CT Application(s) Summary Report

- **Protocol title:** A Randomized, Double-blind, Placebo-controlled, Multicenter Study to Assess the Safety and Efficacy of Inclacumab in Participants with Sickle Cell Disease Experiencing Vaso-occlusive Crises
- **Protocol code number:** GBT2104-131 (C5361001)
- **Public Registry Number:** Eudra-CT: 2020-005286-13
- **Version:** Protocol Amendment 4 (Version 5.0)
- **Date:** 13 July 2023

- **Investigational Medicinal Product being tested:**

Biological ■ **Pharmaceutical** ☐ **Innovative** ☐

Herbal medicine ☐ **Medical device** ☐

- **Sponsor:** Global Blood Therapeutics, Inc. "GBT", a wholly owned subsidiary of Pfizer Inc.
- **Indication:** Sickle Cell Disease Experiencing Vaso-occlusive Crises
- **Investigator's brochure (IB)**

Version:3.0
Date: 31 March 2022

Name of all Sites:

1. MASRI CRC, Faculty of medicine, Ain shams university
2. Abou El Resh and CRC , Faculty of medicine, Cairo University (3 sites)
3. Faculty of Medicine, Mansoura University Hospital
4. Faculty of medicine, Zagazig university
5. CRC , Faculty of medicine , Alexandria university (2 sites)
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- **EDA approval date:**

1. Initial (Protocol V 3.0 and IB edition 2.0): 14/06/2022
2. Amendment approval for IB edition 3.0: 15/11/2022

3. Amendment approval for Protocol Amendment 3.1 (Egypt) and Global 4.1: 21/02/2023
4. IMPD V3.0 Amendment approval: 17/01/2023
5. Shelf-life extension and re-labelling amendment approval: 08/06/2023
6. Amendment approval for Protocol Amendment 4 (Version 5.0): 25/03/2024

- **Summary of pre-clinical studies:**

1. Primary Pharmacology

1.1. In Vitro Studies

1.1.1. Binding Affinity to Human P-selectin

Affinity of inclacumab to its target P-selectin was determined using a Biacore assay by flowing soluble P-selectin (a truncated form of the membrane-bound P-selectin) over immobilized inclacumab. Binding affinity was found to be high with a dissociation constant (Kd) value of 9.5 nM, which is similar to crizanlizumab in an intraexperimental comparison.

1.1.2. Selectivity for Human P-selectin Versus E- and L-selectin

The selectivity of inclacumab against the other members of the selectin family (E- and L-selectin) was tested by determining the binding of the antibody to E- and L-selectin immobilized to microtiter plates as well as by its binding to cell lines expressing P-, E-, or L-selectin, respectively. Inclacumab bound to P-selectin transfectants > 3,000-fold more specifically than to E- and L-selectin transfectants when comparing the concentration of drug required for obtaining half-maximal effective concentration (EC50) of inclacumab to the transfectants.

1.1.3. Cross-reactivity with Other Species

Binding and functional assays were conducted to assess cross-reactivity of inclacumab with P-selectin across multiple species (mouse, rat, guinea pig, rabbit, minipig, and cynomolgus monkey).

In the FACS binding assay, inclacumab binding to thrombin-activated platelets expressing P-selectin was evaluated. The rosetting assay was used to assess inhibitory activity by measuring platelet adhesion to HL60 cells. Results showed no cross-reactivity with P-selectin from mouse, rat, guinea pig, rabbit, or minipig. However, inclacumab demonstrated significant binding to cynomolgus monkey P-selectin and inhibited P-selectin-mediated adhesion of monkey platelets to HL60 cells, with an IC50 of 0.30 ± 0.04 µg/mL (in vitro).

1.1.4. Mechanistic Studies

In functional cell-based in vitro assays, inclacumab inhibited P-selectin-mediated adhesive functions. It reduced adhesion of HL60 cells (PSGL-1-expressing) to both immobilized full-length human P-selectin and thrombin-activated human platelets, with IC50 values of 0.18 ± 0.06 µg/mL and 0.09 ± 0.03 µg/mL, respectively. This anti-adhesive effect was confirmed in whole blood from humans and cynomolgus monkeys, where inclacumab inhibited platelet–leukocyte aggregate (PLA) formation induced by platelet agonists such as TRAP . It also inhibited PLA formation triggered by ADP, collagen, and arachidonic

acid, indicating that its effect is independent of platelet activation pathways. Aspirin alone showed no effect on PLA formation, while the combination of inclacumab and aspirin produced inhibition comparable to inclacumab alone. Additionally, inhibition of PLA formation by inclacumab was confirmed in cynomolgus monkeys following single subcutaneous (SC) dosing and both single and multiple intravenous (IV) administrations.

