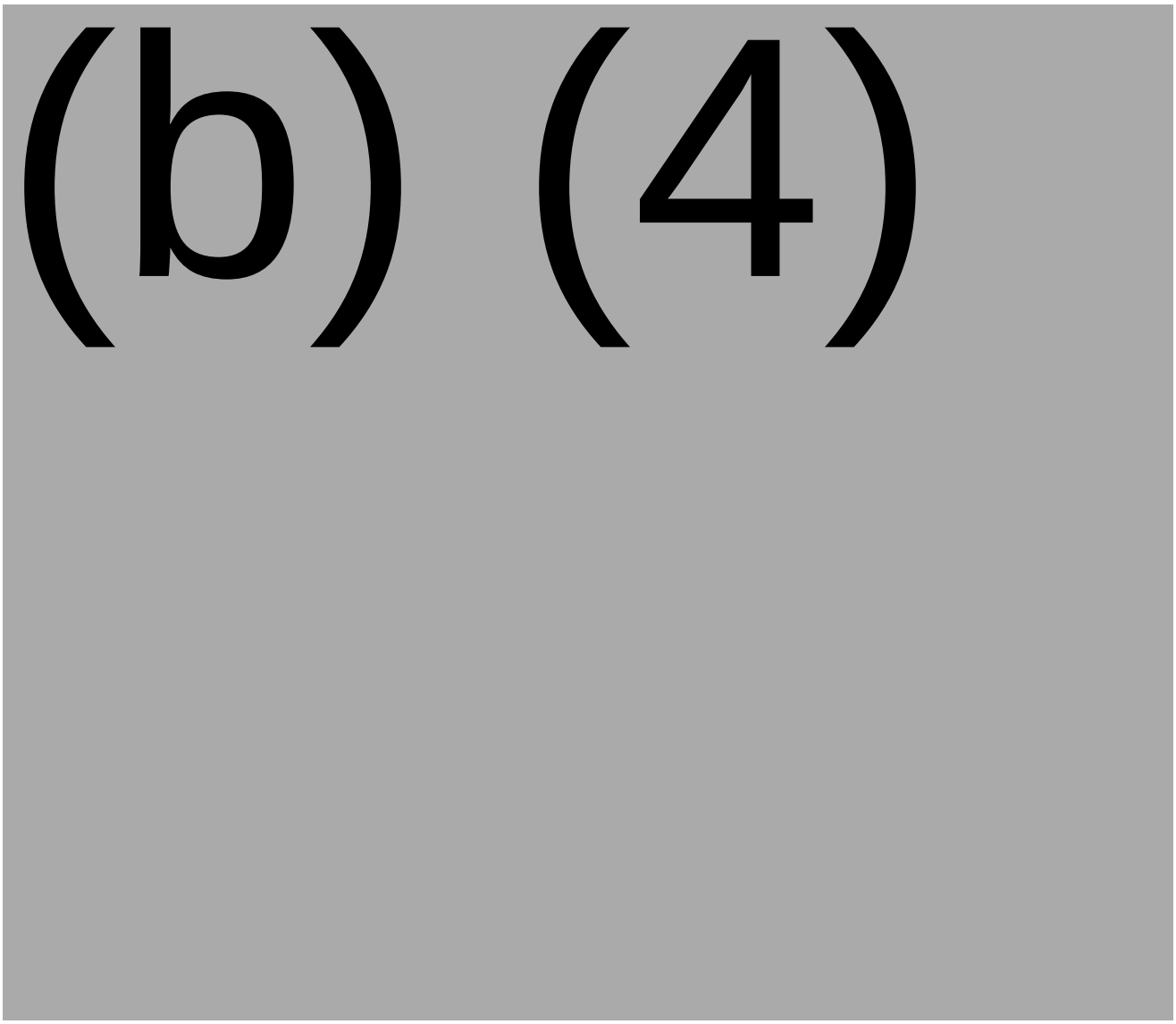
*Review of the following assays identified significant issues which were communicated to Portola (Portola has agreed to resolve these deficiencies but did not provide a complete response at the time of the writing of this memorandum):*

4. *Validation of the TFPI activity assay was not provided. In particular, interference of this method with ANDEXXA or FXa inhibitors was not studied.*

5. *Portola claimed that an* (b) (4) *to assess free TFPI antigen was used in place of the TFPI activity assays in the Phase 2 and 3 clinical trials. However, the replacement of TFPI activity assay with an antigen-based method was not described in the Clinical Study Reports nor was the method validated for this purpose. In particular, a correlation between TFPI activity and free TFPI antigen level was not established.*

6. *Different versions of the critically important PD assays for anti-TFPI activity and TGA were used in the Phase 1, 2 and 3 clinical trials without support of properly performed bridging studies. As a result, an incorrect interpretation of results obtained in the pivotal safety and efficacy Phase 3 trials was provided in the BLA. Specifically, the Phase 3 assay was later shown to be very sensitive to the inhibition of TFPI activity by ANDEXXA. Because Portola*

*did not evaluate the Phase 3 TGT method prior to use, the procoagulant effect arising from TFPI inhibition was incorrectly presented as evidence for the reversal of anti-FXa activity in various documents, including the pre-BLA briefing documents, BLA summary sections, Clinical Study Reports and Portola's publications including the NEJM publication* [54].




7. *Contrary to repeated claims of having done so, Portola has neither used nor developed assays to measure antibodies that inactivate endogenous FX and FXa. Portola claimed that pharmacodynamics test such as anti-FXa activity, TGT and clotting times can be used to detect neutralizing antibodies against FX and FXa but these methods were not validated for the detection of anti-FX antibodies. Portola plans to develop an assay to detect antibodies that can inhibit the activities of FX and FXa. However, Portola claims that no retained samples exist that can be used for the evaluation by this test when it will be developed.*

[54] 19 July 2016 Amendment Sequence 60: File 1.11.3 Clinical Information Amendment - TFPI and Additional Items.pdf

8. *No methods to detect antibodies that can bind (b) (4) were developed. Portola explained that this is not needed because ANDEXXA will be administered once in a life time. However, Portola is now planning to investigate the repeated administrations of ANDEXXA. In addition, a (b) (4) impurity of possible CHO (b) (4) origin may be present in the FDP. Therefore, I recommend developing the method for the detection of (b) (4) antibodies.*

# 9. Summary of Issues Identified During the BLA Review

The following substantive issues were identified during the review of the ANDEXXA BLA:

A. CMC (Product office) deficiencies:

1. The data on process development and validation are 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, data on batch consistency, comparability of (b) (4) and (b) (4) batches, and stability data.

   CBER performed a PLI of the (b) (4) facility from (b) (4) covering the manufacturing of (b) (4) FDP release testing. CBER issued a Form FDA 483 with four observations. Responses to observation 1 "process validation is incomplete", are not acceptable and the inspection is not closed. Portola provided additional data on 30 June and 8 July 2016. In these responses, repeated OOS for (b) (4) of andexanet alfa observed for (b) (4) batches at release, in stability studies and in-process intermediates were linked to the presence of (b) (4) impurities in ANDEXXA intermediates. The identity of the (b) (4) impurities and the capability of the purification process to remove these impurities are under intense investigation. Portola's responses confirm that the process validation remains incomplete.

   On 30 June 2016, in an attempt to partially mitigate the increase in the (b) (4) during the purification process, Portola introduced a new Critical Process Parameter, (b) (4), but this parameter remains poorly controlled at this time [55]. (b) (4) will install new equipment required to control the (b) (4) at the point of use. The earliest date when this action can be completed is 15 November 2016 based on equipment lead time from the vendor. After that, the change to control temperature will have to be assessed for its impact on process validation.

   On 11 July 2016, Portola informed the FDA that new intermediate hold time validation protocol VAL-30291-01 was initiated. According to Portola, the new study is more representative of the manufacturing scale, and will challenge the process at the maximum limits of the process step hold times. Portola proposes to submit the final validation report by the end of October 2016.

[55] 30 June 2016 Amendment Sequence 48; file 1.11.1 (b) (4)-483 Responses (Amended).pdf

In addition, an apparent non-compliance with CGMP was identified during the review of FDP PPQ report 414-21-04-001-SR2 submitted on 16 April 2016. The PPQ series was manufactured using a (b) (4) scheme in which an FDP batch was produced by (b) (4). The (b) (4) FDP batch in the PPQ series, (b) (4), was produced by (b) (4). (b) (4) had a release OOS for the (b) (4) and was not released to-date while the resulting FDP (b) (4) was tested within (b) (4) specifications and released. In addition, a total of (b) (4) of (b) (4) batch (b) (4) were used to make all (b) (4) PPQ FDP batches although the validated final fill for the (b) (4) is only (b) (4), which indicates that batch (b) (4) was OOL for a key operating parameter of the (b) (4) process. The use of (b) (4) non-conformant (b) (4) batches was approved by QA of the FDP facility, (b) (4) in (b) (4), and the deviations were not documented in the FDP PPQ report.

2. The available stability data are not sufficient to support the proposed shelf-life because only 6 months of real-time data for (b) (4) FDP were provided using the proposed quantitative lot release and stability analytical methods. Up to 12 months of (b) (4) data derived from the earlier versions of the lot release methods are available but the data were not analyzed quantitatively. The data obtained from the (b) (4) material to support the shelf-life are not acceptable because a side-by-side comparison under accelerated stability conditions demonstrated (b) (4) of (b) (4) batches as evidenced by a (b) (4) of formation of the (b) (4).

3. The Comparability Protocol (CP) for the implementation of proposed manufacturing changes (scaled-up process referred to as (b) (4)) is also deficient. We received Portola's responses and a revised CP on 24 June 2016. FDA identified several deficiencies in the revised CP including absence of well-defined criteria of comparability, and does not consider the CP to be suitable to support a downgrade of the supplement to add (b) (4) for the manufacture of (b) (4) FDP.

4. The release specifications of (b) (4) FDP for excipients, identity, and impurities are deficient and the release methods are not fully validated. Andexanet alfa is a mutated coagulation factor product manufactured at large scale, formulated at high concentration and administered at high doses. The inclusion of excipient specifications and enhanced identity tests ((b) (4) and characterization of (b) (4) modifications, e.g., (b) (4)) will provide assurance of consistent product quality to compensate for the limited manufacturing experience. IRs were sent on 2 April and 22 June 2016 requesting specifications to be established based upon results from all relevant studies and manufactured batches. Portola proposed to develop new release assays by 31 October 2016, 2.5 months after the goal date.

   For the remaining release methods, the proposed specifications for the (b) (4) are based on retrospective analysis of (b) (4) batches and release data for (b) (4) batches. Because (b) (4) batches were demonstrated to contain (b) (4) levels of (b) (4), use of (b) (4) data is no longer acceptable. FDP specifications are based on (b) (4) specifications which is not acceptable.

The release specification for endotoxins in the FDP is very close to the compendial infusion limit for endotoxins and can be exceeded because Portola is considering the use of higher doses in the future. On 8 July 2016, Portola responded that the current endotoxin method at (b) (4) is not suitable for supporting a lower specification [56]. Portola proposed to develop new method and specification by 31 October 2016.

In addition, deficiencies in the (b) (4) release method for purity were identified by the FDA in-support testing group. FDA found that a slight change in analytical assay conditions reveals presence of (b) (4) of andexanet alfa. This brings into question the suitability of Portola's test to assess product purity because their (b) (4) analysis shows only (b) (4). These results indicate the need for additional product characterization and method validation. The FDA did not have time to communicate these findings to Portola because Portola had not submitted the product samples and standards to us by the agreed upon dates, on 30 June and 22 July 2016, respectively.

5. The potency standard is not properly qualified and the consistency of product potency in the event of future standard replacement is not assured. The determination of potency and its specification which is expressed as "percent of a reference standard" is not suitable for the control of the unitage because there is no assurance of the stability and consistency of the reference standard. On 2 April and again on 22 June 2016, Portola was asked to develop a potency unit for andexanet alfa. Portola proposed to develop product-specific units and establish specifications by 31 October 2016, 2.5 months after the goal date. Additional requests about primary potency standard were submitted by the FDA in-support testing group.

   On 15 July 2016, Portola informed the FDA that the current reference standard manufactured on 26 March 2015 is no longer available. New reference standard was established on 2 May 2016 against the previous lot and no bridging studies between the predecessor standards were conducted. It is apparent that Portola does not maintain a primary reference standard for ANDEXXA. This practice is unacceptable because there is no assurance of the conservation and control of the unitage for the potency of the product.

B. Validation of bioanalytical methods used in the clinical studies – not resolved:

1. Portola failed to properly qualify and bridge different versions of TFPI activity and TFPI antigen assays, and the Thrombin Generation Test assays (TGT) used in the Phase 1, Phase 2 and Phase 3 studies. On 19 July 2016, Portola provided preliminary analysis of a subset of retained clinical samples which confirmed the differences in results obtained by the different versions of these assays. The investigations are on-going and no date for completion was provided.

