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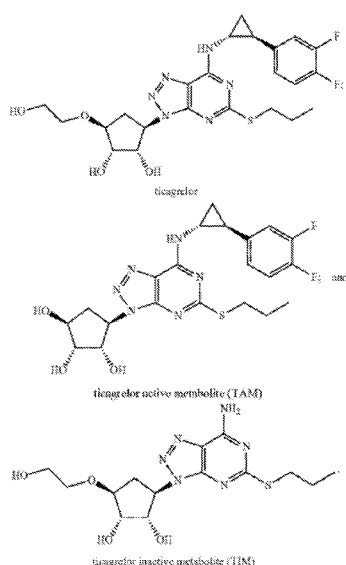
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(54) Title: METHODS OF REVERSING TICAGRELOR ACTIVITY

(57) Abstract: The present disclosure provides a method of reversing ticagrelor-associated bleeding in a patient by administering an antibody or fragment thereof that binds to ticagrelor.

Figure 1



Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
- *of inventorship (Rule 4.17(iv))*

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- *with sequence listing part of description (Rule 5.2(a))*

METHODS OF REVERSING TICAGRELOR ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of Provisional U. S. Application No. 62/733,892, filed September 20, 2018, Provisional U.S. Application No. 62/806,225, filed February 15, 2019, and Provisional U.S. Application No. 62/836,373, filed April 19, 2019, the contents of each of which are incorporated by reference in their entireties for all purposes.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: a computer readable format copy of the sequence listing (filename: PHAS_037_01WO_SeqList_ST25.txt, date recorded: September 20, 2019, file size 44 kilobytes).

BACKGROUND

[0003] Acute Coronary Syndrome (ACS) describes a range of conditions associated with sudden reduced blood flow to the heart, including unstable angina and myocardial infarction, or heart attack. ACS is caused by the inappropriate formation of clots in the coronary arteries. These blood clots are made up primarily of platelets, small lens-shaped cells found in the blood that normally aggregate at sites of injury to help stop bleeding. According to the Centers for Disease Control and Prevention, approximately 790,000 Americans have a heart attack every year, and heart attacks are a leading cause of death in the developed world.

[0004] The primary treatment for ACS is the use of antiplatelet drugs to prevent the worsening of existing clots or to reduce the formation of additional clots. These clots can occur in the heart or in stents that are placed in the blocked coronary artery to keep the blood vessel open or elsewhere in the body. Without antiplatelet drugs, patients are at a significantly increased risk of recurrent heart attacks, stroke and death. The standard of care for ACS patients is dual antiplatelet therapy, or DAPT, which is a combination of aspirin and an inhibitor of a specific receptor found on platelets known as the P2Y₁₂ receptor. This combination, started after a patient experiences a heart attack or other manifestation of ACS, has been shown to significantly reduce platelet aggregation and clot formation and reduce the frequency of recurrent heart attacks, stroke and death.

[0005] While the antiplatelet drugs used in DAPT therapy have proven effective at improving overall outcomes in ACS patients, their suppression of blood clotting increases patients' risk of major bleeding. Bleeding events in patients on antiplatelet therapy, which can occur spontaneously or as a result of injury or surgery, are classified as minor or major. In the 18,000-patient clinical trial, Platelet Inhibition and Patient Outcomes, or PLATO, conducted by AstraZeneca, ticagrelor was shown to be superior to the antiplatelet drug clopidogrel, marketed under the brand name Plavix, in reducing recurrent heart attack, stroke and death in patients with ACS (Wallentin *et al.* 2009). However, in both treatment groups, 11% to 12% of patients in the trial suffered major bleeding events, and in 5.8% of patients, these major bleeding events were fatal or life-threatening. The causes of bleeding varied in the trial population. In approximately 3% of the patients on ticagrelor, major bleeding events were spontaneous and not related to any medical procedure, whereas approximately 9% of patients on ticagrelor developed major bleeding that was related to procedures like coronary artery bypass surgery, or CABG (Wallentin *et al.* 2009). Although the trial protocol recommended that patients who needed CABG stop taking ticagrelor for one to three days prior to surgery, nearly half of all ticagrelor patients needed surgery urgently and could not wait the up to three days for ticagrelor's effect to dissipate so normal blood clotting could be restored. Overall, up to 80% of CABG patients in the trial suffered a major or life-threatening bleeding event related to the surgery, and for those who needed urgent surgery and could not wait three days for ticagrelor to wash out, approximately 50% experienced a fatal or life-threatening bleeding event (Held *et al.*, 2011). While some of this risk was likely associated with patients' underlying conditions, the overall bleeding risk is significantly increased by antiplatelet drugs, and the current US prescribing information for ticagrelor suggests suspension of ticagrelor treatment for five days prior to surgery.

[0006] Despite the increased bleeding risk, antiplatelet drugs, along with anticoagulant drugs which are used to prevent clots in veins, represent some of the most widely prescribed drugs in the United States due to their lifesaving effects. While both of these classes of drugs increase the risk of bleeding, reversal agents have been developed for anticoagulant drugs, but to date, no reversal agents exist for antiplatelet drugs. In the absence of a reversal agent, physicians have limited treatment options, and sometimes administer platelet transfusions, which are unproven in this setting. The ability to quickly reverse the antiplatelet activity of ticagrelor and restore normal

clotting would increase its safety, both in instances of major bleeding as well as in situations where surgical or other medical interventions associated with bleeding are urgently needed.

[0007] The three oral antiplatelet P2Y₁₂ receptor antagonist drugs prescribed in DAPT therapy are clopidogrel, marketed under the brand name Plavix, prasugrel, marketed under the brand name Effient, and ticagrelor, marketed under the brand names Brilinta and Brilique. Unlike clopidogrel and prasugrel that permanently bind to and inhibit the target receptors on platelets, ticagrelor binds to the P2Y₁₂ receptor in a transient manner, quickly cycling on and off the receptor. This transient binding of ticagrelor presents a unique opportunity to develop a specific reversal agent for ticagrelor, whereas the permanent binding of the other drugs to the receptor precludes a reversal agent from being developed.

[0008] Ticagrelor is considered the best-in-class P2Y₁₂ antiplatelet agent because it has demonstrated superior efficacy compared to clopidogrel. Yet, the side-effects of ticagrelor treatment can cause spontaneous bleeding. Further, due to its anti-platelet activity, ticagrelor treatment can be dangerous in patients requiring emergency surgery, and lead to increased blood loss.

[0009] Ticagrelor may also be used in patients with unstable angina, in patients with stable ischemic heart disease, in patients with sickle cell disease, including pediatric patients, patients with atrial fibrillation, patients with coronary arterial disease, patients with peripheral arterial disease, patients with ischemic stroke, patients with one or more coronary stents, patients with carotid artery stents, patients with stents following an intracranial aneurysm, patients with arterio-venous fistulae created for hemodialysis. Ticagrelor may also be used in patients with type 2 diabetes mellitus.

SUMMARY OF THE INVENTION

[0010] The present disclosure provides methods of reversing the activity of ticagrelor using a human Fab fragment that binds to ticagrelor with high affinity and specificity to reverse ticagrelor's antiplatelet activity.

[0011] In some aspects, the present disclosure provides a method of reversing ticagrelor-associated bleeding, or the risk of said bleeding, in a patient in need thereof comprising administering to said patient a composition comprising an effective amount of a pharmaceutical

composition comprising an antibody or fragment thereof that binds to ticagrelor ((1*S*,2*S*,3*R*,5*S*)-3-[7-{{[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino}-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol) or a metabolite or derivative thereof.

[0012] In some embodiments, the antibody or a fragment thereof comprises complementarity-determining region (CDR) combinations selected from the group consisting of:

[0013] a) SEQ ID NO:53 (VH CDR1), SEQ ID NO:54 (VH CDR2), SEQ ID NO:55 (VH CDR3), SEQ ID NO:58 (VL CDR1), SEQ ID NO:59 (VL CDR2), and SEQ ID NO:60 (VL CDR3);

[0014] b) SEQ ID NO:63 (VH CDR1), SEQ ID NO:64 (VH CDR2), SEQ ID NO:65 (VH CDR3), SEQ ID NO:68 (VL CDR1), SEQ ID NO:69 (VL CDR2), and SEQ ID NO:70 (VL CDR3); and

[0015] c) SEQ ID NO:73 (VH CDR1), SEQ ID NO:74 (VH CDR2), SEQ ID NO:75 (VH CDR3), SEQ ID NO:78 (VL CDR1), SEQ ID NO:79 (VL CDR2), and SEQ ID NO:80 (VL CDR3).

[0016] In some embodiments, the antibody or a fragment thereof comprises a combination of heavy chain variable region (VH) and light chain variable region (VL) sequences selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:57; SEQ ID NO:62 and SEQ ID NO:67; and SEQ ID NO:72 and SEQ ID NO:77.

[0017] In some embodiments, the patient has been administered ticagrelor before administration of the anti-ticagrelor antibody or fragment thereof.

[0018] In some embodiments, the antibody or fragment thereof is a Fab and the patient is administered a dose between about 1 g and about 48 g. In some embodiments, the dose is between about 9 g to about 18 g of the Fab. In some embodiments, the patient is administered a dose of about 1 g, about 3 g, about 9 g, about 18 g, about 24 g, about 30 g, about 36 g or about 48 g of the Fab.

[0019] In some embodiments, the pharmaceutical composition is administered to the patient intravenously. In some embodiments, pharmaceutical composition is administered intravenously over about 15 minutes to about 36 hours.

[0020] In some embodiments, the pharmaceutical composition is administered in two or more segments. In some embodiments, the first segment is a bolus. In some embodiments, the

administration rates for each of the segments differ. In some embodiments, the administration rates for each of the segments differ for successive segments of the infusion. In some embodiments, the pharmaceutical composition is administered in three or more segments, wherein the administration rates for each of the segments differ for successive segments of the infusion. In some embodiments, the pharmaceutical composition is administered in the following schedule: 12 g infused over 10 minutes, followed by 12 g over 6 hours, followed by 12 g over 18 hours.

[0021] In some embodiments, the pharmaceutical composition comprises about 50 mg/mL to about 200 mg/mL of the anti-ticagrelor antibody or fragment thereof, about 5 mM to about 50 mM histidine/histidine hydrochloride buffer, about 100 mM to about 300 mM sucrose, and about 0.01% (w/v) to about 1.0% (w/v) polysorbate 80, pH 5.5 to 6.5. In some embodiments, the pharmaceutical formulation comprises 100 mg/mL of the anti-ticagrelor antibody or fragment thereof, 25 mM histidine/histidine hydrochloride buffer, 290 mM sucrose, and 0.05% (w/v) polysorbate 80, pH 6.0. In some embodiments, the pharmaceutical formulation is diluted in isotonic saline for administration.

[0022] In some embodiments, the ticagrelor-associated bleeding is major bleeding. In some embodiments, the major bleeding is characterized by being life-threatening, potentially leading to clinically significant disability, requiring surgery to control the bleeding, requiring treatment with blood products, or is acute bleeding associated with a clinically important drop in hemoglobin. In some embodiments, the patient requires surgery or intervention. In some embodiments, the patient requires urgent surgery or intervention. In some embodiments, the urgent surgery or intervention is known to be associated with a significant risk of bleeding, such as coronary artery bypass surgery, has an adverse surgical outcome if bleeding is not carefully controlled, neurological, ophthalmologic, or joint replacement surgery, associated with risk of experiencing perioperative events; or in a patient at high risk of thrombosis if dual antiplatelet therapy is withheld preoperatively. In some embodiments, the patient requires elective surgery or intervention which is known to be associated with a significant risk of bleeding. In some embodiments, the patient is at risk of developing, or has been diagnosed with Acute Coronary Syndrome (ACS). In some embodiments, the patient is at risk of developing, or has been diagnosed with a disease selected from the group consisting of myocardial infarction (MI), unstable angina, stable ischemic heart disease, in sickle cell disease, including pediatric patients, atrial fibrillation, coronary arterial

disease, peripheral arterial disease, ischemic stroke, one or more coronary stents, carotid artery stents, stents following an intracranial aneurysm, and arterio-venous fistulae created for hemodialysis.

[0023] In some embodiments, the patient is a pediatric patient. In some embodiments, the pediatric patient is younger than 18 years old. In some embodiments, the pediatric patient is younger than 2 years old.

[0024] In some embodiments, the patient is an adult patient. In some embodiments, the adult patient is between 18 and 64 years old inclusive. In some embodiments, the patient is over 64 years old. In some embodiments, the patient is between 65 and 80 years old inclusive.

[0025] In some embodiments, the patient has been administered aspirin (acetylsalicylic acid).

[0026] In some embodiments, administration of the antibody or fragment thereof reverses ticagrelor activity. In some embodiments, administration of the antibody or fragment thereof restores platelet function. In some embodiments, administration of the antibody or fragment thereof restores platelet aggregation. In some embodiments, administration of the antibody or fragment thereof restores platelet aggregation to at least 80% of baseline. In some embodiments, administration of the antibody or fragment thereof restores platelet aggregation within 1 minute to 60 minutes of administration. In some embodiments, administration of the antibody or fragment thereof restores platelet aggregation within 5 minutes of administration. In some embodiments, administration of the antibody or fragment thereof provides a sustained restoration of platelet aggregation. In some embodiments, the restoration of platelet aggregation is sustained for at least 12 hours after administration. In some embodiments, the restoration of platelet aggregation is sustained for at least 16 hours after administration. In some embodiments, the restoration of platelet aggregation is sustained for at least 24 hours after administration.

[0027] In some embodiments, the patient has been administered ticagrelor and one or more additional drugs that impact ticagrelor exposure in the patient. In some embodiments the patient has been administered ticagrelor and one or more additional drugs that inhibit the activity of cytochrome P450 isoform 3A (CYP3A), leading to increased exposure to ticagrelor. In some embodiments the patient has been administered ticagrelor and one or more additional drugs that induce the activity of CYP3A, leading to reduced exposure to ticagrelor.

[0028] In some embodiments the patient has taken an overdose of ticagrelor.

BRIEF DESCRIPTION OF THE FIGURES

[0029] **Figure 1** shows the structure of Ticagrelor and metabolites of ticagrelor.

[0030] **Figure 2** shows mean inhibition of platelet aggregation (IPA) in Cohort 4. Administration of 1g PB2452 intravenously does not reverse ticagrelor action.

[0031] **Figure 3** shows mean inhibition of platelet aggregation (IPA) in Cohorts 4 and 6. Intravenous administration of 3 g or 9 g PB2452 over 30 minutes causes complete restoration of platelet function by 30 minutes.

[0032] **Figure 4** shows inhibition of platelet aggregation (IPA) in Cohort 7. Administration of PB2452 causes ticagrelor reversal for 16 hours.

[0033] **Figure 5** shows inhibition of platelet aggregation (IPA) in Cohorts 8, 9, and 10. Administration of PB2452 causes ticagrelor reversal for about 24 hours.

[0034] **Figures 6A-B** show an exemplary enrollment and study flowchart. Healthy subjects were screened and randomized according the flow diagram shown. To ensure that full cohorts were randomized for each dose cohort, an excess of subjects were screened prior to each cohort enrollment (A). Subject check-in, randomization, and discharge schedule is shown (B).

[0035] **Figures 7A-B** show the onset and duration of ticagrelor reversal. Shown is the observed increase in platelet aggregation measured by LTA in the PB2452 and placebo groups (Cohorts 4-6) administered ascending doses of PB2452 intravenously for 30 minutes (A). Subjects in Cohorts 7-10 were administered fixed 18 g doses of PB2452 with infusion durations of 8, 12, and 16 hours in Cohorts 7, 8, and 9/10, respectively (B). The -48 hr timepoint shows platelet aggregation prior to administration of ticagrelor.

[0036] **Figures 8A-B** show the onset and duration of ticagrelor reversal by VerifyNow and VASP ELISA. Shown are mean platelet function analyses for Cohort 7-10 subjects treated with PB2452 (open markers) or placebo (solid circles) measured at multiple timepoints pre- and post-PB2452 (or placebo) infusion. Time 0 represents mean platelet function after 48 hr pretreatment with ticagrelor, but prior to PB2452 infusion. Mean platelet aggregation (PRU) measured by VerifyNow is shown in (A). Mean VASP ELISA PRI is shown in (B).

[0037] **Figures 9A-C** show correlation analyses between platelet function assays measured by LTA, VerifyNow, and VASP ELISA. Pearson and Spearman correlation analyses were performed

between LTA measured aggregation and VerifyNow PRU in (A), between LTA measured aggregation and VASP ELISA PRI (B), and between VerifyNow PRU and VASP ELISA PRI (C). All timepoints were included. r values represent correlation coefficients. $P < 0.0001$ for all analyses.

[0038] Figures 10A-C show the normalization of normal platelet function post-administration of PB2452. The degree of reversal delivered by PB2452 was assessed by comparing platelet function at multiple timepoints post-PB2452, with baseline platelet function assessed in all subjects prior to ticagrelor pretreatment (-48 hr). For platelet aggregation assessed by LTA, normal platelet function post-reversal was considered to be $\geq 80\%$ of baseline platelet aggregation as shown by the dotted line (A). For VerifyNow platelet reactivity units (PRU), normal platelet function post-reversal was considered to be ≥ 180 PRU as shown by the dotted line (B). For P2Y₁₂ receptor signaling measured by the VASP platelet reactivity index (PRI), normal platelet function post-reversal was considered to be $\geq 80\%$ of baseline PRI as shown by the dotted line (C).

[0039] Figures 11A-D show the pharmacokinetics of PB2452 and ticagrelor. Shown are the circulating drug concentrations of ascending doses of PB2452 over time in Cohorts 4, 5, and 6 (A), the circulating drug concentrations of total ticagrelor over time in Cohorts 4, 5, and 6 (B), the circulating drug concentrations over time of fixed 18 g doses of PB2452 with extended infusion times in Cohorts 7-10 (C), and the circulating drug concentrations of total ticagrelor over time in Cohorts 7-10 (D).

[0040] Figures 12A-B show platelet aggregation with low-dose ADP. Shown are Cohorts 7-10 mean platelet aggregation data measured by LTA using 5 μ M ADP as the P2Y₁₂ agonist (A), and Cohorts 7-10 mean platelet aggregation data using 20 μ M ADP as the P2Y₁₂ agonist (B).

DETAILED DESCRIPTION

[0041] Ticagrelor works by binding to the P2Y₁₂ receptor on platelets, thereby preventing adenosine diphosphate, or ADP, from causing platelet aggregation. Ticagrelor binds transiently to the P2Y₁₂ receptor, cycling on and off, allowing anti-ticagrelor agents, such as PB2452, a human Fab fragment that binds to ticagrelor, to bind to free ticagrelor, thereby preventing ticagrelor's activation of the receptor and removing ticagrelor from circulation. With ticagrelor bound to PB2452 or removed, ADP can once again bind the P2Y₁₂ receptor and induce platelet aggregation.

Anti-ticagrelor agents

[0042] In some aspects, the present disclosure provides agents that bind to ticagrelor ((1*S*,2*S*,3*R*,5*S*)-3-[7-{{[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino}-5-(propylthio)-3*H*-[1,2,3]-triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol) or a metabolite or derivative thereof.

[0043] In some embodiments, the ticagrelor and/or metabolite thereof is depicted in **Figure 1**. In some embodiments, the ticagrelor metabolite is an active metabolite.

[0044] In some aspects, the agent that binds to ticagrelor and/or a metabolite thereof is an antibody or a fragment thereof. In some embodiments, the antibody or fragment thereof is selected from, but is not limited to, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a single chain Fv (scFv), a single domain antibody, a Fab, a F(ab')₂, a single chain diabody, an antibody mimetic, an antibody variable domain, a camelid antibody (also known as V_{HH} or nanobody). In some embodiments, the antibody comprises a scFv. In some embodiments, the antibody or a fragment thereof comprises a Fab. In some embodiments the antibody mimetic is an adnectin molecule, an affibody molecule, an affilin molecule, an affimer molecule, an affitin molecule, an alphabody molecule, an anticalin molecule, an aptamer molecule, an armadillo repeat protein molecule, an atrimer molecule, an avimer molecule, a designed ankyrin repeat protein molecule (DARPin) molecule, a fynomer molecule, a knottin molecule, a knottin molecule, a Kunitz domain inhibitor molecule, a monobody, a nanoCLAMP molecule, or a nanofitin molecule.

[0045] In further embodiments of the above aspects and embodiments, the antibody or a fragment thereof comprises a heavy chain variable region (VH) sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:12, SEQ ID NO:22, SEQ ID NO:32, SEQ ID NO:42, SEQ ID NO:52, SEQ ID NO:62, and SEQ ID NO:72; and a light chain variable region (VL) sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27, SEQ ID NO:37, SEQ ID NO:47, SEQ ID NO:57, SEQ ID NO:67, and SEQ ID NO:77. In some embodiments, the antibody comprises a combination of VH and VL sequences selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:7; SEQ ID NO:12 and SEQ ID NO:17; SEQ ID NO:22 and SEQ ID NO:27; SEQ ID NO:32 and SEQ ID NO:37; SEQ ID NO:42 and SEQ ID NO:47; SEQ ID NO:52 and SEQ ID NO:57; SEQ ID NO:62 and SEQ ID NO:67; and SEQ ID NO:72 and SEQ ID NO:77. In further embodiments, the antibody comprises a combination of VH

and VL selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:57; SEQ ID NO:62 and SEQ ID NO:67; and SEQ ID NO:72 and SEQ ID NO:77.

[0046] In further embodiments of the above aspects and embodiments, the antibody or a fragment thereof comprises framework regions (FR) and complementarity-determining regions (CDRs) 1, 2, and 3 of a heavy chain variable region and a light chain variable region, wherein the CDR1, CDR2, and CDR3 sequences of the heavy chain variable region comprise, SEQ ID NO:3 (CDR1), SEQ ID NO:4 (CDR2), and SEQ ID NO:5 (CDR3); SEQ ID NO:13 (CDR1), SEQ ID NO:14 (CDR2), and SEQ ID NO:15 (CDR3); SEQ ID NO:23 (CDR1), SEQ ID NO:24 (CDR2), and SEQ ID NO:25 (CDR3); SEQ ID NO:33 (CDR1), SEQ ID NO:34 (CDR2), and SEQ ID NO:35 (CDR3); SEQ ID NO:43 (CDR1), SEQ ID NO:44 (CDR2), and SEQ ID NO:45 (CDR3); SEQ ID NO:53 (CDR1), SEQ ID NO:54 (CDR2), and SEQ ID NO:55 (CDR3); SEQ ID NO:63 (CDR1), SEQ ID NO:64 (CDR2), and SEQ ID NO:65 (CDR3); or SEQ ID NO:73 (CDR1), SEQ ID NO:74 (CDR2), and SEQ ID NO:75 (CDR3); and wherein the CDR1, CDR2, and CDR3 sequences of the light chain variable region comprise, SEQ ID NO:8 (CDR1), SEQ ID NO:9 (CDR2), and SEQ ID NO:10 (CDR3); SEQ ID NO:18 (CDR1), SEQ ID NO:19 (CDR2), and SEQ ID NO:20 (CDR3); SEQ ID NO:28 (CDR1), SEQ ID NO:29 (CDR2), and SEQ ID NO:30 (CDR3); SEQ ID NO:38 (CDR1), SEQ ID NO:39 (CDR2), and SEQ ID NO:40 (CDR3); SEQ ID NO:48 (CDR1), SEQ ID NO:49 (CDR2), and SEQ ID NO:50 (CDR3); SEQ ID NO:58 (CDR1), SEQ ID NO:59 (CDR2), and SEQ ID NO:60 (CDR3); SEQ ID NO:68 (CDR1), SEQ ID NO:69 (CDR2), and SEQ ID NO:70 (CDR3); or SEQ ID NO:78 (CDR1), SEQ ID NO:79 (CDR2), and SEQ ID NO:80 (CDR3). In further embodiments, the antibody comprises a combination of CDR regions selected from the group consisting of: SEQ ID NO:53 (VH CDR1), SEQ ID NO:54 (VH CDR2), SEQ ID NO:55 (VH CDR3), SEQ ID NO:58 (VL CDR1), SEQ ID NO:59 (VL CDR2), and SEQ ID NO:60 (VL CDR3); SEQ ID NO:63 (VH CDR1), SEQ ID NO:64 (VH CDR2), SEQ ID NO:65 (VH CDR3), SEQ ID NO:68 (VL CDR1), SEQ ID NO:69 (VL CDR2), and SEQ ID NO:70 (VL CDR3); and SEQ ID NO:73 (VH CDR1), SEQ ID NO:74 (VH CDR2), SEQ ID NO:75 (VH CDR3), SEQ ID NO:78 (VL CDR1), SEQ ID NO:79 (VL CDR2), and SEQ ID NO:80 (VL CDR3).

[0047] In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of SEQ ID NO:73 (VH CDR1), SEQ ID NO:74 (VH CDR2), SEQ ID NO:75 (VH CDR3), SEQ ID NO:78 (VL CDR1), SEQ ID NO:79 (VL CDR2), and SEQ ID NO:80 (VL CDR3).

