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 & Thomas Maruna (DBCD/OBRR/CBER)

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Subject: Final review of the (b) (4) data on structural integrity and molecular interaction in Portola's biologics license application (BLA) for Coagulation Factor Xa (Recombinant), Inactivated [ANDEXAA]

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BACKGROUND

Andexanet alfa is a recombinant analog of human Coagulation Factor (F) Xa that lacks the Gla-domain and has its catalytic serine residue replaced with alanine ((b) (4)). Andexanet alfa 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 is catalytically inactive and has no measureable enzymatic activity. However, it retains the ability to bind small molecule FXa inhibitors with high affinity despite the mutation of the active site.

SCOPE OF THE REVIEW

This review evaluates the data provided by Portola that characterizes the thermodynamics of apixaban, (b) (4) , edoxaban, and rivaroxaban binding with (b) (4) lots of (b) (4) and (b) (4) lots of (b) (4) using (b) (4) . Amongst the differences between the two processes, (b) (4) is formulated to (b) (4) in the final DP lyophilization buffer, whereas (b) (4) to 10 mg/mL.

(b) (4) directly measures the (b) (4) upon ligand binding to a macromolecule. Binding curves are generated by (b) (4)

(b) (4) . Based on the (b) (4) and ΔH, the Gibbs energy of binding (ΔG) and entropy (ΔS), can also be determined using the following equations:

(i) (b) (4)

(ii) (b) (4)

Once (b) (4) are calculated, a complete thermodynamic picture, which in turn allows a comprehensive assessment of the nature of the FXa inhibitor binding characteristics.

REAGENTS AND PROTEIN LOT NUMBERS

All experiments were performed using (b) (4) . (b) (4) was supplied by (b) (4) . (b) (4) for (b) (4) is manufactured by (b) (4), and supplied by (b) (4) . (b) (4) used for (b) (4) are (b) (4) supplied by (b) (4) .

Small molecule FXa inhibitors:

- Apixaban (Lot (b) (4)) was supplied by (b) (4) .
- (b) (4)
- Edoxaban (Lot (b) (4)) was supplied by (b) (4) .
- Rivaroxaban (Lot (b) (4)) was manufactured and supplied by (b) (4) .

Andexanet alfa ((b) (4)) process and lot numbers:

i. (b) (4)
ii. (b) (4)

REVIEW

Binding of andexant alfa to Factor Xa Inhibitors

From one (b) (4) experiment, one can derive the protein's binding stoichiometry, binding affinity and enthalpy. Below is the data provided by Portola to support andexanet alfa activity and binding stechiometry to four small molecules FXa inhibitors: apixaban, (b) (4) , edoxaban, and rivaroxaban.
Binding affinities (Kd) between (b) (4) lots (Table 1) and small molecules are comparable, with Kd (b) (4) for apixaban, (b) (4) , rivaroxaban, and a slightly (b) (4) Kd of (b) (4) for edoxaban.

Table 1. Binding affinity and stoichiometry between Andexanet Alfa and inhibitors.

(b) (4)

The thermodynamic signatures of all FXa inhibitors indicate that the binding is enthalpically driven (Figure 1), with ΔH (b) (4) for (b) (4), edoxaban, rivaroxaban, and slightly higher for apixaban (b) (4). It should be noted that there is also significant favorable entropy contribution. (b) (4) and rivaroxaban seem to have the same binding entropy, while edoxaban and especially apixaban show more favorable entropy contributions.

Figure 1. Summary of thermodynamic parameters determined by (b) (4) results.

(b) (4)

Factor Xa inhibitors generally bind in an L-shaped conformation, and occupy the anionic FXa S1 pocket lined by Asp189, Ser195, and Tyr228 and the aromatic S4 pocket lined by residues Tyr99, Phe174, and Trp215. However, only the structures of rivaroxaban and apixaban in complex with FXa are available (PDB ID 2W26 and 2P16, respectively) and therefore, the nature of the interactions for the other two FXa inhibitors cannot be determined. Despite the lack of structural information, it could be speculated, based on the

thermodynamic signatures, that (b) (4) and rivaroxaban exhibit similar interactions, including their effects on FXa conformational changes and formation or dissolution of hydrogen bonds. Moreover, lots of the same drug product display considerable consistency in their thermodynamic profiles.

(b) (4) data provided by Portola has several important implications in terms of protein activity and structural integrity:

(i) The data confirm that all variants in each lot (those with (b) (4) of andexanet alfa) used for final DP formulation (n = 1) can bind all the small molecule FXa inhibitors examined in this study.

(ii) The results suggest that the lots from (b) (4) in this sudy are comparable; there are no differences in the proximity of the small FXa inhibitors binding pockets that would affect protein-FXa inhibitor binding.

Binding of andexanet alfa to Tissue Factor Pathway Inhibitor

Since andexant alfa also binds Tissue factor Pathway Inhibitor (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 andexanet alfa with TFPI was also not examined by this method.

RECOMMENDATION

I recommend issuance of a complete response (CR) letter summarizing the deficiencies regarding the use (b) (4) to characterize the interactions between andexanet alfa, its (b) (4) molecular forms, and different ligands. I also recommend Portola to implement (b) (4) assessment as a standard procedure during andexanet alfa manufacture.

Items to be incorporated 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 high c-value. 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 - Kd, ΔH, TΔS, ΔG and n