[56] 8 July 2016 Amendment Sequence 55, file: 1.11.1 Quality Information Amendment.pdf

2. Because of the deficiencies in the qualification of the methods used in the clinical studies, the interpretation of the results in pharmacodynamics studies, and in turn the description in the *Prescribing Information* are incorrect. Specifically, the magnitude and duration of the inhibition of TFPI activity by andexanet alfa was underestimated. The role of the inhibition of TFPI activity by andexanet alfa in the elevation of thrombin generation was underestimated. On 19 July 2016 [57] Portola provided a preliminary analysis of the retained samples from the Phase 3 clinical trial using a TF-independent TGT which confirmed that, contrary to the Clinical Study Reports (CSRs) and clinical summaries in the IND and the BLA, the sustained TGT elevation observed for up to 24 hours after andexanet alfa administration was related more to the inhibition of TFPI activity by andexanet alfa rather than the reversal of FXa inhibitory activity by andexanet alfa. As a consequence of these new findings, the CSRs should be amended with new safety and efficacy interpretations and the *Prescribing Information* sections *12 CLINICAL PHARMACOLOGY* and *14 CLINICAL STUDIE* should be substantially rewritten. These investigations are not completed at this time as Portola has finished evaluating only one third of the retained samples and no statistical analyses were performed. No date for the submission of a completed study report and the amended CSRs was provided.

3. The methods used to assess immunogenicity in patient samples are deficient and do not permit the adequate assessment of safety of andexanet alfa in the clinical trials. Portola has not developed assays to detect ADAs that may neutralize endogenous coagulation Factors X and Xa. Development of neutralizing antibodies against endogenous proteins is a potential serious adverse event, and the FDA had requested Portola to develop these assays during the pre-IND meeting on 16 June 2009 (CRMTS #7089, Ref. PS000698). In the original IND submitted on 15 March 2012, Portola had included a commitment to develop these assays, but Portola now states that the assays for neutralizing antibodies against FX and FXa activities were replaced with the analysis of the pharmacodynamics assay data. However, Portola did not validate the pharmacodynamics assays for interference with neutralizing antibodies. As a result, Portola's claims about the immunogenic safety of andexanet alfa in the *Prescribing Information*, *Risk Management Plan* (1.16.1 Risk Management) are not supported at this time. In addition, Portola is not able to assess the unwanted immune responses during the on-going clinical trials [58] as required by the *FDA Guidance for Industry - Immunogenicity Assessment for Therapeutic Protein Products*. Portola proposed to develop new methods to assess immunogenicity by 31 October 2016. Portola claims that retained samples from previous clinical trials are no longer available and the new methods will be introduced for the ongoing Phase 3b/4 study.

   Portola's clinical studies have not assessed the immunogenic potential of process-related impurities, such as CHO (b) (4), because ANDEXXA was expected to be used once in a life time of the patient. However, repeated administrations of ANDEXXA are now planned in Phase 3b/4 clinical trials and

[57] 19 July 2016 Amendment Sequence 60: File 1.11.3 Clinical Information Amendment - TFPI and Additional Items.pdf
[58] FDA Guidance for Industry - Immunogenicity Assessment for Therapeutic Protein Products

(b) (4) impurities of possible CHO (b) (4) origin could possibly be present in (b) (4) FDP. Therefore, Portola should also develop the assays to detect anti-CHO antibodies in patients.

# 10. Chemistry, Manufacturing and Controls - Conclusion

Because ANDEXAA is developed for an indication that addresses an urgent unmet medical need, our CMC review team has been working closely with Portola to facilitate the development of this product in accordance with FDA's guidance on *Expedited Programs for Serious Conditions* since our first pre-IND meeting in 2009. There were multiple meetings and correspondences on CMC issues since then even after the submission of the BLA in December 2015.

It was not until the submission of the BLA that the review team has a chance to study the actual data related to the product and manufacturing process, and to learn the details of the manufacturing process and its capability and state of control. During the BLA review and PLI in (b) (4), we identified several manufacturing deficiencies which were not previously reported to the FDA. Multiple extensive information requests were sent to Portola throughout this review. In a series of CMC amendments received in the month of July 2016, Portola also acknowledged multiple new deficiencies in the validation of the manufacturing process and analytical methods; and indicated that it will not be able to address these deficiencies by the PDUFA goal date of 17 August 2016. Portola stated that several investigations and method validation studies are expected to be completed by 31 October 2016, and a number of critical investigations into the sources of (b) (4) impurities have only been started at this time. In addition, Portola's contract manufacturer for the ANDEXXA BDS, (b) (4) in (b) (4), is currently modifying its equipment in an effort to improve the control over the critical process parameters. Equipment modification to (b) (4) at the point of use is scheduled for completion on 15 November 2016 and its implementation will likely require an additional process validation study.

Other deficiencies were identified in the *Comparability Protocol* (CP) which Portola plans to use to implement the scaling up of the manufacturing process. Most significant, Portola has not specified definite acceptance criteria of comparability in the protocol. In addition, other deficiencies were noted in stability data, establishment of reference standards as it is related to potency assignment and unitage conservation, suitability and qualification of assays to assess immunogenicity, and consideration of the mechanism of action of andexanet alfa based on the totality of evidence presented in the various modules of the BLA.

At this time, from a basic assessment of the CMC information provided thus far, the manufacturing process of ANDEXXA is not considered to be adequately validated and sufficiently controlled to ensure consistent manufacture of the commercial product that meets the release specifications. The CMC information do not support the quality and safety of ANDEXXA to be used for the urgent reversal of anticoagulation with direct FXa inhibitors.

In summary, I found the CMC information inadequate to support the quality, identity, purity, potency and safety of ANDEXXA, and recommend issuing a *Complete Response* (CR) *Letter* in which all CMC deficiency items will be listed.

# 11. Proposed CMC Deficiency Items to be included in the *Complete Response Letter*

We have completed our review of all submissions made relating to this BLA with the exception of the labeling amendment dated 8 July 2016, amendments with promotional materials dated 11 July, 12 July, 18 July, 04 and 12 August 2016, the clinical and preclinical amendment dated 19 July, and the clinical protocol amendments dated 4 August and 5 August 2016 . The scope of this letter does not encompass dosing regimens of longer than 2 hours.

Based on the current status of review, we have concluded that we cannot grant final approval because of the deficiencies outlined in this document. In your complete response to this letter you may reference applicable sections of the amendments that have not yet been reviewed and we will address those sections accordingly.

CMC

We acknowledge that ANDEXAA is a breakthrough therapy developed for an indication that addresses an urgent unmet medical need. As such, FDA is committed to working with Portola to advance your manufacturing program. We have submitted multiple requests for information (IRs), and we have received your responses. We have determined that these responses to our IRs are incomplete The information needed for approval is outlined below in detail:

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:

    a. 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 (b) (4) 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)

(b) (4) *including the* (b) (4) *that may lead to increased* (b) (4) *product percentage*." In the 17 July 2016 amendment to the BLA, you explained that several investigations on (b) (4) impurities are ongoing and acknowledged that "*As of yet, we have not identified the source of the* (b) (4) *in the upstream process.*" Please note that impurity clearance 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.

b. 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) impurities:

   1. Analysis of consecutive BDS 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).

   2. 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) of the (b) (4) and a (b) (4) of (b) (4) in the (b) (4) when comparing materials from (b) (4) to those from (b) (4).

   3. 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).

   4. 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.

c. 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)

(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.

d. During the PLI, we observed that (b) (4) were associated with a (b) (4) 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.

e. Complete the validation of hold times for process intermediates during the manufacture of the (b) (4) and demonstrate the control over the (b) (4) and other quality attributes of the (b) (4). 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.

f. 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) batche(b) (4) and Out of Limit (OOL) (b) (4) batch (b) (4) with conforming (b) (4) batches to manufacture (b) (4) PPQ FDP batches that met specifications as described below:

1. According to the aforementioned deviation investigation *DEV-1632*, (b) (4) batch (b) (4) ((b) (4) number (b) (4) ) was not released because the release testing for the (b) (4) ((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) batches (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) 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) out of specification batches with batches meeting specifications in order to meet acceptance criteria is not considered to be acceptable GMP.

2. 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.

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.

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 (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 were 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:

   a. 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).

   b. In reference to our IR dated 07 April 2016 and your 20 April, 08 July and 29 July 2016 responses, which are incomplete,

      1. 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).

2. Validate the analytical methods and establish release specifications for the excipients mannitol, sucrose, and Polysorbate 80. Please also qualify all compendial analytical methods used for the release of raw materials intended for FDP formulation.

3. 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) and 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) "

c. 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) lyophilized (FDP) formulations of (b) (4) materials. Please also explain why the product is treated with (b) (4) before (b) (4) . The treatment reduces (b) (4) , and in turn gives results that are not representative of the actual composition of the product.

d. 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*".

e. In reference to our IR dated 01 June 2016 and your 15 June and 19 July 2016 responses which are incomplete, develop a potency 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.

f. 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.

g. 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 a representative (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.

h. 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:

    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 increase 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.

    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.

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) 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.

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.

i. 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.

j. Please note that your justifications for specifications should explain how the finalized specifications 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..

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:

a. Provide your reference standard qualification protocol for review.

b. Qualify and establish (b) (4) 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.

c. Qualify the reference standards independently for both the direct and the indirect potency assays.

d. 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.

e. 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.

f. 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.

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.

   To demonstrate product stability over time:

   a. 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.

b. 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 (b) (4) is related to the (b) (4) detected by the new (b) (4) methods.

c. 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) 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.

d. 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 24-hour period.

5. Please address the following deficiencies in in-process control parameters:

a. 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 (b) (4).

b. 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.

c. List the validated (b) (4) FDP fill volume ranges for the commercial (b) (4), the expected scaled-up (b) (4) (known as (b) (4)) and the Gen2 process at Lonza. In your response, 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 1 FDP batch
v. Number of vials per FDP batch

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:

a. 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 $K_d$ 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 $K_d$ 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.

b. Use (b) (4) to investigate the interactions of the (b) (4) of andexanet alfa with TFPI.

c. Investigate the sensitivity of the (b) (4) method to evaluate the (b) (4) 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 - $K_d$, ΔH, TΔS, ΔG and n.

7. (b) (4) of your FDP (b) (4) samples, including (b) (4) batches of lyophilized drug product, (b) (4) lot of pre-lyophillized solution and the "reference standard", which we analyzed by (b) (4) using a (b) (4) all show (b) (4), in addition to (b) (4) for (b) (4), when (b) (4) is replaced by (b) (4) in the (b) (4). Please identify the proteins in these (b) (4).

8. In reference to the latest version of the Comparability Protocol (CP) for post-approval changes for (b) (4) FDP manufacture submitted on 21 June 2016, which also included the manufacturing history for the (b) (4) process, we find that the CP cannot be approved as currently designed. The following deficiencies need to be addressed:

a. Drug Substance Protocol:

(b) (4)

Given the manufacturing history of (b) (4) and the numerous deviations resulting in (b) (4) failures and lot terminations, the (b) (4) process does not appear to be in a state of control. In addition, including additional (b) (4) (more than needed) to account for anticipated failures is not acceptable CGMP.

2. Deviation investigation DEV-2188- (b) (4), Lot (b) (4), reviewed on inspection of (b) (4), documents an OOS for (b) (4) in the (b) (4), and (b) (4) failures in multiple downstream steps and final (b) (4). Because the root cause was determined to be a (b) (4) cleaning failure, please provide the completed investigation and any corrective actions associated with deviation DEV-2188-A86U03 since at the time of the inspection ((b) (4)), the investigation was still in progress.

3. Investigate the impact of (b) (4) manufacturing trends on operating parameters by studying the differences in trends and bias in process parameters for (b) (4) and (b) (4) batches. In this analysis, study the yields for every unit operation of (b) (4) process as well as the overall yield as (b) (4).

4. Study the comparability of (b) (4) and (b) (4) batches using representative pharmacodynamics assays used in the clinical trials, including the TGT.

b. Drug Product Protocol

1. In your response to IR item 5 provided in Amendment 61, page 4, paragraph 3, the following was noted "*up to* (b) (4) *are used of the total* (b) (4) *on lyophilizer* (b) (4) *and of the total* (b) (4) *on lyophilizers* (b) (4) ". Given the difference in the number of (b) (4) between the lyophilizers, these lyophilizers do not appear to be equivalent as initially claimed. In addition, to date only (b) (4) runs have been performed on lyophilizer (b) (4) and only (b) (4) runs have been performed on lyophilizers (b) (4). Based on this information, we do not agree with the validation strategy proposed in the revised CP regarding the number and type of lots run to date to show comparable results between lyophilizer (b) (4) vs (b) (4). Please comment.

Given that (b) (4) does not appear to be in a state of control as evidenced by the manufacturing history provided for (b) (4), we strongly advise that the CP be withdrawn from the BLA and that the post-approval changes to (b) (4) FDP be submitted as a Prior Approval Supplement after BLA approval.