In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of SEQ ID NO:72 and SEQ ID NO:77. In some embodiments, the antibody or fragment thereof comprising the amino acid sequences of SEQ ID NO: 72 and SEQ ID NO: 77 is PB2452 (MEDI2452). In some embodiments, the antibody or fragment thereof comprises SEQ ID NO: 81. In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of a VH region and a CH region. In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of a VH region and a CH1 region. In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of SEQ ID NO: 72 and a CH1 region. In some embodiments, the antibody or fragment thereof comprises the amino acid sequence of SEQ ID NO: 83. In some embodiments, the antibody or fragment thereof comprises the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 84. In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of a VL and a CL region. In some embodiments, the antibody or fragment thereof comprises the amino acid sequences of SEQ ID NO: 77 and a CL region. In some embodiments, the antibody or fragment thereof comprises the amino acid sequence of SEQ ID NO: 85. In some embodiments, the antibody or fragment thereof comprises the amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO: 86. In some embodiments, the antibody or fragment thereof comprising the amino acid sequences of SEQ ID NO: 83 and SEQ ID NO: 85 is PB2452 (MEDI2452).

[0048] In some embodiments, the antibodies or fragments thereof of the disclosure include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the antibody or fragment thereof.

[0049] The present disclosure provides antibodies or fragments thereof that comprise, or alternatively consist of, variants (including derivatives) of the VH domains, VH CDRs, VL domains, and VL CDRs described herein, which antibodies immunospecifically bind to ticagrelor or a derivative or metabolite thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino

acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. In specific embodiments, the variants encode substitutions of VHCDR3. In a preferred embodiment, the variants have conservative amino acid substitutions at one or more predicted non-essential amino acid residues. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (e.g., the ability to bind ticagrelor or a derivative or metabolite thereof). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (e.g., ability to immunospecifically bind ticagrelor or a derivative or metabolite thereof) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0050] In another embodiment, an antibody or fragment thereof of the disclosure that immunospecifically binds to ticagrelor, a derivative, or a metabolite thereof comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the VL domains. In another embodiment, an antibody of the disclosure that immunospecifically binds to ticagrelor, a derivative, or a metabolite thereof comprises, or alternatively consists of, a

polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the VL CDRs. In another embodiment, an antibody of the disclosure that immunospecifically binds to ticagrelor, a derivative, or a metabolite thereof comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the VL CDR3s. Nucleic acid molecules encoding these antibodies are also encompassed by the disclosure.

[0051] In another embodiment, an antibody or fragment thereof of the disclosure that immunospecifically binds to ticagrelor, a derivative, or a metabolite thereof comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the VH domains. In another embodiment, an antibody of the disclosure that immunospecifically binds to ticagrelor, a derivative, or a metabolite thereof comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the VH CDRs. In another embodiment, an antibody of the disclosure that immunospecifically binds to ticagrelor, a derivative, or a metabolite thereof comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the VH CDR3s. Nucleic acid molecules encoding these antibodies are also encompassed by the disclosure.

[0052] PB2452 is a recombinant human IgG1 λ monoclonal Fab antibody fragment that binds specifically to ticagrelor and TAM. PB2452 was obtained by optimization of a human anti-ticagrelor antibody using phage display, from libraries that were generated by randomizing amino acids in the variable heavy or variable light chain complementarity-determining region 3s followed by affinity selection and screening. *See* US 2016/0130366 which is incorporated by reference

herein in its entirety for all purposes. PB2452 is produced in *E. coli* cells and is purified using a 4-step chromatography process.

[0053] In some embodiments, the antibody or fragment thereof binds to ticagrelor and neutralizes the anti-platelet aggregation activity of ticagrelor and TAM, thus restoring ADP-induced platelet aggregation in the presence of ticagrelor and TAM.

[0054] In some embodiments, the terminal half-life of the antibody or fragment thereof in a subject is about the same as the terminal half-life of ticagrelor and TAM. In some embodiments the antibody terminal half-life is from about 4-24 hours (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours). In some embodiments the terminal half-life is from about 4-12 hours (e.g., 4, 5, 6, 7, 8, 9, 10, 11, or 12 hours). In some embodiments, the terminal half-life in a subject is between about 6-9 hours. In some embodiments, the terminal half-life in a subject is between about 6-7 hours. In some embodiments, the terminal half-life is about 6.9 hours.

[0055] In some embodiments, the distribution half-life of the antibody or fragment thereof in a subject is about the same as the distribution half-life of ticagrelor and TAM. In some embodiments the distribution half-life is from about 0.1 to 2 hours (e.g., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, or 2 hours). In some embodiments the distribution half-life is from about 0.1 to 1 hour (e.g., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 hour). In some embodiments, the distribution half-life is about 0.89 hours.

[0056] In some embodiments, the antibody or fragment thereof provides for a rapid onset of activity. For example, in embodiments the antibody time to onset or the time to neutralize ticagrelor and TAM mediated platelet inhibition, is from about 5-120 minutes, or from about 5-60 minutes. In some embodiments, the time to onset is less than 60 minutes. In some embodiments, the time to onset is about 30 minutes. In some embodiments, the time to onset is less than about 30 minutes. In some embodiments, the time to onset is less than about 10 minutes. In some embodiments, the time to onset is less than about 5 minutes.

[0057] In some embodiments, the antibody or fragment thereof provides for sustained inhibition of ticagrelor and TAM activity. In some embodiments, the inhibition of ticagrelor and TAM activity by the antibody or fragment thereof is sustained for about 2 to about 48 hours (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or 48 hours). In some embodiments, the

sustained inhibition of ticagrelor and TAM activity is dose dependent. In some embodiments, the sustained inhibition of ticagrelor and TAM activity is dependent on the dose administered in a bolus before IV infusion. In some embodiments, the sustained inhibition of ticagrelor and TAM activity is dependent on the dose administered via IV infusion.

[0058] In some embodiments, the antibody or fragment thereof of the present disclosure exhibits both rapid (e.g. within 5 minutes of administration) and sustained (e.g. up to 24 or 48 hours) inhibition of ticagrelor and TAM activity.

[0059] In some embodiments, the antibody or fragment thereof is administered to the subject in need thereof immediately after the last ticagrelor administration. In some embodiments, the antibody or fragment thereof is administered to the subject in need thereof within about 1 hour to 120 hours after the last ticagrelor administration. In some embodiments, the antibody or fragment thereof is administered to a subject in need thereof within about 1 hour to 72 hours after the last ticagrelor administration. In some embodiments, the antibody or fragment thereof is administered to the subject in need thereof within about 1 hour to 24 hours (e.g. about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours).

[0060] In some embodiments the antibody or fragment thereof has a PK/PD profile that provides for a rapid offset of activity, such that, for example, a subject who has been administered the antibody may recommence with the prescribed ticagrelor therapy. In some embodiments, a subject who has received an antibody disclosed herein (e.g., by i.v. infusion) may receive or restart ticagrelor therapy within six hours following the administration of the antibody. In some embodiments, a subject who has received an antibody or fragment thereof disclosed herein (e.g., by i.v. infusion) may receive or restart ticagrelor therapy within twelve hours following the administration of the antibody. In some embodiments, a subject who has received an antibody disclosed herein (e.g., by i.v. infusion) may receive or restart ticagrelor therapy within twenty-four hours following the administration of the antibody.

PB2452 activity

[0061] Without being bound by theory, PB2452 binds to ticagrelor with an affinity that is 100 times stronger than ticagrelor's affinity for the P2Y₁₂ receptor. This high affinity enables PB2452

to bind to free ticagrelor, resulting in a rapid reversal of ticagrelor's effect and restoration of platelet activity.

[0062] The chemical starting point for the development of ticagrelor was adenosine triphosphate (ATP), and ticagrelor retains an adenosine-like core. To confirm the specificity of PB2452 for ticagrelor and ticagrelor active metabolite (TAM), its binding to ATP, ADP, and adenosine was evaluated. To further confirm PB2452 specificity, a structural database for marketed drugs was interrogated for molecules that have any structural similarity to ticagrelor. From this *in silico* analysis, a panel of 12 compounds was selected (fenofibrate, nilvadipine, cilostazol, bucladesine, regadenoson, cyclothiazide, cyfluthrin, lovastatin, linezolid, simvastatin, cangrelor, and pantoprazole). The selectivity of PB2452 was determined by competition binding with PB2452 to biotinylated ticagrelor. No inhibition of PB2452 binding to biotinylated ticagrelor was found for ATP, ADP, adenosine, or the 12 structurally related compounds. Thus, PB2452 binds with high affinity and selectivity to ticagrelor and TAM.

[0063] In some embodiments, the anti-ticagrelor antibody or fragment thereof reverses, prevents, inhibits, or reduces ticagrelor or TAM activity. In some embodiments, this ticagrelor or TAM activity is selected from, but not limited to, decreasing ADP-induced platelet aggregation and/or binding to the P2Y₁₂ receptor. In some embodiments, administration of the anti-ticagrelor antibody or fragment thereof restores ADP-induced platelet aggregation and/or binding to the P2Y₁₂ receptor. In some embodiments, the anti-ticagrelor antibody that restores ADP-induced platelet aggregation and/or binding to the P2Y₁₂ receptor is PB2452. *See* US 2016/0130366 which is incorporated by reference herein in its entirety for all purposes.

Methods of Treatment

[0064] In some aspects, the present disclosure provides a method of reversing, inhibiting, decreasing, or preventing ticagrelor or TAM activity comprising administering pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need. The reversal, inhibition, decrease, or prevention of ticagrelor or TAM activity may be measured by any means known in the art, including, for example by measuring free or total ticagrelor in blood samples

[0065] In some embodiments, administration of the pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof restores platelet aggregation. In some embodiments, administration of the pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof inhibits the binding of ticagrelor or TAM to the P2Y₁₂ receptor. In some embodiments, the anti-ticagrelor antibody or fragment thereof is PB2452.

[0066] In some aspects, the present disclosure provides a method of restoring platelet aggregation comprising administering pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need. In some aspects, the present disclosure provides a method of decreasing blood loss in a patient receiving ticagrelor comprising administering pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need. In some embodiments, administration of the pharmaceutical composition of the present disclosure inhibits ticagrelor-associated bleeding in a patient.

[0067] In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need clears ticagrelor and/or TAM from the patient's body. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need reduces the amount of ticagrelor and/or TAM in a patient's blood. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need reduces the amount of ticagrelor in a patient's blood by about 100% to about 5% compared to baseline amounts of ticagrelor and/or TAM. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need reduces the amount of ticagrelor in a patient's blood serum by about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10% or about 5% compared to baseline amounts of ticagrelor and/or TAM.

[0068] In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject reduces the amount of free ticagrelor and/or TAM in the patient's body. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need reduces the amount of free ticagrelor and/or TAM in a patient's blood. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a

subject in need reduces the amount of free ticagrelor and/or TAM in a patient's blood by about 100% to about 15% compared to baseline amounts of ticagrelor and/or TAM. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need reduces the amount of free ticagrelor and/or TAM in a patient's blood by about 99.9%, about 99.8%, about 99.7%, about 99.6%, about 99.5%, about 99.4%, about 99.3%, about 99.2%, about 99.1%, about 99%, about 98%, about 97%, about 96%, about 95%, about 94%, about 93%, about 92%, about 91%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10% or about 5% compared to baseline amounts of ticagrelor and/or TAM. In some embodiments, ticagrelor or TAM activity is reversed, inhibited, decreased, or prevented for about 1 hour to about 2 days. In some embodiments, ticagrelor or TAM activity is reversed, inhibited, decreased, or prevented for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 36 hours or about 48 hours. In some embodiments, this reversal, inhibition, decrease, or prevention is observed at the time points disclosed herein.

[0069] In some embodiments, administration of the pharmaceutical compositions disclosed herein reverses, inhibits, decreases, or prevents ticagrelor or TAM activity in a subject compared to an untreated subject. In some embodiments, ticagrelor or TAM activity is reversed, inhibited, decreased, or prevented by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% or about 100% compared with activity in an untreated subject. In some embodiments, this reversal, inhibition, decrease, or prevention is observed at the time points disclosed herein.

[0070] In some embodiments, administration of the pharmaceutical compositions disclosed herein reverses inhibition of platelet aggregation in a subject compared to an untreated subject on ticagrelor. In some embodiments, the inhibition of platelet aggregation is reversed by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% or about 100% compared with inhibition of platelet aggregation in an

untreated subject on ticagrelor. In some embodiments, this reversal of inhibition of platelet aggregation is observed at the time points disclosed herein.

[0071] In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject restores platelet aggregation in the patient's blood. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood to about 100% to about 15% compared to baseline levels of normal platelet aggregation. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood to about 100%, about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10% or about 5% of baseline levels of normal platelet aggregation. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood to about 80% or greater of baseline levels of normal platelet aggregation. In some embodiments, platelet aggregation is restored to at least 80% of baseline levels of normal platelet aggregation in about 1 hour to about 2 days after administration. In some embodiments, platelet aggregation is restored at about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 36 hours or about 48 hours. In some embodiments, this restoration is observed at the time points disclosed herein.

[0072] In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject restores platelet aggregation in the patient's blood as measured by the VerifyNow P2Y₁₂ (also known as the VerifyNow PRU_{Test}) assay method (Accriva/Instrumentation Laboratory, San Diego CA). In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood as measured by the VerifyNow to about 50 to about 250 platelet reactivity units (PRU). In some embodiments,

administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood as measured by the VerifyNow to about 250 platelet reactivity units (PRU), to about 240 PRU, to about 230 PRU, to about 220 PRU, to about 210 PRU to about 200 PRU, to about 190 PRU, to about 180 PRU, to about 170 PRU, to about 160 PRU, to about 150 PRU, to about 140 PUR, to about 130 PRU, to about 120 PRU, to about 110 PRU, to about 100 PRU, to about 90 PRU, to about 80 PRU, to about 70 PRU, to about 60 PRU, to about 50 PRU. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood to at least 180 PRU. In some embodiments, platelet aggregation is restored to at least 180 PRU in about 1 hour to about 2 days after administration. In some embodiments, platelet aggregation is restored to at least 180 PRU at about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 36 hours or about 48 hours. In some embodiments, this restoration is observed at the time points disclosed herein.

[0073] In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject restores platelet aggregation in the patient's blood as measured by the vasodilator-stimulated phosphoprotein (VASP) method. In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood as measured by VASP to about 50% to about 150% baseline platelet reactivity index (PRI). In some embodiments, administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood as measured by VASP to about 150% baseline platelet reactivity index (PRI), to about 140% baseline PRI, to about 130% baseline PRI, to about 120% baseline PRI, to about 110% baseline PRI, to about 100% baseline PRI, to about 90% baseline PRI, to about 80% baseline PRI, to about 70% baseline PRI, to about 60% baseline PRI, to about 50% baseline PRI. In some embodiments,

administration of pharmaceutical compositions comprising an anti-ticagrelor antibody or fragment thereof to a subject in need restores platelet aggregation in a patient's blood to at least 100% baseline PRI. In some embodiments, platelet aggregation is restored to at least 100% baseline PRI in about 1 hour to about 2 days after administration. In some embodiments, platelet aggregation is restored to at least 100% baseline PRI at about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 36 hours or about 48 hours. In some embodiments, this restoration is observed at the time points disclosed herein.

Patient Populations

[0074] The anti-ticagrelor antibodies or fragments thereof of the present disclosure may be administered to any patient in need. In some embodiments, the patient is at risk of, or has been diagnosed with, Acute Coronary Syndrome (ACS). In some embodiments, the patient is at risk of, or has been diagnosed with myocardial infarction (MI). In some embodiments, the patient has a history of MI. In some embodiments, the patient is receiving, or has received ticagrelor. In some embodiments, the patient is receiving, or has received ticagrelor along with another anti-platelet therapy, such as aspirin.

[0075] In some embodiments, the patient has unstable angina, stable ischemic heart disease, in sickle cell disease, including pediatric patients, atrial fibrillation, coronary arterial disease, peripheral arterial disease, ischemic stroke, one or more coronary stents, carotid artery stents, stents following an intracranial aneurysm, or arterio-venous fistulae created for hemodialysis.

[0076] In some embodiments, the patient has type 2 diabetes mellitus. In some embodiments, the patient has type 2 diabetes mellitus and coronary disease. In some embodiments, the patient has type 2 diabetes mellitus with a history of percutaneous coronary intervention.

[0077] In some embodiments, the patient is at higher risk or increased rate of bleeding associated with ticagrelor treatment. In some embodiments, this ticagrelor-associated bleeding is gastrointestinal bleeding. In some embodiments, this ticagrelor-associated bleeding is intracranial

bleeding, or intracranial hemorrhage (ICH). In some embodiments, this ticagrelor-associated bleeding is as a result of traumatic injury, such as a road traffic accident. In some embodiments, the ticagrelor-associated bleeding is categorized as major bleeding. Major bleeding will include any bleeding event which is judged by the treating physician to require reversal. This includes, but is not limited to, bleeding events which are life-threatening, potentially leading to clinically significant disability, requiring surgery to control the bleeding, requiring treatment with blood products, or acute bleeding associated with a clinically important drop in hemoglobin levels.

[0078] In some embodiments, the patient requires urgent surgery or intervention. Urgent surgery or intervention is defined as requirement for a surgical operation or medical procedure associated with a risk or perioperative bleeding in a situation where it is not medically advisable to withhold ticagrelor five days. Requirement for urgent surgery may include, but is not limited to, patients in any of the following clinical situations: undergoing surgery or procedures known to be associated with a significant risk of bleeding (such as coronary artery bypass surgery); undergoing surgery or procedures which may have an adverse surgical outcome if bleeding is not carefully controlled (such as neurological, ophthalmologic, or joint replacement surgery); at risk of experiencing perioperative events such as shock, myocardial infarction or stroke if significant perioperative bleeding occurs (especially in elderly patients or those with co-morbidities); at high risk of thrombosis if dual antiplatelet therapy is withheld preoperatively (such as patients with recent coronary stent placement).

[0079] In some embodiments, the patient has begun experiencing bleeding before administration of an anti-ticagrelor antibody or fragment thereof. In some embodiments, the patient has not begun experiencing bleeding before administration of an anti-ticagrelor antibody or fragment thereof. In some embodiments, the patient requires surgery, and thus poses increased risk of bleeding due to ticagrelor treatment. In some embodiments, the surgery is urgent surgery. In some embodiments the surgery is emergent surgery.

[0080] In some embodiments, the patient is an adult. In some embodiments, the adult patient between 30 and 100 years old or more. In some embodiments, the adult patient is over 40 years old, over 50 years old, over 60 years, over 70 years old, over 80 years old, or over 90 years old. In some embodiments, the adult patient is between 50-64 years old. In some embodiments, the adult

patient is between 65-75 years old. In some embodiments, the patient is defined as older (*e.g.* between the ages of 50 and 64 years old inclusive). In some embodiments, the patient is defined as elderly (*e.g.* over the age of 65 years or between the ages of 65 and 80 years old inclusive). In some embodiments, the older or elderly patient has been pretreated with ticagrelor and aspirin. In some embodiments, older or elderly patients experience higher exposure to ticagrelor and/or a lower response to ticagrelor compared to younger subjects.

[0081] In some embodiments, the patient is a young adult. In some embodiments, the young adult patient between 18 and 30 years old or more. In some embodiments, the patient is a pediatric patient under 18 years of age. In some embodiments, the patient is a pediatric patient under 2 years of age. In some embodiments, the pediatric patient or young adult patient has sickle cell disease.

Pharmaceutical Compositions and Administration

[0082] The present disclosure provides pharmaceutical compositions including an anti-ticagrelor antibody or fragment thereof with one or more pharmaceutically acceptable excipients and/or diluents. In some embodiments, the anti-ticagrelor antibody or fragment thereof is PB2452.

[0083] The formulations of the present disclosure may include any appropriate excipient known in the art. Exemplary excipients include, but are not limited to, amino acids such as histidine, glycine, or arginine; glycerol; sugars, such as sucrose; surface active agents such as polysorbate 20 and polysorbate 80; citric acid; sodium citrate; antioxidants; salts including alkaline earth metal salts such as sodium, potassium, and calcium; counter ions such as chloride and phosphate; sugar alcohols (*e.g.* mannitol); preservatives; sugar alcohols (*e.g.* mannitol, sorbitol); and buffering agents. Exemplary salts include sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium phosphate dibasic, sodium phosphate monobasic, sodium phosphate, and potassium phosphate.

[0084] In certain embodiments, the formulation may include from about 5 mM histidine/histidine hydrochloride buffer to about 100 mM histidine/histidine hydrochloride buffer. In some embodiments, the formulation includes about 50 mM histidine/histidine hydrochloride buffer, about 40 mM histidine/histidine hydrochloride buffer, about 30 mM histidine/histidine

hydrochloride buffer, about 25 mM histidine/histidine hydrochloride buffer, about 20 mM histidine/histidine hydrochloride buffer, or about 15mM histidine/histidine hydrochloride buffer.

[0085] In certain embodiments, the formulation may include from about 100 mM sucrose to about 1 M sucrose. In some embodiments, the formulation may include about 150 mM sucrose, about, about 200 mM sucrose, about 250 mM sucrose, about 290 mM sucrose, about 300 mM sucrose, about 350 mM sucrose, about 400 mM sucrose, or about 500 mM sucrose.

[0086] In certain embodiments, the formulation may include a surfactant. In some embodiments the surfactant is a non-ionic surfactant. In some embodiments, the non-ionic surfactant is polysorbate 80. In some embodiments, the formulation may include from about 0.01 % w/v polysorbate 80 to about 1.00% w/v polysorbate 80. In some embodiments, the formulation may include about 0.01% w/v, about 0.02% w/v, about 0.03% w/v, about 0.05% w/v, about 0.06% w/v, about 0.07% w/v, about 0.08% w/v, about 0.09% w/v, or about 0.1% w/v polysorbate 80.

[0087] In certain embodiments, the formulation may include from about 10 mg/mL anti-ticagrelor antibody or fragment thereof to about 200 mg/mL anti-ticagrelor antibody or fragment thereof. In some embodiments, the formulation may include about 10 mg/mL, about 20 mg/mL, about 30 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/ml, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, or about 150 mg/mL anti-ticagrelor antibody or fragment thereof.

[0088] In some embodiments, the formulation includes 100 mg/mL of the anti-ticagrelor antibody or fragment thereof, 25 mM histidine/histidine hydrochloride buffer, 290 mM sucrose, and 0.05% (w/v) polysorbate 80, pH 6.0.

[0089] The formulation can be stored frozen, refrigerated or at room temperature. The storage condition may be below freezing, such as lower than about -10°C , or lower than about -20°C , or lower than about -40°C , or lower than about -70°C . Storage conditions are generally less than the room temperature, such as less than about 32°C , or less than about 30°C , or less than about 27°C , or less than about 25°C , or less than about 20°C , or less than about 15°C . In some embodiments, the formulation is stored at $2^{\circ}\text{-}8^{\circ}\text{C}$. For example, the formulation may be isotonic with blood or have an ionic strength that mimics physiological conditions

[0090] In some embodiments, the formulation is formulated at physiological pH. In some embodiments, the formulation is formulated at a pH in the range of about 5.5 to about 8.5. In some embodiments, the formulation is formulated at a pH in the range of about 6.0 to about 8.0. In some embodiments, the formulation is formulated at a pH in the range of about 6.5 to about 7.5. In some embodiments, the formulation is formulated at a pH of 7.5. In some embodiments, formulations with a lower pH demonstrate improved formulation stability compared to formulations at a higher pH. In some embodiments, formulations with a higher pH demonstrate improved formulation stability compared to formulations at a lower pH.

[0091] In some embodiments, the formulation is stable at storage conditions. Stability can be measured using any appropriate means in the art. Generally, a stable formulation is one that shows less than a 5% increase in degradation products or impurities. In some embodiments, the formulation is stable for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about one year, or at least about 2 years or more at the storage conditions. In some embodiments, the formulation is stable for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, or at least about one year or more at 25 °C.

[0092] In some aspects, the formulation is a lyophilized product. In some embodiments, the formulation is a lyophilized product containing about 1 g to about 36 g of the anti-ticagrelor antibody or fragment thereof. In some embodiments, the formulation is a lyophilized product containing about 6 g of the anti-ticagrelor antibody or fragment thereof. In some aspects, following reconstitution with water for injection, the product may be further diluted into 0.9% saline for intravenous (iv) infusion. In some embodiments, the product is not lyophilized and is further diluted into 0.9% saline for intravenous (iv) infusion.

[0093] In one aspect, the formulations of the disclosure are pyrogen-free formulations which are substantially free of endotoxins and/or related pyrogenic substances. Endotoxins include toxins that are confined inside a microorganism and are released only when the microorganisms are broken down or die. Pyrogenic substances also include fever-inducing, thermostable substances (glycoproteins) from the outer membrane of bacteria and other microorganisms. Both of these substances can cause fever, hypotension and shock if administered to humans. Due to the potential

harmful effects, even low amounts of endotoxins must be removed from intravenously administered pharmaceutical drug solutions. The Food & Drug Administration (“FDA”) has set an upper limit of 5 endotoxin units (EU) per dose per kilogram body weight in a single one hour period for intravenous drug applications (The United States Pharmacopeial Convention, Pharmacopeial Forum 26 (1):223 (2000)). In certain specific aspects, the endotoxin and pyrogen levels in the composition are less than about 1 EU/mg, or less than about 0.1 EU/mg, or less than about 0.01 EU/mg, or less than about 0.001 EU/mg. In some embodiments, the endotoxin and pyrogen levels in the composition are 0.0138 EU/mg or less.

[0094] When used for *in vivo* administration, the formulations of the disclosure should be sterile. The formulations of the disclosure may be sterilized by various sterilization methods, including sterile filtration, radiation, etc. In one aspect, the formulation is filter-sterilized with a pre-sterilized 0.22-micron filter. Sterile compositions for injection can be formulated according to conventional pharmaceutical practice as described in “Remington: The Science & Practice of Pharmacy”, 21st ed., Lippincott Williams & Wilkins, (2005).

