10 April 2020

Glen Pierce
Vice President, Medical
World Federation of Hemophilia

Radoslav F. Kaczmarek
Chair, CPSSAC
World Federation of Hemophilia

Dear Glen Pierce and Radoslav Kaczmarek,

Thank you for your letter received by email on 6 April 2020. We share your concern relating to potential interaction between the coagulopathy associated with some COVID-19 cases and new hemostatic agents in clinical development or recently approved. This is especially relevant to non-factor replacement therapies (NFRT) such as emicizumab, the anti-TFPI antibodies (BAY1093884, concizumab, marstacimab and MB1113) and the antithrombin knock-down small interfering RNA product, fitusiran. Indeed, emicizumab treatment has been associated with multiple cases of disseminated intravascular coagulation (DIC), interpreted as thrombotic microangiopathy, when used in conjunction with FEIBA. The anti-TFPI antibodies have been found to cause elevation of D-dimer levels and arterial thrombosis in clinical trials in persons with hemophilia (PwH), without concomitant use of any other hemostatic agent, leading to two programs being terminated. It has become clear that NFRTs can have serious adverse effects on their own and in combination with other factors. Assessing the interaction between NFRTs and the cytokine storm associated with some severe cases of COVID-19 is of paramount importance at this time.

Some might argue that this is especially true for our drug, SerpinPC, a specific inhibitor of activated protein C (APC). APC is known to have anti-inflammatory and cytoprotective effects exerted through cleavage of protease-activated receptor-1 (PAR-1) on endothelial cells. Inhibition of this activity exacerbates the effect of the cytokine storm induced by sub-lethal doses of lipopolysaccharide (LPS) in mice, leading to DIC, organ failure and death (Xu J, et al., J Thromb Haemost. 2009, 7(5):851-6). In addition, recombinant APC (Xigris™, Eli Lilly) was for a brief time approved for treatment of sepsis on interim clinical findings, although was later shown to be ineffective and to be counter-indicated in some cases because of bleeding. Due to the perceived risk of APC inhibition in the context of inflammation and the hypercoagulable state induced by a cytokine storm, ApcinteX has conducted extensive preclinical and clinical assessments of the potential effect of SerpinPC on inflammation and thrombosis.

As far as we are aware, SerpinPC is the only NFRT agent to be tested for its interaction with the effects of an LPS-induced cytokine storm. Chuck Esmon had previously reported that antibodies against mouse PC and APC (MPC1609 and MAPC1591, respectively) had differing effects on wild-type (WT) mice given sublethal doses of LPS (Xu J, et al., J Thromb Haemost. 2009, 7(5):851-6). MPC1609 binds to the Gla-domain of PC and APC preventing association with the endothelial cell protein C receptor (EPCR). MAPC1591 binds only to APC in a manner that prevents its cleavage of factors (f) Va and VIIIa. When these antibodies were given to mice at 10mg/kg before administration of a sublethal dose of LPS (one that causes transient, recoverable septicemia), only MC1609 worsened the cytokine storm and caused the death of all mice. MAPC1591, on the other
hand, appeared to be somewhat protective, at least no worse than vehicle. This study demonstrated that inhibition of the interaction between PC/APC and EPCR exacerbates the cytokine storm leading to organ failure and death, but inhibition of the anticoagulant/proteolytic activity of APC does not.

We obtained these antibodies from Chuck Esmon and recreated his experiment, including 10mg/kg SerpinPC to the conditions. Again, MPC1609 worsened the inflammation resulting in the death of all mice. In contrast, SerpinPC did not cause lethality in this model, nor did it cause an increase in IL-6 or serum-BUN levels relative to vehicle control. Indeed, SerpinPC appeared somewhat protective, even when compared to MAPC1591. It is important to note that the dose of SerpinPC used in this experiment is 8-times the highest dose planned in the single ascending dose (SAD) part of our clinical trial and is about 50-times the dose we currently anticipate using in the six-month repeat dose part of our study (Part 2). These results have been presented at several international conferences, and you may recall having seen them.