9. The Proven Acceptable Ranges and Normal Operating Ranges for (b) (4) and (b) (4) indicated for the lyophilization cycle parameters used for the FDP manufacturing are not supported by the process validation provided in the BLA. Results of (b) (4) lab-scale experiments were provided in amendment 50 (received 1 July 1 2016); however, there was no justification for how the lab-scale studies support the lyophilization parameter ranges at commercial scale. Please provide a detailed plan to support these ranges at commercial scale.

10. In regards to the CCIT for stability samples performed by (b) (4), which was incomplete, please provide the following:

    a. Specific details of the "point of failure" control that was used

    b. Clarify if (b) (4) analysis was performed for product filled vials on stability.

    c. Provide details, SOPs etc. of the (b) (4) process and how operators are qualified to perform visual inspection.

    d. Results of the (b) (4) study (in the presence of the product), which was noted in your response to IR item 5 in Amendment 50 (received 01 July 2016), to be conducted at (b) (4) and stability determined by (b) (4) on Days (b) (4).

11. In regards to the Container Closure Integrity (CCIT) method performed at (b) (4), please provide details, SOPs, etc. in reference to the qualification of the operators that perform (b) (4). Include a description of course 04-01-C001,

which was used for the qualification of operators noted in your response to IR item 5 in Amendment 50, received 01 July 2016.

12. Regarding (b) (4) equipment cleaning validation, please provide the following:
    a. Data to support the cleaning efficacy of the (b) (4) .
    b. Validation data to support the cleaning and storage of all (b) (4) . In addition, please indicate the frequency in monitoring the (b) (4) during storage.

13. 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) ), and report the results in (b) (4) .

b. 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.

c. 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 (b) (4) in stability studies.

d. 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.

    ii. Test retained clinical samples for anti-Factor X and anti-Factor Xa inhibitory antibodies and (b) (4) antibodies.

13. 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 (i) the in-house TF-activated (b) (4) (TF(b) (4)) method used in the Phases 1 and 2 clinical trials, (ii) the commercially available TF-activated CAT (TF-CAT), and (iii) the in-house (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.

    Please also address the following examples of incorrect presentation and interpretation of TGT data in the BLA:

    a. 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.

    b. 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-RFU 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(b) (4) 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.

    c. 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 (b) (4) and time to thrombin (b) (4), respectively. These findings suggest that the effect of ANDEXXA is not represented by the presented parameter of the TGT method, (b) (4).

14. 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:

    a) 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.

    b) 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:

        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 contact coagulation pathway-activated 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;

        ii. Re-analyze your TF-activated TGT assay data using the parameters suitable for evaluation of TFPI effect. For example, your data suggest that (b) (4) is significantly less sensitive than the *thrombin* (b) (4) to the procoagulant effect of TFPI inhibition by ANDEXXA. The use of a single parameter, e.g., (b) (4), could therefore be misleading;

        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

        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;

        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

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);

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.

vi. Please also reference the communication from FDA on 1 June 2016, which you have not yet addressed.

# 12. Information Requests Submitted During BLA Review Cycle

## 07 December 2015 Pre-BLA Request for Immunogenicity Methods Validation Report

Ms. Castillo,

CBER has the following request for additional information pertaining to IND 15809:

1. Please provide the validation reports and Standard Operating Procedures for the immunogenicity methods used in the clinical studies to detect binding and neutralizing antibodies to Andexanet; endogenous human factor X and factor Xa; and impurities, such as CHO (b) (4) .

Please respond by December 11, 2015.

## 09 December 2015 Follow-up Request for Immunogenicity Methods Validation Report

This is the follow-up Information request under IND 015089:

1. With reference to Clinical Study Protocol 15-507 dated 09 June 2015, you stated that "blood specimens will continue to be evaluated for antibodies against andexanet and against fX and fXa. Samples that are positive for antibodies will be further assayed for the ability to neutralize the activity of andexanet, fX or fXa." Therefore, please provide the validation reports and Standard Operating Procedures for the assays for neutralizing (inhibitory) antibodies against human coagulation factor X and factor Xa. Please also provide the Standard Operating Procedure for the assay for neutralizing (inhibitory) antibodies against andexanet.

2. Since you reported positive titers of binding antibodies in at least 30 subjects, please provide the results from these samples, or any others that you tested, from both the confirmatory immunogenicity assays and the assays for neutralizing (inhibitory) antibodies against andexanet and against human coagulation factors X and Xa.

Please respond by December 15, 2015.

NOTE: Reminder was submitted on 06 January 2016 as follows:

From: Maruna, Thomas
Sent: Wednesday, January 06, 2016 11:18 AM
To: Janice Castillo

Cc: Ovanesov, Mikhail V.
Subject: RE: Information Requested - IND 15089 - Please Respond by December 11. 2015

Ms. Castillo,

We have not received a response to this IR. Please provide your rationale for missing the deadline.

Respectfully,

LT Thomas J. Maruna, USPHS, MSc, MLS(ASCP)

## 09 December 2015 Clarification of Pre-BLA Review of Immunogenicity Methods

From: Maruna, Thomas
Sent: Wednesday, December 09, 2015 1:52 PM
To: Janice Castillo
Subject: RE: Information Requested - IND 15089 - Please Respond by December 11. 2015

The CMC reviewer has stated that he has already started his "BLA" review based on what was submitted under the IND, but from a management perspective, the Dec 11th deadline (should he choose to stick with it) will make little difference since there is no active review clock on the BLA. If you are unable to meet the deadline stipulated for this IR, or any IR sent in the future for that matter, simply reply to my email proposing an alternative date. We will negotiate the deadline from there depending on the stage of review and what has been requested.

## 25 January 2016 Pre-Filing Information Request

We determined that the following information is necessary to continue our review:

1. Please provide an estimated completion date for the ongoing qualification of the sterility test for the final drug product (FDP), and for the submission of the final report to the BLA.

2. Please provide the full reports of the method bridging studies including, but not be limited to, Report AD-2015-001-007, Version 3 (referenced in section 3.2.S.4.5).

3. Please clarify which of the FDP release test methods were validated using only the (b) (4) .

4. Please provide information on the source of the *sterile Water for Injection* you used in the validation of the test methods for the establishment of the FDP specifications and the analysis of FDP batches.

5. Please submit a Pharmacovigilance (risk management) Plan.

The review of this submission is on-going and issues may be added, expanded upon, or modified as we continue to review this submission.

Please submit your responses as an amendment to this file by January 29, 2016 referencing the date of this request.

## 17 February 2016 Information Request Regarding Module 3 Quality and Validation of Bioanalytical Methods

We determined that the following information is necessary to continue our review:

**1.** With reference to **Module 3: Quality**, please provide the following:

a. An explanation of the batch numbering system, including information regarding any (b) (4) or intermediates and batch size or scale **(Section 3.2.S.2.2)**;
b. The container closure system(s) used for storage of the drug substance (DS) (details in **Section 3.2.S.6.**); and storage and shipping conditions for the DS **(Section 3.2.S.2.2)**;
c. In-process control testing for (b) (4) **(Section 3.2.S.4.2)**;
d. Stability protocol and data, including those for (b) (4), to support the stability and shipping conditions of the DS **(Section 3.2.S.7.3)**;
e. Description and status update on the validation studies to support the in-process hold-times in the manufacture of the Drug Product (DP) **(Section 3.2.P.3.5)**;
f. Standard Operating Procedures (SOPs) in **Sections 3.2.S.4** and **3.2.P.5** for all the analytical methods used for the release of the (b) (4) DP, respectively;
g. Update on the *Adventitious Agents Safety Evaluation* report **(Section 3.2.A.2)** with information on measures to assure sterility, which should include, but not be limited to, the description of sterility testing and measures to prevent and control potential contamination;
h. Analysis and risk assessment of the extractables and leachables in **Sections 3.2.S.3.2** and **3.2.P.5.5 *Impurities*** for materials used in the manufacture of the (b) (4) DP, respectively;
i. Document AD-2015-001-007 Version 3 referenced in **Section 3.2.S.4.5**. Please also correct any links to this document.

2. With reference to Clinical Study Protocols, e.g., protocol 15-507 dated 09 June 2015, in which you stated that "*blood specimens will continue to be evaluated for antibodies against andexanet and against fX and fXa. Samples that are positive for antibodies will be further assayed for the ability to neutralize the activity of andexanet, fX or fXa*", please

a. Develop and validate assays to measure the activity of the antibodies that inhibit the activities of endogenous human Factors X and Xa. For example, the anti-Factor X inhibitor assay should be based on the (b) (4) assay for Factor X activity, and the results should be presented in (b) (4) of anti-Factor X activity;

b. Assess how the presence of the anti-Factor Xa inhibitory antibodies may interfere with the assays used to evaluate the pharmacodynamics, pharmacokinetics, and immunogenicity in the clinical studies;
c. Test the retained clinical samples for anti-Factor X and anti-Factor Xa inhibitory antibodies;
d. Provide a timeline for the completion of the activities described in items 2.a., 2.b. and 2.c.

3. With reference to **Section 5.3.1.4.**, please provide the following:
    a. A table summarizing all the analytical methods used in the assessment of pharmacokinetics, pharmacodynamics and immunogenicity in the clinical studies. For each of the methods and its respective method revisions, please include the following information: the principle and intended use of the method, the protocol number and title of the clinical studies in which the method was used, and the date of introduction of the method;
    b. Information to support the comparability of different versions of a method that was changed between or during the clinical studies. The methods should include, but not be limited to, the thrombin generation assay.

The review of this submission is on-going and issues may be added, expanded upon, or modified as we continue to review this submission.

Please submit your responses as an amendment to this file by March 3, 2016 referencing the date of this request.

## 07 April 2016 Combined Information Request

**CMC (Product)** – Please respond by April 20, 2016

7. You used (b) (4) to characterize the thermodynamics and stoichiometry of the interaction between andexanet alfa and (b) (4). Please expand the study to include rivoroxaban, edoxaban and apixaban. Specifically, please repeat the (b) (4) experiments presented in BLA section 3.2.S.3.1.19 *Elucidation of Structure and Other Characteristics* and IND section 3.2.S.3.1.11, using all four inhibitors (b) (4), rivaroxaban, edoxaban and apixaban and representative (b) (4) batches from (b) (4) ((b) (4) batches) and (b) (4) ((b) (4) batches). Please submit the final study report as an amendment to the BLA by 17 June 2016.

8. In the specifications of the (b) (4) Drug Product (DP), you have not provided a parameter(s) to monitor (b) (4) of the protein. Your data for characterization of andexanet alfa (section 3.2.S.3.1) indicate that the protein has at least (b) (4) sites for (b) (4), which are (b) (4), respectively (Table 3.2.S.3.1-7). Therefore, the theoretical (b) (4). However, in Table 3.2.S.3.1-8, you reported a ratio of (b) (4), indicating that (b) (4) of the (b) (4), and/or (b) (4) of the protein is incomplete. In addition, the information provided in Figure

3.2.S.3.1.1-3 is not consistent with your analytical data because it does not show (b) (4), but does show (b) (4) other sites and only (b) (4) on the molecule. Therefore, please correct Figure 3.2.S.3.1.1-3 to show all (b) (4) sites with the respective (b) (4) positions and provide a clear assessment of the (b) (4) of the (b) (4) on the protein in the eCTD file.

9. The proposed release specifications of (b) (4) DP for identity, (b) (4) modifications and excipients are deficient. Andexanet alfa is a mutated coagulation factor product manufactured at large scale, formulated at high concentration and administered at high doses. To provide assurance of consistent product quality and to compensate for the limited manufacturing experience, please develop new (b) (4) DP release assays and propose release specifications to control the following parameters:

    c. identity by protein structure, e.g., the (b) (4) method described under *Justification of Specification* section 3.2.S.4.5.2.6;
    d. (b) (4) content; and
    e. identity and quantity of excipients - sucrose, mannitol and Polysorbate 80

10. In the specifications of the (b) (4) DP (e.g., section 3.2.P.5.1), the Direct and Indirect Potencies are expressed in percentage units relative to a reference standard. However, the use of percentage unit is not suitable for the evaluation of the stability of the product because the stability of the reference standard is not established. To establish a reliable reference standard throughout the life-cycle of the product, please develop a potency unit that is traceable to international reference preparations distributed by the (b) (4). In this case, the potency unit could be defined as follows: "(b) (4)" Please update the specifications of the DS and DP accordingly.

11. In the *Justification of Specifications* of the (b) (4) DP (sections 3.2.S.4.5 and 3.2.P.5.6, respectively), you have not provided an assessment of the critical quality attributes of the product and their relative importance (such as arbitrary scores) for the product's safety and efficacy. Considering our comments above (1-3), please provide these data and update the eCTD file accordingly.