Inclacumab inhibited leukocyte adhesion to a platelet monolayer with IC50 values of 4.2–5.2 µg/mL (plasma concentration), depending on shear rate. Leukocyte adhesion was reduced by >80% at ~20 µg/mL (shear rate 65 s^{-1}), and >95% inhibition (near-complete suppression) was achieved at 10 µg/mL on collagen-deposited platelets.

In a model mimicking leukocyte–endothelial interactions at inflammatory sites, inclacumab inhibited leukocyte adhesion to activated endothelial cells with an IC50 of 0.9 µg/mL (shear rate 65 s^{-1}). It showed differential effects across leukocyte subtypes, with strongest inhibition in granulocytes (96% ± 1% at 3 µg/mL), including neutrophils and eosinophils; monocytes (81% ± 7%) and lymphocytes (37% ± 18%) were less affected. Overall, results from the human whole blood flow system demonstrate that inclacumab effectively inhibits cell adhesion, supporting its potential to mitigate key steps in Vaso-occlusive crises (VOCs) in sickle cell disease (SCD).

1.2. In Vivo Studies

The inhibition of PLA formation shown in in vitro assays was confirmed after treatment of cynomolgus monkeys with inclacumab. After a single SC dose (4 mg/kg), inclacumab inhibited ex vivo TRAP-induced PLA formation with an IC50 < 2µg/mL, consistent with the in vitro IC50 of 1.4 ± 0.1 µg/mL. ≥80% suppression of PLA formation was sustained for at least 28 days after dosing. In the 13-week toxicology study (IV doses: 2, 10, 50 mg/kg), both circulating PLA levels and ex vivo TRAP-induced PLA formation were fully inhibited to background levels at all doses, with no recovery between doses (7-day interval), consistent with a half-life >15–18 days. In the 39-week toxicology study, inhibition of ex vivo TRAP-induced PLA formation was confirmed, though the effect on non-activated circulating PLAs was less pronounced. In an allergen-challenged cynomolgus monkey model, inclacumab (3.5 and 5 mg/kg IV) significantly reduced formation of platelet–monocyte, platelet–neutrophil, and platelet–eosinophil aggregates. Overall, these in vivo studies demonstrate that P-selectin blockade by inclacumab effectively inhibits PLA formation and related cell–cell interactions, supporting PLAs as a proof-of-mechanism biomarker for clinical evaluation in sickle cell disease (SCD).

2. Secondary Pharmacodynamics

2.1. Fc-mediated Effector Functions

Inclacumab, an IgG4 variant with an FC mutation, does not induce Fc-mediated effector functions. In ELISA assays, inclacumab did not bind complement factors C1q or C3, unlike the parent antibody (huMAb LC1004-002, IgG1). Consistently, it did not cause complement-dependent cytotoxicity (CDC) in P-selectin-expressing human platelets or endothelial cells at concentrations up to 50 µg/mL, as shown by absence of calcein release . This indicates a low likelihood of complement activation

in humans. Regarding Fcγ receptor interactions, inclacumab did not bind to FcγRI (CD64)-transfected cells and showed only background-level binding to human granulocytes, monocytes, and natural killer cells, in contrast to the parent antibody. It also failed to induce antibody-dependent cellular cytotoxicity (ADCC), with no lysis of P-selectin-expressing endothelial cells in the presence of NK cell-enriched PBMCs.
Overall, inclacumab lacks both CDC and ADCC activity, which supports its suitability as a therapeutic agent for P-selectin-related pathologies.

2.2. Effect of Inclacumab on Platelet Activation and Platelet Aggregation

The effect of inclacumab on platelet activation was investigated at concentrations of up to 100 µg/mL. Inclacumab neither caused nor enhanced the expression of GPIIb/IIIa, an early marker of platelet activation detected by FACS analysis. Inclacumab also did not induce or increase the expression of phosphatidylserine on the outer surface of platelets, a late event of platelet activation as determined by annexin V binding. These data indicate that inclacumab intrinsically has no platelet activation inducing or enhancing activity. In human washed platelets or platelet-rich plasma collected from healthy donors, inclacumab did not induce auto-aggregation of platelets at tested concentrations up to 50 µg/mL using an optical aggregometer. Moreover, inclacumab did not enhance or inhibit ADP-, ADP plus epinephrine-, collagen, or TRAP-induced platelet aggregation at tested concentrations up to 200 µg/mL.These in vitro data suggest **that inclacumab is unlikely to exert undesired effects on platelet activation and aggregation.**

3. Safety Pharmacology

Potential effects of inclacumab on the cardiovascular, respiratory, and central nervous systems were evaluated in 13-week and 39-week IV toxicity studies in cynomolgus monkeys at doses of 2, 10, and 50 mg/kg/week. Assessments included respiratory rate, cardiovascular parameters (ECG and blood pressure), and central nervous system evaluations using modified Irwin test–based neurobehavioral observations and neurological examinations.
Results: No inclacumab-related adverse effects were observed in any system up to the highest tested dose. The NOEL (No Observed Effect Level) was 50 mg/kg/week.

