M E M O R A N D U M
Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Biologics Evaluation and Research

To: File of STN 125586/0 & Jean Gildner (CBER/OTAT/DRPM/RPMBI)

From: Wojciech Jankowski (CBER/OTAT/DPPT/HB)

Through: Tim Lee, Chief (CBER/OTAT/DPPT/HB)

Basil Golding, Director (CBER/OTAT/DPPT)

Subject: Final review of the ITC data on structural integrity and molecular interaction in Portola's biologics license application (BLA) for Coagulation Factor Xa (Recombinant), Inactivated [Andexxa]

CC: Mikhail Ovanesov (CBER/OTAT/DPPT/HB)
Zuben Sauna (CBER/OTAT/DPPT/HB)

BACKGROUND

Andexxa is the proprietary name for Portola's recombinant analog of human Coagulation Factor Xa (FXa) product, which lacks the Gla-domain, and has its catalytic serine residue replaced with alanine (b) (4) Andexxa is composed of (b) (4) amino acids (AA) with an approximate molecular weight of 41 kDa based on the cDNA sequence. Due to the (b) (4) mutation, it has no measureable enzymatic activity. However, it retains the ability to bind small-molecule FXa inhibitors with high affinity. This allows Andaxxa the potential to be used clinically in patients treated with rivaroxaban and apixaban, when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding.

It is important to note that Andexxa has two independent mechanisms of action. In the Andexxa molecule, the serine residue responsible for the FXa proteolytic activity was replaced with alanine, and the gamma-carboxyglutamic acid (Gla) domain responsible for procoagulant lipid binding was genetically removed. The aim was to prevent the activation of blood coagulation, but retain the protein's ability to bind FXa inhibitors. Since FXa and Andexxa bind to FXa inhibitors with comparable affinities, Andexxa competes with FXa for these inhibitors, and increases the activity of FXa in blood. Andexxa's second physiological target, like FXa, is Tissue Factor Pathway Inhibitor (TFPI), an endogenous protein which, in complex with FXa, FVIIa and Tissue Factor, acts as the only known inhibitor against the initiation of blood coagulation via the Tissue Factor (extrinsic) pathway. Because FXa's Gla domain is needed for TFPI to form the quaternary complex

to exert inhibition, the binding of Andexxa to TFPI interferes with the inhibitory action, thereby allows the activation of blood coagulation to proceed.

SCOPE OF THE REVIEW

A Complete Response (CR) letter for the BLA was issued to Portola on 17 August 2016. In this memorandum, I summarize my review of Portola's responses to the CR letter and subsequent information requests (IRs). Specifically, I reviewed the data provided by Portola that characterized the thermodynamics of the interactions between Andexxa or its (b) (4) and:

(i) small-molecule inhibiotrs of FXa (apixaban, betrixaban, edoxaban, and rivaroxaban)
(ii) Tissue Factor Pathway Inhibitor (TFPI)

I also evaluated the results of (b) (4) and potency assays following (b) (4) of Andexxa.