12. In the specifications of the DP (section 3.2.P.5.1), please clarify which compound corresponds to the parameter "Concentration by (b) (4)." Please revise this parameter to "Protein Concentration by (b) (4)."

13. In the specifications for the (b) (4) DP under "Test/Test Method" for compendial methods, please refer to the specific chapters of the compendia (e.g., (b) (4) for (b) (4), *etc.*).

14. Your March 3, 2016 response to our February 17, 2016 request to develop assays for anti-drug antibodies that may bind or neutralize endogenous Coagulation Factors X and Xa is not acceptable. 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 FDA 2014 *Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products.* To ensure protection of clinical 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 had requested Portola to develop these assays during the pre-IND meeting on 16 June 2009 (CRMTS # 7089, Ref. PS000698), and Portola had included a commitment to develop these assays in the original IND submitted on 15 March 2012. You reiterated this commitment in your Clinical Study Protocol 15-507 dated 09 June 2015. To comply with FDA requirements and your prior commitments, you must develop and validate assays for antibodies that inhibit the activities of endogenous human Factors X and Xa. For example, the anti-Factor X inhibitory antibody assay should be based on the (b) (4) assay for Factor X activity, and the results should be presented in (b) (4) of anti-Factor X activity. By April 12, 2016, please provide a timeline for the analytical studies you will conduct to comply with this request. In addition, please include this timeline in *Clinical Study Protocol 15-507* and inform the clinical investigators accordingly.

15. Your March 3, 2016 response to our February 17, 2016 request to assess the interference of anti-Factor Xa inhibitory antibody on the pharmacodynamics, pharmacokinetics, and immunogenicity assays is not acceptable. For example, you need to validate the assays for dRVVT, thrombin generation, PT, aPTT and ACT for antibody interference. This information is required to support the claim of lack of immunogenic response with neutralizing activity for Factors X and Xa, which you made in the *Prescribing Information*, *Risk Management Plan* (1.16.1 Risk Management), *Clinical Study Protocols* and your March 3, 2016 response to our information request. *FDA Draft Guidance for Industry: Assay Development for Immunogenicity Testing of Therapeutic Proteins* also instructs you to study the interference of anti-Factor Xa inhibitory antibodies with all binding immunogenicity assays. By April 5, 2016, please provide a timeline for the analytical studies you will conduct to comply with this request.

16. In your March 3, 2016 amendment, *Table A1-2: Antibody Assays*, you stated that assays for anti-andexanet, anti-Factor X and anti-Factor Xa antibody were first used on January 1, 2013. However, the data on these antibodies were reported as early as September 19, 2012, in an information package for the End-of-Phase 1 meeting. Please explain this inconsistency and provide detailed information on any immunogenicity assays used prior to January 1, 2013.

17. Regarding the two thrombin generation assays described in your March 3, 2016 amendment (the original Portola's method and the currently used commercially available CAT method) used in Phase 1, Phase 2 and Phase 3/4 clinical trials, your justification for assay comparability presented in the March 3, 2016 response is not acceptable. The sensitivity of the thrombin generation assay to the action of pro- and anti-coagulant molecules is known to

depend on (b) (4) . Therefore, please provide a side-by-side comparison of the (b) (4) thrombin generation assays to demonstrate the comparability of responses to the activities of the study drugs (including but not be limited to andexanet alfa, (b) (4) , rivoroxaban, edoxaban and apixaban and their combinations) and antibodies (including inhibitory antibodies to Factor X and andexanet alfa). In addition, the original Portola assay utilized a substantially higher level of tissue factor reagent ((b) (4) in the commercial CAT reagent), suggesting that the Portola assay is less sensitive to tissue factor-dependent anti-TFPI action of andexanet alfa. Since the sensitivity to TFPI inhibition has been previously demonstrated by the CAT method, please use CAT to repeat studies of anti-TFPI action of andexanet as described in NC-12-0451-R0001 *PRT064445 activity and interaction with fXa-EGR*.

18. The comparability protocols for the proposed manufacturing changes are deficient. You need to provide clear and specific information on the manufacturing changes that should include, but not be limited to, the rationale for the changes; knowledge and understanding of the process the changes are involved in; supporting information; comparability study design and protocol; test methods, justification and validation protocol for the quality attributes to be tested; test methods and acceptance criteria; and data analysis strategy including statistic assessment. Please note that deficiencies in the comparability protocol, if not addressed adequately, will negatively affect the outcome of the BLA.

## 13 April 2016 Record of 08 April 2016 Mid-Cycle Communication Telecon

### 3. Discipline Review Concerns

a. Chemistry, Manufacturing and Controls

i. We have concern for immunotoxicity and potential for ANDEXXA to elicit binding and/or neutralizing antibodies to endogenous Factor X or Factor Xa. We request that you continue to develop your assays to address this potentially significant safety concern.

ii. Given that clinical data showed that ANDEXXA reacts differently with the various Factor Xa inhibitors, we ask you to expand the (b) (4) study to include all 4 inhibitors - (b) (4) , rivaroxaban, edoxaban and apixaban and representative (b) (4) batches from (b) (4) ((b) (4) batches) and (b) (4) ((b) (4) batches).

iii. We note that your proposed release specifications of (b) (4) DP (DP) for identity and excipients are deficient. ANDEXXA is a mutated coagulation factor product manufactured at large scale, formulated at high concentration and administered at high doses. We need thorough testing of the (b) (4) DP to assure consistency of the manufacturing process and product quality.

iv. Similarly, we ask that you enhance the characterization of (b) (4) modifications of andexanet alfa, specifically (b) (4). We need an explanation for the low ratio of (b) (4) of the (b) (4).

v. We note that your definition of potency for ANDEXXA "percent of a reference standard" is not suitable for the control of the unitage because there is no assurance of the stability of the reference standard. We ask you to develop a potency unit for ANDEXXA that is traceable to the international reference preparations distributed by the (b) (4). For example, the unit can be defined as follows: "(b) (4)"

vi. Your March 3, 2016, response to our February 17, 2016, request to assess the interference of anti-Factor Xa inhibitory antibody on the pharmacodynamics, pharmacokinetics, and immunogenicity assays is not acceptable. We have sent you a detailed explanation of the deficiency and what you need to do to address it.

vii. Regarding the (b) (4) thrombin generation assays described in your March 3, 2016, amendment (the original Portola's method and the currently used commercially available CAT method) used in phase 1, phase 2 and phase 3/4 clinical trials, your justification for assay comparability presented in the March 3, 2016, response is not acceptable. We have sent you a detailed explanation of the deficiency and what you need to do to address it.

viii. You did not provide sufficient stability data to support the proposed shelf-life of (b) (4) DP manufactured using (b) (4). Although real-time stability data demonstrated no negative trends, the results from the accelerated stability studies suggest product degradation. We will re-assess the proposed shelf-life when Portola submits additional stability data on Day 120, 16 April 2016.

ix. The comparability protocols for the proposed manufacturing changes are deficient. You need to provide clear and specific information for the manufacturing changes that should include, but not be limited to, the rationale for the changes, knowledge and understanding of the process the changes are involved in, supporting information, comparability study design and protocol, test methods, justification and validation protocol for the quality attributes to be tested, test methods and acceptance criteria, and data analysis strategy including statistic assessment. Please note that deficiencies in the comparability protocol, if not addressed adequately, will negatively affect the outcome of the BLA review.

x. We have also identified several less significant deficiencies regarding the validation of the manufacturing process and analytical methods, which we will convey to you via Information Requests. We will probably have additional questions and

comments for you after we review the information on process development and validation, which should arrive on April 16, 2016; and the pre-license inspection on (b) (4) .

xi. The sensitivity of the Container Closure Integrity Testing (CCIT) performed for the primary container is not adequate. Please note that the positive control, in which the stopper was (b) (4) does not adequately simulate a critical leak defect. To support the sensitivity, we recommend that the defect diameter be as small as reasonably possible.

These questions have been communicated to Portola in an IR on April 6, 2016. Portola inquired about a possibility for a teleconference to discuss this IR. Product reviewers agreed to meet with Portola on the basis of reviewers' and Portola's availability.

## 31 May 2016 Information Request and Advise to Portola Regarding Revised Comparability Protocol

Information Request and Advise to Portola Regarding their 11 May 2016 request to discuss the revised Comparability Protocol "Andexanet Alfa (PRT064445) (b) (4) to (b) (4) Resulting Drug Product

We have determined that the following information is necessary to continue our review:

We will not comment on the appropriateness of the proposed review category until we have a chance to review the completed Comparability Protocol (CP). As of now, your revised CP "Comparability Protocol Andexanet Alfa (PRT064445) (b) (4) to (b) (4) Resulting Drug Product" is still deficient, and will not support a downgrade of the submission for (b) (4) from a *Prior Approval Supplement* to a *CBE-30 Supplement*.

We have reviewed your revised CP submitted in Amendment 27 to STN 125586/0 dated 29 April 2016. Your revised CP is to support changes in the manufacturing processes of the (b) (4) Drug Product (DP), specifically those related to the use of (b) (4) , the use of (b) (4) new lyophilizers, and additional (b) (4) in the lyophilizers. As discussed during our teleconference on 23 May 2016, we have summarized for you the following deficiencies in the form of an information request:

**1. Drug Substance:**

(b) (4)

1 page has been determined to be not releasable: (b)(4)

(b) (4)

**2. Drug Product:**

a. The CP does not include a detailed approach as to how the lyophilizers will be validated such as a description of a bracketing strategy detailing the number of runs per lyophilizer and a justification for this strategy. The CP indicates that (b) (4) DP produced from DS from (b) (4) will be performed; and there is no justification provided for why this is sufficient to demonstrate consistency for addition of (b) (4) lyophilizers and additional use of (b) (4) .

b. The CP does not provide a description of the testing that will be performed to demonstrate the lyophilizers are equivalent. The CP states that the lyophilizers are demonstrated to be equivalent, but there were no details of how the lyophilizers were shown to be equivalent (i.e., specific listing of testing performed and the acceptance criteria as it relates to the lyophilizer operating parameters, specifically, the allowable variance in operating parameters between lyophilizers for determining equivalency).

c. The CP does not define a product sampling plan for the lyophilization runs (i.e., details of sampling at pertinent (b) (4) from each lyophilizer and the number of samples to be taken and tested at each location). Please note that routine release testing is not acceptable to demonstrate consistency of the process for the new lyophilizers.

d. The CP does not address validation of aseptic processing for the (b) (4) additional lyophilizers.

e. The CP does not address how the cleaning and sterilization of the (b) (4) additional lyophilizers will be validated.

f. The CP does not include a detailed description of the data that will be provided to support the follow up supplement. For example, for the validation of additional lyophilizers and (b) (4) , we would expect to review the following:

   - Product testing results of the extended sampling of the lyophilization runs
   - Lyophilization cycle graphs, monitoring the (b) (4) during the lyophilization runs
   - Results of IQ/OQ testing and other testing performed demonstrating equivalency of the lyophilizers
   - Results of media fills performed with the additional lyophilizers

- Results of cleaning and sterilization validation of the additional lyophilizers

Based on the lack of a detailed plan (protocol), we do not agree with your assessment that (b) (4) DP lot is sufficient to support the follow up supplement. Generally, for addition of multiple lyophilizers, we expect a bracketing strategy such as (b) (4), which is (b) (4) runs in one lyophilizer to demonstrate consistency, and (b) (4) run in each of the other additional lyophilizers (demonstrated as equivalent) for further confirmation the process is consistent. In demonstrating PQ of additional lyophilizers, the use of placebo with product vials located at pertinent locations for testing may be acceptable if the placebo adequately represents and is scientifically justified that all the relevant physical characteristics of the drug product under conditions that the drug product will see during lyophilization.

Please be advised that the CP covering changes to the DS and DP manufacturing processes must be very detailed and outline specifically the data that will be provided to support the subsequent CBE-30 supplement. If the CP is deficient, this can negatively impact the review process and the outcome of your BLA. Additionally, in the event that we approve the CP and allow a downgrade of the submission for (b) (4), if the subsequent CBE-30 supplement does not contain all the supporting information, as specified in the CP or if the results fail to meet the acceptance criteria and conditions specified in the CP, the submission will be upgraded to a *Prior Approval Supplement*. Please refer to the Draft Guidance "Comparability Protocols for Human Drugs and Biologics: Chemistry, Manufacturing and Controls Information, April 2016" for additional information in regards to the expectations for Comparability Protocols.

The review of this submission is on-going and issues may be added, expanded upon, or modified as we continue to review this submission.

You are required to submit your responses as an amendment to this file by close-of-business, Friday, **June 21, 2016**.

## 01 June 2016 Information Request About Anti-TFPI Action

b. In an 18 April 2016 email to the office director, Portola explained that the elevation of thrombin generation (TG) over the pre-inhibitor treatment baseline was mediated by the inhibition of tissue factor pathway inhibitor (TFPI) activity, as evidenced from a lack of such an elevation in a (b) (4) TG assay which was used in the clinical studies as a control. This new information explains inconsistencies in the biomarker results between the clinical and preclinical studies, and prompts us to examine more closely the results in the clinical studies regarding the duration of the procoagulant effect and the risk of thrombogenicity.