[0095] In some embodiments, the pharmaceutical composition is formulated for intravenous administration. In some embodiments, the formulation is administered intravenously over about 5 minutes to 48 hours. In some embodiments, the formulation is administered in any appropriate volume. In some embodiments, the formulation is administered in a total volume of about 30 mL to about 2L. In some embodiments, the formulation is administered in a total volume of about 30 mL, about 40 mL, about 50 mL, about 100 mL, about 125 mL, about 150 mL, about 175 mL, about 200 mL, about 225 mL, about 250 mL, about 275 mL, about 300 mL, about 400 mL, about 500 mL, about 1L, about 1.5 L, or about 2L. In some embodiments, the formulation is administered intravenously over about 30 minutes in a total volume of about 250 mL. In some embodiments, the formulation is first administered as a bolus, followed by a longer infusion. In some embodiments, the longer infusion following the bolus is about 4 hours. In some embodiments, the longer infusion following the bolus is about 8 hours. In some embodiments, the longer infusion following the bolus is about 12 hours. In some embodiments, the longer infusion following the bolus is about 18 hours. In some embodiments, the longer infusion following the bolus is about 24 hours. In some embodiments, the longer infusion following the bolus is about 36 hours.

[0096] In some embodiments, the concentration of anti-ticagrelor antibody or fragment thereof in the formulation varies between 0.4 mg/mL up to 72 mg/mL in a single IV infusion, 250 mL to be delivered over 30 minutes to 12 hours in doses of 0.1 g, 0.3 g, 1.0 g, 3 g, 9 g, 180 g, 24 g, 30 g, 36 g or 48 g or intermediate doses between 9 to 48 g. In some cases, a portion of the therapeutic composition is infused (up to about 12 g) at a faster rate (equivalent to a bolus) for the first 5-20 minutes of the infusion.

[0097] In some embodiments, the anti-ticagrelor antibody or fragment thereof is stored in one or more glass vials and subsequently transferred to an infusion bag for administration. In some embodiments, the anti-ticagrelor antibody or fragment thereof is stored in one or more glass vials and subsequently transferred to a syringe for administration using a syringe pump. In some embodiments, the anti-ticagrelor antibody or fragment thereof is stored in pre-filled syringe for administration using a syringe pump. In some embodiments, the anti-ticagrelor antibody or fragment thereof is stored in an IV container, such as Baxter Galaxy Liquid Premix System or Baxter Galaxy Frozen Premix System.

[0098] In some embodiments, the antibody or fragment thereof is administered to effect rapid and prolonged reversal of ticagrelor activity. In some embodiments, the infusion rate remains constant over the entire infusion. In some embodiments, the infusion rate varies over the infusion time. In some embodiments, a greater amount of the pharmaceutical composition is administered first in the infusion, and the amount is tapered during the rest of the infusion.

[0099] In some embodiments, the infusion duration lasts between about 5 minutes and about 36 hours. In some embodiments, the infusion regimen is selected from, but not limited to infusion of about 3 g to about 36 g at a constant infusion rate over about 1 hour to about 24 hours, infusion of about 3 g over about 5 minutes, followed by infusion of about 15 grams over about 8 hours, infusion of about 6 g over about 15 minutes, followed by infusion of about 6 grams over about 3 hours, followed by infusion of about 6 g over about 8.75 hours, infusion of about 6 g over about 15 minutes, followed by infusion of about 6 grams over about 4 hours, followed by infusion of about 6 g over about 12 hours, infusion of about 6 g over about 10 minutes, followed by infusion of about 6 grams over about 3 hours, followed by infusion of about 6 g over about 13 hours, infusion of about 12 g over about 10 minutes, followed by infusion of about 12 grams over about 6 hours, followed by infusion of about 12 g over about 18 hours.

[00100] In some embodiments, if rapid reversal of ticagrelor activity is desired (e.g. during an active bleed in a patient), the antibody or fragment thereof of the present disclosure may be administered according to the below:

[00101] 1. about 3 g to about 6 g infused over about 5 to about 15 minutes, followed by about 3 g to about 6 g infused over about 1 to about 3 hours, followed by about 3 g to about 6 g infused over about 3 to about 8 hours.

[00102] 2. about 3 g to about 6 g infused over about 5 to about 15 minutes, followed by about 6 g to about 12 g infused over about 1 to about 3 hours, followed by about 6 g to about 12 g infused over about 3 to about 8 hours.

[00103] 3. about 3 g to about 6 g infused over about 5 to about 15 minutes, followed by about 6 g to about 12 g infused over about 1 to about 3 hours, followed by about 1 g/hour infused over up to about 24 to about 48 hours.

[00104] 4. about 9 g infused over about 5 to about 30 minutes, followed by about 1 g/hr to about 3 g/hr infused over about 3 to about 8 hours.

[00105] 5. about 9 g to about 24 g infused over about 1 to about 4 hours.

[00106] In some embodiments, if the patient is about to undergo surgery, the antibody or fragment thereof of the present disclosure may be administered according to the below:

[00107] 1. about 3 to about 6 g infused over about 5 to about 30 minutes, followed by about 3 to about 6 g infused over about 3 to about 6 hours, followed by about 1 g/hr infused over up to about 12 to about 24 hours;

[00108] 2. about 3 to about 6 g infused over about 5 to about 15 minutes, followed by about 3 to about 6 g infused over about 1 to about 3 hours, followed by about 1 g/hr infused over about 12 to about 24 hours.

[00109] 3. about 9 g to about 24 g infused over about 1 to about 4 hours.

[00110] In some embodiments, if the patient is taking one or more additional drugs that impact ticagrelor exposure, such as drugs that inhibit the activity of cytochrome P450 isoform 3A (CYP3A), leading to increased exposure to ticagrelor, or the patient has taken an overdose of ticagrelor, the antibody or fragment thereof of the present disclosure may be administered according to the below:

[00111] 1. about 6 to about 12 g infused over about 5 to about 30 minutes, followed by about 6 to about 12 g infused over about 3 to about 6 hours, followed by about 6 to about 12 g infused over up to about 12 to about 24 hours;

[00112] 2. about 6 to about 12 g infused over about 5 to about 15 minutes, followed by about 6 to about 12 g infused over about 1 to about 3 hours, followed by about 0.5 - 1 g/hr infused over about 12 to about 24 hours.

[00113] 3. about 18 g to about 36 g infused over about 3 to about 6 hours.

[00114] The formulations may conveniently be presented in unit dosage form and may be prepared by any method known in the art of pharmacy. Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present disclosure may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient (e.g., "a therapeutically effective amount"). The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00115] Suitable dosages may range from about 1000 mg to about 36 g, or from about 9 g to about 24 g, or from about 9 g to about 15 g, or from about 1g to about 3 g. In some embodiments, the dose may be about 1000 mg, about 3 g, about 9 g, about 18 g, about 24 g, about 30 g, about 36 g, or about 48 g. In some embodiments, the amount of anti-ticagrelor antibody or fragment thereof administered to a patient depends on the amount of ticagrelor the patient has received. In some embodiments, the amount of anti-ticagrelor antibody or fragment thereof administered to a patient depends on the body weight of the patient.

[00116] The dose of anti-ticagrelor antibody or fragment thereof administered will be that dose which causes the reversal of ticagrelor-induced platelet disaggregation in 95% of the simulated patient population, with reversal taken to be the reversal of the platelet disaggregation to less than 10% of baseline.

[00117] In some embodiments, the patient has been administered at least 180 mg ticagrelor. In some embodiments, the patient has been administered a loading dose of at least 180 mg ticagrelor with 90 mg subsequently administered twice a day. In some embodiments, the patient has been administered ticagrelor at least three days prior to administration of an anti-ticagrelor antibody or fragment thereof. In some embodiments, the patient is administered ticagrelor at the same time as administration of an anti-ticagrelor antibody or fragment thereof. In some embodiments, the patient has been administered an overdose of ticagrelor.

[00118] Note that the disclosure similarly contemplates that formulations suitable for diagnostic and research use may also be made. The concentration of active agent in such formulations, as well as the presence or absence of excipients and/or pyrogens can be selected based on the particular application and intended use.

[00119] It should be understood that singular forms such as “a,” “an,” and “the” are used throughout this application for convenience, however, except where context or an explicit statement indicates otherwise, the singular forms are intended to include the plural. All numerical ranges should be understood to include each and every numerical point within the numerical range, and should be interpreted as reciting each and every numerical point individually. The endpoints of all ranges directed to the same component or property are inclusive, and intended to be independently combinable.

[00120] The term “about” when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric indication. For example, the language “about 50” covers the range of 45 to 55.

[00121] As used herein, the word “include,” and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the materials, compositions, devices, and methods of this technology. Similarly, the terms “can” and “may” and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features. Although the open-ended term “comprising,” as a synonym of terms such as including, containing, or having, is used herein to describe and claim the disclosure, the present technology, or embodiments thereof, may

alternatively be described using more limiting terms such as “consisting of” or “consisting essentially of” the recited ingredients.

[00122] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present disclosure, the preferred methods and materials are described herein.

EXAMPLES OF NON-LIMITING EMBODIMENTS OF THE DISCLOSURE

[00123] Embodiments, of the present subject matter disclosed herein may be beneficial alone or in combination, with one or more other embodiments. Without limiting the foregoing description, certain non-limiting embodiments of the disclosure, numbered 1-42 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered embodiments may be used or combined with any of the preceding or following individually numbered embodiments. This is intended to provide support for all such combinations of embodiments and is not limited to combinations of embodiments explicitly provided below.

[00124] Embodiment 1. A method of reversing ticagrelor-associated bleeding, or the risk of said bleeding, in a patient in need thereof comprising administering to said patient a composition comprising an effective amount of a pharmaceutical composition comprising an antibody or fragment thereof that binds to ticagrelor ((1*S*,2*S*,3*R*,5*S*)-3-[7-{{(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl}amino}-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol) or a metabolite or derivative thereof.

[00125] Embodiment 2. The method of embodiment 1, wherein the antibody or a fragment thereof comprises complementarity-determining region (CDR) combinations selected from the group consisting of:

a) SEQ ID NO:53 (VH CDR1), SEQ ID NO:54 (VH CDR2), SEQ ID NO:55 (VH CDR3), SEQ ID NO:58 (VL CDR1), SEQ ID NO:59 (VL CDR2), and SEQ ID NO:60 (VL CDR3);

b) SEQ ID NO:63 (VH CDR1), SEQ ID NO:64 (VH CDR2), SEQ ID NO:65 (VH CDR3), SEQ ID NO:68 (VL CDR1), SEQ ID NO:69 (VL CDR2), and SEQ ID NO:70 (VL CDR3); and

c) SEQ ID NO:73 (VH CDR1), SEQ ID NO:74 (VH CDR2), SEQ ID NO:75 (VH CDR3), SEQ ID NO:78 (VL CDR1), SEQ ID NO:79 (VL CDR2), and SEQ ID NO:80 (VL CDR3).

[00126] Embodiment 3. The method of embodiment 1 or 2, wherein the antibody or a fragment thereof

[00127] comprises a combination of heavy chain variable region (VH) and light chain variable region (VL) sequences selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:57; SEQ ID NO:62 and SEQ ID NO:67; and SEQ ID NO:72 and SEQ ID NO:77.

[00128] Embodiment 4. The method of any of embodiments 1-4, wherein the patient has been administered ticagrelor before administration of the anti-ticagrelor antibody or fragment thereof.

[00129] Embodiment 5. The method of any of embodiments 1-5, wherein the antibody or fragment thereof is a Fab and the patient is administered a dose between about 1 g and about 48 g.

[00130] Embodiment 6. The method of embodiment 5, wherein the dose is between about 9 g to about 18 g of the Fab.

[00131] Embodiment 7. The method of embodiment 5 or 6, wherein the patient is administered a dose of about 1 g, about 3 g, about 9 g, about 18 g, about 24 g, about 30 g, about 36 g or about 48 g of the Fab.

[00132] Embodiment 8. The method of any of embodiments 1-7, wherein the pharmaceutical composition is administered to the patient intravenously.

[00133] Embodiment 9. The method of embodiment 8, wherein the pharmaceutical composition is administered intravenously over about 15 minutes to about 36 hours.

[00134] Embodiment 10. The method of any of embodiments 1-9, wherein the pharmaceutical composition is administered in two or more segments.

[00135] Embodiment 11. The method of embodiment 10, wherein the first segment is a bolus.

[00136] Embodiment 12. The method of embodiment 10 or 11, wherein the administration rates for each of the segments differ.

[00137] Embodiment 13. The method of any of embodiments 10-12, wherein the administration rates for each of the segments differ for successive segments of the infusion.

[00138] Embodiment 14. The method of any of embodiments 10-13, wherein the pharmaceutical composition is administered in three or more segments, wherein the administration rates for each of the segments differ for successive segments of the infusion.

[00139] Embodiment 15. The method of any of embodiments 5-14, wherein the pharmaceutical composition is administered in the following schedule: 12g infused over 10 minutes, followed by 12g over 6 hours, followed by 12g over 18 hours.

[00140] Embodiment 16. The method of any of embodiments 1-15, wherein the pharmaceutical composition comprises about 50 mg/mL to about 200 mg/mL of the anti-ticagrelor antibody or fragment thereof, about 5 mM to about 50 mM histidine/histidine hydrochloride buffer, about 100 mM to about 300 mM sucrose, and about 0.01% (w/v) to about 1.0% (w/v) polysorbate 80, pH 5.5 to 6.5

[00141] Embodiment 17. The method of embodiment 16, wherein the pharmaceutical formulation comprises 100 mg/mL of the anti-ticagrelor antibody or fragment thereof, 25 mM histidine/histidine hydrochloride buffer, 290 mM sucrose, and 0.05% (w/v) polysorbate 80, pH 6.0.

[00142] Embodiment 18. The method of embodiment 16 or 17, wherein the pharmaceutical formulation is diluted in saline for administration.

[00143] Embodiment 19. The method of any of embodiments 1-18, wherein the ticagrelor-associated bleeding is major bleeding.

[00144] Embodiment 20. The method of embodiment 19, wherein the major bleeding is characterized by being life-threatening, potentially leading to clinically significant disability, requiring surgery to control the bleeding, requiring treatment with blood products, or is acute bleeding associated with a clinically important drop in hemoglobin.

[00145] Embodiment 21. The method of any of embodiments 1-20, wherein the patient requires urgent surgery or intervention.

[00146] Embodiment 22. The method of embodiment 21, wherein the urgent surgery or intervention is known to be associated with a significant risk of bleeding, such as coronary artery bypass surgery, has an adverse surgical outcome if bleeding is not carefully controlled,

neurological, ophthalmologic, or joint replacement surgery, associated with risk of experiencing perioperative events; or in a patient at high risk of thrombosis if dual antiplatelet therapy is withheld preoperatively.

[00147] Embodiment 23. The method of any of embodiments 1-22, wherein the patient is at risk of developing, or has been diagnosed with Acute Coronary Syndrome (ACS).

[00148] Embodiment 24. The method of any of embodiments 1-23, wherein the patient is at risk of developing, or has been diagnosed with a disease selected from the group consisting of myocardial infarction (MI), unstable angina, stable ischemic heart disease, in sickle cell disease, including pediatric patients, atrial fibrillation, coronary arterial disease, peripheral arterial disease, ischemic stroke, one or more coronary stents, carotid artery stents, stents following an intracranial aneurysm, and arterio-venous fistulae created for hemodialysis.

[00149] Embodiment 25. The method of any of embodiments 1-24, wherein the patient is a pediatric patient.

[00150] Embodiment 26. The method of embodiment 25, wherein the pediatric patient is younger than 18 years old.

[00151] Embodiment 27. The method of embodiment 26, wherein the pediatric patient is younger than 2 years old.

[00152] Embodiment 28. The method of any of embodiments 1-24, wherein the patient is an adult patient.

[00153] Embodiment 29. The method of embodiment 28, wherein the adult patient is between 18 and 64 years old inclusive.

[00154] Embodiment 30. The method of embodiment 28, wherein the patient is over 65 years old.

[00155] Embodiment 31. The method of embodiment 30, wherein the patient is between 65 and 80 years old inclusive.

[00156] Embodiment 32. The method of any of embodiments 1-31, wherein the patient has been administered aspirin (acetylsalicylic acid).

[00157] Embodiment 33. The method of any of embodiments 1-32, wherein administration of the antibody or fragment thereof reverses ticagrelor activity.

- [00158] Embodiment 34. The method of embodiment 33, wherein administration of the antibody or fragment thereof restores platelet function.
- [00159] Embodiment 35. The method of embodiment 34, wherein administration of the antibody or fragment thereof restores platelet aggregation.
- [00160] Embodiment 36. The method of embodiment 35, wherein administration of the antibody or fragment thereof restores platelet aggregation to at least 80% of baseline.
- [00161] Embodiment 37. The method of embodiment 35 or 36, wherein administration of the antibody or fragment thereof restores platelet aggregation within 1 minute to 60 minutes of administration.
- [00162] Embodiment 38. The method of embodiment 37, wherein administration of the antibody or fragment thereof restores platelet aggregation within 5 minutes of administration.
- [00163] Embodiment 39. The method of any of embodiments 35 to 38, wherein administration of the antibody or fragment thereof provides a sustained restoration of platelet aggregation.
- [00164] Embodiment 40. The method of embodiment 39, wherein the restoration of platelet aggregation is sustained for at least 12 hours after administration.
- [00165] Embodiment 41. The method of embodiment 40, wherein the restoration of platelet aggregation is sustained for at least 16 hours after administration.
- [00166] Embodiment 42. The method of embodiment 41, wherein the restoration of platelet aggregation is sustained for at least 24 hours after administration.
- [00167] This disclosure is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1- A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of PB2452 With and Without Ticagrelor PreTreatment in Healthy Volunteers

- [00168] **Primary Objective:** 1) To evaluate the safety and tolerability of single ascending intravenous (IV) doses of PB2452 with or without oral ticagrelor; 2) To evaluate the effect of

single ascending doses of PB2452 on ticagrelor antiplatelet activity using light transmittance aggregometry (LTA).

[00169] Secondary Objectives: 1) To determine the pharmacokinetics of ascending doses of IV PB2452 in the presence and absence of ticagrelor; 2) To determine the pharmacokinetics of ticagrelor and its active metabolite TAM in the presence and absence of PB2452; 3) To assess the effectiveness of a single IV PB2452 dose in reversing ticagrelor antiplatelet activity by measuring P2Y₁₂ reaction units (PRU) with VerifyNow™ P2Y₁₂ assay and platelet reactivity index (PRI) with vasodilator stimulated phosphoprotein (VASP) phosphorylation assay by enzyme-linked immunosorbent assay (ELISA); 3) To evaluate the pharmacokinetics and pharmacodynamics of restarting a single dose of oral ticagrelor 24 hours after IV PB2452 administration if a sixth dose of ticagrelor is given; 4) To evaluate the immunogenicity potential of PB2452

[00170] Exploratory Objectives: To evaluate the effect of PB2452 on the pharmacokinetic (PK) profile of unbound ticagrelor and unbound TAM plasma concentrations.

[00171] Study design and methodology: This is a Phase 1, first-in-human, randomized, double-blind, placebo-controlled, single ascending dose, sequential group study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of PB2452 with and without ticagrelor pretreatment when administered to healthy male and female subjects. All references to study drug within the content of the protocol apply to PB2452 or matching placebo.

[00172] This study will have up to 10 cohorts and up to a total of approximately 80 subjects. The starting dose of PB2452 will be 100 mg and the planned doses for subsequent cohorts are 300, 1000, 3,000, 9,000, and 18,000 mg. Other doses may also be tested and may exceed 18,000 mg.

[00173] The study will consist of a screening period (Days -45 to -4), check-in/pretreatment (Day -3 to Day -1), an in-house treatment period (Days 1 through 3), and follow-up visits (Days 4, 7, and 28 [+2 days]). Subjects will receive an IV dose of study drug on Day 1. On Day 1, subjects who meet all of the inclusion criteria and none of the exclusion criteria will be randomly assigned to receive PB2452 or placebo in a ratio of 3:1 in all treatment cohorts:

[00174] Cohorts 1 to 3: For the initial cohort (Cohort 1), 4 healthy subjects will be randomly assigned in a 3:1 ratio of active treatment to placebo (3A:1P) to receive a single 100 mg IV dose of study drug over 30 minutes. For the second cohort (Cohort 2), 4 healthy subjects will be randomly assigned (3A:1P) to receive a single 300 mg IV dose of study drug over 30 minutes. For

the third cohort (Cohort 3), 4 healthy subjects will be randomly assigned (3A:1P) to receive a 1,000 mg IV dose of study drug over 30 minutes.

[00175] Cohorts 4 to 6: Provided no safety concerns arise in previously dosed cohorts, 8 subjects in each of Cohorts 4 through 6 will be randomly assigned in a 3:1 ratio (6A:2P) to receive a single IV dose of study drug within approximately 2 hours after the 5th dose of ticagrelor pretreatment. For ticagrelor pretreatment, subjects will receive an oral loading dose of 180 mg ticagrelor in the morning (Day -2), followed by 90 mg ticagrelor orally every 12 hours for 4 additional doses, prior to administration of a single IV dose of study drug within approximately 2 hours after the 5th ticagrelor dose (Day 1). Subjects in each of Cohorts 4 through 6 will receive ticagrelor pretreatment, as described above, and a single IV dose of 1,000, 3,000, or 9,000, or 18,000 mg of study drug, respectively, over 30 minutes. Cohorts 4 through 6 will be dosed sequentially following the safety and dose-escalation assessment of each preceding dose cohort.

[00176] Cohorts 7 to 10: For Cohort 7, following ticagrelor pretreatment, subjects will be randomly assigned in a 3:1 ratio (6A:2P) to receive a single IV dose of study drug. The intravenous infusion will be initiated 2 hours after the 5th ticagrelor dose and will administer PB2452 continuously, but at different rates during the infusion period as follows: 3,000 mg over 5 minutes, followed by 15,000 mg for 7 hours 55 minutes.

[00177] For cohort 8, the infusion rates during the infusion period will be as follows: 6,000 mg over 15 minutes, followed by 6,000 mg for 3 hours, followed by 6,000 mg for 8.75 hours. For cohort 9, the infusion rates during the infusion period will be as follows: 6,000 mg over 15 minutes, followed by 6,000 mg for 4 hours, followed by 6,000 mg for 12 hours. For cohort 10, the infusion rates during the infusion period will be as follows: 6,000 mg over 10 minutes, followed by 6,000 mg for 3 hours, followed by 6,000 mg for 13 hours.

[00178] Subjects in Cohorts 1 through 3 will check in to the clinical site on Day -1. On Day 1, subjects will receive a single IV dose of study drug in the morning. Subjects will be discharged from the clinical site on Day 3 and will return for follow-up visits on Days 4, 7, and 28 (+2 days).

[00179] Subjects in Cohorts 4 through 10 will check into the clinical site on Day -3. In the morning on Day -2, subjects will begin pretreatment with ticagrelor. On Day 1, subjects will receive a single IV dose of study drug in the morning. Subjects in Cohorts 4 through 8 (and also Cohort 9 if they do not receive a 6th dose of ticagrelor) will be discharged from the clinical site on

Day 3 and will return for follow-up visits on Days 4, 7, and 28 (+2 days). Cohort 10 will be discharged from the clinical site on Day 7 and will return for their final follow up visit on Day 28 (+2 days).

[00180] On Day 2, subjects in Cohort 9 may receive an additional dose of ticagrelor in the morning 24 hours after the initiation of the study drug infusion. In this case subjects will be discharged from the clinical site on Day 4 and will return for follow-up visits on Days 7 and 28 (+2 days). If the 6th ticagrelor dose is not given they will be discharged from the unit on Day 3 and will return for follow-up visits on Days 4, 7, and 28. The decision on whether to administer this 6th dose of ticagrelor will be made based upon review of data from prior cohorts.

[00181] Plasma samples for PK and pharmacodynamics (PD) analysis of PB2452, ticagrelor, and its active metabolite, TAM, and urine samples for the PK analysis of ticagrelor and TAM will be collected at specified intervals up to 28 days after dosing. Hour 0 will be the initiation of the study drug infusion for all cohorts.

[00182] Safety and tolerability will be carefully monitored throughout the study. Immunogenicity will be determined in all subjects at baseline and for up to 28 days following administration of the study drug.

[00183] **Sentinel Dosing:** Dosing for Cohort 1 (first exposure of PB2452 in humans) will proceed with two sentinel subjects initially randomly assigned to receive a single IV dose of study drug. Blinded safety data from the sentinel subjects up to 24 hours following the study drug infusion will be reviewed by the investigator before the remaining 2 subjects in Cohort 1 are dosed. The remaining subjects will be dosed at least 24 hours after the sentinel subjects. Additionally, dosing for Cohort 4 (first exposure of the combination of PB2452 with ticagrelor in humans) will proceed with 2 sentinel subjects pre-treated with ticagrelor prior to receiving a single IV dose of PB2452 or placebo randomized in a 1:1 ratio of active to placebo. Blinded safety data from the sentinel subjects up to 24 hours following the ticagrelor and study drug administration will be reviewed by the investigator before the remaining 6 subjects in Cohort 4 are dosed. The remaining subjects will be dosed at least 24 hours after the sentinel subjects.

[00184] **Dose Escalation:** A safety review committee (SRC) will be formed for blinded reviews of safety (e.g., clinical laboratory results, adverse events [AEs], 12-lead electrocardiograms [ECGs], vital signs) and available PK data through Day 4 for each dose cohort.