REVIEW

Binding of Andexxa to Factor Xa Inhibitors

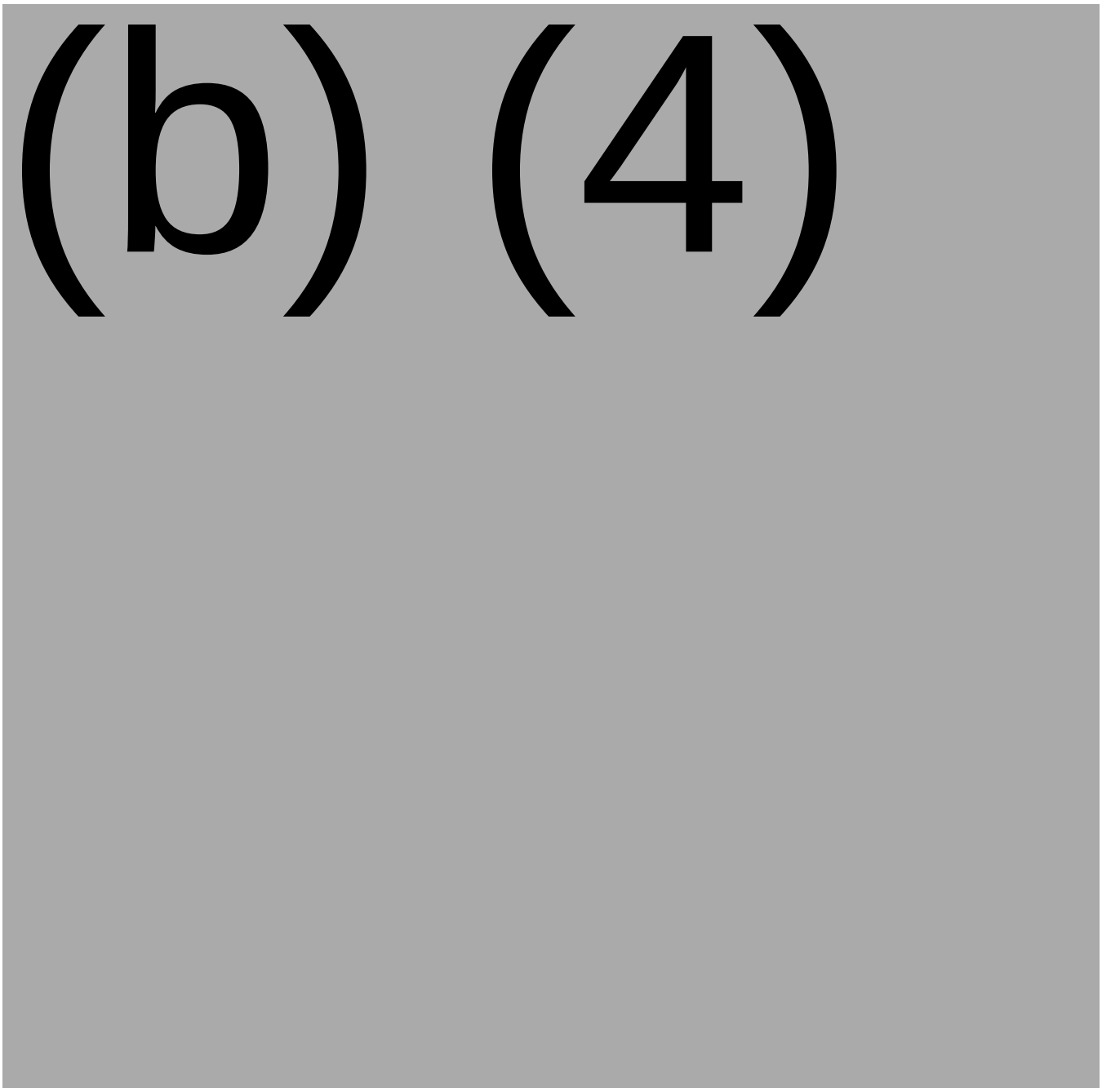
Previously, Portola had evaluated binding of the Andexaa (b) (4) ((b) (4)) to four small-molecule FXa inhibitors. The data confirmed that:

(i) all variants in each lot (those with (b) (4) of Andexxa) used for final drug product (DP) formulation (n = 1) can bind all the small-molecule FXa inhibitors examined in this study.

(ii) the (b) (4) lots from (b) (4) used in this sudy are comparable; there are no differences in the proximity of the small-molecule FXa inhibitors binding pockets as determined by (b) (4) that would affect the binding of the protein to FXa inhibitor.

Additional thermodynamic parameters were determined for interactions between Andexxa and all four small-molecule FXa inhibiors using (b) (4). These parameters for different lots of Andexaa (b) (4) manufactured using (b) (4) are provided in Table 1.

(b) (4)



These additional experiment results are consistent with results previously provided by Portola in the original BLA.

Since Andexxa also binds TFPI, and inhibits its action in the control of hemostasis, it is important to examine the thermodynamic and stoichiometric characteristcs of this interaction. This information was not provided in the BLA. Simlarly, the interactions of the various (b) (4) forms of Andexxa with TFPI were also not examined using (b) (4). Therefore, these deficiencies were included in the CR Letter.