   The finding that the inhibition of TFPI was contributing to procoagulant activity observations in the clinical studies suggests that: **(i)** the *Clinical Study Reports* need to be updated with all results available to Portola so that we can consider all the evidence; **(ii)** the transient reversal of anti-FXa activity may not contribute directly to the sustained procoagulant effect as inferred from the sustained increase in TG; **(iii)** the effect of the TFPI activity inhibition is more significant than it was previously thought and the TFPI activity

data was not submitted for anticoagulated patients; and **(iv)** the TG assays used in the clinical and preclinical spiking studies may not be adequately qualified for the evaluation of andexanet's effects.

Because TFPI inhibition is potentially thrombogenic and is not as transient as anti-FXa activity reversal, it is necessary to measure the duration and magnitude of both outcomes of the effect of andexanet. Please provide additional data on anti-FXa activity reversal and TFPI activity changes as described below:

i. To investigate the relationship between the duration and magnitude of TG elevation and the reversal of anti-FXa activity, please

    i. Use retained samples from the Phase 2 and 3 studies to determine (b) (4) TG (a TG activated by the contact coagulation pathway) after andexanet dosing by bolus and bolus plus infusion;
    ii. Evaluate the time courses of tissue factor (TF)-activated TG and contact-activated TG by plotting the graphs side-by-side for each healthy volunteer in these studies;
    iii. Apply the same statistical criteria you previously used in the Phase 3 study for TF-activated TG analyses to characterize the elevation of TF-independent TG levels.

ii. To investigate the pharmacodynamics of TFPI inhibition and risk of thrombosis, please

    i. Determine the TFPI activity in retained samples from the 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 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.

    ii. Please describe all known thrombotic events (at least 8) and related deaths observed in the ANNEXA 4 study in their potential relationship to the expected anti-TFPI action of andexanet (potentially more than one day in patients with renal impairment), as well as the magnitude of anticoagulation (concentration of anti-FXa inhibitor) at the time of andexanet administration and during the expected or observed decrease in TFPI activity.

    iii. Please discuss the following potential thrombogenic mechanisms related to TFPI activity inhibition:

        1. The risk of disseminated intravascular coagulation following TFPI inhibition in patients who have circulating TF in blood, as was suggested in your 18 April 2016 communication regarding blood-borne TF activity in bleeding patients in ANNEXA 4 study.

2. The TFPI-dependent restoration of thrombosis observed in a rabbit model of recurrent arterial thrombosis under the control of anticoagulant therapy (Ragni et al. Circulation 2000;102(1):113-7) and rabbit model of venous rethrombosis after lysis (Kaiser and Fareed. Thromb Haemost. 1996;76(4):615-20)
3. The loss of TFPI control over initiation of thrombotic events at the sites of TF exposure which may include atherosclerotic plaques, cancer cells and vascular injuries, for example in trauma patients, during surgery and in catheter-related events.

iii. To address the apparent deficiency in your prior conclusions from the analytical method qualification and preclinical studies that the effect of TFPI inhibition may be insignificant,

i. Please explain why the preclinical studies using human plasma spiked with andexanet *in vitro* were not able to predict the TFPI-inhibition-dependent TG elevation seen in plasma samples from individuals receiving andexanet *in vivo*. Although on average a (b) (4) elevation in TG above the baseline was documented in the Phase 3 clinical studies, the spiking studies reported only a (b) (4) increase in TG above the pre-treatment baseline in plasma samples with or without a fully reversed anti-FXa activity (Figures 3-3 and 3-4 in preclinical report NC-15-0659-R0001, Figures 1 and 2 in report NC-12-0451-R0001, and Figure 1 in report NC-12-0452-R0001).

   Please consider the possibility of laboratory artifacts (including matrix effects such as inhibition of thrombin generation by excipients), the impact of plasma levels of TFPI, FXa inhibitor and andexanet which may have been different in the clinical versus spiked preclinical studies (e.g., use CAT to measure TG in normal plasma spiked with (b) (4) of andexanet in the presence of (b) (4) of rivaroxaban, in the presence and absence of inhibitory anti-TFPI antibody), and the impact of assay conditions, including but not be limited to assay temperature, plasma dilution factor, stability of plasma samples before and after andexanet spiking, and concentration of TF. Please also provide raw (b) (4) data collected by the (b) (4) (relative (b) (4) units versus time for each (b) (4) ) for the above figures in reports NC-15-0659-R0001, NC-12-0451-R0001, and NC-12-0452-R0001;

ii. Please provide all qualification data for the TFPI activity and the (b) (4) TG assays used in the Phase 1, 2 and 3 studies. These data were not submitted in the BLA, nor were they included in the responses to our 17 February 2016 Information Request (question # 3 provided in your 03 March 2016 amendment to the BLA);

iii. Please confirm that the assays used for the determination of TFPI activity in plasma samples were investigated for the interference with FXa inhibitors

and, if needed, please develop methods based on the competition with an anti-TFPI antibody to allow for the detection of TFPI activity in a matrix that contains anti-FXa activity. For example, a commercially available antibody may be obtained from the manufacturer of your TFPI antigen assay which uses an anti-TFPI monoclonal antibody targeting a Factor Xa binding epitope on TFPI.

iv. To permit a meaningful evaluation of TG data in the Phase 1 and 2 versus Phase 3 studies, please evaluate the differences between the three versions of the clinical TG assays ((b) (4) , TF-activated CAT and (b) (4) TG) in their sensitivities to the anti-FXa activity of each FXa inhibitor and the anti-TFPI action of andexanet;

v. With reference to preclinical Study # NC-12-0439-R0001, please explain your conclusion that the absence of increases in the TAT and PF1.2 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 suggests 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;

vi. 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) of rivaroxaban contributes to less than a (b) (4) decrease in andexanet binding to TFPI on endothelial cells, suggesting that rivaroxaban may provide no protection from TFPI inhibition (b) (4) 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.

iv. To facilitate our review of all collected data related to the mechanisms of action of andexanet as they are related to its safety and efficacy, please

i. Submit a list of all clinical and preclinical investigations on andexanet you have initiated but have not reported in the BLA, regardless of their GLP status, status of completion or perceived relevance to this discussion;

ii. Provide 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: (i) (b) (4) TG results mentioned in your 18 April 2016 communication, (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), (iii) the evidence of the normal (not elevated) PF1.2 and D-dimer levels mentioned in the above sources and patent WO 2013123248 A1, and (iv) the TG (b) (4) results in the Phase 1 studies in the absence of spiked anti-FXa inhibitors;

iii. Please submit a timeline for the planned addendums to provide new interpretations in view of the new collected information about the anti-TFPI action of andexanet and its reflection by the TG data and preclinical studies.

c. Your 03 March 2016 response to our 17 February 2016 IR to establish the comparability between the different versions of the TG assay is not acceptable because your hypothesis that the TG (b) (4) and CAT methods are similar appears to contradict the available data. In preclinical study NC-12-0451-R0001, the TG (b) (4) method was found to be similar to a (b) (4) TG assay while in the clinical studies the CAT method was found to be different from the (b) (4) TG method. Analysis of the clinical study data presented in Table A1-5 provided in your 03 March 2016 amendment demonstrates that in the apixaban studies, andexanet TG (b) (4) was elevated above the pre-apixaban baseline by 29% (Study 12-502, Module 1) and CAT was elevated by 66% (Study 14-503 Part 1) and 40% (Study 14-503 Part2).

In the rivaroxaban studies, TG (b) (4) was elevated by 15% (Study 12-502, Module 2) and CAT was elevated by 30% (Study 14-504 Part 1) and 39% (Study 14-504 Part 2). In contrast to the differences in TG elevation, TG (b) (4) and CAT were inhibited to a similar degree by apixaban (50% inhibition in both methods) and rivaroxaban (80% in TG (b) (4) and 71% in CAT). Please provide results of a side-by-side analytical comparability study for a meaningful comparison of the duration of TG normalization in the Phase 1-2 and Phase 3-4 studies.

3. With reference to TFPI inhibition by andexanet, please provide data to support the consistency of anti-TFPI activity action in andexanet alfa batches. Specifically,

   a. Please repeat the (b) (4) experiments presented in the BLA section 3.2.S.3.1.19 *Elucidation of Structure and Other Characteristics* and IND section 3.2.S.3.1.11, using TFPI and representative (b) (4) batches from (b) (4) ((b) (4) batches) and (b) (4) ((b) (4) batches);
   b. Please investigate the interaction of the (b) (4) of andexanet with TFPI because an increase in the (b) (4) was observed in (b) (4) but not (b) (4) studies (reference is made to accelerated stability comparability studies) and in batches manufactured on (b) (4) ;

Please develop an anti-TFPI potency assay and compare the results of this assay with the TG-based anti-TFPI activity method because the TG assays were used to assess andexanet activity in clinical trials.

## 07 June 2016 Advice and Information Request Regarding (b) (4) Responses

Advice and Information Request regarding (b) (4) responses to the observations in the form 483 issued during Pre License Inspection (b) (4):

1. On behalf of the agency, please submit the following comments to (b) (4).

    a. With reference to your response to Observation item # 1:

    We disagree with your conclusion that the process validation for (b) (4) is complete because at least one process parameter, (b) (4), was not investigated during the completed process qualification studies. We also disagree with your proposal to widen the specification limits for the (b) (4) before the completion of your investigations into the effect of (b) (4) on (b) (4) performance, and the effect of the (b) (4) on TFPI inhibition. In addition, we disagree with your conclusion that the process performance qualification series for (b) (4) is complete because (b) (4) was not in a state of control during the (b) (4) inspection. We acknowledge your commitment:

    - To update the Process Validation documents POL-1510 *Policy for Process Design – Stage 1 of Process Validation* and POL-1512 *Policy for Continued Process Verification (CPV) – Stage 3 of Process Validation* with new information about all currently understood process parameters;
    - To complete the investigations on the effect of (b) (4) on the performance of the (b) (4) steps in the Characterization Protocol (b) (4)-CP-054;
    - To complete the trend investigation into the levels of (b) (4) that exceeded the in-process limit (IPL);
    - To re-assess the in-process limit for (b) (4) levels in the (b) (4);
    - To complete the investigation on the increased levels of the (b) (4) observed during the small-scale validation study on hold times (VAL-30234-01);
    - To update the master batch record (MBR) to include instructions for more accurate temperature measurements and implement control on (b) (4) at the point of use.

Please submit the following documents in an amendment to the BLA by 30 June 2016:

1. The final reports and supporting documentation for the above listed studies which you have committed to perform,
2. The *Final Report* for the *At-scale* (b) (4) *Study* (VAL-30230-02.1, approved 11 May 2016), and
3. All related Deviation Reports, either closed or open (including DEV-1484, DEV-1498, DEV-1573, and DEV-1632).

In addition, please prepare an amendment to the (b) (4) process validation report with an explanation of the new control strategy for process parameters.

b. With reference to your response to Observation item # 4:

Your response is not acceptable because your proposal does not address the root cause of your failure to follow your standard operating procedure for deviation management. Your delay in opening an official deviation record is due to your practice of consulting your client before official documentation of the deviation. Please ensure the timely initiation and accurate maintenance of manufacturing records by opening deviation records promptly prior to your communication with anyone outside of (b) (4) .

2. The Agency recommends a teleconference (with the inspection team) to discuss any questions or concerns about this Information Request and our expectations for a revised response to your 483 observations. We can make ourselves available tomorrow 08 June 2016 or Thursday 09 June 2016. If you would like a teleconference, please communicate a proposed time on either day so that we can confirm our availability.