Dose escalation to successive cohorts will be based upon safety of the preceding cohort. The investigator will make a recommendation on whether to proceed to the next predefined dose level, pause dosing for review of additional safety and/or PK data, or to adjust the dose of the next dose cohort. The decision to adjust or pause the dose or proceed to the next cohort will be made by the SRC. The safety data will be reviewed in a blinded manner and must be deemed acceptable to the SRC prior to dosing of the next higher dose group.

[00185] Based on the review of safety and PK data, if available, the SRC may choose to repeat a dose level, administer a dose less than the previous dose, or escalate to a dose lower than the next planned dose. The SRC has the authority to make the decision to proceed with dose escalation after review of the available safety data regarding any DLTs.

[00186] Stopping Criteria: After completion of Day 4 for each dose cohort, the SRC will review and assess all available safety (e.g., clinical laboratory results, AEs, ECGs, vital signs), tolerability, and available PK data to make a dose escalation decision for next dose cohort. Dose escalation will be suspended if any of the following scenarios occur after confirmation of receipt of PB2452 and review by the SRC:

[00187] Any preclinical or clinical events that, in the opinion of the SRC, contraindicate further dosing of additional subjects with PB2452;

[00188] Any serious adverse event (SAE) in a dose cohort;

[00189] Data from the previous dose cohort indicating safety concerns for the next cohort to be dosed at a higher level, such as unanticipated responses (e.g., clinically significant changes in clinical laboratory data, ECGs, cardiac telemetry, vital signs, or physical examinations);

[00190] Two or more subjects in a dose cohort experience any DLT, or 1 subject experiences a grade 2 or higher AE (DLT) that in the opinion of the SRC warrants suspension of dose escalation;

[00191] Two or more subjects have $>3\times$ upper limit of normal (ULN) of either alanine aminotransferase (ALT) or aspartate aminotransferase (AST), or $> 2 \times$ ULN for bilirubin or alkaline phosphatase where no other reason can be found to explain the increases; or

[00192] One or more subjects experiences a grade 2 or higher infusion-related reaction despite having been pre-medicated for infusion-related reactions.

[00193] Dose escalation may also be suspended if, in the opinion of the SRC or sponsor, any other significant safety or tolerability issues are identified in the comprehensive review of available data that warrant further evaluation before additional subjects are dosed. This may include emerging nonclinical data, clinically relevant AEs, or relevant data from other sources indicating safety concerns even if the event(s) per se does not meet the protocol-specified definition of a dose-limiting toxicity. The SRC has the authority to make the decision to proceed with dose escalation after review of the available safety data regarding SAEs and other stopping criteria.

[00194] INCLUSION CRITERIA:

[00195] 1) The subject is male or female between 18 and 50 years of age, inclusive for Cohorts 1-10 male or female subjects.

[00196] 2) The subject has a body mass index between 18 and 35 kg/m² and a weight of ≥ 50 kg but ≤ 120 kg, inclusive, at screening.

3) The subject is considered by the investigator to be in good general health as determined by medical history, clinical laboratory test results, vital sign measurements, 12-lead ECG results, and physical examination findings at screening.

[00197] 4) Female subjects of childbearing potential must not be pregnant, lactating, or planning to become pregnant before 3 months after the last dose of study drug, and have a negative serum pregnancy test at screening and check-in. Female subjects of childbearing potential must use 2 effective methods of birth control (i.e., oral, implantable, patch, or injectable contraceptives in combination with a condom, hormone-containing intrauterine device that has been in place for at least 2 months prior to screening in combination with a condom, double-barrier method [i.e., condoms, sponge, diaphragm, or cervical cap with spermicidal gels or cream], or vasectomy for male subjects or male partners of female subjects) from 30 days before study drug administration through the end of the study. Women are considered not to be of childbearing potential if they have fulfilled one of the following criteria: documentation of irreversible surgical sterilization (i.e., hysterectomy, or bilateral oophorectomy [not tubal ligation]), or postmenopausal (defined as amenorrhea for 12 consecutive months following cessation of all exogenous hormonal treatments, and documented plasma follicle-stimulating hormone level >40 IU/mL, or amenorrhea for 24 consecutive months). Male subjects with partners of childbearing potential must agree to use

appropriate and effective measures of contraception (e.g., condom plus diaphragm with spermicide; condom plus spermicide) during the study and for 30 days after the last dose of study drug, and to refrain from donating sperm for at least 7 days prior to the dose of study drug and until at least 90 days following the last dose of study drug.

[00198] 5) The subject agrees to comply with all protocol requirements.

[00199] 6) The subject is able to provide written informed consent.

[00200] EXCLUSION CRITERIA

[00201] 1. History of any clinically significant acute or chronic disease or medical disorder.

[00202] 2. History or presence of gastrointestinal, hepatic (with the exception of Gilbert's syndrome), or renal disease or renal insufficiency (i.e., estimated glomerular filtration rate <60 ml/min/1.73 m²), or any other condition known to interfere with absorption, distribution, metabolism, or excretion of drugs.

[00203] 3. Any clinically significant illness, medical/surgical procedure, or trauma within 4 weeks of the administration of study drug or any planned surgical procedure that will occur during the study (from screening through the Day 28 follow up visit).

[00204] 4. Any clinically significant abnormal findings in physical examination, vital signs, laboratory assessments, and ECG parameters identified during screening or check-in. Note: abnormal test results may be repeated once for confirmation.

[00205] Specific vital sign exclusionary criteria occurring after 10 minutes of supine rest are any of the following:

[00206] Systolic blood pressure >150 mm Hg;

[00207] Diastolic blood pressure >90 mm Hg; or

[00208] Heart rate <50 or >100 beats per minute.

[00209] Specific exclusionary criteria for ECG parameters at screening or check-in are any of the following:

[00210] Prolonged Fridericia-corrected QT interval (QTcF) >450 milliseconds (msec), shortened QTcF <340 msec, or pause >3 seconds, or family history of long QT syndrome;

[00211] Prolonged PR (PQ) interval >240 msec, intermittent second- or third-degree atrioventricular (AV) block or AV dissociation, or shortened PR interval <120 msec;

- [00212] Incomplete, full, or intermittent bundle branch block (QRS <110 msec with normal QRS and T wave morphology is acceptable if there is no evidence of left ventricular hypertrophy;
- [00213] 5. Any history of arterial or venous thrombosis, including any of the following:
- [00214] History of transient ischemic attack, cardiovascular accident, stroke (ischemic or hemorrhagic), unstable angina, myocardial infarction, or peripheral arterial disease; or
- [00215] History of deep venous thrombosis, pulmonary embolus, thrombophlebitis, or cavernous malformations.
- [00216] 6. Any increased risk of bleeding, including the following:
- [00217] Recent history (within 30 days preceding the first dose of study drug) of gastrointestinal bleeding;
- [00218] Any history of severe head trauma, intracranial hemorrhage, intracranial neoplasm, arteriovenous malformation, aneurysm, or proliferative retinopathy;
- [00219] Any history of intracranial, intraocular, retroperitoneal, or spinal bleeding;
- [00220] Any recent (within 30 days preceding the first dose of study drug) major trauma;
- [00221] History of hemorrhagic disorders that may increase the risk of bleeding (e.g., hemophilia, von Willebrand's disease);
- [00222] Receiving chronic treatment with nonsteroidal anti-inflammatory drugs (including aspirin [greater than 100 mg daily]), anticoagulants, or other antiplatelet agents that cannot be discontinued (including clopidogrel, prasugrel, ticlopidine, dipyridamole, or cilostazol).
- [00223] Have taken, within 30 days of screening, any oral or parenteral anticoagulant, including low molecular-weight heparin;
- [00224] Have taken non-steroidal anti-inflammatory medications, including aspirin within 14 days of screening;
- [00225] 7. The subject has a positive test result for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus types 1 or 2 antibodies at screening.
- [00226] 8. Any ongoing or recent (i.e., during the screening period) minor medical complaints that may interfere with the interpretation of the study data or are considered unlikely to comply with study procedures, restrictions, and requirements as judged by the investigator.
- [00227] 9. Any risk of bradycardic events (e.g., known sick sinus syndrome, atrial fibrillation, or second- or third-degree AV block).

[00228] 10. Concomitant oral or IV therapy with strong CYP3A inhibitors, CYP3A substrates with narrow therapeutic indices, or strong CYP3A inducers, which cannot be stopped within at least 5 half-lives, but not shorter than 10 days, before randomization (a list of examples can be found in Section 6.2).

[00229] 11. Any prescription (excluding hormonal birth control) or over-the-counter medications (except paracetamol [up to 2 g per day], including herbal or nutritional supplements, within 14 days before the first dose of study drug.

[00230] 12. The subject has consumed grapefruit or grapefruit juice, Seville orange or Seville orange-containing products (e.g., marmalade), or alcohol-, or xanthine-containing products within 48 hours before dosing with study drug.

[00231] 13. The subject is participating in any other study or is taking part in a non-medication study which, in the opinion of the investigator, would interfere with the outcome of the study.

[00232] 14. The subject has received another new chemical entity (defined as a compound which has not been approved for marketing) or any marketed or investigational biologic agent within 30 days of the administration of study drug in this study. The period of exclusion begins 30 days after the final dose or 5 half-lives of the experimental medication has elapsed, whichever is longer.

[00233] 15. The subject has involvement with any Sponsor or study site employee or their close relatives (e.g., spouse, parents, siblings, or children whether biological or legally adopted).

[00234] 16. The subject has previously received PB2452 or had been randomized to receive study drug in an earlier cohort for this study.

[00235] 17. The subject is a smoker or has used nicotine or nicotine-containing products (e.g., snuff, nicotine patch, nicotine chewing gum, mock cigarettes, or inhalers) within 3 months before the infusion of study drug.

[00236] 18. The subject has a known or suspected history of drug abuse (including alcohol) or has a positive test result for drugs of abuse, alcohol, or cotinine (nicotine level above 300 ng/mL) at screening or check-in.

[00237] 19. The subject has been involved in strenuous activity or contact sports within 24 hours before the infusion of study drug and while confined in the clinical site.

[00238] 20. The subject has donated blood or plasma within 1 month of screening or any blood donation/loss more than 500 mL during the 3 months prior to the infusion of study drug.

[00239] 21. The subject has a history of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the investigator or history of hypersensitivity to drugs with a similar chemical structure or class to ticagrelor, any biologic therapeutic agent, or any significant food allergy that could preclude a standard diet in the clinical site.

[00240] 22. Concern for the inability of the subject to comply with study procedures and/or follow-up, or, in the opinion of the investigator, the subject is not suitable for entry into the study.

[00241] EVALUATION CRITERIA:

[00242] Safety Assessments: Safety and tolerability will be assessed by monitoring and recording of AEs, clinical laboratory test results (hematology, coagulation, serum chemistry, and urinalysis), vital sign measurements (systolic and diastolic blood pressures, oral body temperature, respiratory rate, and heart rate), 12-lead ECG results, cardiac telemetry monitoring, immunogenicity, and physical examination findings.

[00243] Pharmacokinetic Assessments

[00244] Plasma Collection: Blood samples for PK analysis of PB2452 in plasma will be collected from all subjects at the following time points: before dosing (within 10 minutes prior to the initiation of study drug infusion and up to 28 days after the initiation of study drug infusion. Specific collection times for each cohort are listed in the table below:

Summary of Pharmacokinetic Assessments for PB2452	
Cohorts 1-6	-10 minutes, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72 hours, 7 and 28 days
Cohorts 7-10	-10 minutes, 5 minutes, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 8.25, 9, 10, 11, 12, 16, 20, 24, 32, 48 hours, 7 and 28 days

[00245] Plasma samples for determining total concentration of ticagrelor and its active metabolite, TAM (or ARC-124910XX) will be collected from subjects in Cohorts 4 through 10 before dosing (within 10 minutes prior to the initiation of the study drug infusion [Hour 0], and up to 48 hours following the initiation of study drug infusion. Specific collection times for each cohort are listed in the table below:

Summary of Pharmacokinetic Assessments for ticagrelor and active metabolite TAM	
Cohorts 4-6	-10 minutes, 0.5, 1, 2, 3, 4, 6, 12, 24, and 48 hours
Cohorts 7-10	-10 minutes, 5 minutes, 0.25, 0.5, 1, 2, 3, 6, 8, 10, 12, 14, 16, 20, 24, 32, and 48 hours

[00246] If in Cohort 9, a 6th dose of ticagrelor is administered 24 hours after the study drug infusion, additional PK samples will be collected up to 24 hours following the administration of the 6th ticagrelor dose as noted in the table below:

Summary of Pharmacokinetic Assessments for Ticagrelor and Active Metabolite TAM AFTER a 6th dose of Ticagrelor has been given	
Cohort 9	0.5, 1, 2, 3, 6, 12, and 24 hours AFTER the administration of the 6 th dose of ticagrelor

[00247] Plasma samples for determining unbound ticagrelor and TAM concentrations will be collected from subjects in Cohorts 4 through 10 before dosing (within 10 minutes prior to the initiation of study drug infusion [Hour 0] and up to 48 hours following the initiation of study drug infusion). Specific collection times for each cohort are listed in the table below:

Summary of Pharmacokinetic Assessments for unbound ticagrelor and active metabolite TAM		Summary of Pharmacokinetic Assessments for Unbound Ticagrelor and TAM
Cohorts 4-6	-10 minutes, 0.5, 1, 2, 3, 6, 12, 24, and 48 hours	
Cohorts 7-10	-10 minutes, 5 minutes, 0.25, 0.5, 1, 2, 3, 6, 8, 10, 12, 14, 16, 20, 24, 32, and 48 hours	

[00248] The following plasma PK parameters for PB2452 will be calculated:

[00249] Area under the plasma concentration versus time curve (AUC) from time 0 to the time of the last quantifiable concentration (AUC_{0-t});

[00250] Observed maximum plasma concentration (C_{max});

[00251] Time to reach the observed maximum plasma concentration (T_{max});

[00252] AUC from time 0 extrapolated to infinity (AUC_{0-inf}) (if data permit);

[00253] Terminal elimination half-life (t_{1/2}) (if data permit);

[00254] Apparent clearance (CL) (if data permit);

[00255] The following plasma PK parameters for ticagrelor and TAM will be calculated:

[00256] C_{max};

[00257] T_{max};

- [00258] AUC from time 0 to 12 hours after dosing (AUC0-12);
- [00259] AUC from time 0 to 24 hours after dosing (AUC0-24);
- [00260] AUC from time 0 to 48 hours after dosing (AUC0-48);
- [00261] AUC from time 0 to the time of last quantifiable concentration (Clast) (AUC0-t);
- [00262] AUC0-inf (if data permit);
- [00263] t½ (if data permit);
- [00264] For Cohorts receiving a 6th dose of ticagrelor 24 hours following study drug, PK parameters AUC0-24 and AUC 0-inf (if data permits) will only be calculated. Additional PK parameters might be included.

[00265] Pharmacodynamic Assessments:

Blood samples for PD analysis will be collected from subjects in Cohorts 4 through 10 at the following time points: before dosing (within 60 minutes prior to first ticagrelor dose on Day -2) and again before dosing (within 10 minutes prior to the initiation of the study drug] infusion [Hour 0]), and up to 48 hours after the initiation of study drug infusion. Specific collection times are listed in the table below:	
Summary of Pharmacodynamic Assessments	
Pharmacodynamic	
Cohorts 1-6	Day -2 (60 minutes prior to first ticagrelor dose) and Day 1 (-10 minutes, 0.5, 1, 2, 3, 6, 12, 24 and 48 hours)
Cohorts 7-10	Day -2 (60 minutes prior to first ticagrelor dose) and Day 1 (-10 minutes, 5 minutes, 0.25, 0.5, 1, 2, 3, 6, 8, 10, 12, 16, 20, 24 and 48 hours)
If in cohort 9 a 6th Ticagrelor dose is administered 24 hours after the infusion of study drug, additional PD samples will be collected as follows:	
Cohort 9	Day 2: 1, 2, 6 and 12 hours post 6th ticagrelor dose. Note: A 24 hour post-6th ticagrelor dose PD sample is equivalent to the 48 hour post-study drug sample already being collected.

[00266] The following PD data and parameters will be generated from LTA, P2Y₁₂ reaction units (pRU), and platelet reactivity index (PRI) assays.

[00267] The maximal, final extent of aggregation and area under the curve for up to four platelet agonists, [(20 µM adenosine diphosphate (ADP), 5 µM adenosine diphosphate (ADP), 1.6 mM arachidonic acid (AA) and 15 µM thrombin receptor activating peptide (TRAP)] at each assessment point will be recorded.

- [00268] percent of baseline platelet aggregation;
- [00269] Maximal platelet aggregation;
- [00270] Time to maximal platelet aggregation;
- [00271] Time to 60%, 80%, 90% of baseline platelet aggregation.
- [00272] VerifyNow™ P2Y₁₂: 1) PRU at each assessment point; 2) Percent of baseline in PRU; 3) Maximal PRU; 4) Time to maximal PRU; 5) Time to 60%, 80%, 90% of baseline PRU
- [00273] VASP by ELISA: 1) PRU at each assessment point; 2) Percent of baseline in PRU; 3) Maximal PRU; 4) Time to maximal PRU; 5) Time to 60%, 80%, 90% of baseline PRU
- [00274] STUDY DRUG, DOSAGE, AND ROUTE OF ADMINISTRATION:
- [00275] PB2452:
- [00276] All cohorts: PB2452 (concentration will vary between 0.4 mg/mL up to 72 mg/mL) single IV infusion, 250 mL to be delivered over 30 minutes to 12 hours in escalating doses of 100, 300, 1,000, 3,000, 9,000, 18,000 mg, 24,000 or 36,000 mg or intermediate doses between 9,000 to 36,000 mg. In some cases, the sponsor may elect to infuse a portion of study drug (up to 6 g) at a faster rate (equivalent to a bolus) for the first 5-20 minutes of the infusion.
- [00277] Matching Placebo:
- [00278] All Cohorts: 0.9% sodium chloride single IV infusion, 250 mL to be delivered at a rate matching the defined active infusion duration.
- [00279] Ticagrelor:
- [00280] Cohorts 4 through 10: ticagrelor 90 mg oral tablet (immediate release); administered as 180 mg (2 × 90 mg tablet) loading dose plus 90 mg every 12 hours for 4 additional
- [00281] Cohort 9: may have one additional dose administered as 180 mg ([2 × 90 mg) oral tablets (immediate release)] 24 hours after the initiation of the study drug infusion.

Example 2 - Human Clinical Trial Data – PB2452 reverses ticagrelor activity

[00282] Ticagrelor is an oral P2Y₁₂ inhibitor used with aspirin to reduce the risk of ischemic events in patients with acute coronary syndromes. As with other antiplatelet drugs, spontaneous major bleeding and bleeding associated with urgent invasive procedures are concerns. The

antiplatelet effects of ticagrelor cannot be reversed with platelet transfusion. A rapid-acting reversal agent would be useful.

[00283] In a first-in-human randomized, double-blind, placebo-controlled, healthy volunteer study, intravenous (IV) PB2452, a monoclonal antibody fragment that binds ticagrelor with high affinity, was evaluated as a ticagrelor reversal agent. Platelet function was assessed using light transmission aggregometry (LTA), VerifyNow, and vasodilator stimulated phosphoprotein (VASP) assays before and after 48 hours of ticagrelor administration

[00284] Of the sixty-four subjects randomized, 48 received PB2452 and 16 received placebo. After 48 hours of ticagrelor, platelet aggregation was suppressed by ~80%. Compared with placebo, PB2452 administered as a 10-minute intravenous bolus, followed by 16-hour infusion, significantly restored platelet function measured by multiple assays. Onset of reversal occurred within 5 minutes and was sustained for over 20 hours ($P < 0.0001$, Bonferroni adjusted across all time points for all assays). There was no evidence of rebound in platelet activity after drug cessation. There were no drug-related adverse events.

[00285] PB2452, a specific reversal agent for ticagrelor, provided immediate and sustained reversal of ticagrelor's antiplatelet effects safely and effectively using multiple assays. PB2452 may represent a useful approach to treating or preventing ticagrelor-associated bleeding complications.

Background

[00286] Antiplatelet therapy is an essential part of secondary prevention of cardiovascular events. (Bhatt (2014)). In particular, dual antiplatelet therapy – the combination of aspirin with an oral P2Y₁₂ antagonist – is the predominant approach in patients with acute coronary syndromes, coronary artery stenting, and prior myocardial infarction. (Yusuf 92001); Mehta (2001); Bhatt (2006); Bhatt (2007); Chen (2005); Wiviott (2007); Prasugrel (2012); Wiviott (2013). The three oral P2Y₁₂ receptor antagonists in use are clopidogrel, prasugrel, and ticagrelor. (Koski (2018)). Randomized trials have found that ticagrelor is superior to clopidogrel across the entire spectrum of acute coronary syndromes. (Wallentin (2009); Cannon (2010); James (2011)).

[00287] A limitation of all three oral P2Y₁₂ antagonists is that they increase the risk of bleeding, which persists for several days after cessation. (Plavix prescribing information; Brilinta prescribing information; Effient prescribing information). This creates challenge in patients with

major bleeding, such as intracranial or gastrointestinal hemorrhage. (Ducrocq (2013); Bhatt (2007)). Additionally, patients in need of urgent and especially emergent invasive procedures are also at increased risk of periprocedural bleeding complications. If an emergent procedure is needed, the proceduralist must accept an increased bleeding risk, often after empirically providing platelet transfusions. If urgent, the operator either proceeds and anticipates increased bleeding hazard or postpones the procedure for several days with the attendant risks of delaying a clinically indicated procedure. Society guidelines recommend cessation of oral P2Y₁₂ receptor antagonists at least five days prior to surgery. (Capodanno (2013); Douketis (2012)).

[00288] Currently, there are no reversal agents for P2Y₁₂ receptor antagonists. Unlike the other P2Y₁₂ antagonists, ticagrelor is a reversible inhibitor. Consequently, ticagrelor will bind to P2Y₁₂ on transfused platelets, thereby rendering them ineffective. Therefore, a specific reversal agent for ticagrelor would be desirable.

Trial Design

This study was a single-center, randomized, double-blind, placebo-controlled, single ascending dose study to evaluate the safety, efficacy, and pharmacokinetics of PB2452 in healthy subjects 18 to 50 years of age who were or were not given ticagrelor pre-treatment. As shown in Table 1, ten sequential dose cohorts were evaluated.

Table 1 – Final Study Design

Cohort	Ticagrelor Pretreatment	PB2452 IV regimen	Subjects (Active:Placebo)
1	None	0.1 g 30 min	3A:1P
2		0.3 g 30 min	3A:1P
3		1.0 g 30 min	3A:1P
4	180 mg PO + 90 mg BID x	1.0 g 30 min	6A:2P
5		3.0 g 30 min	6A:2P
6		9.0 g 30 min	6A:2P
7		18 g (3 g 5 min + 15 g 7 hr 55 min)	6A:2P

8	2 days	18 g (6 g 15 min + 6 g 3 hr + 6 g 8hr 45 min)	6A:2P
9		18 g (6 g 15 min + 6 g 4 hr + 6 g 12 hr)	3A:1P
10		18 g (6 g 10 min + 6 g 3 hr + 6 g 13 hr)	6A:2P
g = grams A = active P = placebo			

[00289] Abbreviations: BID, twice daily

[00290] Cohorts 1-3 each enrolled 4 subjects and assessed intravenous doses of 0.1, 0.3, and 1.0 grams of PB2452 infused for 30 minutes in the absence of ticagrelor pre-treatment.

[00291] Subjects in Cohorts 4 to 10 were pre-treated with a 180 mg oral ticagrelor loading dose followed by 90 mg twice daily for 48 hours prior to evaluation of PB2452 at doses of 1.0 to 18 grams. Cohorts 4, 5, and 6 each consisted of 8 subjects randomly assigned to receive 1.0, 3.0, and 9.0 grams of PB2452 or placebo after ticagrelor pretreatment. Cohorts 7 to 10 received an 18-gram fixed dose of PB2452 via various infusion regimens or placebo. Infusions of PB2452 in these cohorts were initiated 2 hours after the last ticagrelor pretreatment dose to coincide with the peak concentration of ticagrelor. (Gurbel (2009)). Subjects in all cohorts were randomized in a 3:1 ratio to receive PB2452 or placebo.

[00292] Subject Eligibility: Eligible subjects were healthy males and female 18 to 50 years of age with a body mass index between 18 and 35 kg/m² and weight from ≥ 50 kg to ≤ 120 kg. Subjects with any contraindication to ticagrelor, medical history suggestive of an increased risk of bleeding, or estimated glomerular filtration rate below 60 mL/min/1.73m² were excluded. Written informed consent was obtained from all subjects.

[00293] *Outcomes*

[00294] The primary efficacy outcome was the effectiveness of PB2452 in reversing the antiplatelet effect of ticagrelor by analyzing platelet aggregation using light transmission aggregometry at multiple time points before and after PB2452 or placebo administration in ticagrelor pretreated subjects. Secondary efficacy outcomes were the effectiveness of ticagrelor reversal assessed using VerifyNow and the vasodilator stimulated phosphoprotein phosphorylation immunoassay (VASP).

[00295] The primary safety outcome was the frequency and severity of treatment emergent adverse events (AEs) associated with PB2452 with or without oral ticagrelor pretreatment. Clinical laboratory test results, vital sign measurements, 12-lead ECG and continuous telemetry results,

and physical examination findings were also assessed at multiple timepoints from screening to the end of the 28-day safety follow-up period. Immunogenicity was assessed before and at 7 and 28 days after PB2452 administration.

