Official Title: Safety and Efficacy of Inhaled Tissue Plasminogen Activator (tPA) for the Acute Treatment of Pediatric Plastic Bronchitis

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CLINICAL TRIAL PROTOCOL

Safety and Efficacy of Inhaled Tissue Plasminogen Activator (tPA) for the Acute Treatment of Pediatric Plastic Bronchitis

Study Agents: recombinant human tissue plasminogen activator (alteplase, Activase®, Genentech, South San Francisco, CA)

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The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations.

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Title

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1 PROTOCOL SUMMARY

Background and Rationale: Plastic bronchitis (PB) is a rare, most often pediatric disease characterized by the formation of obstructive airway casts primarily composed of fibrin. There is presently no FDA-approved pharmacotherapy for PB, but acute exacerbations of the illness are often treated with inhaled tissue plasminogen activator (tPA). To date, this is done somewhat anecdotally because there has been no safety or efficacy testing of this treatment. In addition, there is presently no reliable surrogate marker of adverse drug events. Nevertheless, in the absence of inhaled tPA treatment, PB-induced respiratory distress can be severe, often warranting urgent or emergent bronchoscopy for cast removal, or can sometimes result in respiratory failure. As such there is a significant unmet need for safety and efficacy testing of inhaled tPA and for biomarkers of drug response.

Objectives and Endpoints: This is an open-label, clinical trial of inhaled tPA for the treatment of acute PB. The objectives of this protocol are to: 1) test the safety and efficacy of an inhaled tPA regimen in children with PB; and 2) identify potential candidate biomarkers of inhaled tPA drug response. Safety endpoints will consist of the development of new, active bleeding that is systemic and/or pulmonary and/or new hematuria (defined as gross hematuria). Secondary endpoints of efficacy will also be measured (e.g., frequency of cast production). Urine and blood will also be collected for the development of potential biomarkers of inhaled tPA drug response.

Assessments: Enrolled subjects will be routinely clinically monitored and blood work will be assessed for the development of new, active bleeding that is systemic and/or pulmonary or new gross hematuria. Levels of oxygenation and pulmonary function will be assessed during the study period. We will also include the incidence of expectorated casts as a measurement of efficacy.

Statistical Methods: This is an open-label study of up to 13 subjects with PB. A group of healthy subjects, Fontan subjects without PB, and Fontan subjects with protein losing enteropathy (PLE) will serve as controls for biomarker assay development. The incidence of new, active bleeding events and the frequency of airway cast expectoration will be assessed in subjects with PB.

2 INTRODUCTION

2.1 Indication

The proposed study will investigate the safety and efficacy of inhaled recombinant human tissue-type plasminogen activator (tPA, alteplase, Activase ®, South San Francisco, CA) for the treatment of acute exacerbations of PB. It also seeks to find potential biomarkers of drug response.

2.2 Background and Rationale

Plastic Bronchitis

Plastic bronchitis (PB) is a rare pediatric lung disease characterized by the production of obstructive bronchiolar casts [1]. This condition predominantly occurs in patients with congenital heart disease (CHD), the majority of whom have undergone single ventricle, or Fontan, surgical palliation [2]. Plastic bronchitis has also been reported to occur in patients with previously existing pulmonary diseases including asthma and cystic fibrosis [3].

The clinical presentation of plastic bronchitis typically includes symptoms of dyspnea, tachycardia, hypoxemia, fever, and cough due to partial or full bronchiolar cast obstruction [3-5]. Diagnosis of the condition is typically not made until the affected patient either expectorates a cast or bronchiolar casts are visualized and removed during bronchoscopy [6]. This condition is particularly problematic in patients with underlying CHD as it contributes to mortality and an increased incidence of life-threatening events in this population. Once a patient is diagnosed with PB, acute exacerbations of this otherwise quiescent chronic illness typically occur on a fairly regular basis (e.g., once every 2-3 months). In some situations, acute exacerbations can recur as often as several times a month.