4. Pharmacodynamic Drug Interactions

The potential interaction between inclacumab and aspirin was evaluated in an in vitro platelet aggregation study. Aspirin alone (300 µmol/L) inhibited arachidonic acid- and collagen-induced platelet aggregation, but had no effect on ADP- and TRAP-induced aggregation. The addition of inclacumab (up to 200 µg/mL) after aspirin pre-incubation neither enhanced nor reduced aspirin's inhibitory effects on arachidonic acid- and collagen-induced aggregation, and did not affect ADP- or TRAP-induced aggregation. Overall, these results indicate that inclacumab does not interfere with the anti-platelet effects of aspirin, suggesting it is unlikely to modify aspirin's impact on primary hemostasis.

5. Pharmacokinetics and Drug Metabolism in Animals

The pharmacokinetics (PK) of inclacumab were characterized in cynomolgus monkeys across two IV studies, one SC study, and supported by toxicokinetic data from three toxicity studies.

Inclacumab exhibited low clearance (CL: 0.0011–0.0018 mL/min/kg) and a volume of distribution at steady state (Vss: 38.1–52.7 mL/kg). Plasma concentrations showed biphasic elimination, with a rapid alpha phase (2–3 days) followed by a slower terminal beta phase with a half-life (t½) of 15–18 days after IV administration. Exposure was dose-proportional across 2–50 mg/kg (IV), and plasma levels after repeated dosing were consistent with single-dose predictions, indicating no change in clearance (CL) over time. Overall, inclacumab demonstrates PK characteristics typical of humanized monoclonal IgG antibodies.

6. Toxicology

The nonclinical toxicology program for inclacumab included repeat-dose toxicity studies (up to 39 weeks in cynomolgus monkeys), a rabbit local tolerance study, and additional in vitro toxicity assessments (hemolysis, cytokine release, and tissue cross-reactivity).

The cynomolgus monkey was selected as the primary toxicology species based on demonstrated cross-reactivity of inclacumab with P-selectin and comparable tissue cross-reactivity with human tissues, supporting its relevance for safety evaluation.

Key studies included the 13-week and 39-week GLP-compliant repeat-dose toxicity studies, conducted in accordance with OECD GLP regulations and acceptable to FDA requirements. All pivotal studies were performed at an OECD Mutual Acceptance of Data–compliant CRO.

Inclacumab was administered via the intended clinical route (IV), and toxicokinetic (TK) profiles were assessed in plasma across all repeat-dose studies. Safety pharmacology endpoints were integrated into the 13-week study (cardiovascular, respiratory, CNS assessments), while cardiovascular endpoints were also evaluated in the 39-week study.

6.1. Repeat dose Toxicity

Repeat-dose toxicity of inclacumab was assessed in cynomolgus monkeys using an exploratory range-finding study followed by GLP-compliant 13-week and 39-week IV (bolus) studies.

Across studies, parameters included mortality, clinical observations, body weight, food intake, ophthalmology, ECG, Toxicokinetics, clinical pathology (hematology, coagulation, clinical chemistry, urinalysis), gross and microscopic pathology (including injection site evaluation), and organ weights. Additional assessments included immunophenotyping, T-cell dependent antigen response, and reproductive parameters (sperm analysis, vaginal cytology, menstrual cycles) in the pivotal studies. Safety pharmacology endpoints (CNS and respiratory) were evaluated in the 13-week study, while cardiovascular endpoints were included in both pivotal studies.

Cynomolgus monkeys received 1, 5, or 25 mg/kg IV every 4 days (5 doses total). No mortality or treatment-related effects were observed, and the maximum tolerated dose (MTD) was 25 mg/kg/4 days.

Monkeys received 0, 2, 10, or 50 mg/kg IV once weekly (n=4/sex/group, plus recovery animals). No mortality or inclacumab-related effects were observed. The NOEL was 50 mg/kg/week (highest dose tested).

Monkeys received 0, 2, 10, or 50 mg/kg IV once weekly (n=4/sex/group, plus recovery animals). No mortality or treatment-related effects were observed. The NOEL was 50 mg/kg/week (highest dose tested). Overall, repeat-dose administration up to 39 weeks showed no treatment-related toxicity, with the highest tested dose consistently identified as the NOEL.