[00296] Other secondary outcomes included assessment of the PB2452 and ticagrelor pharmacokinetic profiles and evaluation of PB2452 immunogenicity at day 7 and 28 post-infusion.

Statistical Analysis

[00297] The sample size of 64 was based on clinical and practical considerations and not on a formal statistical power calculation. The sample size was considered sufficient to adequately assess the safety, efficacy, and pharmacokinetic profiles of PB2452 and the pharmacokinetic and pharmacodynamic profiles of ticagrelor. Reversal of antiplatelet effect was evaluated by comparing the mean percent of baseline platelet aggregation between PB2452 and placebo using Wilcoxon rank-sum test. Multiplicity was adjusted using the Bonferroni method. Correlations between each pair of the platelet function tests were evaluated using Pearson's correlation and Spearman's rank correlation coefficient. Mean data from subjects receiving placebo were pooled across cohorts for all presentations. Categorical variables are summarized by their frequencies and percentages, and continuous variables are presented with the number of subjects with non-missing data, mean, standard deviation, median, minimum, and maximum. Descriptive statistics are presented for each cohort, and pooled placebo and PB2452 data. For pharmacodynamic data, platelet aggregation results were compared between each cohort and pooled placebo data (cohorts 4 to 6 and cohort 7 to 10 were pooled separately). All analyses were performed with SAS software, version 9.4 (SAS Institute).

Results

[00298] **Study Population:** A total of 64 subjects were randomized. Of these, 48 received PB2452 and 16 received placebo. Of the 48 receiving PB2452, 21 received the highest dose of 18 grams and 39 received ticagrelor pretreatment. Final study design and subject flow diagrams are shown in **Table 1** and **Figure 6**, respectively. Baseline characteristics of the subjects are provided in **Table 2**.

Table 2 – Baseline Characteristics

	All Placebo (N=16)	All MEDI2452 (PB2452) (N=48)
Age (years), Mean (min-max)	34.0 (8.26)	30.5 (8.76)
Sex, n (%)		
Male	11 (68.8)	23 (47.9)
Female	5 (31.3)	25 (52.1)
Weight (kg), Mean (SD)	86.2 (13.5)	78.2 (14.8)
Height (cm), Mean (SD)	173.3 (6.4)	167.8 (10.2)
BMI (kg/m ²), Mean (SD)	28.6 (3.3)	27.7(4.1)
Ethnicity, n (%)		
Hispanic or Latino	6 (37.5)	22 (45.8)
Not Hispanic or Latino	10 (62.5)	26 (54.2)
Race, n (%)		
White	7 (43.8)	27 (56.3)
Black or African American	8 (50.0)	18 (37.5)
Asian	0	1 (2.1)
American Indian or Alaska Native	1 (6.3)	0
Native Hawaiian or Other Pacific Islander	0	1 (2.1)
Multiple	0	1 (2.1)
Platelet count, Mean (SD) (x1000/uL)	239 (52.1)	253 (46.8)
LTA Platelet aggregation, Mean (SD) (%)	82.1 (7.53)	82.9 (7.49)
VerifyNow P2Y12 PRU, Mean (SD)	226.4 (39.9)	237.7 (36.8)
VASP ELISA PRI, Mean (SD)	89.8 (4.17)	90.2 (3.64)

[00299] **Safety:** Overall PB2452 was considered safe and well-tolerated. A total of 30 treatment-emergent adverse events were reported in 19 of the 64 subjects (29.7%). Of the 48 subjects given PB2452, 17 (35.4%) reported 27 adverse events, whereas 2 of the 16 subjects given placebo (12.5%) reported 3 adverse events (**Table 3**). None of the adverse events was considered related to study drug. There were no study-drug related serious adverse events, dose-limiting toxicities, or infusion-related reactions. There were no deaths or adverse events leading to study drug discontinuation. One subject experienced 2 serious adverse events (alcohol poisoning and acute respiratory failure) after discharge from the clinical site both of which were considered

unrelated to study drug. With the exception of 1 subject in Cohort 3, all subjects with adverse events had received ticagrelor pretreatment. Changes in mean clinical laboratory results, vital sign measurements, and ECG values were similar across treatment groups and when compared with placebo.

Table 3 – Treatment-emergent Adverse Events, Safety Population

Preferred Term	All Placebo (N=16) n (%)	All PB2452 (N=48) n (%)	All Subjects (N=64) n (%)
Total Number of TEAEs	3	27	30
Number of Subjects with at Least 1 TEAE	2 (12.5)	17 (35.4)	19 (29.7)
Infusion site bruising	0	4 (8.3)	4 (6.3)
Medical device site reaction	0	3 (6.3)	3 (4.7)
Infusion site extravasation	0	2 (4.2)	2 (3.1)
Vessel puncture site bruise	0	2 (4.2)	2 (3.1)
Abdominal pain	0	1 (2.1)	1 (1.6)
Acute respiratory failure	0	1 (2.1)	1 (1.6)
Alcohol poisoning	0	1 (2.1)	1 (1.6)
Blood urine present	0	1 (2.1)	1 (1.6)
Conjunctivitis	0	1 (2.1)	1 (1.6)
Contusion	1 (6.3)	0	1 (1.6)
Dizziness	0	1 (2.1)	1 (1.6)
Eyelid irritation	1 (6.3)	0	1 (1.6)
Gastroenteritis	0	1 (2.1)	1 (1.6)
Hematuria	0	1 (2.1)	1 (1.6)
Infusion site reaction	0	1 (2.1)	1 (1.6)
Musculoskeletal chest pain	1 (6.3)	0	1 (1.6)
Nasopharyngitis	0	1 (2.1)	1 (1.6)
Oropharyngeal pain	0	1 (2.1)	1 (1.6)
Pharyngitis streptococcal	0	1 (2.1)	1 (1.6)
Pneumonia aspiration	0	1 (2.1)	1 (1.6)
Skin abrasion	0	1 (2.1)	1 (1.6)
Upper limb fracture	0	1 (2.1)	1 (1.6)

Note: A treatment-emergent AE (TEAE) is defined as any event not present before exposure to study drug or any event already present that worsens in intensity or frequency after the exposure. For analysis purposes, TEAEs are the events with onset on or after initiation of study drug. At each level of subject summarization, a subject is counted once if the subject reported 1 or more events. Percentages are based on the number of subjects in the safety population for each treatment group and overall. Adverse events are coded using MedDRA version 21.0.

Reversal of Ticagrelor

[00300] Ticagrelor reversal was assessed in Cohorts 4 to 10. Subjects were pretreated with ticagrelor for 48 hours prior to receiving PB2452 or placebo. In those receiving placebo, platelet aggregation was suppressed by 80-85% after 48 hours of ticagrelor pretreatment and remained suppressed for an additional 24 hours after ticagrelor was stopped. Platelet function gradually increased between 24 and 48 hours (**Figures 3-5; Figure 7A and 7B**), and no reversal was observed with administration of 1g of PB2452 (**Figure 2**). With PB2452, the first significant reversal of ticagrelor was observed at the 3.0 and 9.0 gram dose levels in Cohorts 5 and 6, respectively (**Figure 7A and 7B**). Significant but transient increases in mean platelet aggregation were observed at 30 minutes, the first time point immediately after completion of the 30 minute infusion. Duration of reversal was dose-dependent and lasted 1 to 3 hours (**Figure 7A**).

[00301] To achieve more rapid and sustained reversal, the total PB2452 dose was increased to 18 grams in Cohorts 7 to 10 to accommodate an initial bolus of PB2452 and longer infusions of 8, 12, or 16 hours. In Cohort 7, a 3.0 gram bolus of PB2452 followed by an 8 hour infusion significantly increased mean platelet aggregation by 2 hours with a duration of approximately 12 hours (**Figure 7B**). In Cohorts 8 to 10, the bolus was increased to 6.0 grams followed by infusions for 12 or 16 hours. With the larger bolus, reversal was achieved within 5 minutes of initiation of the infusion and was sustained for 16 to 24 hours (**Figure 7B**).

[00302] The statistically significant immediate and sustained reversal measured using the primary efficacy assessment of aggregometry was confirmed with VerifyNow and VASP (**Figure 8A and 8B**). Correlation analyses between each of the three platelet function test results demonstrated highly significant correlation for all comparisons with r values ≥ 0.81 and ≥ 0.91 for Pearson's and Spearman's analyses, respectively ($P < 0.0001$ for all comparisons, **Figure 9**).

[00303] To assess the extent to which normal platelet function was restored, post-PB2452 platelet function was compared with pre-ticagrelor baseline platelet function using all three assays. PB2452 restored mean platelet aggregation to within 80% of baseline at all post-treatment time points up to 20 hours (**Figure 10A**). Mean VerifyNow platelet reactivity revealed rapid and sustained normalization of platelet function with PRU ≥ 180 for 24 hours (**Figure 10B**). When restoration of P2Y₁₂ receptor signaling was assessed with VASP, PRI was restored to nearly 100% of baseline within 5 minutes and sustained for 20-24 hours (**Figure 10C**).

[00304] To determine whether acute ticagrelor reversal causes a potentially prothrombotic rebound increase in platelet aggregation, platelet aggregation in response to a low concentration of ADP was determined (5 μ M instead of the usual 20 μ M). Additionally, platelet aggregation in response to other agonists including arachidonic acid and thrombin receptor activating peptide was also tested. As expected, 5 μ M ADP elicited a lower response than 20 μ M ADP (**Figure 12A and 12B**), and the responses to arachidonic acid and thrombin receptor activating peptide after reversal were similar to baseline before ticagrelor or PB2425 administration (data not shown).

Pharmacokinetics

[00305] Pharmacokinetic analysis of PB2425 demonstrated dose-linear increases in mean exposure across the dose range of 0.1 to 9.0 grams (**Figure 11A**). The estimated distribution half-life and terminal half-life were 0.86 hours and 6.9 hours, respectively, with an estimated clearance of 1.88 L/min and a volume of distribution of approximately 2.9 L, suggesting PB2452 confinement to the vascular compartment. In subjects receiving ticagrelor alone, total ticagrelor concentrations peaked at 900 ng/mL approximately 2 hours after an oral dose. In the presence of PB2452, the mean concentration of circulating ticagrelor increased by approximately 2 – 5.6-fold (**Figure 11B**). At the 18 gram dose level with 8- to 16-hour infusions, PB2452 exposure was extended for 12-24 hours (**Figure 11C**), and mean ticagrelor concentrations increased by 5.6 fold compared with those in subjects given ticagrelor alone (**Figure 11D**). These increases in ticagrelor exposure appeared to be dependent on PB2452 dose and likely reflect tight binding between PB2452 and ticagrelor and the subsequent redistribution of extra-vascular ticagrelor into the vascular compartment.

Immunogenicity

[00306] Of the 48 subjects given PB2452, 21 (43.8%) had detectable anti-drug antibodies, of whom 15 (31.3%) had pre-existing (pre-dose) antibodies and 6 (12.5%) were positive post-dosing, albeit with low titers of 40 (n=5) or 160 (n=1). In subjects assigned to placebo, 3 of 16 (18.8%) tested positive, with 2 having pre-existing antibodies. All antibody titers were low and had no observed effect on PB2452 safety or efficacy.

Discussion

[00307] This study demonstrated that intravenous infusion of the monoclonal antibody fragment PB2452 significantly reversed the antiplatelet effects of ticagrelor as measured using

multiple sensitive assays of platelet function. Within the healthy population studied, there were no serious adverse events or infusion reactions associated with PB2452. Therefore, PB2452 represents an effective means to reverse ticagrelor's antiplatelet effect in patients with bleeding or in urgent need of hemostasis during invasive procedures.

[00308] The ability to reverse the action of the novel oral anticoagulants has been a major advance in anti-thrombotic therapy. Currently, there is no effective way to reverse the antiplatelet effects of oral P2Y₁₂ antagonists such as ticagrelor, other than to hold the drug and wait 3 to 5 days for its effects to dissipate, which is problematic in patients with life-threatening bleeding or at high risk of thrombosis. Treatment guidelines and ticagrelor prescribing information state to wait at least 5 days prior to surgical procedures. Waiting this long is impossible in patients who need emergent surgery and may be inadvisable in a patient needing urgent surgery. Unfortunately, platelet transfusion is not useful for patients taking ticagrelor because the drug will bind to the fresh platelets. (Godier (2015); Teng (2016)). Therefore, there is a need for a ticagrelor reversal agent.

[00309] PB2452 is a recombinant human IgG1 monoclonal antibody antigen-binding fragment that binds with high specificity to ticagrelor its active metabolite AR-C124910XX. (Buchanan (2015)). With its high affinity, PB2452 can neutralize free ticagrelor and drug bound to the P2Y₁₂ receptor, which without being bound by theory, explains the observed rapid reversal with PB2452. The mechanism of action is specific to ticagrelor only and will not work on clopidogrel or prasugrel, which are irreversible P2Y₁₂ receptor antagonists, nor is any other off-target binding be anticipated.

Conclusion

[00310] Intravenous PB2452 reverses the antiplatelet effects of ticagrelor. Administration of PB2452 may be a useful strategy for ticagrelor reversal in patients with serious bleeding or requiring urgent surgery or other invasive procedures.

Example 3 - Study to Evaluate the Safety, Tolerability, PK and PD of PB2452 in Older and Elderly Subjects Pre-dosed with Ticagrelor and Acetylsalicylic acid

[00311] The safety, tolerability, pharmacokinetics and pharmacodynamics of PB2452 will be compared to matching placebo, in older and elderly patients with ticagrelor and acetylsalicylic

acid (ASA) pretreatment in a Phase 2A, randomized, double-blind, placebo-controlled, single dose, sequential group study. Various dose levels and administration regimens will be administered to older (ages 50 to 64 years) and elderly (ages 65 to 80 years) male and female subjects.

[00312] Up to 5 dose levels and/or administration regimens will be evaluated in up to 5 cohorts. Each cohort will include 8 to 12 subjects randomized in a 3:1 ratio (PB2452:placebo).

[00313] The initial cohort (Cohort 1) will include approximately 8 subjects ages 50 to 64 years pretreated with ASA + ticagrelor who will be randomized to receive 18 grams (g) of PB2452 or matching placebo administered as an initial 6 g bolus infused over 10 minutes, followed by 12 g infused over the next 15 hours and 50 minutes to complete a 16 hour regimen. This initial regimen was shown to be safe and well tolerated in healthy young adults (18 to 50 years) in a prior Phase 1 study, and provided immediate and sustained reversal of the antiplatelet effects of ticagrelor.

[00314] Following completion of Cohort 1, subsequent cohorts may test the same, higher or lower dose levels, and/or different infusion regimens of PB2452 or matching placebo in the same population as in Cohort 1, or in different populations such as elderly subjects (65 to 80 years old).

[00315] The study will consist of a Screening period (Days -45 to -4), a Check-in day (Day -3) and Pretreatment Period, an on-site Randomization/Treatment day (Day 1), 3 days on-site for treatment and safety monitoring, a Follow-up Visit (Day 7), and a Final Follow-up visit (Day 28 [± 2 days]). Seven days prior to Randomization (Day -7), subjects will be administered 81 mg ASA orally once daily (QD) until the final dose on the morning of Day 1 before receiving study drug. A ticagrelor 180 mg oral loading dose will be administered on the morning of Day -2 followed by 90 mg every 12 hours until the 5th dose has been administered on the morning of Day 1. A 6th dose of ticagrelor may be administered 24 hours after the initiation of study drug in a subsequent cohort.

[00316] In the morning on Day -2, subjects will begin pretreatment with ticagrelor as described in the preceding paragraph. On Day 1, subjects will be randomized in a ratio 3:1 (PB2452:placebo), to receive an IV dose of PB2452 or placebo 2 hours following the 5th ticagrelor

dose. Subjects may be discharged from the clinical site between Days 3 and 7 inclusive and will return for a Follow-up visit on Day 7, if already discharged, and on Day 28 (± 2 days).

[00317] STUDY DRUG, DOSAGE, AND ROUTE OF ADMINISTRATION:

[00318] PB2452: PB2452 IV infusion will be administered on Day 1 for up to 48 hours. The total dose for each subject will not exceed 30 g. The infusion rate will not exceed 18 g over 30 minutes and the concentration will not exceed 24 g in 250mL. Subjects will not receive more than 250 mL of study drug infusion within any 1-hour period.

[00319] Matching Placebo: 0.9% sodium chloride single IV infusion, to be delivered at a rate and volume matching the active infusion.

[00320] Ticagrelor: Ticagrelor 90 mg oral tablet (immediate release) will be administered as a 180 mg (2×90 mg tablets) loading dose plus 90 mg every 12 hours for 4 additional doses. In one or more subsequent cohorts following cohort 1, subjects may also receive an additional single oral dose of 90 mg ticagrelor 24 hours after the initiation of the study drug infusion (6th ticagrelor dose).

[00321] Aspirin (acetylsalicylic acid; ASA): Aspirin (ASA) 81 mg oral tablet (enteric coated) will be administered daily between Day -7 and in the morning before receiving study medication on Day 1. Subjects may resume ASA after discharge from the study. Subjects entering the study who are already taking ASA daily must be willing to document a daily 81 mg dose between Day -7 and Day 1 and must suspend further ASA doses until discharge from the clinical facility.

[00322] EVALUATION CRITERIA:

[00323] Safety Assessments: Safety and tolerability will be assessed by monitoring and recording of AEs, clinical laboratory test results (hematology, coagulation, serum chemistry, and urinalysis), vital sign measurements (SBP and DBP, oral body temperature, respiratory rate [RR], and HR), 12 lead ECG, immunogenicity, biomarkers and physical examination findings.

[00324] Immunogenicity: Blood/serum samples will be screened for the presence of binding anti-drug antibodies (ADAs) at Check-in and on Days 1, 7 and 28 (± 2 days).

[00325] Pharmacodynamic Assessments:

[00326] PD data and parameters will be generated from PRU, LTA, and PRI assays:

[00327] VerifyNow®P2Y₁₂:

- [00328] • PRU at each assessment point
- [00329] • Percent of reversal in ticagrelor antiplatelet activity by PRU at each assessment point
- [00330] • Maximum PRU
- [00331] • Time to maximum PRU
- [00332] • Maximum PRU within 4 hours
- [00333] • Time to ≥ 180 PRU
- [00334] • Time to ≥ 200 PRU
- [00335] • Time to ≥ 220 PRU
- [00336] • Time to 60%, 80%, and 90% of reversal in PRU within 30 minutes or 4 hours
- [00337] LTA:
- [00338] • The maximum and final extent of aggregation for up to 4 platelet agonists, (20 μ M adenosine diphosphate [ADP], 5 μ M ADP, 1.6 mM arachidonic acid [AA], and 15 μ M thrombin receptor activating peptide [TRAP]), will be recorded at each assessment point.
- [00339] • will be recorded at each assessment point.
- [00340] • Percent of reversal in ticagrelor antiplatelet aggregation
- [00341] • Maximum platelet aggregation
- [00342] • Time to maximum platelet aggregation
- [00343] • Maximum platelet aggregation within 4 hours
- [00344] • Time to 60%, 80%, and 90% of reversal in platelet aggregation
- [00345] • Number and percent of subjects achieving 60%, 80%, and 90% of reversal in platelet aggregation within 30 minutes or 4 hours
- [00346] VASP by ELISA:
- [00347] • PRI at each assessment point;
- [00348] • Percent of reversal in PRI at each assessment point;
- [00349] • Maximum PRI;
- [00350] • Time to maximum PRI;
- [00351] • Maximum PRI within 4 hours;
- [00352] • Time to 60%, 80%, and 90% of reversal in PRI;

[00353] • Number and percent of subjects achieving 60%, 80%, and 90% of reversal in PRI within 30 minutes or 4 hours.

[00354] For Cohorts receiving a 6th dose of ticagrelor 24 hours following study drug, PD parameters will be calculated for Days 1 and Day 2 separately.

[00355] Pharmacokinetic Assessments:

[00356] Blood Collection for Plasma: Blood samples will be drawn at appropriate timepoints and plasma concentration of PB2452 at each sampling timepoint will be assessed. The following PK parameters will be calculated:

[00357] • Area under the plasma concentration versus time curve (AUC) from time zero to the time of the last quantifiable concentration ($AUC^{0-\tau}$)

[00358] • Observed maximum plasma concentration (C_{max})

[00359] • Time to reach the observed maximum plasma concentration (T_{max})

[00360] • AUC from time zero to 24 hours post-dose (AUC_{0-24})

[00361] • AUC from time zero to 48 hours post-dose (AUC_{0-48})

[00362] • AUC from time zero extrapolated to infinity ($AUC_{0-\infty}$; if data permit)

[00363] • Terminal elimination half-life ($t_{1/2}$; if data permit)

[00364] • Clearance (CL; if data permit)

[00365] Plasma PK concentration for ticagrelor and the metabolite TAM at each sampling timepoint will be assessed. The following PK parameters will be calculated:

[00366] • C_{max}

[00367] • T_{max}

[00368] • $AUC_{0-\tau}$

[00369] • AUC from time zero to 12 hours post-dose (AUC_{0-12})

[00370] • AUC_{0-24}

[00371] • AUC_{0-48}

[00372] • $AUC_{0-\infty}$; if data permit

[00373] • $t_{1/2}$; if data permit

[00374] For Cohorts receiving a 6th dose of ticagrelor 24 hours following study drug initiation, AUC_{0-48} will not be calculated for neither plasma PB2452 nor ticagrelor/TAM. The remaining PK parameters may be calculated for Days 1 and 2 separately.

[00375] Urine Collection Pooled urine samples to assess urine PB2452, ticagrelor, and TAM concentrations will be collected over these intervals: before dosing (within 60 minutes prior to the first ticagrelor dose on Day -2) and 0 to 6, 6 to 12, and 12 to 24 hours. In patients receiving a 6th dose of ticagrelor, pooled urine samples to assess urine ticagrelor and TAM concentrations will be collected over these intervals beginning with the 6th ticagrelor dose: 0 to 6, 6 to 12, 12 to 24 hours.

[00376] Pharmacokinetic parameters for PB2452, ticagrelor, and TAM concentrations in urine for all subjects in the PK population to be calculated are:

[00377] • Total amount of drug excreted in urine at 24 hours after dosing (A_{e24}) and at 48 hours after dosing (A_{e48})

[00378] • Total amount of drug excreted in urine from time t_1 to t_2 ($A_{e(t_1-t_2)}$) hours when the values of t_1 to t_2 are 0 to 6, 6 to 12, 12 to 24 and 24 to 48 hours

[00379] • Fraction excreted in urine from 1 to 24 hours after dosing (F_{e24}) and from 1 to 48 hours after dosing (F_{e48})

[00380] • Renal clearance (CL_r) for 24 hours after dosing

[00381] For Cohorts receiving a 6th dose of ticagrelor 24 hours following study drug initiation, F_{e48} and A_{e24-48} will not be calculated. Other urine PK parameters may be calculated for Day 1 and 2 separately.

Example 4 - Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of PB2452 with High-Dose Ticagrelor Pretreatment in Healthy Subjects

[00382] A cohort of healthy subjects pretreated with 180 mg of oral ticagrelor twice daily for 48 hours was randomized in a 3:1 ratio (PB2452:placebo) to a dose and regimen of PB2452 or placebo. The dose regime of PB2452 was 12 g infused over 10 minutes, followed by 12 g over 6 hours, followed by 12 g over 18 hours. Platelet function was determined as described above.

INCORPORATION BY REFERENCE

[00383] All publications, patents, and patent publications cited are incorporated by reference herein in their entirety for all purposes.

[00384] This application incorporates by reference the following publication in its entirety for all purposes: US 2016/0130366.

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CLAIMS

1. A method of reversing ticagrelor-associated bleeding, or the risk of said bleeding, in a patient in need thereof comprising administering to said patient a composition comprising an effective amount of a pharmaceutical composition comprising an antibody or fragment thereof that binds to ticagrelor ((1*S*,2*S*,3*R*,5*S*)-3-[7-{{(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl}amino}-5-(propylthio)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol) or a metabolite or derivative thereof.

2. The method of claim 1, wherein the antibody or a fragment thereof comprises complementarity-determining region (CDR) combinations selected from the group consisting of:

a) SEQ ID NO:53 (VH CDR1), SEQ ID NO:54 (VH CDR2), SEQ ID NO:55 (VH CDR3), SEQ ID NO:58 (VL CDR1), SEQ ID NO:59 (VL CDR2), and SEQ ID NO:60 (VL CDR3);

b) SEQ ID NO:63 (VH CDR1), SEQ ID NO:64 (VH CDR2), SEQ ID NO:65 (VH CDR3), SEQ ID NO:68 (VL CDR1), SEQ ID NO:69 (VL CDR2), and SEQ ID NO:70 (VL CDR3); and

c) SEQ ID NO:73 (VH CDR1), SEQ ID NO:74 (VH CDR2), SEQ ID NO:75 (VH CDR3), SEQ ID NO:78 (VL CDR1), SEQ ID NO:79 (VL CDR2), and SEQ ID NO:80 (VL CDR3).

3. The method of claim 1, wherein the antibody or a fragment thereof comprises a combination of heavy chain variable region (VH) and light chain variable region (VL) sequences selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:57; SEQ ID NO:62 and SEQ ID NO:67; and SEQ ID NO:72 and SEQ ID NO:77.

4. The method of claim 1, wherein the patient has been administered ticagrelor before administration of the anti-ticagrelor antibody or fragment thereof.

5. The method of claim 1, wherein the antibody or fragment thereof is a Fab and the patient is administered a dose between about 1 g and about 48 g.