Although limited patient populations affected by this disease state have been identified, the pathophysiology of PB itself remains poorly understood. Casts produced in this disease state are reportedly composed of fibrin, mucin, or a combination of both depending on the underlying disease state of the patient [1, 3]. Previous case reports on cardiac patients with PB reported that casts consisted primarily of mucin and were generally acellular [1]. However, recent evidence shows that casts produced by patients with CHD consist primarily of fibrin and small cellular infiltrates of lymphocytes and macrophages with little or no mucin [1, 7, 8].

Treatment of Plastic Bronchitis

Current treatment for PB remains largely anecdotal, and information on the clinical efficacy of the pharmacologic interventions utilized in this population is lacking [1]. The general response to acute exacerbations of PB involves the initiation of inhaled mucolytics, inhaled and systemic corticosteroids, and bronchoscopic removal of obstructive casts in cases of severe respiratory distress [5, 9, 10]. Various case reports have also noted the use of macrolides [3, 9], bronchodilators [3, 9], anticoagulants [5, 10], hypertonic saline [3], and fibrinolytics [3, 5, 7, 9, 10].

Alteplase
The use of tPA to reduce cast burden in PB has become an area of focus in both the clinical and laboratory setting due to the fibrinolytic properties that it possesses. As stated previously, bronchiolar casts in individuals with underlying CHD are most often composed of fibrin [1]. Subsequently, the use of inhaled fibrinolytics, specifically tPA, could potentially be more efficacious in reducing cast burden than the mucolytics that are frequently being used to treat this condition.

tPA (alteplase, Activase®, recombinant human tissue-type plasminogen activator; Genentech, South San Francisco, CA) is a serine protease that is primarily utilized to dissolve thrombus associated with myocardial infarction (MI) or acute ischemic stroke (AIS) [11-13]. tPA is a fully-glycosylated, single polypeptide chain of 527 amino acids (molecular weight ~70kDa) produced through the transfection of Chinese hamster ovary cells (CHO) with the cDNA of endogenous human tPA [11]. The recombinant protein is structurally identical to the endogenous tissue plasminogen activator (tPA) from which it is produced.

tPA is typically systemically administered for the treatment of MI and AIS. Pharmacokinetic models project tPA infusion into a central compartment containing the liver, with first order transfers between the central and peripheral compartments. The half-life of tPA is characterized by a short yet dominant α-phase ($t_{1/2\alpha}$=3-4 minutes, AUC_{α}= 66-88%), followed by a second β-phase that is longer in duration ($t_{1/2\beta}$=15.4-88 minutes) [14-16]. Elimination from the central compartment follows Michaelis-Menten kinetics, with first-order elimination at low plasma concentration levels and zero-order elimination at higher plasma concentrations [17]. Multiple mechanisms of elimination have been identified, the majority of which involve catabolic hepatic processes [17, 18]. Clearance generally begins with the binding of tPA to one of two hepatic receptor systems, which triggers receptor-mediated endocytosis of the serine protease. The two main hepatic receptor systems that have been implicated in this process include low-density lipoprotein receptor-related protein-1 (LRP1) and mannose receptors. Fucose receptors on Kupffer cells also aid in tPA clearance from the systemic circulation, but to a far less extent [18]. Presently, the mechanism of tPA clearance from the lungs following inhalation is unknown. However, LRP1 in the lungs is capable of receptor mediated endocytosis of tPA suggesting the lungs possess the ability to clear tPA from the airways [19].

tPA exhibits fibrinolytic activity through its direct activation of plasminogen to plasmin, in turn leading to plasmin-mediated fibrinolysis [11, 12, 20]. tPA is highly fibrin-specific, and demonstrates a low affinity for plasminogen in the absence of fibrin [11].

2.3 Hypothesis
We hypothesize that a 5mg Q6h regimen of inhaled tPA (alteplase, Activase ®, Genentech, South San Francisco, CA) will be safe and efficacious for the reduction of airway cast burden associated with acute exacerbations of plastic bronchitis.

