

From: Maruna, Thomas
Sent: Wednesday, June 01, 2016 12:09 PM
To: 'Janice Castillo'
Cc: Ovanesov, Mikhail V.
Subject: 01-Jun-2016 Information Request - BLA 125586.0 - Response Required by 15-June-2016

Importance: High

Portola Pharmaceuticals Inc.
Attention: Ms. Janice Castillo
June 1, 2016
Sent by email

Dear Ms. Castillo:

We are reviewing your December 17, 2015 biologics license application (BLA) for the following:

STN	Name of Biological Products
125586/0	Coagulation Factor Xa (Recombinant), Inactivated

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

1. 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:

- a. 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) [REDACTED] 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.
- b. 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.
- c. 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 (TF-(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.
- d. 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.
2. 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.

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 15, 2016**.

The action due date for these files is August 17, 2016.

If you have any questions, please contact me.

Respectfully,

Thomas J. Maruna, MSc, MLS(ASCP), CPH
Lieutenant, U.S. Public Health Service
Senior Regulatory Management Officer
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