6. The method of claim 5, wherein the dose is between about 9 g to about 18 g of the Fab.
7. The method of claim 5, wherein the patient is administered a dose of about 1 g, about 3 g, about 9 g, about 18 g, about 24 g, about 30 g, about 36 g or about 48 g of the Fab.
8. The method of any of claims 1-7, wherein the pharmaceutical composition is administered to the patient intravenously.
9. The method of claim 8, wherein the pharmaceutical composition is administered intravenously over about 15 minutes to about 36 hours.
10. The method of claims 8 or 9, wherein the pharmaceutical composition is administered in two or more segments.
11. The method of claim 10, wherein the first segment is a bolus.
12. The method of claims 10 or 11, wherein the administration rates for each of the segments differ.
13. The method of claim 12, wherein the administration rates for each of the segments differ for successive segments of the infusion.
14. The method of any of claims 10-13, wherein the pharmaceutical composition is administered in three or more segments, wherein the administration rates for each of the segments differ for successive segments of the infusion.
15. The method of any of claims 5-14, wherein the pharmaceutical composition is administered in the following schedule: 12g infused over 10 minutes, followed by 12g over 6 hours, followed by 12g over 18 hours.
16. The method of claim 1, wherein the pharmaceutical composition comprises about 50 mg/mL to about 200 mg/mL of the anti-ticagrelor antibody or fragment thereof, about 5 mM to about 50 mM histidine/histidine hydrochloride buffer, about 100 mM to about 300 mM sucrose, and about 0.01% (w/v) to about 1.0% (w/v) polysorbate 80, pH 5.5 to 6.5
17. The method of claim 16, wherein the pharmaceutical formulation comprises 100 mg/mL of the anti-ticagrelor antibody or fragment thereof, 25 mM histidine/histidine hydrochloride buffer, 290 mM sucrose, and 0.05% (w/v) polysorbate 80, pH 6.0.

18. The method of claim 16 or 17, wherein the pharmaceutical formulation is diluted in saline for administration.
19. The method of claim 1, wherein the ticagrelor-associated bleeding is major bleeding.
20. The method of claim 19, wherein the major bleeding is characterized by being life-threatening, potentially leading to clinically significant disability, requiring surgery to control the bleeding, requiring treatment with blood products, or is acute bleeding associated with a clinically important drop in hemoglobin.
21. The method of claim 1, wherein the patient requires urgent surgery or intervention.
22. The method of claim 21, wherein the urgent surgery or intervention is known to be associated with a significant risk of bleeding, such as coronary artery bypass surgery, has an adverse surgical outcome if bleeding is not carefully controlled, neurological, ophthalmologic, or joint replacement surgery, associated with risk of experiencing perioperative events; or in a patient at high risk of thrombosis if dual antiplatelet therapy is withheld preoperatively.
23. The method of claim 1, wherein the patient is at risk of developing, or has been diagnosed with Acute Coronary Syndrome (ACS).
24. The method of claim 1, wherein the patient is at risk of developing, or has been diagnosed with a disease selected from the group consisting of myocardial infarction (MI), unstable angina, stable ischemic heart disease, in sickle cell disease, including pediatric patients, atrial fibrillation, coronary arterial disease, peripheral arterial disease, ischemic stroke, one or more coronary stents, carotid artery stents, stents following an intracranial aneurysm, and arterio-venous fistulae created for hemodialysis.
25. The method of claim 1, wherein the patient is a pediatric patient.
26. The method of claim 25, wherein the pediatric patient is younger than 18 years old.
27. The method of claim 26, wherein the pediatric patient is younger than 2 years old.
28. The method of claim 1, wherein the patient is an adult patient.
29. The method of claim 28, wherein the adult patient is between 18 and 64 years old inclusive.
30. The method of claim 28, wherein the patient is over 65 years old.

31. The method of claim 30, wherein the patient is between 65 and 80 years old inclusive.
32. The method of claim 4, wherein the patient has been administered aspirin (acetylsalicylic acid).
33. The method of any of claims 1-32, wherein administration of the antibody or fragment thereof reverses ticagrelor activity.
34. The method of claim 33, wherein administration of the antibody or fragment thereof restores platelet function.
35. The method of claim 34, wherein administration of the antibody or fragment thereof restores platelet aggregation.
36. The method of claim 35, wherein administration of the antibody or fragment thereof restores platelet aggregation to at least 80% of baseline.
37. The method of claim 35 or 36, wherein administration of the antibody or fragment thereof restores platelet aggregation within 1 minute to 60 minutes of administration.
38. The method of claim 37, wherein administration of the antibody or fragment thereof restores platelet aggregation within 5 minutes of administration.
39. The method of any of claims 35-38, wherein administration of the antibody or fragment thereof provides a sustained restoration of platelet aggregation.
40. The method of claim 39, wherein the restoration of platelet aggregation is sustained for at least 12 hours after administration.
41. The method of claim 40, wherein the restoration of platelet aggregation is sustained for at least 16 hours after administration.
42. The method of claim 41, wherein the restoration of platelet aggregation is sustained for at least 24 hours after administration.

FIGURES

Figure 1

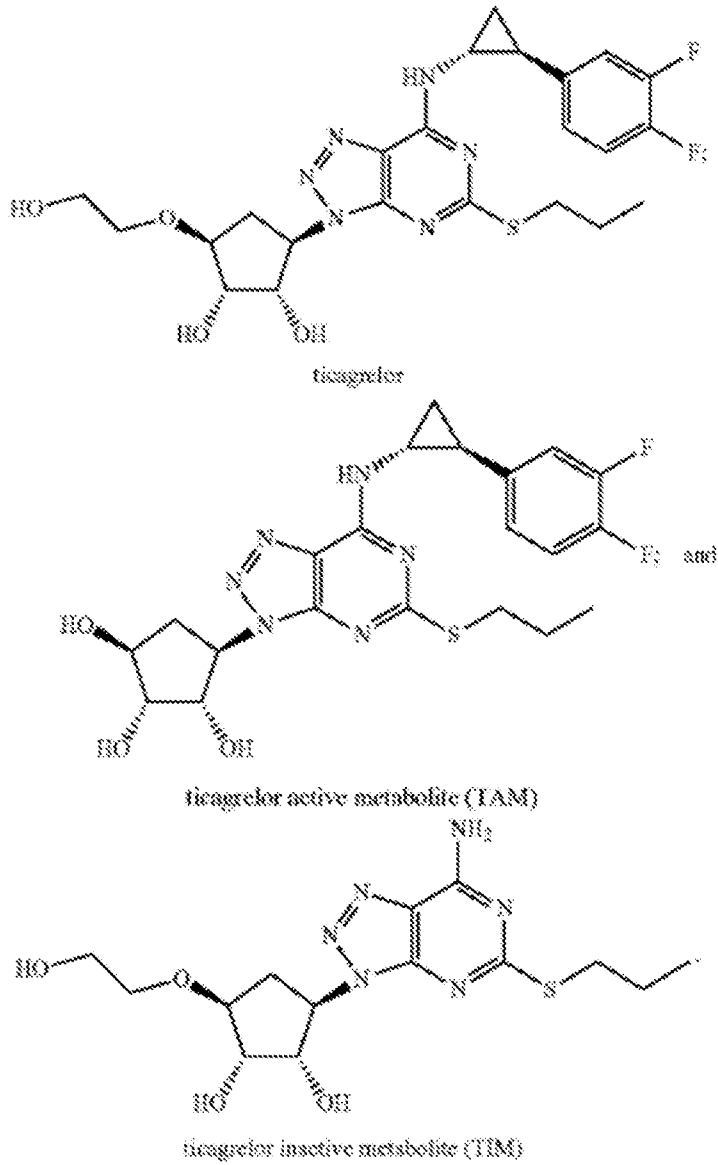
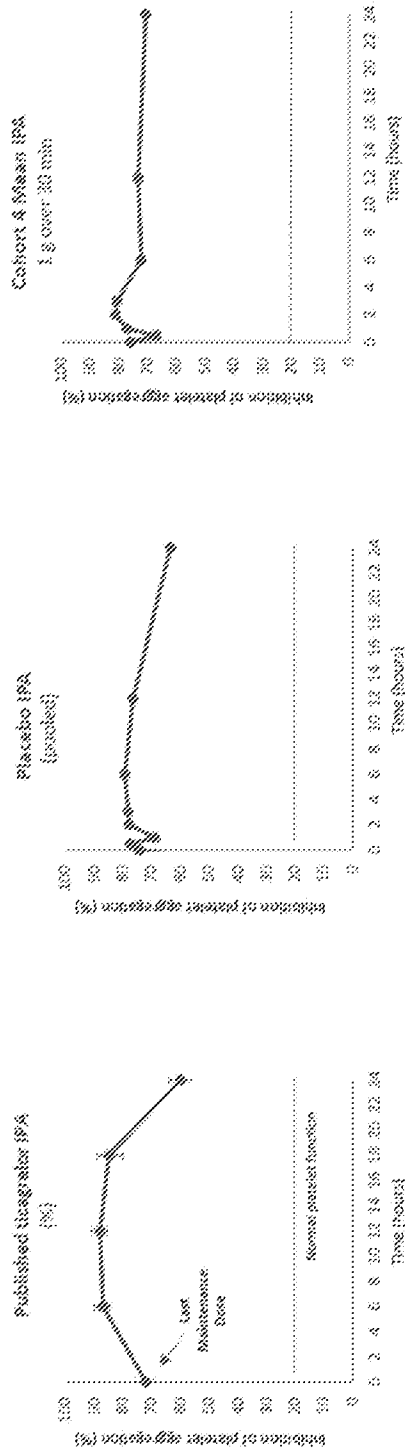


Figure 2

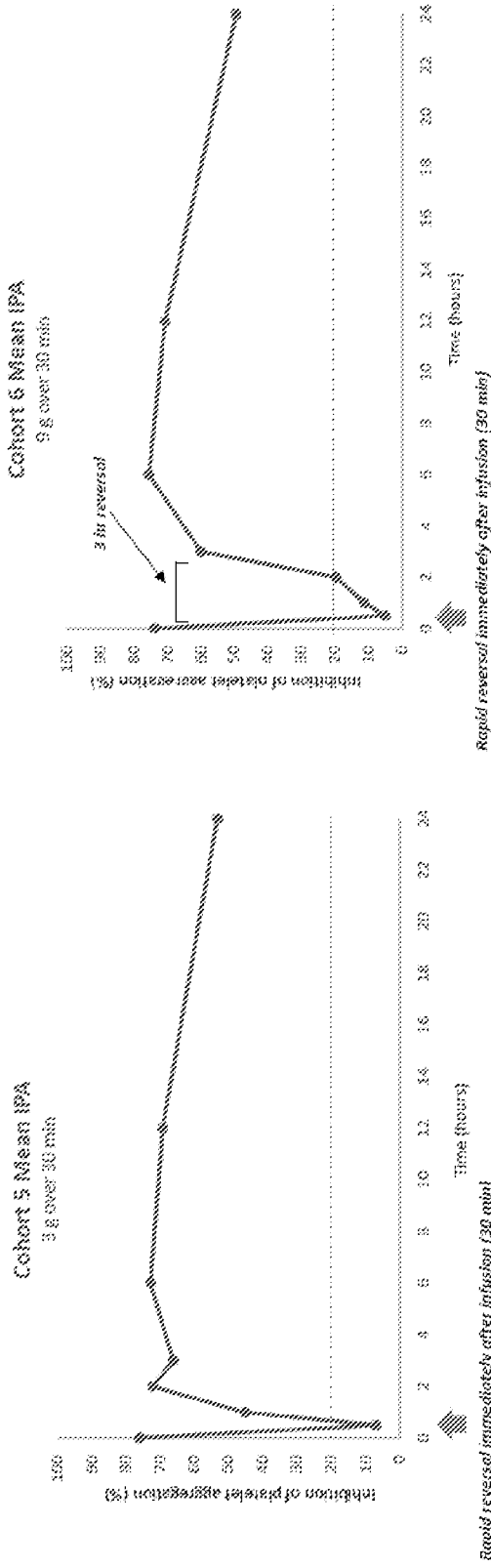


ONSET-ONSET study, September 2015

- * IPA assesses ticagrelor’s inhibition of platelet aggregation
 - IPA peaks at 2 - 4 hours after last oral dose at 80 - 90% inhibition (left graph)
 - Complete PB2452 reversal = IPA reduction to <20%
- * SAD subjects are pre-dosed with ticagrelor for 48 hours prior to PB2452 IV administration
 - As expected, no reversal observed with 1 g of PB2452

Figure 3

Complete and Rapid Reversal Achieved in Cohorts 5 and 6 3 g or 9 g delivered as 30 minute IV infusion

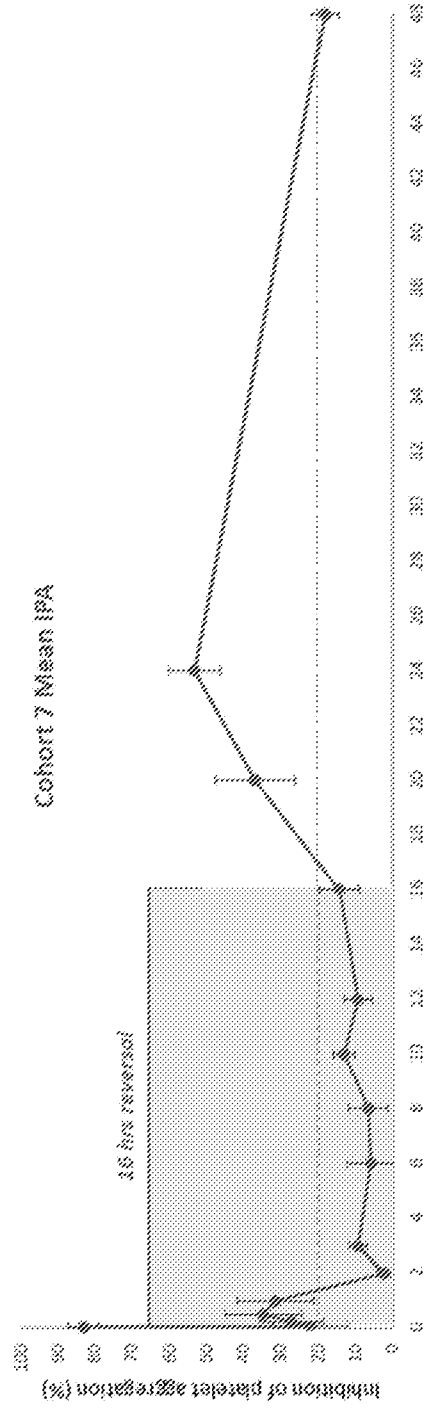


Cohorts 5 and 6: Rapid, complete reversal with short duration when given as 30 minute IV infusion

- * Complete restoration of platelet function at first sampling time point - 30 min, faster than expected
- * Speed to reversal critical for patients with active bleeding
- * Duration of response was dose-dependent

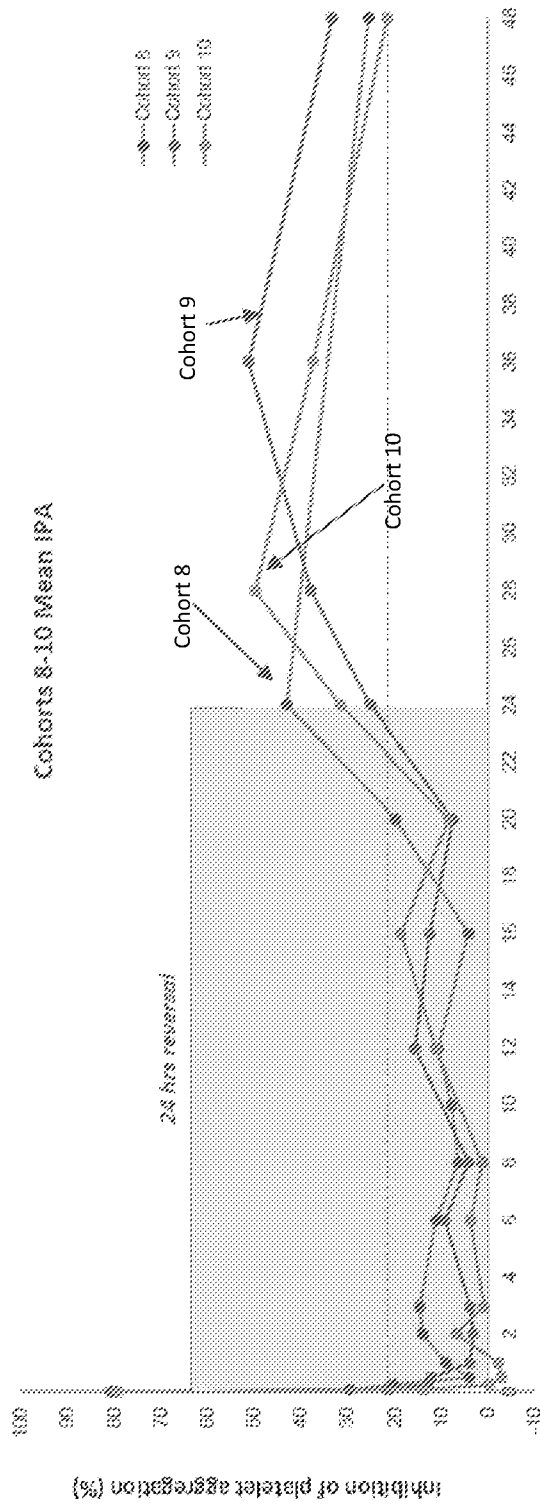
Figure 4

Complete, Sustained Reversal Achieved in Cohort 7 3g 5 min bolus followed by 15g 7h 55 min IV infusion



- * Clean safety profile: no drug-related AEs
- * Reversal in 3 subjects by 5 min
- * 16 hr of reversal is clinically important however would like to extend duration of effect
- * Verify Now correlates well to IPA

Figure 5
Cohorts 8, 9 and 10



Cohorts 8, 9, and 10 achieved study objectives, immediate reversal and sustained duration

- * Clean safety profile: no drug-related AEs or SAEs
- * Duration of reversal extended to 24 hours, significantly beyond Cohort 7 (16 hours)

Figure 6A

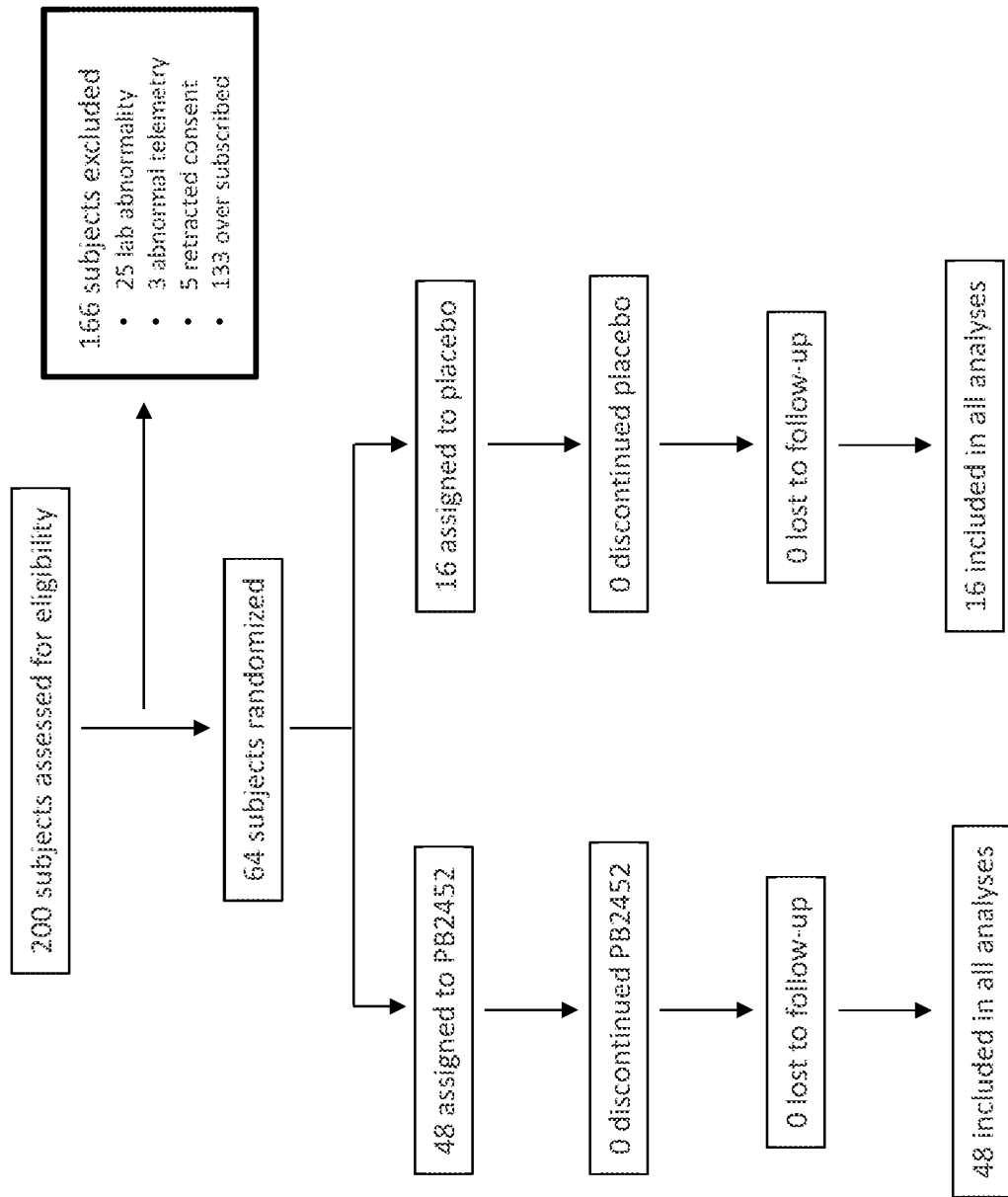


Figure 6B

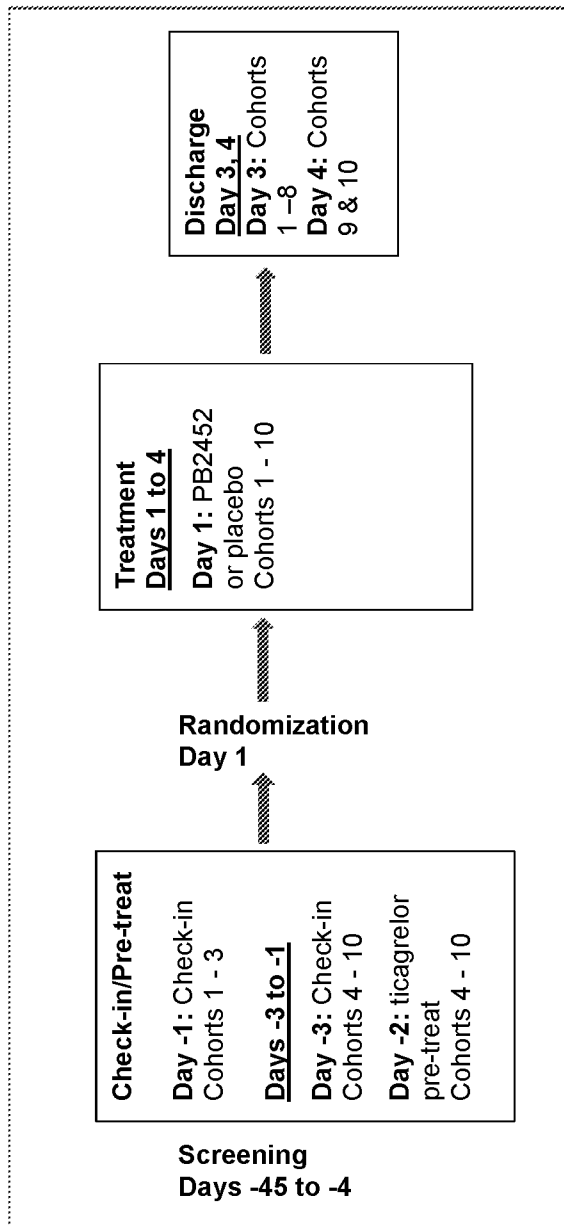
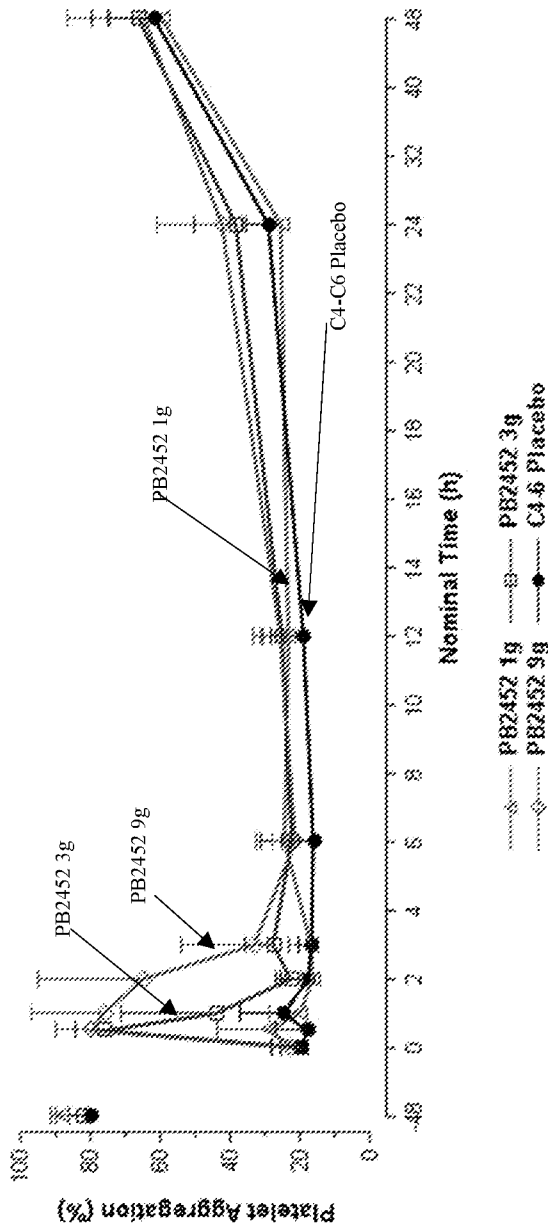