6.2. Reproductive and Developmental Toxicity

Potential effects of inclacumab on male and female fertility were evaluated in sexually mature cynomolgus monkeys in the 13-week IV toxicity study. Assessment of potential effects on male fertility included testicular size and sperm parameters (number, motility, and morphology), while assessments on female fertility included vaginal cytology and menstrual cycles. No inclacumab-related effects on these parameters were observed. No microscopic changes were observed in the testes and ovaries in both the 13-week and 39-week IV toxicity studies in cynomolgus monkeys, further supporting no effects inclacumab has on male and female fertility.

There were no inclacumab-related maternal or developmental effects when pregnant monkeys were administered vehicle or inclacumab at 50 mg/kg via bolus IV injection once weekly beginning on GD20 through delivery (approximately 21 doses) in an ePPND toxicity study with a 3-month postpartum period. The NOEL was 50 mg/kg.

6.3. Local Tolerance

A local tolerance study was conducted in New Zealand White (NZW) rabbits (3 males/group) to evaluate injection site effects of inclacumab.

In the IV dose group, the marginal vein of the right ear received vehicle (0 mg/kg) and the left ear received 20 mg/kg inclacumab IV. In the paravenous group, vehicle was administered at two sites adjacent to the marginal vein of the right ear, while the left ear received 5 mg inclacumab per site to simulate accidental perivascular administration.

Across both groups, there were no mortality or inclacumab-related effects. Evaluations of clinical signs, injection site reactions, body weight, and both macroscopic and microscopic pathology showed no irritation or tissue damage at injection sites.

6.4. Other Toxicity Studies

Other toxicity studies were conducted in vitro with inclacumab and included evaluation of hemolysis, cytokine release, and tissue cross-reactivity. The hemolysis evaluation supports nonclinical and clinical studies with IV administration. Inclacumab in human blood in vitro had no effects on hemolysis, plasma turbidity, or plasma precipitation up to the highest test concentration of 7.5 mg/mL. Inclacumab did not lead to cytokine release or neutrophil activation in human blood up to the highest test concentration of 200 µg/mL. In the tissue cross-reactivity studies using cynomolgus monkey and human tissues, specific reactivity of inclacumab was restricted to cells in which P-selectin expression was expected. No other reactivities or cross reactivities were observed.

- **Summary of previous clinical studies:**

Study No.	Study Design	Population	Evaluation	Dose, Duration	Study Status
Phase I Studies					

BP21112	Randomized, double-blind, single ascending dose, placebo-controlled, parallel study	Healthy participants aged 18-65 years 56 total/42 inclacumab treated 35 M/21 F 23–63 years	Safety, tolerability, PK, PD (PLA, soluble P-selectin)	Single dose of inclacumab from 0.03 to 20 mg/kg, administered by IV infusion	Completed
BP21617	Randomized, double-blind, multiple ascending dose, placebo-controlled, parallel study	Healthy participants aged 18–65 years Participants with PAD age 45–75 years Healthy: 32 total/26 inclacumab treated 16 M/16 F 25–62 years PAD: 39 total/28 inclacumab treated 29 M/10 F 55–75 years	Safety , tolerability, PK, PD (PLA, soluble P-selectin), markers of disease activity	Inclacumab 0.3 (PAD participants only), 3, 7 or 20 mg/kg (healthy participants & PAD participants), by IV infusion 28 days apart for 3 months	Completed

BP22563	Open-label, 2-cohort, 2- period, 1- sequence crossover study	Healthy participants aged 18–60 years 21 total/18 inclacumab treated 17 M/4 F 23–60 years	EAUClast and Emax of anti-IIa, anti-Xa, aPTT, TFPI, safety , tolerability, PK, PD	Single IV bolus of unfractionated heparin 5000 IU or single SC enoxaparin 40 mg alone or after single IV infusion of inclacumab 20 mg/k	Completed
BP28134	Open label, parallel, 3-dose group, single-dose study	Japanese and Caucasian healthy participants aged 18-55 years 62 total/62 inclacumab treated 31 Japanese and 31 Caucasian 31 M/31 F 21–55 years	Safety , tolerability, PK, PD (PLA, soluble P-selectin)	Single dose of inclacumab 0.03, 3, or 20 mg/kg, administered by IV infusion	Completed
GBT2104-111	Open label, SAD study	Healthy participants aged 18-65 years 15 total/15 inclacumab	Safety, tolerability, PK, and PD of inclacumab following IV infusion in	Single dose of 20 or 40 mg/kg, IV infusion	Completed