## 22 June 2016 Information Request Regarding Release Specifications

1. With reference to your 20 April 2016 responses to our Information Request dated 06 April 2016,

a. Your proposal to develop specifications and validate new (b) (4) method after October 2016 is not acceptable because this will preclude the FDA from reviewing the information before the goal date. We recommend you to continue to develop your current (b) (4) method which is already partially validated. Please introduce release specifications for identity by protein structure using your current (b) (4) method; and submit the specifications and justifications to the BLA by 01 August 2016. Please also commit to completing the validation studies of this method by 31 October 2016; and re-evaluate the release specifications after you have obtained data from (b) (4) batches of (b) (4) drug product; or one year post licensure, whichever comes first.

b. We disagree with your statement that "(b) (4) content is also not thought to affect the PK/PD of andexanet". Please use the available data obtained with the assays of your choice to introduce release specifications for the (b) (4) acid content by 01 August 2016. Please also commit to completing the validation studies of these methods by 31 October 2016 and re-evaluate the release specifications after you have obtained data from (b) (4) batches of (b) (4) drug product; or one year post licensure, whichever comes first.

c. We disagree with your proposal to monitor the concentrations of excipients with the in-process control and surrogate assays. Andexanet alfa is administered at high doses, which poses concerns of potential toxicity in patients who are sensitive to sucrose and mannitol. Please introduce specifications for sucrose and mannitol by 01 August 2016. Please also commit to completing the validation studies of these methods by 31 October 2016; and re-evaluate the release specifications after you have obtained data from (b) (4) batches of drug product; or one year post licensure, whichever comes first.

d. We acknowledge your commitment to "develop and validate a potency unit based on the reference units of fXa activity" and "will perform feasibility studies by modifications of the assays currently used for direct and indirect fXa inhibitors". However, it is imperative to introduce a product-specific unit prior to product licensure because as we have noted in the Information Request dated 06 April 2016, the use of percentage unit is not suitable for the evaluation of the stability of the product because the stability of the reference standard is not established. Therefore, we disagree with your proposal to delay characterization of the reference standards. By 01 August 2016, please assign a direct potency and an indirect potency of your primary product-specific standard. It can be arbitrarily assigned as 1 direct unit/mL and 1 indirect unit/mL, respectively; and this unitage can then be used to set your release specifications accordingly. In addition, please apply this unitage to evaluate the potencies of all of your reference standards - primary, secondary or working - in direct and indirect units in side-by-side studies by 31 October 2016.

2. With reference to your justification for specification for endotoxins in the Drug Product (b) (4) ) which is derived from a maximum dose of (b) (4) individual, please note that this specification limit is very close to the compendial infusion limit for endotoxins. Since you are considering the use of higher doses in the future, please revise this specification based on the manufacturing capability.

3. Please include endotoxin values in the Certificate of Analysis for Drug Product batches (b) (4) and (b) (4) .

## 28 June 2016 Information Request Regarding Polysorbate 80 Specification

1. On April 20, 2016, you indicated that you are developing a method for the detection and quantitation of Polysorbate 80 in andexanet alfa. In addition to our request for sucrose and mannitol, submitted on 22 June 2016, please include acceptance limits for Polysorbate 80 in the drug product specification by August 1, 2016, and commit to completing the method validation by October 31,

2016 and re-evaluate the acceptance limits after you have obtained data from (b) (4) batches of drug product or one year post licensure, whichever comes first.

## 30 June 2016 Advice Regarding Release Methods Deficiencies

Ms. Janice Castillo:

Thank you for acknowledging that our requests are reasonable and we in turn appreciate that you are working on the assays needed to add to the release specifications. Although the determination of criticality of quality attributes is part of the review process, we do in general consider all parameters included in release specifications to be critical.

Please note that the referenced information requests were reiterations of our earlier communications with you so most of the issues are not new. In addition, these requests are in line with FDA review practices that additional issues would be added, expanded upon or modified as we continue to review your submission. It is not possible for us to cover all topics of review in pre-submission meetings because we do not have all the information to determine what are deficient and what additional information is needed to remedy them.

To facilitate our discussion on your proposal, we ask that you address each of our information request items thoroughly and submit your rationales for new proposals on the development and introduction for the specific methods and specifications in an amendment to the BLA. We are now extending your response date to 8 July 2016 so that you can have time to better prepare your responses. If there is need for further discussion, we can then schedule a teleconference after we have reviewed your responses.

## 08 July 2016 Information Request Regarding (b) (4) and Procedures

. In your response to Observation Item 1b in Form FDA 483, you stated that the out-of-specification (OOS) results for the (b) (4) of andexanet alfa in batch (b) (4) were caused by acceptance criterion that did not fully represent assay and process variability. You also stated that the (b) (4) are expected to be fully functional, and that your proposal to widen the specification is based on process capability and assay precision. However, your response did not address the cause(s) for the (b) (4) in the levels of these (b) (4) observed (i) at several unit operations, (ii) after the introduction of (b) (4), and (iii) over time in stability studies. Therefore, please provide additional explanations on the following items:

    a. Please explain how the available clinical data support the (b) (4) in the acceptance criterion of the (b) (4) from (b) (4) to (b) (4). Please compare the ranges of (b) (4) levels in the (b) (4) batches used in the completed Phase 1-3 clinical trials to those in the (b) (4) batches used in the ongoing clinical trials.

b. With reference to your Final Investigation Report for deviation DEV-1632, please describe your investigation on the sources and levels of (b) (4) responsible for the formation of the (b) (4) throughout the manufacturing process and during storage of the (b) (4) lyophilized Final Drug Product (FDP). In addition, please describe your investigation on the identity of the (b) (4) responsible for the (b) (4) of the protein, and the corrective and preventive actions implemented to remove the (b) (4).

c. Please provide a summary of risk assessment of the (b) (4), which should include, but not be limited to, the impact of the (b) (4) on the purity, quality, potency, and stability as they are related to the safety and effectiveness of the product. In addition, please list the levels of (b) (4) at release in all your manufactured (b) (4) FDP lots and those enrolled in the ongoing stability studies at all available time points, including any OOS stability results (above 17).

d. Please summarize the data you have collected to date to evaluate the impact of the (b) (4) on (i) the reversal of anti-FXa activity of andexanet alfa, (ii) its interactions with TFPI, and (iii) its circulatory half-life. Please explain the data you have collected on the purified (b) (4) presented in Figure 3.2.S.3.1-13 of the BLA.

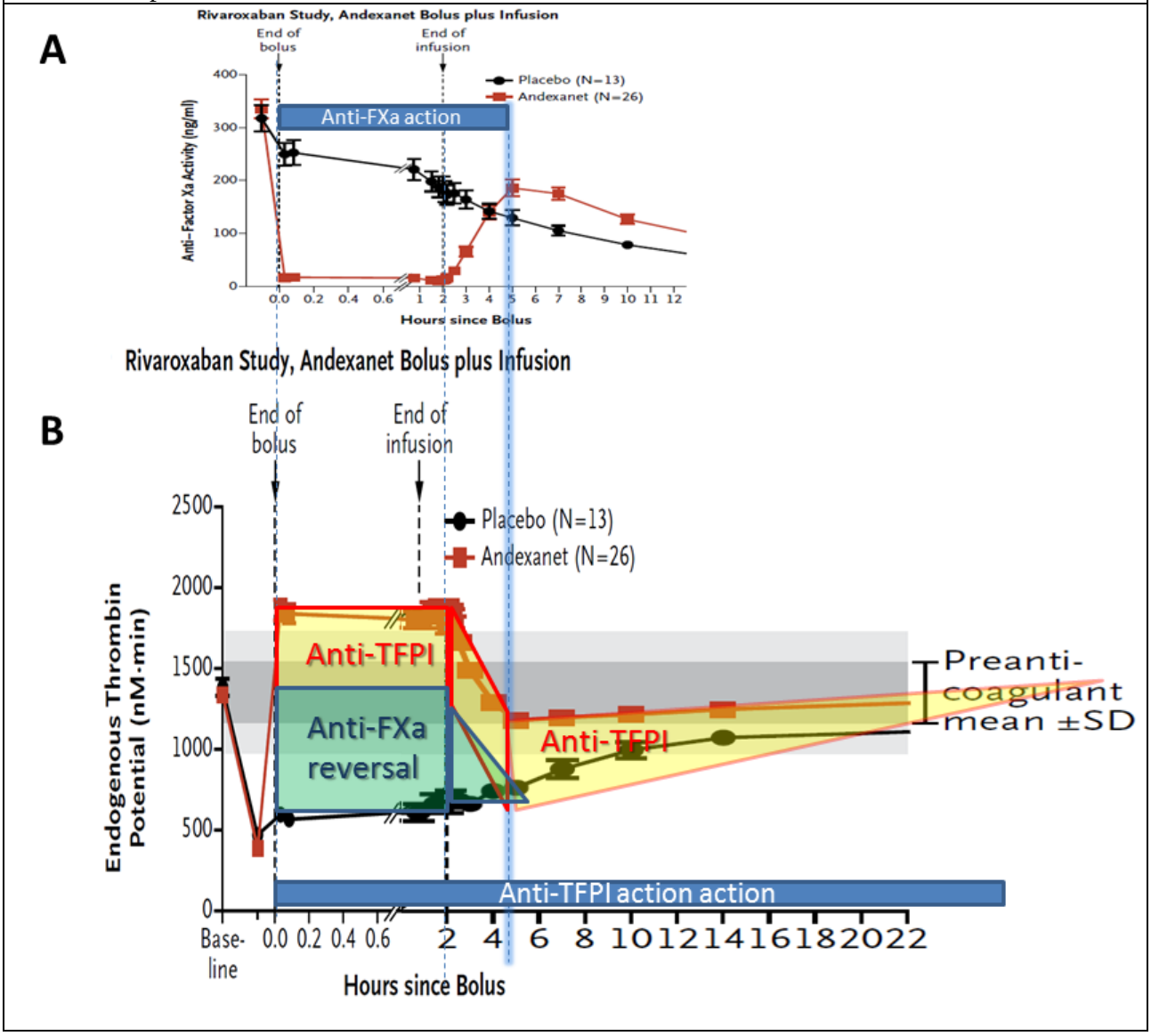
e. Please justify the specification limit of (b) (4) based on the data from the available BDS and FDP batches. Please note that the levels of (b) (4) in all (b) (4) batches, except batch (b) (4), were within the (b) (4) specification limit at release, and that method variability was ruled out as a source of OOS result for batch 1 (b) (4). Your investigations suggest that the increase in the (b) (4) in batch (b) (4) was caused by the exceptionally (b) (4) used in the Capto Adhere step, which means that batch (b) (4) is outside the proposed commercial process capability. Also, please note that method precision should be established and controlled by analytical tools such as repeat testing and system suitability controls.

f. Regarding your lot release process, please specify the date on which the proposed commercial (b) (4) FDP release specifications (for all release assays) and associated stability specifications were introduced. If you are using release specifications which differ from those described in the BLA, please explain the difference.

# 13. APPENDIXES

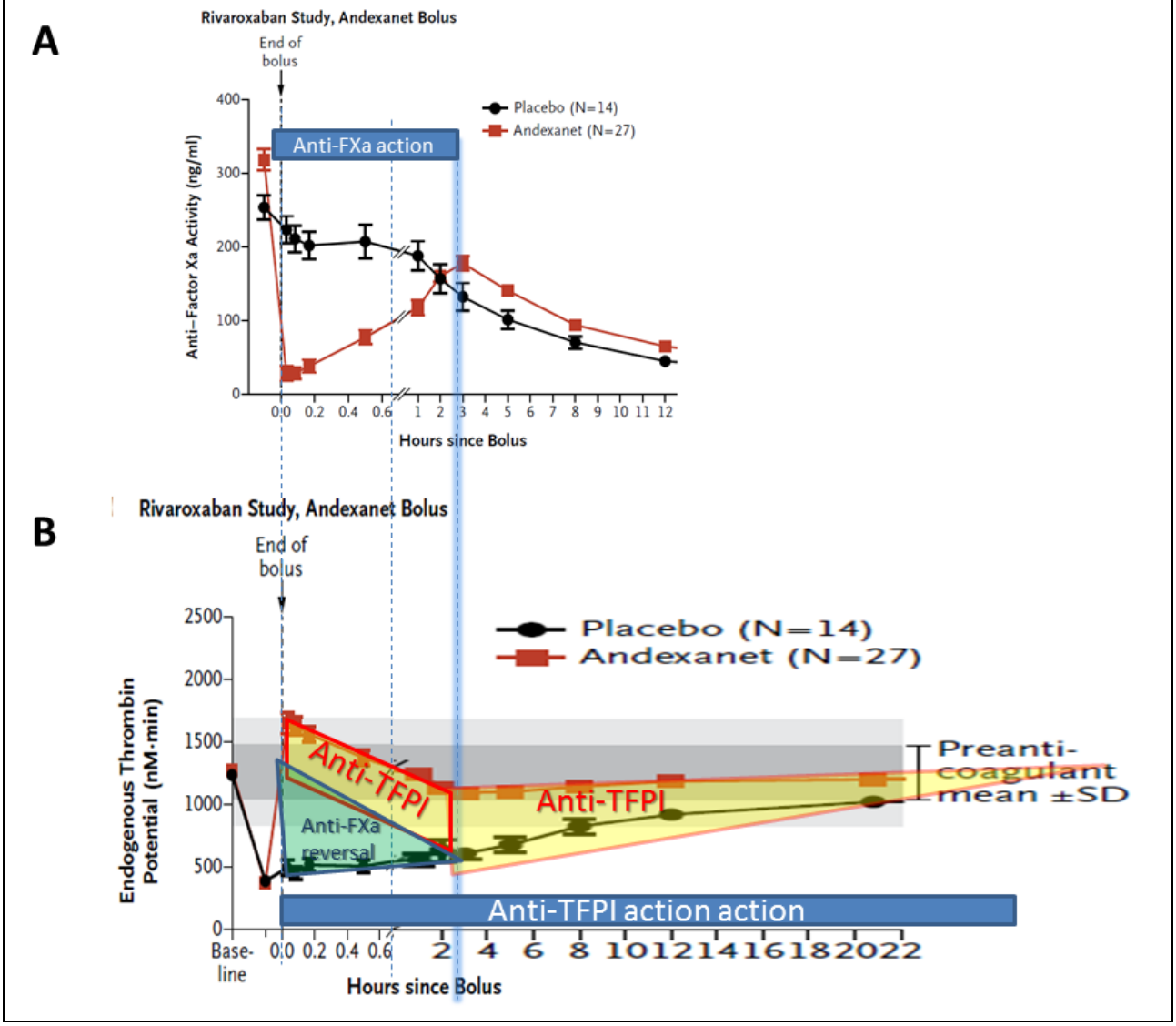
## 13.1. Appendix A: Supplemental Figures

**Supplemental Figure 1: Estimates of the relative contributions of anti-FXa reversal and TFPI inhibition actions of ANDEXXA to the observed elevation of TGT in the Phase 3 studies (<u>FXa inhibitor: rivaroxaban, ANDEXXA: bolus plus infusion</u>).** Time-course of anti-Xa activity reversal (Panel A) and TGT elevation (Panel B) are reproduced with modifications from the NEJM paper[59]. The difference between the TGT values in ANDEXXA and placebo-treated volunteers should be a result of two actions: anti-FXa activity reversal which can bring the TGT to the pre-treatment baseline and the TGT which elevates the TGT over this baseline.