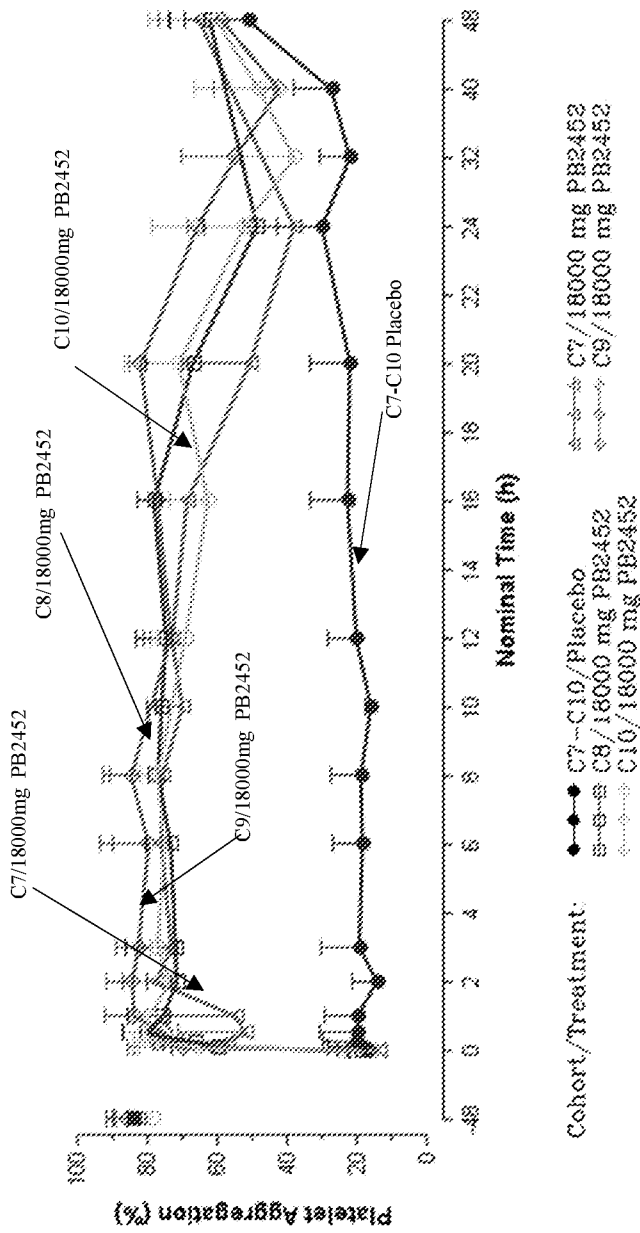
Figure 7A



P values by timepoint for each cohort

Cohort	0.5 hr	1 hr	2 hr	3 hr	6 hr	12 hr	24 hr	48 hr
4	1	1	1	1	1	1	1	1
5	0.0216	1	1	0.1515	1	1	1	1
6	0.0216	0.0433	0.0433	0.0866	1	1	0.5844	1

Figure 7B



P values by timepoint for each cohort

Cohort	5min	0.25hr	0.5hr	1hr	2hr	3hr	6hr	8hr	10hr	12hr	16hr	20hr
7	0.040	0.040	0.131	0.037	0.040	0.019	0.019	0.019	0.152	0.019	0.019	0.224
8	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.152	0.019	0.019	0.019
10	0.043	0.020	0.020	0.020	0.020	0.020	0.020	0.020	.	0.020	0.020	0.020

Due to the small sample size for cohort 9 (n=3), statistical testing was not performed.

Figure 8A

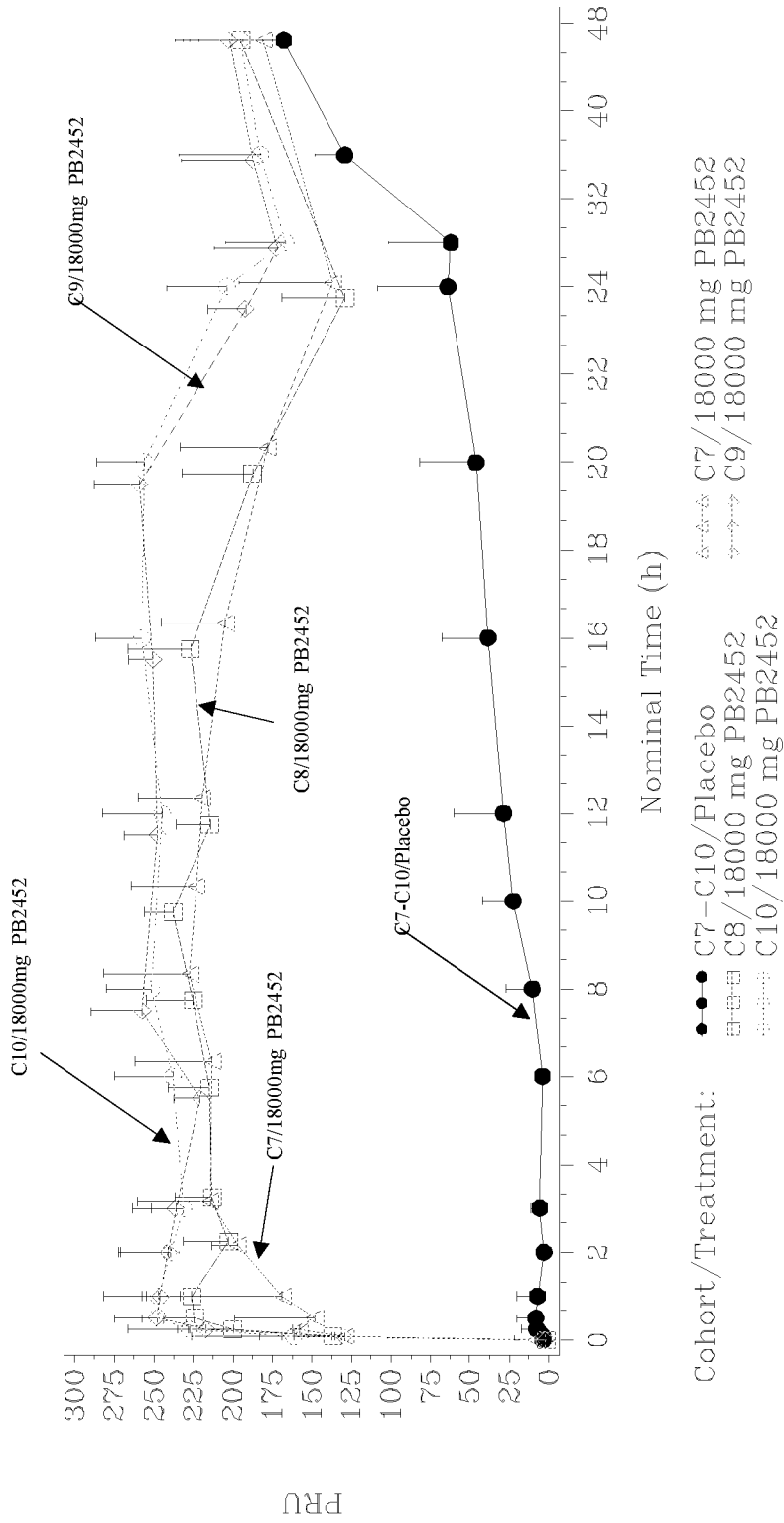
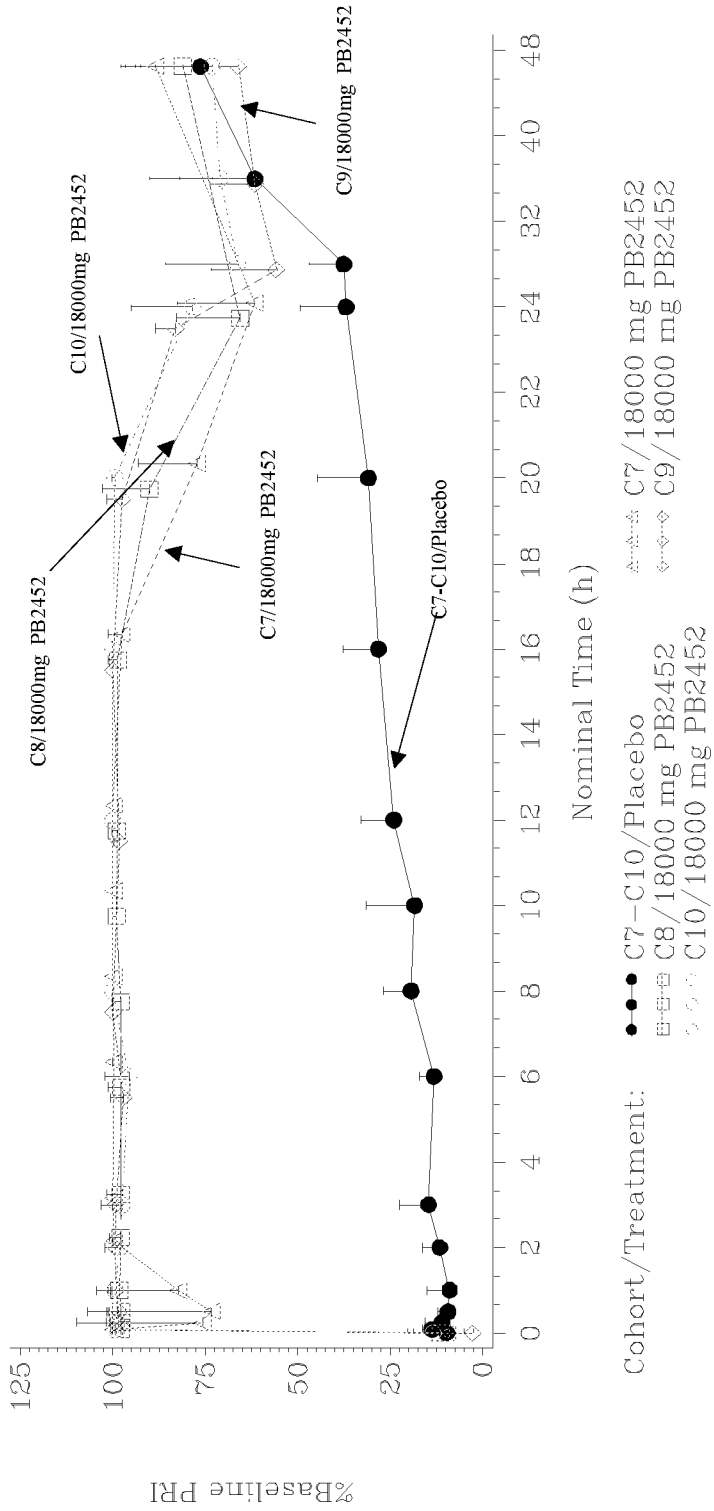


Figure 8B



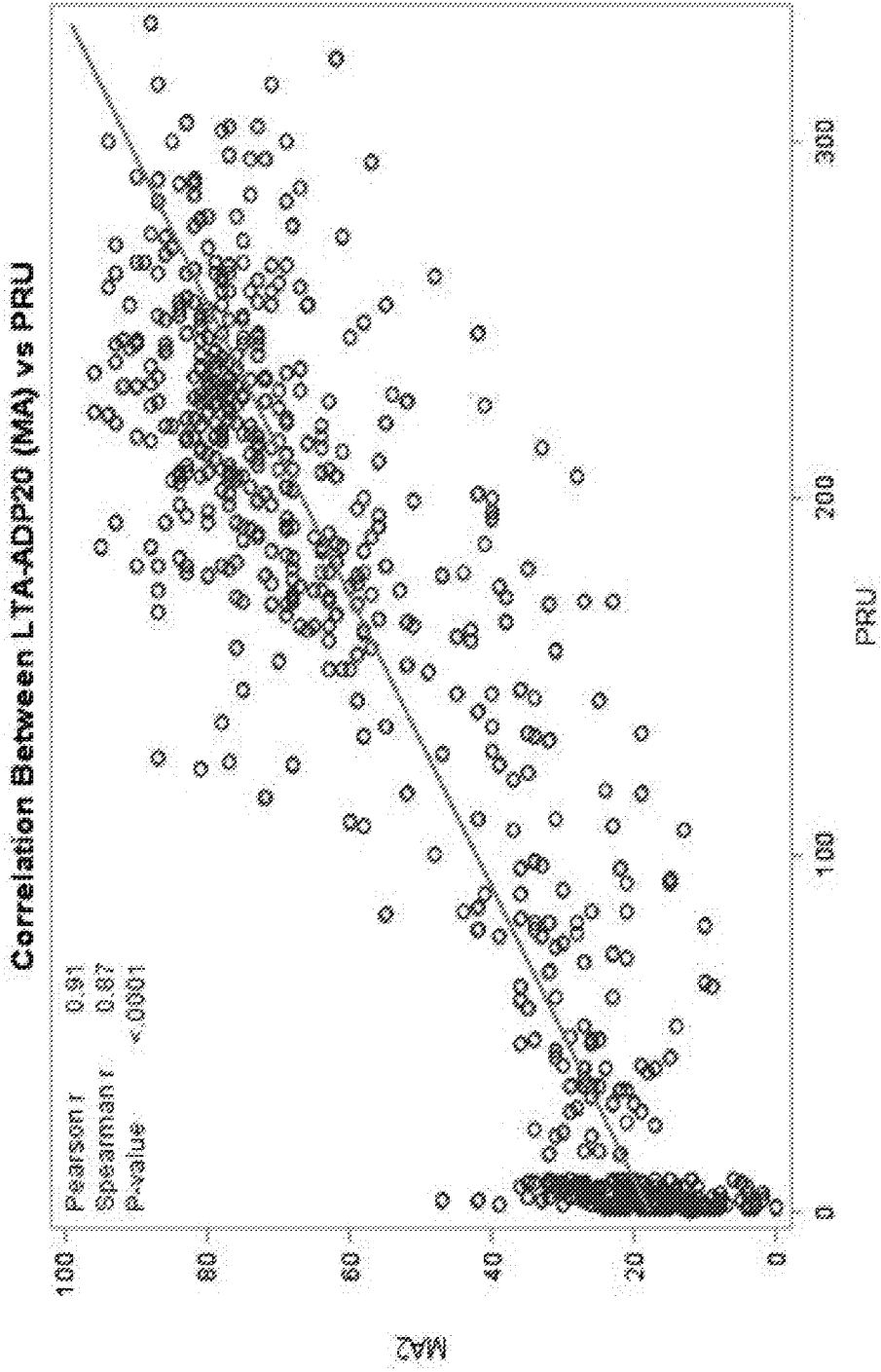
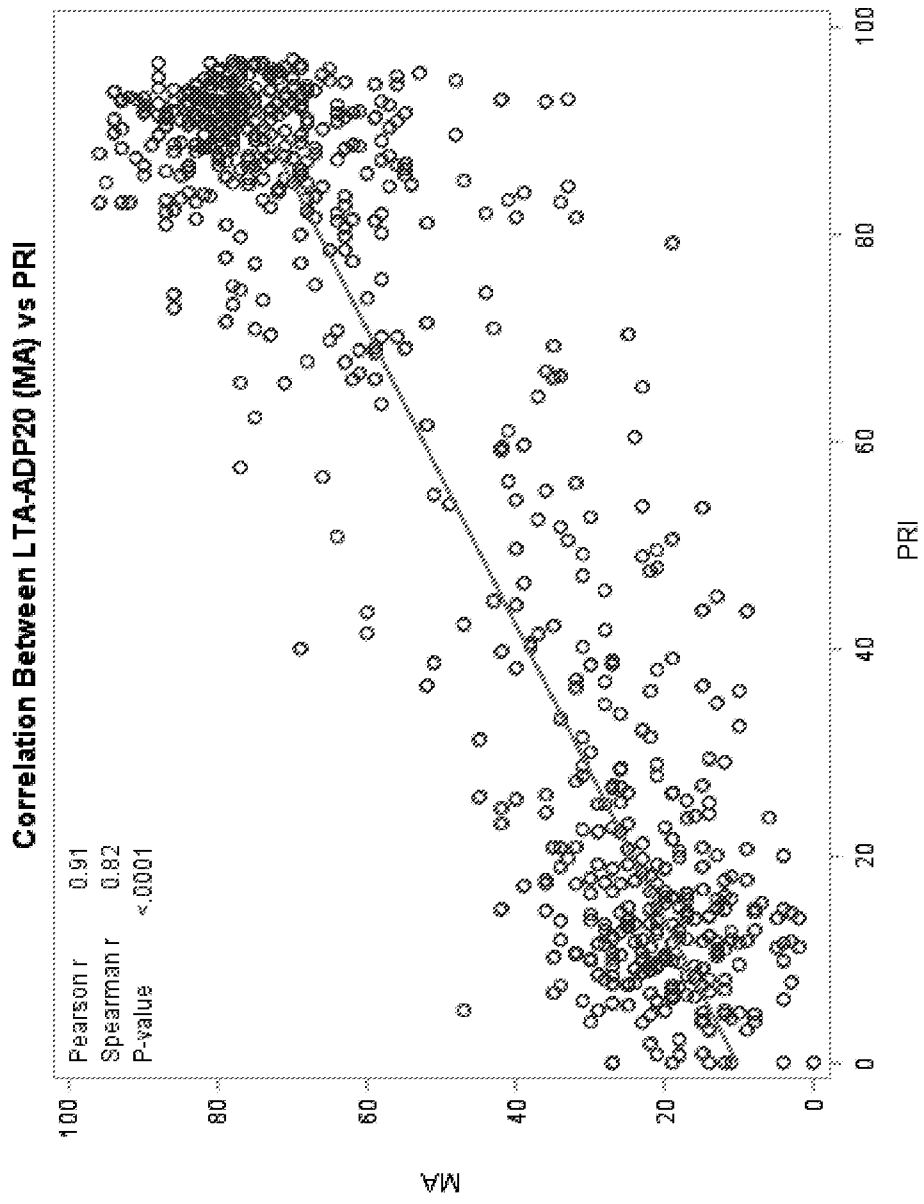