		treated 6 at 20 mg/kg and 9 at 40 mg/kg 8 M/7 F 22 to 52 years	healthy participants		
Phase II Studies					
BP25601	Randomized, double-blind, placebo-controlled study comparing inclacumab 20 mg/kg vs placebo	Participants undergoing CABG surgery aged 18-85 years 384 total/188 inclacumab treated 335 M/49 F 37.7 – 83.6 years	Safety , efficacy, tolerability, incidence of MACE, renal failure and multi-organ failure, PK, PD, biochemical markers (hs-CRP, GDF-15, P-selectin, lipids)	Inclacumab 20 mg/kg or placebo administered IV as ≥ 1-hour infusion once every 4 weeks for a total of 9 infusions	Completed
BP25619	Randomized, double-blind, placebo-controlled study comparing single-doses of inclacumab 5 and 20 mg/kg vs placebo	Participants with NSTEMI undergoing PCI aged 18–85 years 544 total/ 355 inclacumab	Safety , tolerability, incidence of MACE, renal failure, PK, PD (soluble P-selectin), biochemical markers (hs-CRP, GDF-	Inclacumab 5 or 20 mg/kg or placebo administered IV as ≥1-hour infusion prior to PCI	Completed

		treated 396 M/148 F 27.9 – 84.4 years	15, P-selectin, lipids)		

The following is the result of the above-mentioned studies:

1. **Clinical Pharmacokinetics**

The pharmacokinetics (PK) of inclacumab in humans were evaluated after **20-minute to 2-hour IV infusions (0.03–40 mg/kg)** in healthy participants and in patients with peripheral and coronary vascular disease.

Inclacumab shows **Cmax at or shortly after the end of infusion**, followed by a **rapid distribution phase** and a **linear elimination phase at concentrations >10 µg/mL**. At concentrations **<10 µg/mL**, faster clearance is observed, consistent with **target-mediated drug disposition (TMDD)**.

Exposure was generally **dose proportional from 10–40 mg/kg**, and **greater than dose proportional from 0.03–10 mg/kg**. With repeated dosing (**3, 7, or 20 mg/kg IV every 28 days for 3 infusions**), an accumulation ratio of approximately **1.6** was observed in healthy subjects.

The **mean terminal half-life (t½)** was **15.2 days** after a single 40 mg/kg dose. The **volume of distribution at steady state (Vss)** is limited and approximates plasma volume, consistent with typical **human IgG monoclonal antibody behavior**.

2. **Clinical Pharmacodynamics**

Two primary pharmacodynamic (PD) markers used to evaluate inclacumab activity were **ex vivo TRAP-activated platelet–leukocyte aggregate (PLA) formation** and **surface plasmon resonance (SPR) P-selectin inhibition**.

In IV studies (0.03–20 mg/kg), inclacumab produced **dose-dependent inhibition of ex vivo TRAP-induced PLA formation**, with longer duration of effect at higher doses. In **Phase 1 Study GBT2104-111**, a single **20 or 40 mg/kg dose** in healthy participants resulted in **sustained inhibition of PLA formation for up to 23 weeks post-dose**. PK/PD analyses showed that **near-maximal PLA inhibition occurred at plasma concentrations >10 µg/mL**, with similar effects observed in both healthy participants and those with peripheral artery disease (PAD).

In the same study (GBT2104-111), a single **20 or 40 mg/kg dose** also produced **>90% inhibition of SPR-measured P-selectin binding for up to 12 weeks post-dose**.

3. **Drug Interactions**

No PK interactions are expected with inclacumab. The absence of interaction between inclacumab and unfractionated heparin has been demonstrated in a clinical drug-drug interaction study (BP22563).

4. Safety

In completed clinical studies, inclacumab (formerly RO4905417) was **generally safe and well tolerated** up to **20 mg/kg IV** in healthy participants and patients with peripheral and coronary vascular disease . A total of **148 healthy participants** and **28 participants with PAD** were exposed. In two Phase 2a CAD studies, **543 participants** received either a single dose (**5 or 20 mg/kg**) or repeated dosing (**20 mg/kg every 4 weeks up to 9 infusions**).

Safety findings:

- No dose–response relationship for adverse events (AEs) in healthy participants; infusions were generally well tolerated.
- Two healthy participants developed anti-inclacumab antibodies without allergic reactions or impact on PK.
- In CAD Study **BP25619 (NSTEMI)**, AE profiles were similar between placebo and inclacumab groups.
- Six deaths occurred in the inclacumab groups (4 at 5 mg/kg, 2 at 20 mg/kg), all cardiovascular in nature and assessed as unrelated to treatment.
- No meaningful effects were observed on laboratory parameters, vital signs, ECGs, infections, bleeding, or renal failure.
- Three participants developed treatment-emergent anti-drug antibodies at Day 120, with no PK impact.
- In CAD Study **BP25601 (CABG, up to 9 infusions of 20 mg/kg)**, AE profiles were similar between placebo and treatment groups.
- Four deaths occurred (2 per group), and SAEs were slightly higher in the inclacumab 20 mg/kg group, with no treatment-related safety signals identified.
- No effects on clinical safety parameters were observed; two participants developed antibodies without PK impact.
- In Phase 1 Study **GBT2104-111**, single IV doses of **20 or 40 mg/kg** in 15 healthy participants were **well tolerated with no safety concerns**.