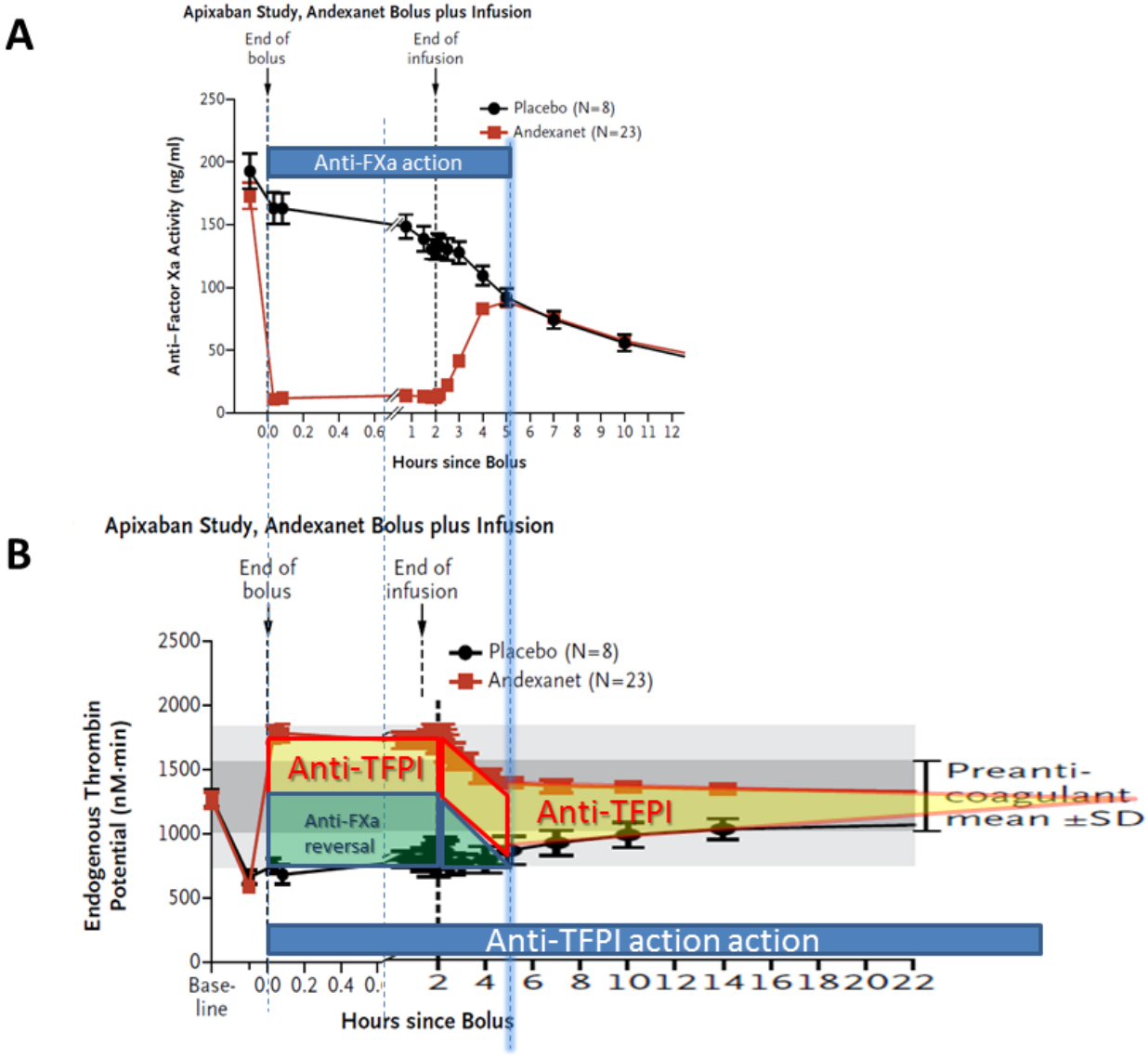

[59] Siegal DM et al. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. N Engl J Med. 2015 Dec 17;373(25):2413-24

**Supplemental Figure 2: Estimates of the relative contributions of anti-FXa reversal and TFPI inhibition actions of ANDEXXA to the observed elevation of TGT in the Phase 3 studies (<u>FXa inhibitor: rivaroxaban, ANDEXXA: bolus only</u>).** Time-course of anti-Xa activity reversal (Panel A) and TGT elevation (Panel B) are reproduced with modifications from the NEJM paper[60]. The difference between the TGT values in ANDEXXA and placebo-treated volunteers should be a result of two actions: anti-FXa activity reversal which can bring the TGT to the pre-treatment baseline and the TGT which elevates the TGT over this baseline.



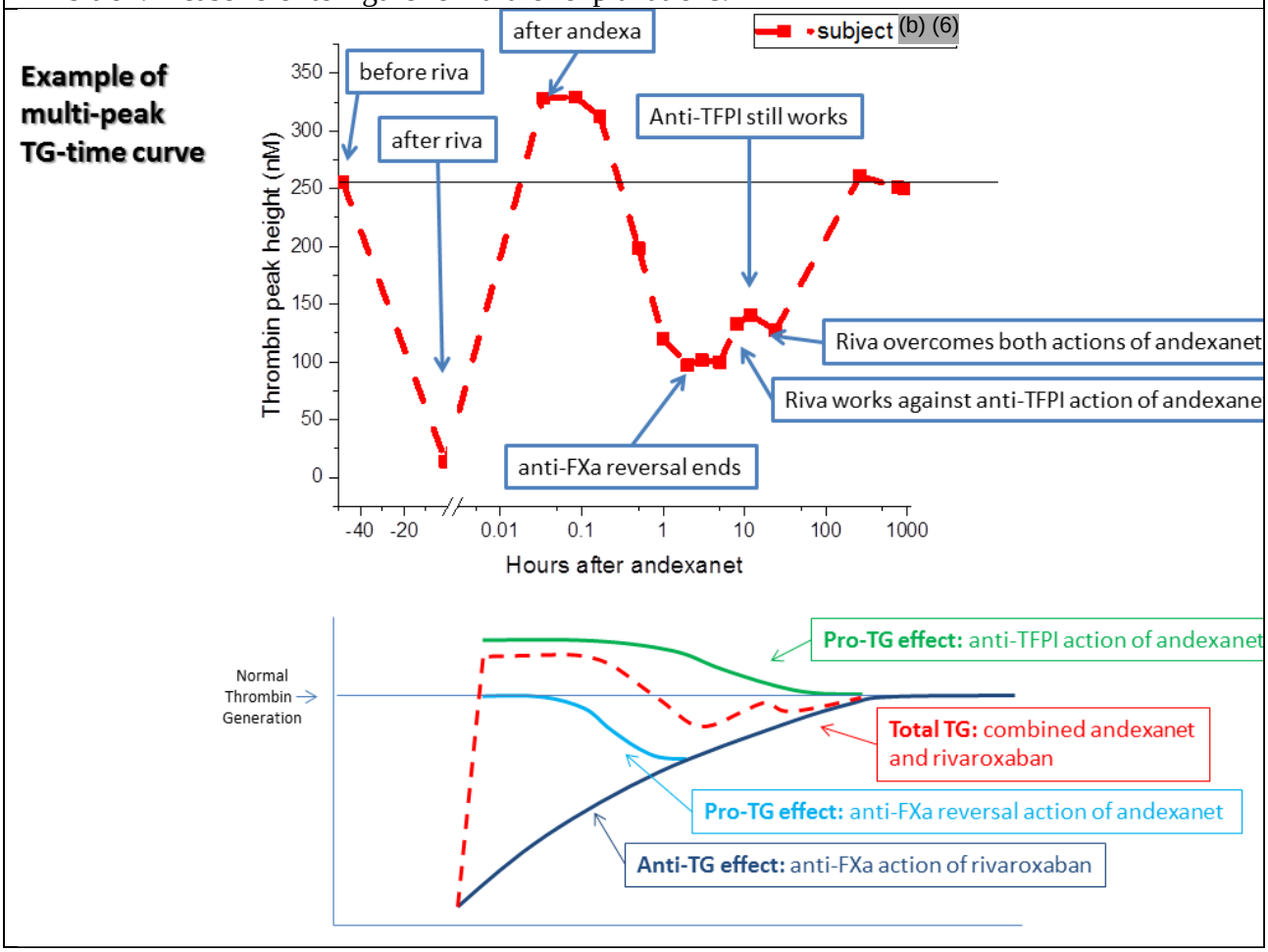

[60] Siegal DM et al. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. N Engl J Med. 2015 Dec 17;373(25):2413-24

**Supplemental Figure 3: Estimates of the relative contributions of anti-FXa reversal and TFPI inhibition actions of ANDEXXA to the observed elevation of TGT in the Phase 3 studies (FXa inhibitor: apixaban, ANDEXXA: bolus plus infusion).** Time-course of anti-Xa activity reversal (Panel A) and TGT elevation (Panel B) are reproduced with modifications from the NEJM paper[61]. The difference between the TGT values in ANDEXXA and placebo-treated volunteers should be a result of two actions: anti-FXa activity reversal which can bring the TGT to the pre-treatment baseline and the TGT which elevates the TGT over this baseline.




[61] 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

**Supplemental Figure 4: Time-course of TGT changes in one healthy volunteer (subject (b) (6)) demonstrates a complex behavior.** Note that TGT is decreased after rivaroxaban administration, increased after ANDEXXA administration, slowly decreases as ANDEXXA is washed out, then goes up briefly again at 10 hours after ANDEXXA before going down again at 24 hours and finally returns to the pre-treatment level. This complex behavior may indicate the changing balance between the two activities of ANDEXXA: anti-FXa activity reversal and TFPI inhibition. Please refer to figure for further explanations.



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

## *13.3. Appendix C: Dr. John Curnutte's 18 April 2016 email about the biomarkers used in clinical trials*

**From:** John Curnutte
**Sent:** Monday, April 18, 2016 4:21 AM
**To:** Epstein, Jay
**Cc:** Bill Lis; Alex Gold
**Subject:** Follow-up to Our Call on April 11

Dear Jay,

Thank you for the good discussion in our telecom last Monday with you, Bindu, Bill, and me. I wanted to get back to you about several major concerns you and Bindu highlighted in the call. They are of such importance to our current discussions that I wanted to make sure you had a top-level summary of the critical data regarding each. From my notes of the call, these are the major issues:

1. Concern that a 2-hour infusion may not be a clinically meaningful duration for reversal
2. Concern that thrombin generation levels in healthy volunteer studies during andexanet infusion were elevated above the normal rang
3. Concern about the anti-fXa PD biomarker and that it is not as validated as ECT is for dabigatran and idarucizumab
4. Clarity on the standards for adjudication of hemostasis in the ANNEXA-4 study

Let me quickly deal with #4 first. We did receive an Information Request on April 6 and submitted a comprehensive response to the Agency on April 13. The Charter (and the hemostasis scoring criteria) for the Independent Adjudication Committee are in the BLA. We have now provided extensive source documents pertaining to the adjudication of the ANNEXA-4 patients including adjudicator scoring documents/notes, central lab reports on the CT's/MRI's from the ICH patients, and radiology reports. I think these should provide a clear view of the criteria and results used in the rigorous adjudication process.

I agree with you and Bindu that Issues 1-3 above are all very important. They are ones that, over the past 3-5 years, we have considered, analyzed, experimented on, and discussed with the Agency. Moreover, they were heavily considered – with FDA guidance – in the design and implementation of the Phase 1, 2, 3, and 3b/4 studies. Let me share with you a top-level summary of the scientific and clinical data that address these concerns.