Items in the CR Letter

1. With reference to our IR dated 01 June 2016 and your 15 June 2016 response which we deem 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 its interactions with 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 degradation of ANDEXXA and consider including the (b) (4) assay in the (b) (4) release specifications. Establish acceptance criteria for its interactions with direct FXa inhibitors for these thermodynamics and stoichiometry parameters - Kd, ΔH, TΔS, ΔG and n

Portola's responses to the CR Letter (CRL) are reporoduced below:

Portola response, in *italics*, to CRL item 1a:

The data that shows that (b) (4) and (b) (4) lots of andexanet alfa bind to TFPI with similar enthalpy and stoichiometry can be found in a report (NC-17-0804-R0001) entitled, (b) (4) Binding interactions between Andexanet alfa and Tissue factor pathway inhibitor (TFPI) using Generation 1 (b) (4), located in Section 3.2.S.3.1 Elucidation of Structure and other Characteristics.

Review summary

In the report NC-17-0804-R0001, Portola characterized and assessed the thermodynamic properties, affinity and stoichiometry of TFPI binding to Generation 1 (b) (4) and (b) (4). The lots used in this study are described in Table 1a.1, and the results are presented in Table 1a.2.

Table 1a.1. Lots used to characterize binding between Andexanet Alfa and TFPI

Manufacturing Process	Portola Lot No.	Lot No.	Date of (b) (4) Manufacturing	Formulation

(b) (4)	12 Sep 2014	(b) (4)
	24 Sep 2014	
	20 Oct 2014	
	16 Dec 2014	10 mg/mL in (b) (4) Tris, (b) (4) arginine HCl, 2% sucrose, 5% mannitol, 0.01% Polysorbate 80, pH 7.8
	31 Jul 2015	
	12 Aug 2015	

(b) (4)

Reviewer's comment

Using (b) (4) as a test method to measure TFPI binding interactions, I found that all Generation 1 (b) (4) and (b) (4) lots can be considered comparable for stoichiometry and enthalpy. The stoichiometries (TFPI:Andexxa) are always slightly below (b) (4), possibly due to small errors in determining the concentration of TFPI. The interactions indicate that the Andexxa (b) (4) is fully functional with respect to its intended mode of action, i.e., high affinity binding to FXa inhibitors.
The response from Portola to the comment in the CRL item 1a is acceptable.

Portola response to CRL item 1b:

See report NC-17-0801-R0001 Potency of (b) (4) *Variants of Andexanet alfa found in Section 3.2.S.3.1 Elucidation of Structure and other Characteristics.*