Overall, Inclacumab demonstrated an acceptable safety profile across studies, with **no consistent treatment-related safety signals identified**.

5. Marketing Experience

Inclacumab is not approved or marketed in any country.

• **Protocol:** A Randomized, Double-blind, Placebo-controlled, Multicenter Study to Assess the Safety and Efficacy of Inclacumab in Participants with Sickle Cell Disease Experiencing Vaso-occlusive Crises

Phase: III

- Sickle cell disease is an inherited monogenic RBC disorder characterized by chronic hemolytic anemia, Vaso-occlusion, and progressive end-organ damage resulting, in life-long disability and early death . Beginning in childhood, patients suffer unpredictable and recurrent episodes of severe pain, referred to as VOCs, often leading to significant psychosocial and occupational disability. In addition to painful VOCs, ongoing hemolysis, Vaso-occlusion and ischemia-reperfusion lead to progressive tissue injury from which no organ system is spared. Multiple pathophysiologic mechanisms likely contribute to the systemic vasculopathy and pain crises, caused by cell adhesion. The abnormally enhanced interactions among erythrocytes, endothelial cells, leukocytes, and platelets at the core of SCD pathophysiology result in abnormal blood flow. In addition, the adhesion of sickle erythrocytes and leukocytes to the endothelium results in the formation of heterocellular aggregates, vascular obstruction, and tissue ischemia . Although the adhesion of leukocytes and sickle erythrocytes to the endothelium can involve multiple adhesion molecules, P-selectin (CD62P) has been found to initiate the adhesion cascade and be necessary for the Vaso-occlusive process.

- Inclacumab (formerly known as RO4905417) is a recombinant human monoclonal antibody (huMAb) of the immunoglobulin (Ig)G4 subclass directed against human P-selectin (CD62P), which is being developed by Global Blood Therapeutics, Inc. (GBT), for the treatment of sickle cell disease (SCD).

-Inclacumab binds to P-selectin, which is a cell adhesion molecule produced by endothelial cells and platelets. Upon activation of these cells (e.g., by thrombin, cytokines, complement components, hypoxia, and heme), P-selectin is translocated to the cell surface where it binds to its primary ligand P-selectin glycoprotein ligand-1 (PSGL-1) and mediates leukocyte recruitment by platelets (contributing to formation of platelet-leukocyte aggregates) or endothelial cells. The same mechanism is also responsible for abnormal rolling and adhesion of sickle red blood cells (RBC) to the endothelium, initiating acute vascular occlusion and chronically impairing microvascular blood flow in patients with SCD. The rapid transfer of P-selectin to the surface of endothelial cells and initiatory role of P-selectin in sickle RBC adhesion together suggest an important role for endothelial P-selectin in acute Vaso-occlusion and chronic microvascular blood flow. Inclacumab binding to P-selectin and prevention of P-selectin binding to its ligands is the putative mechanism by which inclacumab prevents the initiation and propagation of Vaso-occlusion and associated clinical manifestations of Vaso-occlusive crisis (VOC), organ damage, and stroke

in patients with SCD through inhibition of cell-cell interaction between sickled RBCs, leukocytes, platelets and/or endothelial cells.

Objective(s):

The **primary objective** of this study is to evaluate the safety and efficacy of treatment every 12-week with inclacumab to reduce the incidence of VOCs in participants with SCD.

Additional objectives of the study are to evaluate the PK and PD of inclacumab, the presence of anti-drug antibodies (ADAs), and changes in quality of life (QOL).

Endpoint(s):

Primary Efficacy Endpoint	The primary efficacy endpoint for the study is the rate of VOCs during the 48-week treatment period. A VOC is defined as an acute episode of pain that: • Has no medically determined cause other than a Vaso-occlusive event, and Results in a visit to a medical facility (hospitalization, emergency department, urgent care center, outpatient clinic, or infusion center), or results in a remote contact with a healthcare provider; and • Requires parenteral narcotic agents, parenteral nonsteroidal anti-inflammatory drugs (NSAIDs), or an increase in treatment with oral narcotics. Complicated VOCs of acute chest syndrome (ACS), hepatic sequestration, splenic sequestration, and priapism that meet the requirements listed above will be included in the primary endpoint.
Secondary Efficacy Endpoints	The secondary efficacy endpoints for the study are the following: • Time to first VOC during the 48-week treatment period. • Time to second VOC during the 48-week treatment period. • Proportion of participants with no VOCs during the 48-week treatment period. • Rate of VOCs that required admission to a healthcare facility and treatment with parenteral pain medication during the 48-week treatment period where admission includes:

	A hospital admission, or an admission to an emergency room, observation unit, or infusion center for ≥ 12 hours, or 2 visits to an emergency room, observation unit, or infusion center over a 72 hour period. • Number of days of inpatient hospitalization for a VOC during the 48-week treatment period.
Safety Endpoints	Safety endpoints for the study are the following: • Incidence of treatment-emergent adverse events (TEAEs). • Change from Baseline in laboratory assessments (complete blood count [CBC], chemistry, and coagulation).
Exploratory Endpoints	The exploratory endpoints for the study are the following: • Rate of all SCD-related urgent care visits to clinic, emergency room, and hospital during the 48-week treatment period. Proportion of total days missed from school or work due to SCD during the 48-week treatment period. • Rate of complicated VOCs (defined in Section 5.1) during the 48-week treatment period. • Rate of red blood cell (RBC) transfusions during the 48-week treatment period. • Rate of inpatient hospital admissions for any reason during the 48-week treatment period. • Number of days of inpatient hospitalization for any reason during the 48-week treatment period. • Proportion of participants rated as "very much improved" or "much improved" based on the Patients Global Impression of Change (PGI-C) at Weeks 12, 24, 36, and 48. • Proportion of participants rated as "very much improved" or "much improved" based on the Clinician's Global Impression of Change (CGI-C) at Weeks 12, 24, 36, and 48. • Change from Baseline in the cumulative score for the Adult Sickle Cell Quality of Life Measurement (ASCQ-Me) Pain Impact – Short Form over time to Week 48.

Exploratory Pharmacology Endpoints	The following exploratory pharmacology endpoints will be assessed: • Plasma PK of inclacumab as assessed by population PK analysis using nonlinear mixed-effects modeling. • The incidence of ADA to inclacumab. • Pharmacodynamics including changes in non-activated and TRAP-activated PLAs, PLT P-selectin expression, serum P-selectin inhibition measured by surface plasmon resonance (SPR), and plasma total and free soluble P-selectin (sP-selectin-) over time. • Biomarkers including changes in RBC adhesion (selected sites), genomic markers (optional), protein markers in the blood, urine markers of kidney function, and Voxelotor plasma and whole blood concentrations (as applicable).

Rationale:

- Inclacumab binds to P-selectin, which is a cell adhesion molecule produced by endothelial cells and PLTs. Upon activation of these cells (eg, by thrombin, cytokines, complement components, hypoxia, and heme), P-selectin is translocated to the cell surface where it binds to its primary ligand P-selectin glycoprotein ligand-1 (PSGL-1) in leukocytes and mediates leukocytes recruitment by PLTs or endothelial cells. The same mechanism is also responsible for abnormal adhesion of sRBC to the endothelium, initiating acute vascular occlusion and chronically impairing microvascular blood flow in patients with SCD. Inclacumab binding of P-selectin and prevention of P-selectin binding to its ligands is the putative mechanism by which inclacumab effectively blocks interactions between endothelial cells, PLTs, sRBCs and leukocytes, thereby preventing VOCs.

Design:

-This study was a randomized, placebo-controlled, double-blind, multicenter, parallel-group study to assess the safety and efficacy of inclacumab in reducing the frequency of VOCs in approximately 240 adult and adolescent participants (≥ 12 years of age) with SCD globally.

-Enrollment included participants ≥ 16 years of age.

-Eligible participants were randomized with a 1:1 ratio into one of two treatment arms as follows:

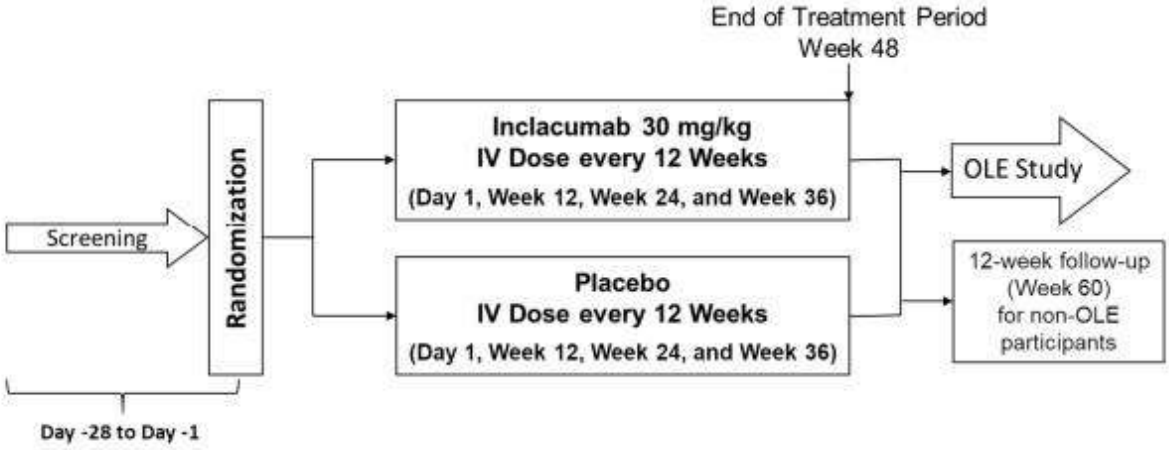
• Inclacumab 30 mg/kg administered intravenously (IV) Q12W (Day 1, Week 12, Week 24, and Week 36); or

