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 BL 125586/0**

From: **Zuben E. Sauna (OBRR/DHRR/LH)**

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

Basil Golding, MD, Director, DHRR

Subject: **Final review of Portola's biologics license application (BLA) for Coagulation Factor Xa (Recombinant), Inactivated [ANDEXAA] with respect to the immunogenicity assessment**

Background

BL 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*.

The proposed indication of ANDEXXA is *for reversing the anticoagulant effect of direct or indirect factor Xa inhibitors in patients experiencing a serious uncontrolled bleeding event* (b) (4) . The active ingredient of ANDEXXA is an inactive variant of human Coagulation Factor (F) Xa produced by recombinant DNA technology in a Chinese Hamster Ovary (CHO) cell line. The product will be available in lyophilized form for intravenous administration after reconstitution with sterile Water for Injection.

Scope

For this BLA, I have reviewed Portola's development of assays to detect potential anti-drug antibodies to the product and the potential development of inhibitory antibodies to endogenous FX and FXa. This review is part of the safety assessment of ANDEXXA from a CMC (Product) perspective.

Immunogenicity assessment plan and risk-assessment

ANDEXXA is being developed as a universal antidote for patients receiving a FXa inhibitor anticoagulant who suffer a major bleeding episode (b) (4) . ANDEXXA is an engineered protein, a mutated analog of human FXa, which is catalytically

inactive but can bind circulating FXa inhibitors with high affinity. Consequently, the product can, in principle, reduce bleeding in individuals treated with anticoagulants whose mode of action is inhibition of FXa.

Portola's safety evaluation in nonclinical and clinical studies is based on the following measurements:

1) Antibodies directed against ANDEXXA itself
2) Antibodies directed against the closely related endogenous protein, human FXa
3) Antibodies directed to the zymogen, FX
4) A bioassay to detect inhibitory antibodies that affect the activities of ANDEXXA, FXa, or FX.

Reviewer's statement: The overall strategy is adequate. However the development and validation of these assays (see below) has been inadequate and is discussed below.

Immunogenicity risk

In assigning immunogenicity risk for a new product, the Agency considers both the product and the manner in which it will be used in the clinic (see for example, *FDA Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products*).

1) From a product standpoint, the risk of immunogenicity of ANDEXXA may be considered low as this protein (nor the endogenous counterpart, FXa) have inherent immunomodulatory properties. Moreover, the active ingredient of the product is a recombinant analogue of a protein of human origin and expressed in Chinese Hamster Ovary (CHO) (i.e., mammalian) cells which is also consistent with low-immunogenicity risk. The Agency does, however, consider engineered proteins that have neo-sequences as being potentially in a slightly higher risk category. In addition, the Gla domain of FXa is deleted in this protein, consequently it is plausible that the region(s) on the molecule, which were shielded by the Gla domain sequence, could now be exposed.

2) From the standpoint of the mode of administration and frequency of use, ANDEXXA is given as a single administration intravenously for an acute event followed immediately by a 120 minute infusion at one of two doses (depending on the specific FXa inhibitor the patient received and the time elapsed since the last dose). ANDEXXA has a very short elimination half-life (~5-7 hours). Taken together, the proposed clinical use of this product suggests a low risk of immunogenicity. This is assuming that the product is relatively homogenous and pure with respective to (b) (4) impurities derived from the CHO cells.

3) Finally, a risk assessment for immunogenicity takes into account the probability of anti-drug antibodies developing and the clinical consequences if they were to develop. For ANDEXXA, based on (1) and (2) above, the probability for the development of antibodies that inhibit FXa is quite low. *However the clinical consequences of antibodies that cross-react and inhibit **endogenous** FXa or FX are very severe*.

Conclusion: As the potential clinical consequences of antibodies that inhibit endogenous FXa or FX are very severe, it is important that the development of ANDEXXA is accompanied by the development of adequately validated, i.e., robust and reliable, assays to detect anti-drug antibodies, i.e., those that binds the andexanet protein and CHO (b) (4); and bio-assays to detect inhibitory antibodies against the product, endogenous FX and endogenous FXa.

Immunogenicity assays

Portola utilized a contract laboratory ((b) (4)) to develop an (b) (4) method to detect and quantify antibodies against ANDEXXA (Report # **NC-15-0643-R0001**). A surrogate positive control was generated by preparing a (b) (4) monoclonal antibodies, (b) (4) of the ANDEXXA protein. Additional assays for the detection of antibodies that recognize the FX and FXa proteins (NC-15-0642-R0001) were developed and validated using FX and FXa purified from human plasma as well as polyclonal antibodies as the surrogate positive controls (Report #s **NC-15-0645-R0001** & **NC-15-0642-R0001,** respectively). In lieu of (b) (4) assays that are commonly used to detect antibodies, Portola used an alternative (b) (4) method employed in the (b) (4) platform technology to gain increased sensitivity and specificity. The method used is acceptable and sufficient data has been provided to validate the method. Patient plasma samples that had a positive result in the initial screening assay were tested in a confirmatory competition assay in which the degree of inhibition of the antibody binding (b) (4) was measured following pre-incubation of the plasma sample in the presence of excess amounts of the appropriate purified protein (ANDEXXA, FX or FXa).