Review summary

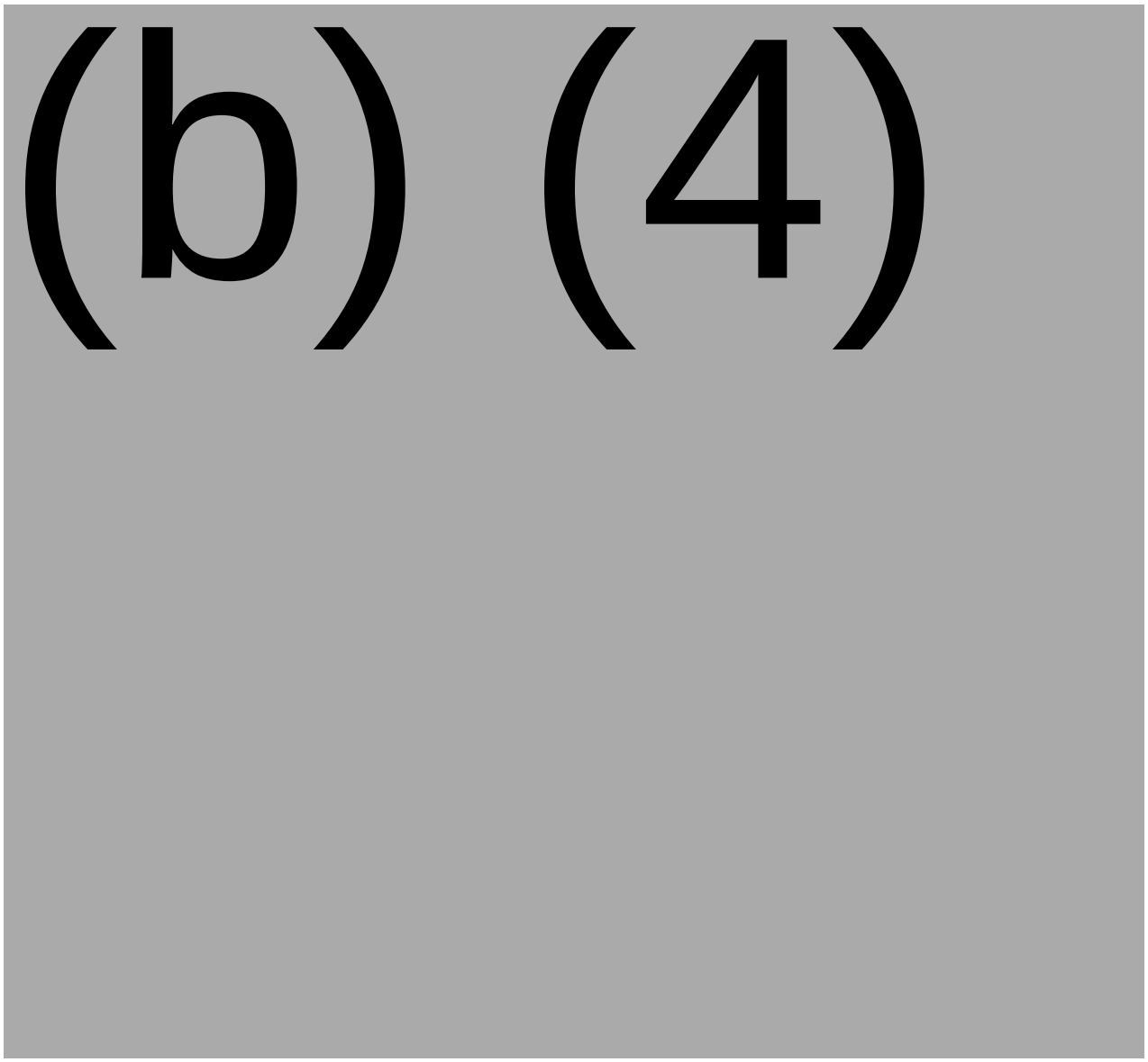
In report NC-17-0801-R0001, Portola evaluated the impact of an (b) (4) variant Andexxa in multiple potency assays. These assays include the release test methods of direct, indirect, and TFPI (b) (4) assays, (b) (4), and tissue factor (TF)-initiated thrombin generation The information about manufacturing lot

used in this study is provided in Table 2a.1.

Table 2a.1. Andexxa lot used to generate (b) (4) varinats

Manufacturing Process	Portola Lot No.	Manufacturing Lot No.	Concentration	Storage Condition
(b) (4)	(b) (4)	(b) (4)	10 mg/mL	(b) (4)

(b) (4) variants of Andexxa were generated using (b) (4) . The (b) (4) (see Figure 2a) illustrates the (b) (4) variants following (b) (4) treatment.



To understand the impact of (b) (4) variants on their interactions with the small-molecule inhibitors, apixaban and rivaroxaban, and TFPI, the thermodynamic parameters for interactions of small-molecule FXa inhibitors with Andexxa were compared for the parent molecules, and samples (b) (4) variant. The results are provided in Tables 2a.2 and 2a.3.

(b) (4)

(b) (4)

Reviewer's comment

The data provided by Portola confirm that the (b) (4) (one of the Andexxa variants) that can be found in the Andexxa (b) (4), has full functionality. Therefore, its binding to small-molecule FXa inhibitors, and TFPI will not be affected by the (b) (4). Portola's response to the CRL item 1b is acceptable.

Portola response to CRL item 1c:

The sensitivity of the (b) (4) method has been used to evaluate the degradation of ANDEXXA as described in the report (b) (4) Binding Interactions of (b) (4) of Andexanet Alfa (NC-17-0799-R0001) which has been included in Section 3.2.S.3.1 Elucidation of Structure. The report characterizes the binding thermodynamics of (b) (4) andexanet alfa (b) (4) to direct factor Xa inhibitors, with Generation 1 (b) (4), using (b) (4). The currently proposed potency assays for release

(including the recently established TFPI (b) (4) assay) are considered sufficient to capture the mechanisms of action of andexanet. In addition, Portola has not been able to identify a contract lab that has the instrumentation available to run the (b) (4) assay under GMP conditions, therefore we will not be able to incorporate (b) (4) into testing as a release assay.