• Placebo administered IV Q12W (Day 1, Week 12, Week 24, and Week 36).

-At the time of randomization, participants were stratified by Baseline hydroxyurea (HU) use (yes; no), number of VOCs (2 to 4; 5 to 10) in the preceding 12 months, and geographic region (North America; sub-Saharan Africa; Europe/rest of world).

-An independent DMC regularly reviewed the totality of accumulated safety data from all ongoing inclacumab studies on an ongoing, unblinded basis, with specific emphasis on adolescent participants.

-Participants that completed the study through Week 48 were provided with the opportunity to enroll in an open-label extension (OLE) study.



• ***Recommendation &/ or*** **Questions & Answers: NA**

• **Abbreviation:**

ACS : Acute chest syndrome
ADA : Anti-drug antibodies
ADCC : Antibody-dependent cellular cytotoxicity
ADP : Adenosine diphosphate
AE : Adverse events
aPTT : Activated partial thromboplastin time
ASCQ-Me : Adult Sickle Cell Quality of Life Measurement
CABG : Coronary artery bypass graft
CAD : Coronary artery disease
CBC : Complete blood count
CDC : Complement-dependent cytotoxicity
CGI-C : Clinician's Global Impression of Change
CL : Clearance

CNS : Central nervous system
CRC : Clinical Research Center
CRO : Contract Research Organization
CSR : Clinical Study Report
CT : Clinical Trial
DMC : Data Monitoring Committee
EAUClast : Area under the effect-time curve
EC50 : Half-maximal effective concentration
ECG : Electrocardiogram
EDA : Egyptian Drug Authority
ELISA : Enzyme-linked immunosorbent assay
Emax : Maximum effect
ePPND : Enhanced prenatal and postnatal development
FACS : Fluorescence-activated cell sorting
FDA : Food and Drug Administration
GBT : Global Blood Therapeutics, Inc.
GD : Gestational day
GDF-15 : Growth Differentiation Factor 15
GLP : Good Laboratory Practice
GPIIb/IIIa : Glycoprotein IIb/IIIa
hs-CRP : High-sensitivity C-reactive protein
HU : Hydroxyurea
IB : Investigator's Brochure
IC50 : Half-maximal inhibitory concentration
IgG : Immunoglobulin G
IgG4 : Immunoglobulin G4
IMP : Investigational Medicinal Product
IMPD : Investigational Medicinal Product Dossier
IV : Intravenous
Kd : Dissociation constant
MACE : Major adverse cardiovascular events
MTD : Maximum tolerated dose
NOEL : No Observed Effect Level
NSAIDs : Nonsteroidal anti-inflammatory drugs
NSTEMI : Non-ST-elevation myocardial infarction
NZW : New Zealand White
OECD : Organization for Economic Co-operation and Development
OLE : Open-label extension
PAD : Peripheral artery disease
PCI : Percutaneous coronary intervention
PD : Pharmacodynamics

PGI-C : Patients Global Impression of Change
PK : Pharmacokinetics
PLA : Platelet–leukocyte aggregate
PLT : Platelets
PSGL-1 : P-selectin glycoprotein ligand-1
Q12W : Every 12 weeks
QOL : Quality of life
RBC : Red blood cells
SAD : Single ascending dose
SAE : Serious adverse event
SC : Subcutaneous
SCD : Sickle cell disease
sP-selectin : Soluble P-selectin
SPR : Surface plasmon resonance
sRBC : Sickle red blood cells
t½ : Terminal half-life
TEAEs : Treatment-emergent adverse events
TFPI : Tissue factor pathway inhibitor
TK : Toxicokinetic
TMDD : Target-mediated drug disposition
TRAP : Thrombin receptor activator peptide
VOC : Vaso-occlusive crisis
Vss : Volume of distribution at steady state