An additional assay was performed on all the samples from subjects for whom antibodies against ANDEXXA were confirmed. Samples from these subjects were tested for the presence of neutralizing antibodies, i.e., antibodies that interfere with the functional activity of ANDEXXA (Report #s **NC-15-0620-R0001 and NC-15-0621-R0001**).

Detection of anti-drug antibodies in clinical studies

In the clinical studies, samples from all subjects treated with either ANDEXXA or placebo were tested for the presence of antibodies against ANDEXXA, FX and FXa. Confirmed anti-drug antibodies (ADAs) against ANDEXXA were observed in all studies except the single ascending dose study, 11-501. The overall incidence of antibodies (any positive sample at any time) increased from the (b) (4) formulation ((b) (4)) to the lyophilized ((b) (4)) drug product (Table 1). There have been no confirmed antibodies against FX or FXa in any study. Additionally, none of the samples with confirmed antibodies against ANDEXXA prevented the binding of ANDEXXA to FXa inhibitors (i.e., they had no neutralizing activity on efficacy).

Table 1: Results of clinical studies showing detection of anti-drug antibodies (ADAs)

Study	ADAs; # patients, (%)	Drug Lot #	Drug Presentation	ADAs by presentation; # patients, (%)

11-501	0/24 (0)	(b) (4)	(b) (4)	2/100 (2)
12-502-M1	2/36 (5.6)			
12-502-M2	0/30 (0)			
12-502-M3	0/12 (0)			
14-506	3/20 (15)		Lyophilized powder	24-28/146 (16.4-19.2)
12-502-M3	2-4/6 (33.3-66.6)*			
12-502-M4 Cohort 3	1/6 (16.7)			
12-502-M4 Cohort 1	3/12 (15)			
12-502-M4 Cohort 2				
14-503 Part 1	3/24 (12.5)			
14-504 Part 1	4/27 (14.8)			
14-503 Part 2	7-8/24 (29.2-33.3)*			
14-504 Part 2	1-2/26 (3.8-7.7)*			

* Some of these subjects showed the presence of ADAs only in samples with a dilution less than (b) (4) which Portola uses as a "minimum required dilution". The range represents two criteria for determining the number of subjects who tested positive to ADAs: (i) All samples that tested positive at ***any*** dilutions were considered or, (ii) A subject was considered positive for ADAs only if a sample with a dilution of 1:10 or higher tested positive.

Conclusions: There are several causes for concern:

1) ADAs were identified in almost all studies (even though these antibodies are claimed to be non-inhibitory).

2) The increase in the percentage of patients developing ADAs when the lyophilized presentation was used which will be the marketed presentation (though it is claimed that these ADAs occur at lower titers).

For the clinical significance of these results, we defer to the Clinical Reviewer. However, in addition from a CMC perspective, we raised several concerns with Portola about the validation of the assays used for the detection of neutralizing ADAs. These concerns are discussed below.

Validation of neutralizing ADA bio-assays to be used in clinical studies

An assessment of the potential risk associated with the development of neutralizing ADAs that may cross-react with and neutralize endogenous FX and/or FXa due to the use of ANDEXXA conducted as per Agency's guidance documents, suggests that even if rare the potential clinical consequences could be very severe (see above). Thus, in communication with Portola, we have repeatedly requested the development and validation of bioassays for measuring neutralizing antibodies to endogenous human Factors X and Xa (IR dated 17 February 2016; responses on 03 March, 20 April, 08 July and 29 July 2016). Specifically, we requested that Portola develop and validate assays for antibodies that inhibit the activities of endogenous human Factors X and Xa. The assays should be based on proven clinically relevant methods, e.g., the (b) (4) , and the results should be presented in (b) (4) .

Recommendation

I recommend the issuance of a Complete Response (CR) Letter to Portola. With respect to the development of bio-assays for immunogenicity, the following should be included in the CR letter:

With 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 we deem incomplete, develop and validate assays to measure the activity of the antibodies that inhibit the activities of endogenous human Factors X and Xa and (b) (4) antibodies. In your response, please address the following requests:

a. 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. The assays should be based on proven clinically relevant methods, e.g., the (b) (4) , and the results should be presented in (b) (4) . FDA had 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.

b. Because (b) (4) impurities are suspected to originate from the CHO cells, which may be present in the FDP as evidenced from the formation of (b) (4) in stability studies, develop an assay to assess the development of (b) (4) antibodies in subjects who have participated in the clinical studies.

c. Use the validated immunogenicity methods to:

    i. Assess how the presence of the anti-Factor Xa and anti-FX inhibitory antibodies and (b) (4) antibodies may interfere with the assays used to evaluate the pharmacodynamics, pharmacokinetics, and immunogenicity in the clinical studies; and

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