It has been reported by several groups that APC circulates at low levels (about 40pM) in both the healthy population and PwH, and it is inhibition of this pre-existing APC that results in the normalization of hemostasis upon treatment with SerpinPC. Because it is a covalent inhibitor, at low doses SerpinPC simply requires more time than at high doses to inhibit the pre-existing APC. This is an ideal profile for a safe prophylactic for PwH. The doses required for efficacy are so low that the secondary functions of APC (signaling and antithrombotic) are not affected. Indeed, at the highest dose planned for the SAD (subcutaneous dose of 1.2 mg/kg), the C_{max} is expected to be 6.3 \mu g/ml (126nM). At this concentration, SerpinPC takes 6 minutes to inhibit half of the APC. New APC is generated on endothelial cells where PC is bound to EPCR and thrombin is bound to thrombomodulin. This newly formed APC must exert its anti-inflammatory/cytoprotective function by cleaving PAR-1 on the same endothelial cell before it dissociates from EPCR to be replaced by another molecule of PC (Kd is 30nM for both, and the PC concentration is 70nM). The residency half-life of APC on EPCR is less than 8 seconds. An inhibition half-life of 6 minutes, therefore, has no impact the ability of newly formed APC to cleave PAR-1, leaving all signaling activities intact.

The newly formed APC that dissociates from EPCR is lost to the anti-inflammatory pool and will never again bind to EPCR (out-competed by PC by a factor of 1,750). It joins the circulating antithrombotic pool that can reach the site of clotting to cleave fV and fVIIIa, shutting down the prothrombinase and intrinsic Xase complexes. This activity is also unaffected at our top clinical dose, since the 6-minute half-time of inhibition is roughly equivalent to the time it would take a newly formed APC molecule to make 8 full trips around the circulatory system (based on 45 seconds for one circuit). The anticipated dose for Part 2 of our clinical study is 6-times lower still. Consequently, in conditions where thrombin generation becomes excessive, the subsequent generation and activity of newly formed APC would not be affected by SerpinPC at any of the doses being used in our trial.

To assess the effect of long-term exposure of SerpinPC, we conducted a 6-month toxicology study in non-human primates. Because of the increase seen in D-dimer levels associated with the anti-TFP antibodies and because of the perceived pro-inflammatory risk of APC inhibition, we monitored D-dimer and cytokine levels in this study. These WT animals have intact hemostatic systems and are a sensitive test of the prothrombotic potential of high doses of SerpinPC delivered over a long period of time. None of the doses, including the highest dose of 10mg/kg, was associated with an elevation in either D-dimer or cytokine levels, nor was any sign of thrombosis noted macro- or microscopically or upon histological examination.
The first part of our clinical trial (Part 1a) was conducted in healthy volunteers in the UK to assess the effect of a single dose of SerpinPC on safety in subjects with intact hemostatic systems, including measurement of D-dimer and cytokine levels. D-dimer levels were closely monitored in these subjects and an increase would triggered an end to dose escalation. Part 1a dosing and all follow-up visits are completed. There were no SerpinPC-related AEs, no increase in cytokine or D-dimer levels and no injection site reactions. Dose escalation switched to PwH (Part 1b) at levels anticipated to provide clinical benefit.

We chose Moldova and Georgia to conduct Parts 1b and 2 because PwH only receive on-demand treatment and the situation in these countries is representative of the unmet need in many parts of the world. So far we have delivered subcutaneous doses of 0.1mg/kg to two subjects with severe hemophilia who suffered bleeding events on a weekly basis. Both subjects are doing well and have not had a bleeding event since treatment (17th and 24th of March), at the writing of this letter. We have been monitoring D-dimer levels and have observed no increase.

Naturally we have considered the impact of COVID-19 on the safety of participants in our clinical trial. We had already tested the interaction between SerpinPC and the cytokine storm/coagulopathy with appropriate controls and demonstrated no interaction. Our preclinical toxicology work also demonstrates that SerpinPC is not prothrombotic in WT animals at high doses, well in excess of those planned for use in our clinical trial. Importantly, we have observed no increase in D-dimer in any subject on trial, either healthy volunteer or PwH.

Our clinical CRO Arensia have dedicated research clinics located across University Hospitals in Eastern Europe and have a track record in drug development that is trusted by the World’s Leading Pharmaceutical and Biotechnology organizations. We are holding weekly meetings with the clinical sites, including the hemophilia clinical staff. In line with the EMA Guidance on the Management of Clinical Trials during the COVID-19 pandemic (Version 2 - 27/03/2020) we are continually reviewing our procedures and the situation, with the safety of the participants in our study as our primary concern.

Yours truly,

James A. Huntington, PhD, FMedSci
ApcinteX, CEO

Trevor P. Baglin, FRCP FRCPath
ApcinteX, CMO