Figure 9A

Figure 9B



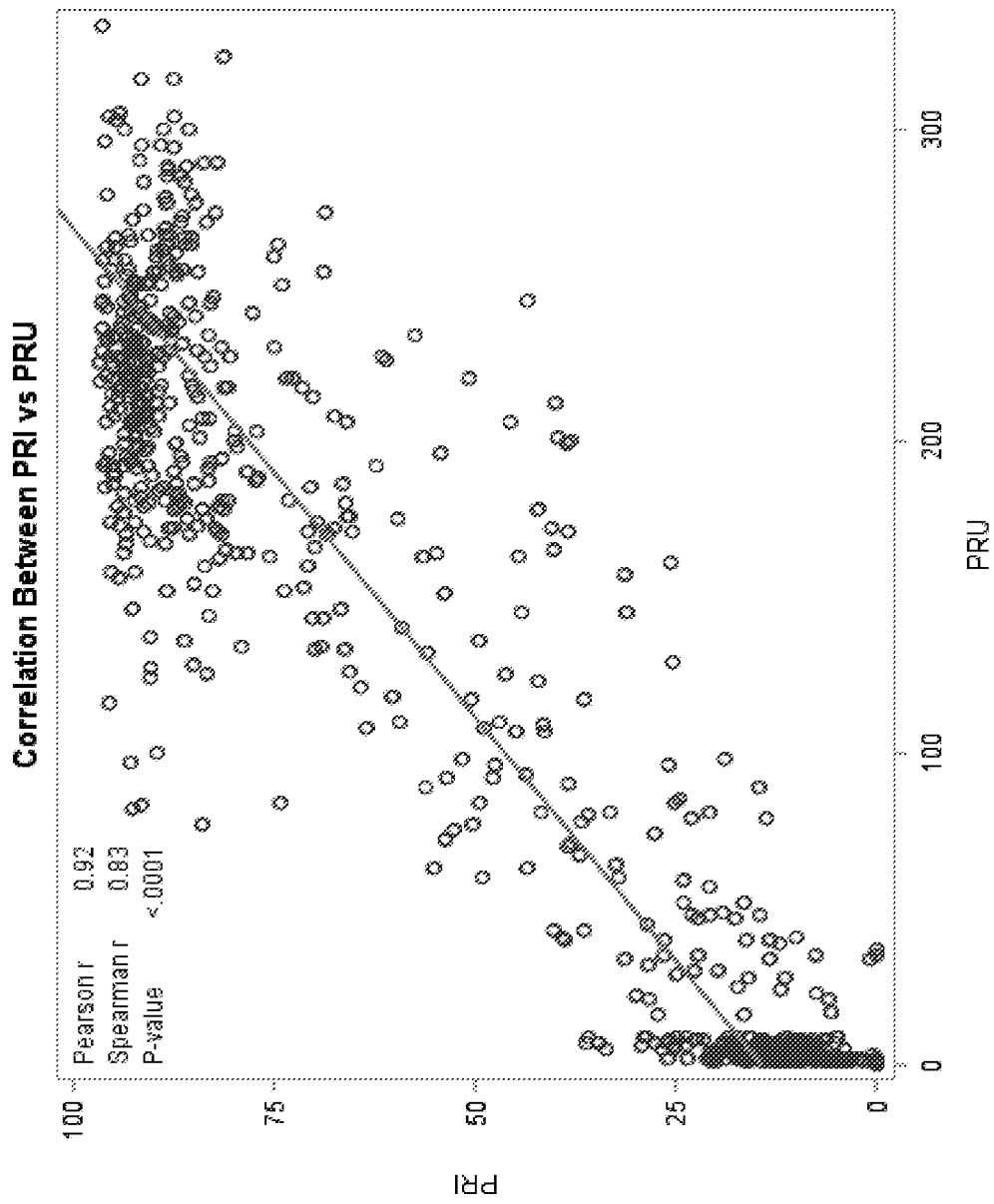


Figure 9C

Figure 10A

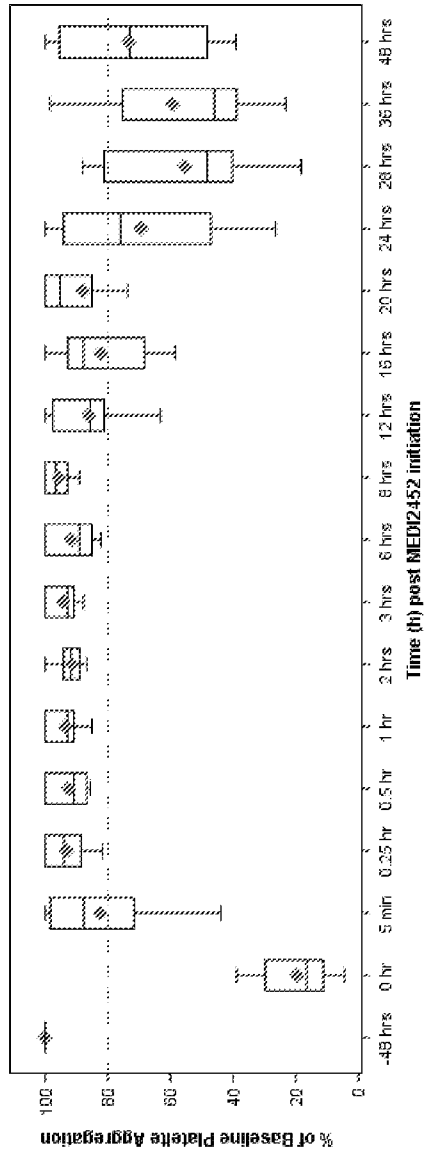


Figure 10B

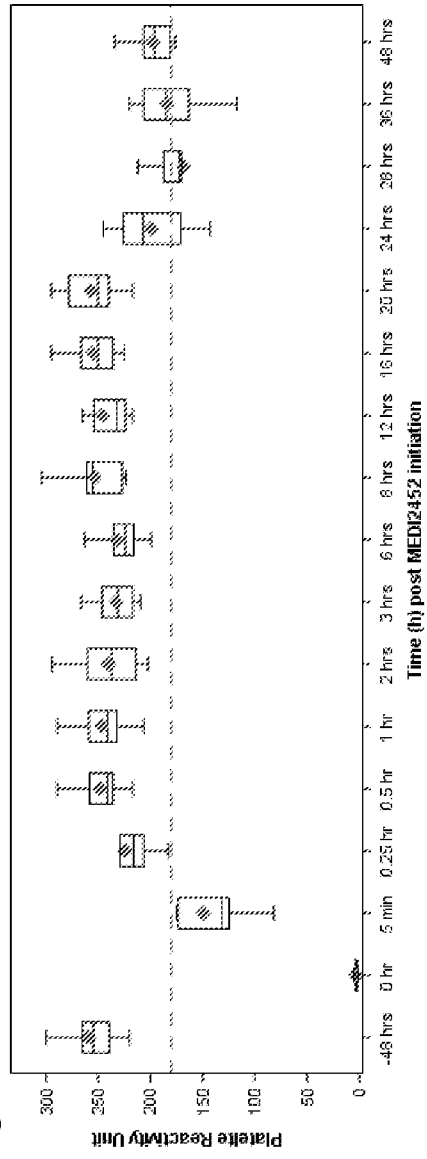


Figure 10C

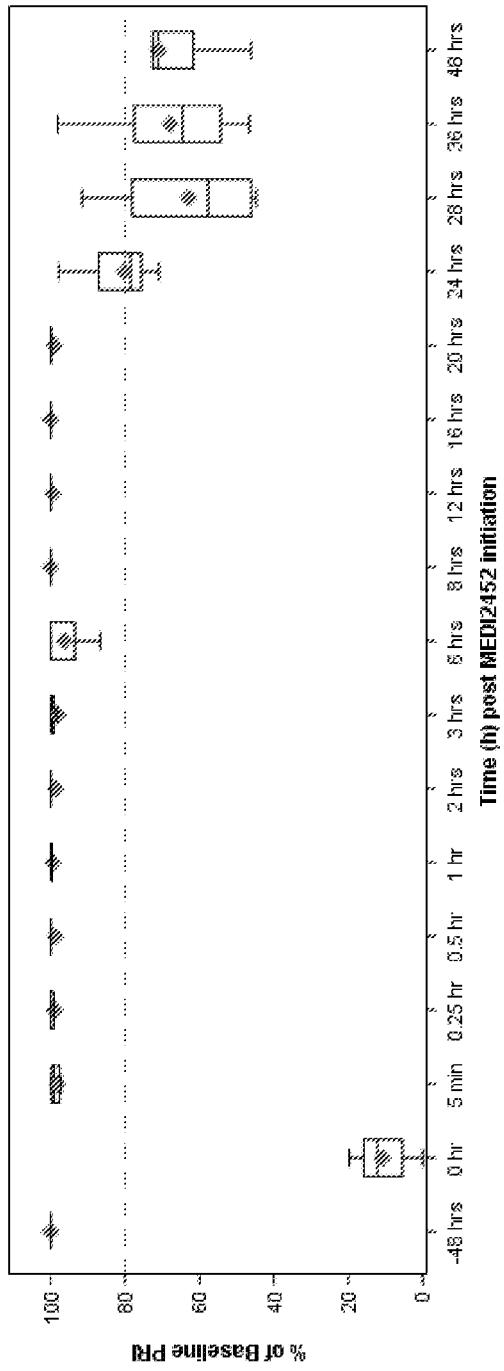


Figure 11A

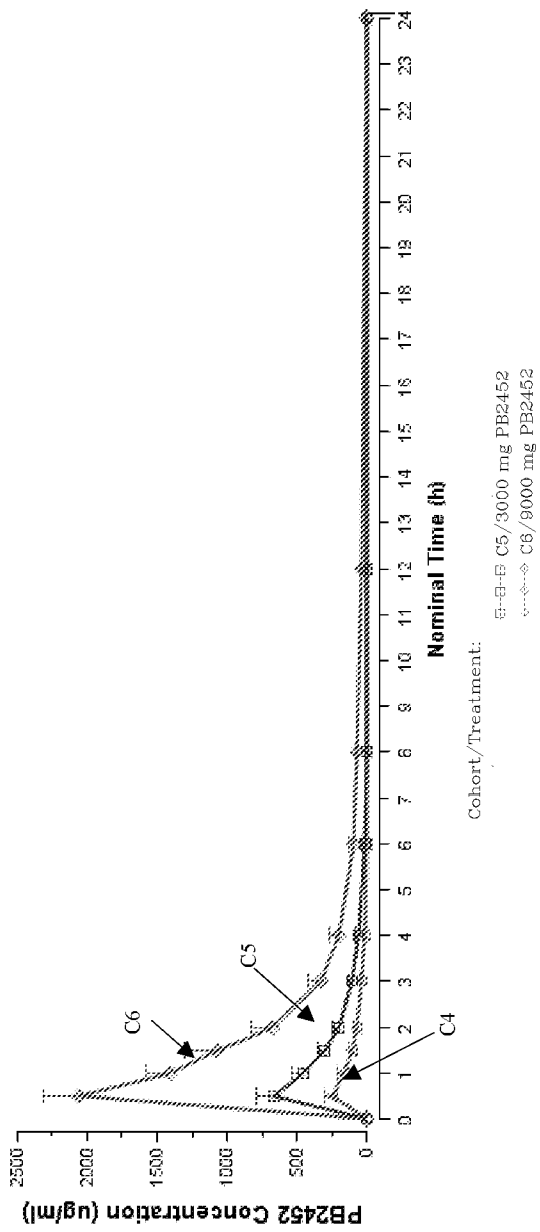


Figure 11B

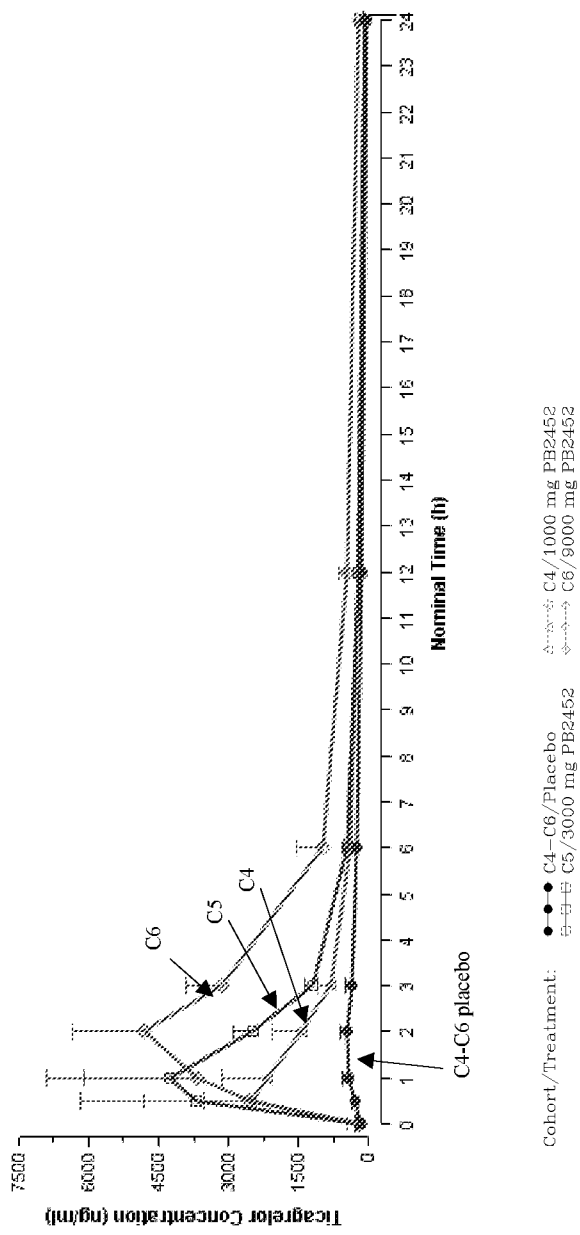


Figure 11C

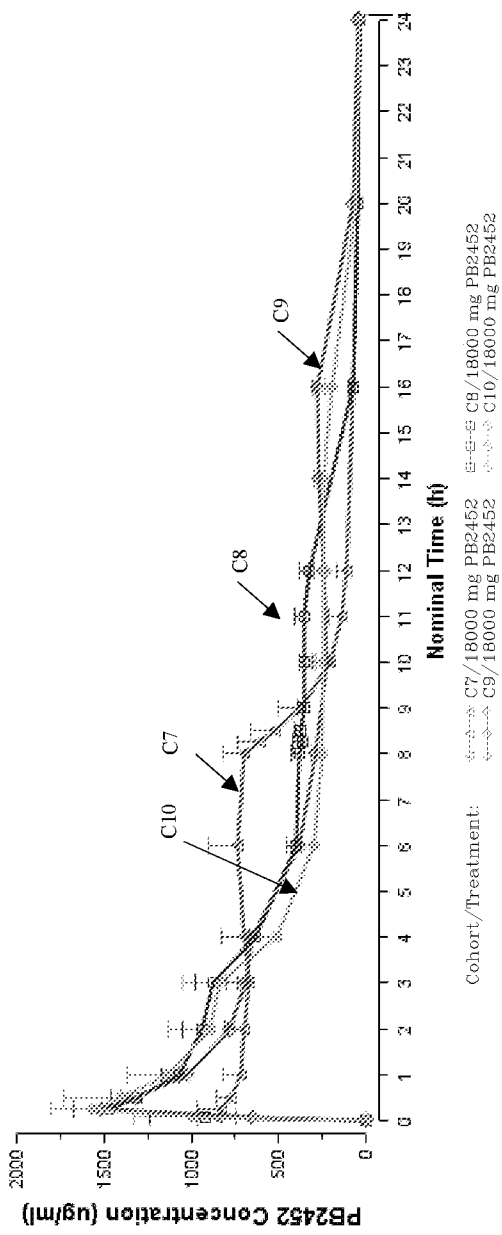


Figure 11D

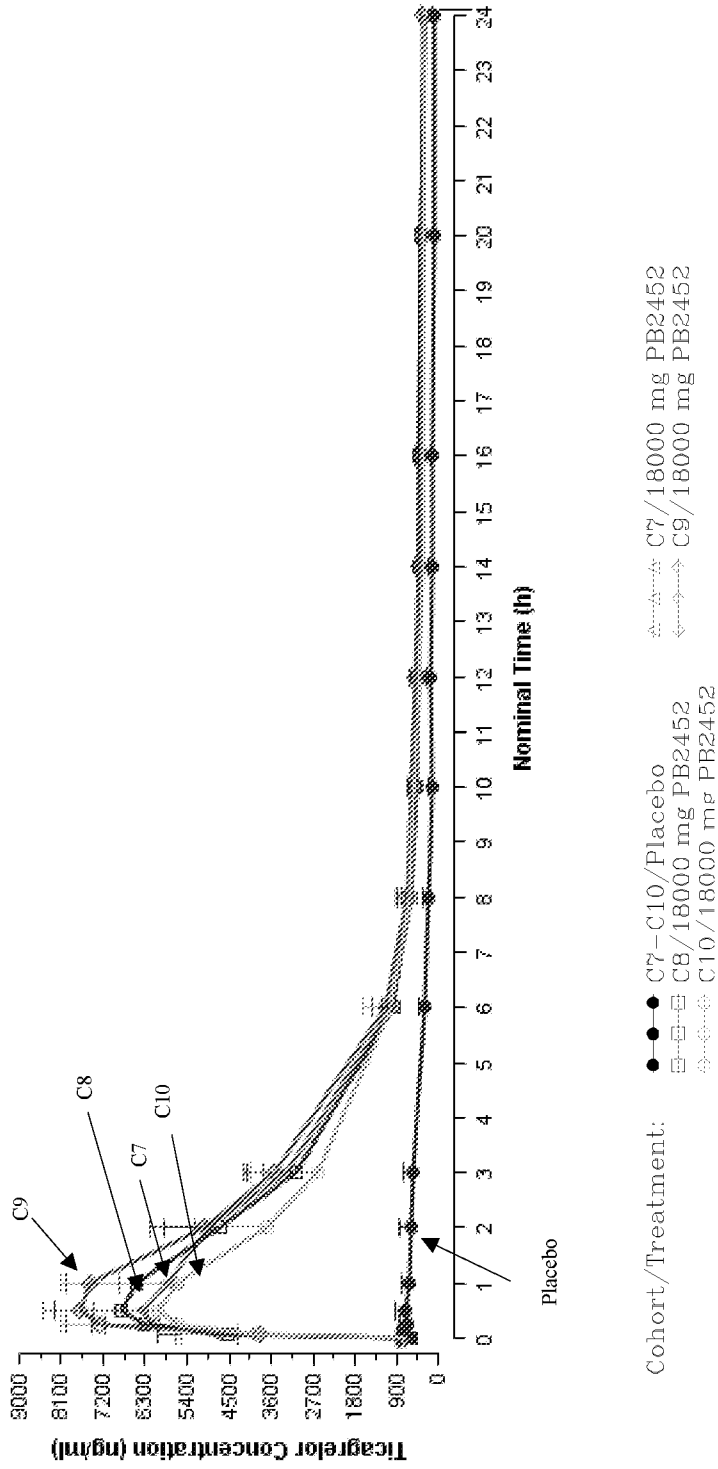


Figure 12A

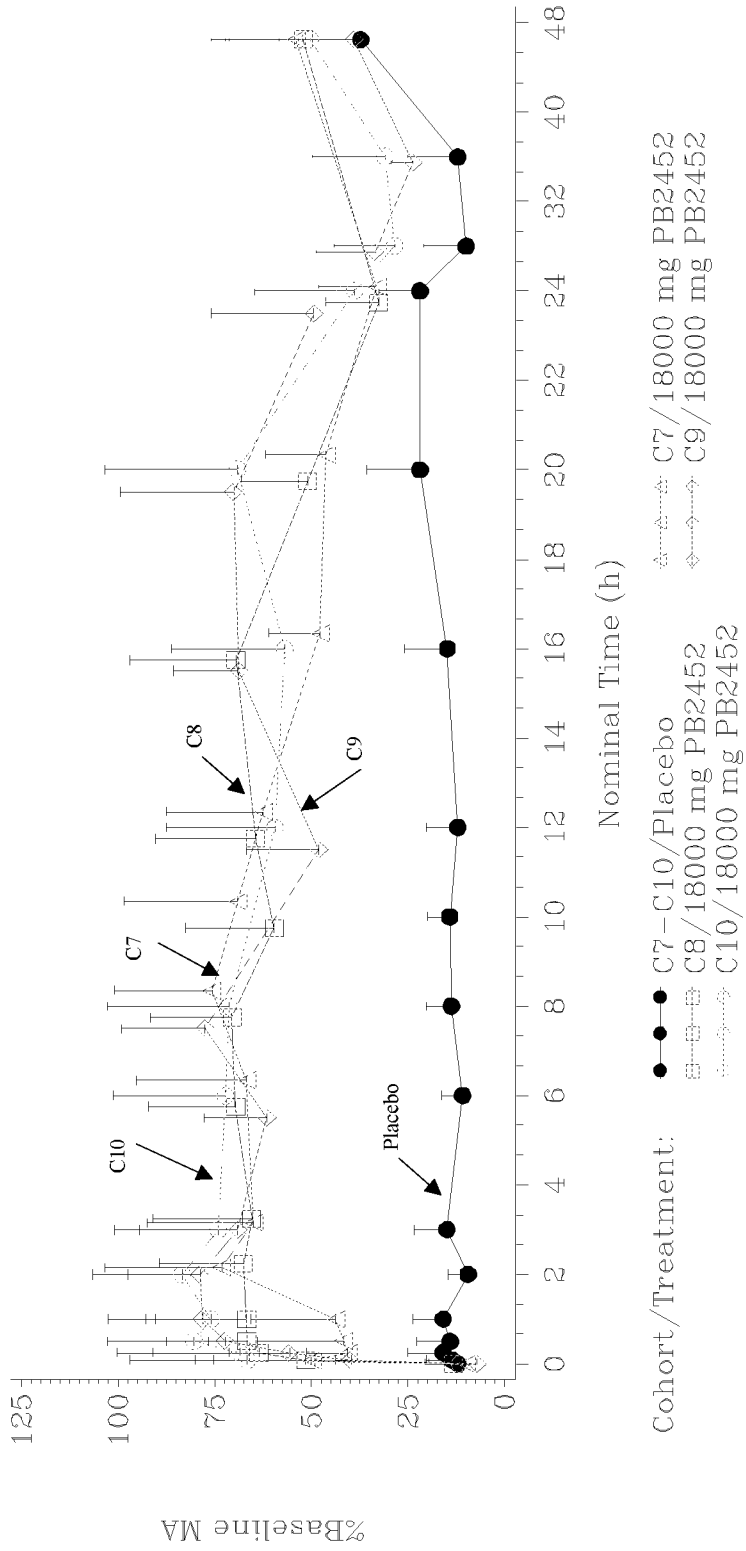
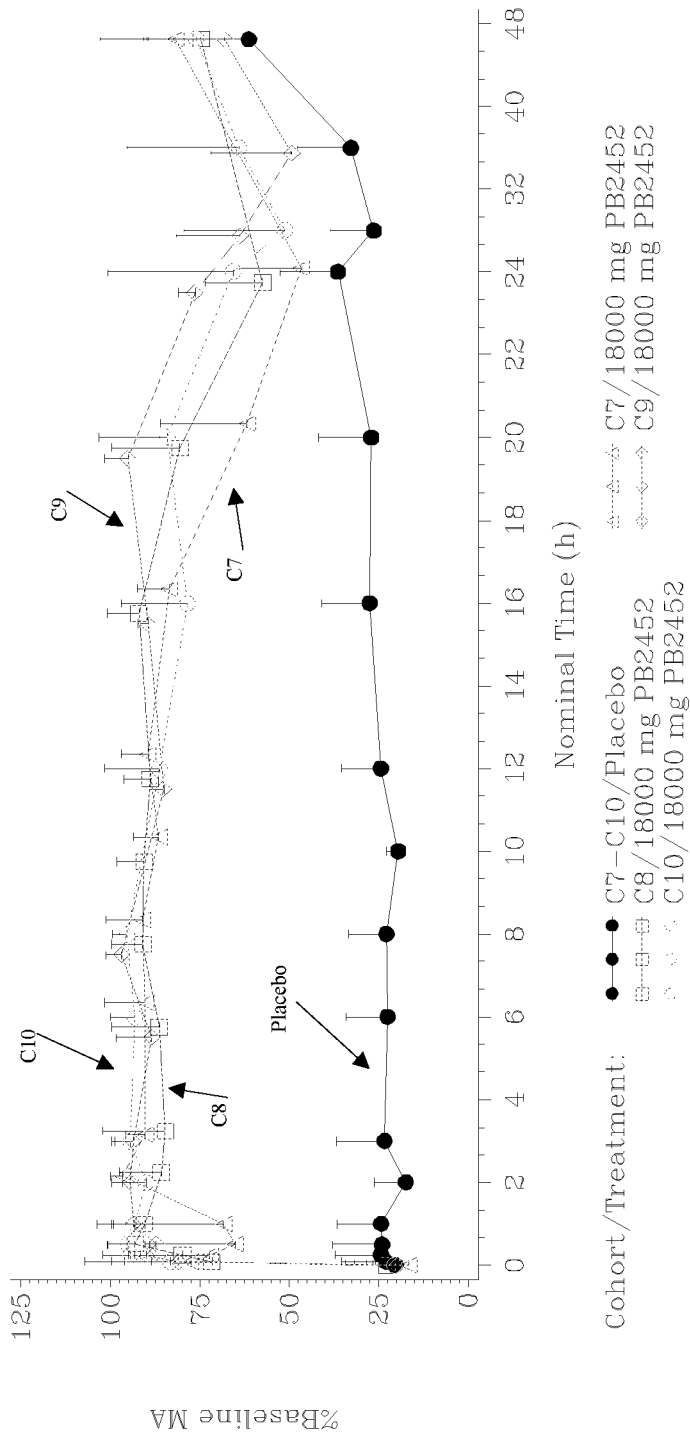


Figure 12B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/52173

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 5/02; A61K 39/395; A61P 7/02, 7/04; C07K 16/44 (2020.01)

CPC - A61B 5/02042; A61K 39/39583; A61P 7/02, 7/04; C07K16/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History documentDocumentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History documentElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2016/0130366 A1 (MEDIMMUNE LIMITED) 12 May 2016; abstract; paragraphs [0016], [0017], [0026], [0097], [0123], [0128], [0129], [0133], [0140], [0232], [0237]; Figure 9; Claim 42	1-7, 8/1-7, 9/8/1-7, 19-24 ----- 16-17, 18/16-17, 25-32
Y	WO 2018/158332 A1 (MEDIMMUNE LIMITED) 7 September 2018; abstract; paragraph [0013], [0024], [0028], [0029], [0030], [0036], [0038], [0073], [0094]	16-17, 18/16-17
Y	WO 2018/116267 A2 (NOVARTIS AG) 28 June 2018; abstract; page 18, 7th paragraph; page 21, 5th paragraph; page 73, 2nd paragraph; page 75, 4th paragraph; page 76, 1st paragraph; page 81, 3rd paragraph	25-31
Y	US 2016/0176912 A1 (ASTRAZENECA AB) 23 June 2016; abstract; paragraph [0002]	32
A	(BUCHANAN, A et al.) Structural and functional characterization of a specific antidote for ticagrelor. Blood. 28 May 2015, Epub 18 March 2015, Vol. 125, No. 22; pages 3484-3490; DOI: 10.1182/blood-2015-01-622928	1-7, 8/1-7, 9/8/1-7, 16-17, 18/16-17, 19-32
A	(JAYASINGHE, R et al.) Dual antiplatelet therapy - management in general practice. Australian Family Physician. October 2013, Vol. 42, No. 10; pages 702-705; PMID: 24130971	1-7, 8/1-7, 9/8/1-7, 16-17, 18/16-17, 19-32

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 January 2020 (09.01.2020)

Date of mailing of the international search report

31 JAN 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/52173

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 10-15, 33-42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-9, 16-32, a heavy chain variable region encompassing SEQ ID NO: 52 (HCVR), with a CDR1 encompassing SEQ ID NO: 53 (VHCDR1), a CDR2 encompassing SEQ ID NO: 54 (VHCDR2), and a CDR3 encompassing SEQ ID NO: 55 (VHCDR3); and a light chain variable region encompassing SEQ ID NO: 57 (LCVR), with a CDR1 encompassing SEQ ID NO: 58 (VLCDR1), a CDR2 encompassing SEQ ID NO: 59 (VLCDR2), and a CDR3 encompassing SEQ ID NO: 60 (VLCDR3) are directed toward a method of reversing ticagrelor-associated bleeding.

-Continued on supplemental page-

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 16-32, antibody encompassing SEQ ID NO: 52 (HCVR), SEQ ID NO: 53 (VHCDR1), SEQ ID NO: 54 (VHCDR2), SEQ ID NO: 55 (VHCDR3), SEQ ID NO: 57 (LCVR), SEQ ID NO: 58 (VLCDR1), SEQ ID NO: 59 (VLCDR2), and SEQ ID NO: 60 (VLCDR3)

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US19/52173

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

The method will be searched to the extent it encompasses an antibody with a heavy chain variable region encompassing SEQ ID NO: 52 (first exemplary HCVR), with a CDR1 encompassing SEQ ID NO: 53 (first exemplary VHCDR1), a CDR2 encompassing SEQ ID NO: 54 (first exemplary VHCDR2), and a CDR3 encompassing SEQ ID NO: 55 (first exemplary VHCDR3); and a light chain variable region encompassing SEQ ID NO: 57 (first exemplary LCVR), with a CDR1 encompassing SEQ ID NO: 58 (first exemplary VLCDR1), a CDR2 encompassing SEQ ID NO: 59 (first exemplary VLCDR2), and a CDR3 encompassing SEQ ID NO: 60 (first exemplary VLCDR3).

Applicant is invited to elect additional antibody(ies), with specified set(s) of heavy and light chain variable region(s), and associated CDR(s) thereof, with specified SEQ ID NO: for each, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and available as an option within at least one searchable claim, to be searched. Additional antibody(ies) will be searched upon the payment of additional fees. It is believed that claims 1, 2 (in-part), 3 (in-part), 4-9, and 16-32 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass an antibody encompassing SEQ ID NO: 52 (HCVR), SEQ ID NO: 53 (VHCDR1), SEQ ID NO: 54 (VHCDR2), SEQ ID NO: 55 (VHCDR3), SEQ ID NO: 57 (LCVR), SEQ ID NO: 58 (VLCDR1), SEQ ID NO: 59 (VLCDR2), and SEQ ID NO: 60 (VLCDR3). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an antibody encompassing a heavy chain variable region encompassing SEQ ID NO: 62 (HCVR), with a CDR1 encompassing SEQ ID NO: 63 (VHCDR1), a CDR2 encompassing SEQ ID NO: 64 (VHCDR2), and a CDR3 encompassing SEQ ID NO: 65 (VHCDR3); and a light chain variable region encompassing SEQ ID NO: 67 (LCVR), with a CDR1 encompassing SEQ ID NO: 68 (VLCDR1), a CDR2 encompassing SEQ ID NO: 69 (VLCDR2), and a CDR3 encompassing SEQ ID NO: 70 (VLCDR3).

No technical features are shared between the antibody sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a method of reversing ticagrelor-associated bleeding, or the risk of said bleeding, in a patient in need thereof comprising administering to said patient a composition comprising an effective amount of a pharmaceutical composition comprising an antibody or fragment thereof that binds to ticagrelor ((1S,2S,3R,5S)-3-[7-(((1R,2S)-2-(3,4-difluorophenyl)cyclopropyl)amino)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol) or a metabolite or derivative thereof; these shared technical features are previously disclosed by US 2016/0130366 A1 to MedImmune Limited (hereinafter 'MedImmune').

MedImmune discloses a method of reversing ticagrelor-associated bleeding, or the risk of said bleeding, in a patient in need thereof (a method of reversing ticagrelor-associated bleeding, or the risk of said bleeding, in a patient in need thereof; paragraph [0026]) comprising administering to said patient a composition comprising an effective amount of a pharmaceutical composition comprising an antibody or fragment thereof that binds to ticagrelor (comprising administering to said patient a composition comprising an effective amount of a pharmaceutical composition comprising an antibody or fragment thereof that binds to ticagrelor; paragraphs [0019], [0026], [0027]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the MedImmune reference, unity of invention is lacking.