**1. Concern that a 2-hour infusion may not be a clinically meaningful duration for reversal**

This touches on a central question for all reversal agents for anticoagulants – how long (duration) and how deep does the reversal need to be to achieve hemostasis. Is it the 24-hour, fairly deep reversal achieved with the 5 gram dose of Praxbind? Is it the 2.5-3 hour duration with the andexanet regimen? An even shorter time? If it is too short, hemostasis may not be achieved and,

if it is, re-bleeding may occur. If it is too long, there may be an increased risk of thrombosis. The following speak to the issue of duration:

a. *The kinetics of clotting* – A definitive hemostatic plug can form within a minute or two and a stable clot can form within minutes if not impeded by an anticoagulant. Thus, it is theoretically possible to achieve hemostasis in minutes.
b. *Andexanet works within seconds* – in vitro, andexanet reverses inhibition of Factor Xa and restore full enzymatic activity within seconds. It does so by rapidly and tightly binding direct Factor Xa inhibitors. Similar kinetics are seen in in vivo models looking at anti-fXa activity – the biomarker that measures directly Factor Xa enzymatic activity.
c. *Animal models* – Three models show that a *single bolus* of andexanet can result in rapid (over minutes) and near-complete hemostasis.
d. *Andexanet Phase 3 data* – Healthy volunteer studies show that a single bolus of andexanet (as well as the bolus + infusion regimen) results in a *durable* correction of thrombin generation that remains in the normal range for > 20 hours (and well above levels seen with placebo) (NEJM paper).
e. *Andexanet Phase 3b/4 (ANNEXA-4) hemostasis data* – In the first 35 patients with major bleeding on apixaban, rivaroxaban, and enoxaparin in the ANNEXA-4 study (included in the 90-day update to the BLA on March 15), *85% of the patients had excellent or good hemostasis* as determined by the Adjudication Committee. As of April 15, 49 patients have now been adjudicated with *87% determined to have excellent/good hemostasis*. Of the 13% (7 patients) who were adjudicated as poor or none, 4 had clear progressive bleeding (1 GI, 1 vaginal, 2 Subdural ICH) and 3 were equivocal. It was anticipated at the study inception that there would be anatomic lesions that would be too large to respond to just a reversal agent. This may be the case here.
f. *ANNEXA-4 re-bleeding data* – In the first 35 patients in the ANNEXA-4 study, *none of the patients had a re-bleeding episode* – i.e., the patient had an excellent/good hemostatic result but then re-bled in the next 12 hours. Among these 35 patients, there were only 3 cases where there was an AE of bleeding reported and these occurred on Days 2, 6, 19 (all were thought to be unrelated to andexanet).
g. *ANNEXA-4 ICH data* – In the first 35 ANNEXA-4 patients, there were 13 ICH (5 intraparenchymal and 8 subdural (ranging from 8-63 cc)). The Adjudication Committee determined that 77% had an excellent/good hemostatic response (with equivalent efficacy in both types of ICH). 10 of the 13 ICH patients were discharged from the hospital in 2-6 days.

Regarding the depth of reversal, the following speak to the issue:

a. *Animal models* – The rat tail transection model with enoxaparin-anticoagulated animals showed near-complete reversal of bleeding with just a 50% decrease in anti-fXa activity to a level (~2.5 IU/mL) that was still a supra-therapeutic level. Rabbit liver laceration models with rivaroxaban and edoxaban showed restoration of hemostasis with only partial lowering of anti-fXa activity (to levels well above the no effect level).
b. *Hemophilia treatment* – Recombinant Factor VIII or IX treatment of major bleeds in hemophilia patients requires only 50-60% restoration of normal levels (per label).

c. *ANNEXA-4 data* – In the first 35 patients, 6 patients presented to the hospital with supra-therapeutic levels of apixaban (487-950 ng/mL) or rivaroxaban (362-862 ng/mL). After andexanet, 5/6 were still at levels (52-411 ng/mL) above the no-effect level. Nonetheless, 5/6 were adjudicated with excellent hemostasis. This excellent response was associated in all cases with a substantial and rapid drop in anti-fXa levels (decreases ranging from 211 to 650 ng/mL) and normalization of thrombin generation.

*Comments:* Based on the nonclinical and early clinical data, the initial concept for andexanet was to treat with a bolus only and reverse anti-fXa levels to somewhere between 1-3X the no effect levels (i.e., 30-90 ng/mL). Since the required duration and depth of reversal were unknown, Portola chose a more conservative approach and added a 2 hour infusion after the bolus. We also set a minimum depth of reversal as one defined as the level of reversal needed to restore thrombin generation back to the normal range. The dosing regimens for rivaroxaban, apixaban, edoxaban, and enoxaparin used in ANNEXA-4 reflect this more conservative approach.

While the ANNEXA-4 study is still in its early stages with a planned enrollment of 250 patients (88 patients enrolled as of April 16), the results thus far suggest that the andexanet bolus + 2 hour infusion regimen is sufficient to result in excellent/good hemostasis in 85% of patients without evidence thus far of re-bleeding. This in accord with the prolonged correction of thrombin generation seen in the Phase 3 healthy volunteer study (>20 hours) and a similar finding in the ANNEXA-4 patients (although thrombin generation data are harder to interpret in bleeding patients).

**2. Concern that thrombin generation levels in healthy volunteer studies during andexanet infusion were elevated above the normal range**

There are several key variables in interpreting the thrombin generation data in the Phase 2, 3, and 3b/4 studies. Here are some of the key considerations:

a. *The thrombin generation (TG) assay* – Normal human plasma on its own does not generate thrombin unless the coagulation cascade is activated via the extrinsic pathway (using Tissue Factor) or the intrinsic pathway (using (b) (4) ). The level of TG is dependent upon the degree of activation which is, in turn, dependent upon the amount of Tissue Factor or (b) (4) added to the assay. In the andexanet clinical trials, the Tissue Factor-activated version of the TG assay is used, although the (b) (4) assay is sometimes used as a control.

*b.* *Effect of Factor Xa inhibitors and andexanet on TG* – Factor Xa inhibitors (direct and indirect) cause a decrease in TG by inhibiting Factor Xa and blocking the cleavage of Prothrombin to Thrombin. TG is restored to normal by andexanet predominantly due to its ability to bind and sequester the Xa inhibitors – this is observed in both the Tissue Factor and (b) (4) formats of the TG assay. Since andexanet also binds to TFPI and therefore removes this "Tissue Factor Pathway Inhibitor" from the patient plasma in the TG assay, the Tissue Factor reagent added to the assay is no longer inhibited by TFPI. As a result, a small "(b) (4)" of extra TG is observed in the assay. This is entirely due to the sequestration of TFPI by

andexanet in the assay mixture. This "(b) (4)" is not seen in the (b) (4) TG assay. If TFPI is removed from human plasma before it is added to the Tissue factor TG assay, the "(b) (4)" is also not seen. Both of these assays – the (b) (4) TG and TFPI-depleted plasma Tissue Factor TG assay – *demonstrate that andexanet on its own has no prothrombotic activity as measured by enhanced thrombin generation.*

c. *Magnitude of the andexanet-TFPI "(b) (4)" in the Tissue Factor version of the TG assay* – In the Phase 3 healthy volunteer studies with andexanet published in the NEJM, the "(b) (4)" was seen with both apixaban and rivaroxaban and with both the bolus and bolus + infusion regimens. The mean TG was on average 7% above the normal range (mean ± 2 SD; defined by the baseline vales for the subjects in the study). This "(b) (4)" was transient as it returned to with 2 SD of the mean within 30 minutes after andexanet administration. In a bleeding situation, this transient small "(b) (4)" is at least directionally and potentially helpful.

d. *ANNEXA-4 TG data* – The TG data from the first 35 patients summarized in the Day 90 update to the BLA do not show this "(b) (4)" or any TG overshoot. Interestingly, many of the bleeding patients present with already "normal" TG even though they have therapeutic levels of anticoagulant and have not yet received andexanet. We hypothesize that this represents the physiologic response to bleeding with activation of the coagulation cascade. The most likely physiologic activator is endogenous Tissue Factor in the bleeding patients – a testable hypothesis that we will pursue later in ANNEXA-4. Since the TG assay used in ANNEXA-4 is the Tissue Factor-driven version of TG, the "normal" levels of TG seen at presentation to the hospital may reflect the effect of the *combined amounts* of the elevated Tissue Factor present in the plasma from these patients *and* the reagent Tissue Factor added to the assay – resulting in a much higher level of Tissue Factor in the assay and, hence, an apparent increase in TG above levels that might be expected.

*Comments:* The mechanism for the "(b) (4)" in TG in the Tissue Factor TG assay is well-understood – TFPI in the plasma is bound to andexanet, resulting in a decreased inhibition of Factor Xa by TFPI-Tissue Factor, leading to higher Factor Xa activity and increased TG. There is no evidence that andexanet on its own increases Factor Xa activity or increases TG.

**3. Concern about the anti-fXa PD biomarker and that it is not as validated as ECT is for dabigatran and idarucizumab**

Based on our review of the SBOA for dabigatran and that for idarucizumab, as well as the literature, the data supporting ECT as a biomarker for Thrombin inhibitor reversal is *highly comparable to that supporting anti-fXa as a biomarker* for Factor Xa inhibitors. Several points for consideration:

a. *Evidence for ECT as a biomarker reasonably likely to correlate with a clinical benefit* – ECT has been shown to correlate closely with the plasma concentration of dabigatran. (Similar results are seen for Dilute Thrombin Time – the other biomarker in the idarucizumab REVERSE AD study.) The plasma concentration of dabigatran correlates with the risk of bleeding. Therefore, ECT (and Dilute Thrombin Time) correlates with the risk of bleeding. This is the justification for using ECT as a biomarker for the idarucizumab REVERSE AD trial and for its approval based on that biomarker.

b. *Evidence for anti-fXa as a biomarker reasonably likely to correlate with a clinical benefit* – In the Briefing Book for the November 13 Type A Meeting, Portola presented a similar argument as outlined above for ECT and dabigatran. We showed Portola data that the plasma concentrations of apixaban, rivaroxaban, and edoxaban correlate closely and linearly with anti-fXa activity. Next we showed that the plasma concentrations of each direct Xa inhibitor correlates with risk of bleeding. The data for these bleeding correlations were obtained from Portola's andexanet partners from their NDA's ((b) (4) rivaroxaban, (b) (4) apixaban, (b) (4) edoxaban). By the same transitive properties used for ECT, we concluded that anti-fXa levels correlate with bleeding risk all the Xa inhibitors and therefore can serve as a biomarker for the ANNEXA studies that is reasonably likely to correlate with a benefit in bleeding. The final FDA minutes from that meeting (dated December 8, 2015) agreed with Portola's conclusion, stating "FDA agrees to accept a BLA for consideration for filing for edoxaban, apixaban, and rivaroxaban as a 'class-effect" (i.e., direct FXa inhibition)."

c. *Performance of anti-fXa biomarker in ANNEXA-4* – To date, data from ANNEXA-4 are available on 35 patients to assess preliminarily the performance of anti-fXa activity as a biomarker (all data are present in the 90-day update to the BLA). The anti-fXa reversal time course was very similar to that seen in the Phase 3 study (NEJM) in 28/35 patients with 1 other roughly similar. 4/35 had very high levels (discussed above) and all had substantial and rapid drops in anti-fXa. 2/35 had no detectable anti-fXa. Thus, the overall performance of the biomarker was in accord with that seen in the Phase 3 and Phase 2 studies. For patients who were adjudicated to have excellent/good hemostasis, there is a good correlation with rapid, large drops in anti-fXa activity (although several did not to reach effect levels because of the extraordinarily high levels at presentation). The converse is not a good correlation – the 5 patients with poor/none did have good drops in anti-fXa activity. While this could be interpreted that the biomarker is not sufficiently robust, this situation was anticipated by Portola (and by the FDA in May 2014 during the discussions on designing the Phase 3b/4 study). Both predicted that there would be cases where anti-fxa levels would fall as expected but inadequate hemostasis would be observed due to anatomic lesions that would require instrumentation/surgery because they were too large for a hemostatic plug to repair.

*Comments:* The anti-fXa biomarker is as strongly validated as ECT and thus far in ANNEXA-4 is performing as expected.

I hope you have found this top level summary helpful, Jay. These are clearly complicated issues but as I hope you see, there is a rich scientific foundation supported by extensive experimental data. These data, in turn, are consistent with the first waves of data coming from the ANNEXA-4 study. The fuller answers to these questions and concerns will be obtained in due course as ANNEXA-4 moves toward completion. Given the high degree of consistency thus far between the science and the clinical data, one prudent course of action is to watch and wait for the eventual results from this confirmatory trial.

I would be glad to discuss any of the matters in this email with you at any time, Jay.

With very best regards,

John

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