Review summary

In report *NC-17-0799-R0001*, Portola evaluated the impact of (b) (4) of Andexxa (b) (4) using (b) (4). Interactions of degraded Andexxa with all four small-molecule FXa inhibitors (apixaban, betrixaban, edoxaban, and rivaroxaban) were evaluated in this study.

Andexxa ((b) (4) -see Table 1c.1) was (b) (4)

Table 1c.1. Andexxa (b) (4) used in the f(b) (4) study

Manufacturing Process	Lot Number	Concentration	Storage Condition
(b) (4)	(b) (4)	10 mg/mL	(b) (4)

A summary of the results of all (b) (4) studies is provided in Table 1c.2.

Table 1c.2. Thermodynamic Parameters for Binding of Rivaroxaban to Andexxa (b) (4) (Parent and (b) (4))

(b) (4)

Reviewer's comment

The results from the (b) (4) study clearly show a significant decrease of binding affinity, and reduction in stochiometry relative to the parent samples.

Based on the above information, the following IR was submitted to Portola on 5 December 2017.

With reference to your report entitled "(b) (4) Binding Interactions of (b) (4) of Andexanet Alfa" (# NC-17-0799-R0001), please provide (b) (4), potency assessment and other available analytical evidence to demonstrate the structural and functional degradation of the (b) (4) sample (b) (4).

Portola's response

The (b) (4) sample of Lot (b) (4), described in Report (# NC-17-0799-R0001), was not extensively characterized, however the structural and functional degradation of the (b) (4) sample can be deduced from the comprehensive (b) (4) study summarized in BLA sections 3.2.S.7.1.3.12 and 3.2.S.7.3. In the (b) (4) studies described in these BLA sections an andexanet alfa (b) (4) sample (Lot (b) (4)) was exposed to (b) (4) for (b) (4) and tested by various analytical methods to characterize the (b) (4). The impact of (b) (4) on the andexanet alfa structure and function was characterized by the release methods (b) (4), and activity by Direct Potency and Indirect Potency. The characterization by these methods confirmed that (b) (4) induced structural changes that include (b) (4). These structural changes resulted in a decrease in the activity of andexanet alfa as determined by the direct and indirect inhibitor potency assays.

Review summary

The impact of (b) (4) on the Andexxa direct and indirect potency (refrenced by Portola in their response to my IR) is provided in Table 2.

Table 2. Percent (b) (4) by direct and indirect potency for lot (b) (4) sample (b) (4) at (b) (4)

(b) (4)

Reviewer's comment

The (b) (4) data by (b) (4) provided in Table 1c.2. and (b) (4) results by direct and indirect potency (see Table 2) cannot be directly compared because of the difference in the (b) (4). However, there is a close association between the (b) (4) of direct potency and indirect percent activity ((b) (4), respectively), and the (b) (4) results for all inhibitors (Apixaban (b) (4), Betrixaban (b) (4), Edoxaban (b) (4) and Rivaroxaban (b) (4)).

Based on the above, it can be assumed that the potency assays for release are considered sufficient to capture the mechanisms of action of Andexxa.

RECOMMENDATION

The results of the studies evaluating Andexxa (parent molecules and samples (b) (4) for the (b) (4) Variant) manufactured using (b) (4) were provided. These studies show that the Andexxa (b) (4) lot used for final DP formulation binds small-molecule FXa inhibitors with high affinity, and in a stoichiometry of 1:1. Thus, all variants are fully active. Moreover, Andexxa manufactured using (b) (4) can bind to TFPI, and shows comparable loss of activity in the (b) (4) studies. Based on the results of these studies, I conclude that that the quality of Andexxa manufactured using (b) (4) is comparable.

Therefore, I recommend approval of the BLA under STN 125586/0 from the perspective of product quality and comparability based on thermodynamic analyses.