2.4 Previous Human Experience
There are no clinical trial data to date available on the use of aerosolized tPA to treat PB. However, aerosolized tPA has been used to treat acute exacerbations of PB, the experiences of which have been recorded in multiple, unrelated case reports [3, 5, 7, 8, 9, 10, 21, 22]. A brief

summary of medical histories associated with the patients described in these case reports is included in **Table 1**. Successful treatment with tPA was achieved in these patients following either inadequate responses to clinical interventions (consisting of pharmacological treatments and bronchoscopy) or concomitantly with other therapies and/or bronchoscopy. No adverse events associated with tPA use were reported in any of the cases.

Table 1. Summary of patient histories excerpted from published case reports of inhaled tPA treatment for plastic bronchitis

Reference	Patient	Underlying Condition/CHD	Age at DFO (months)	Onset of PB from DFO (months)	Fontan Type
[9]	1	DILV, TGA	26	27	Lateral tunnel
[3]	2	UAVCS	60	72	Lateral tunnel
[10]	3	DILV, TGA	15	33	NR
[5]	4	HLHS	66	2	NR
[7]	5	Asthma	N/A	N/A	N/A
[8]	6	HLHS	24	120	NR
[22]	7-10*	HLHS, TGA,	39.4	6	EC (1 case)
[21]	11	HLHS	27	0.75	EC
[21]	12-14*	TA, TGA, TOF	NR	NR	NR
[23]	15	DORV, TGA	72	>12	NR
[24]	16	HLHS	72	108	NR

CHD: congenital heart disease; DFO: date of Fontan operation; PB: plastic bronchitis; DILV: double-inlet left ventricle; TGA: transposition of the great arteries; UAVCS: unbalanced atrial-ventricular canal status; HLHS: hypoplastic left heart syndrome; TA: tricuspid atresia; TOF: Tetralogy of Fallot; EC: extracardiac; NR = not reported; N/A = not applicable; *multiple cases reported.

Three dosing regimens of aerosolized tPA were outlined in five of the case reports summarizing its use [5, 8, 9, 10, 22]. The dosing regimens utilized in patients 2 and 12-14 were not provided [3, 21]. A starting dose of 12 mg tPA was used in patients 1 and 3, followed by a single 10 mg dose one hour after administration of the initial dose [9, 10]. Doses of 5 mg tPA were administered every 2 hours thereafter, totaling four rounds in patient 3 [10]. Therapy was withdrawn in patient 1 due to death attributed to recurrent episodes of hypoxia and hypotension and subsequent central nervous system (CNS) dysfunction [9]. A testing dose of 4 mg tPA and loading dose of 10 mg tPA was utilized in patient 4, with repeated administration of 5 mg tPA every 4 hours thereafter [5]. In patients 5 and 11, tPA was directly instilled into the airway during bronchoscopy [7,21], and in patients 7-10 a regimen of 2.0mg/kg/d in four divided doses was used [22]; patient 11 also received a regimen of inhaled tPA four times daily [21].

Following the initiation of tPA, patient 1 showed a significant improvement in systemic oxygenation saturation to 75-85% from 50-70% within twenty-four hours of treatment [9]. Markedly opened airways were also observed comparatively to bronchoscopies conducted prior to the initiation of tPA. Despite the improvements in clinical status observed following treatment with tPA, the patient displayed signs of severe CNS distress attributed to recurrent hypotension and hypoxia throughout the five days prior to the initiation of treatment with tPA. Subsequently, patient 1 died following the withdrawal of life support.

Patient 2 demonstrated gradual clinical improvement following numerous surgical and

pharmacologic interventions [3]. Atelectasis was partially cleared following the use of inhaled tPA and dornase-alfa. However, selective bronchoscopy was performed to extract a fibrin cast obstructing the left-mainstem bronchus. Inhaled tPA was progressively tapered off after improvements with extubation. High-flow nasal cannula and bi-level positive airway pressure comprised the post-extubation therapies, and airway clearance therapies employed in patient 2 included the use of inhaled dornase-alfa, N-acetylcysteine, 3% hypertonic saline, vest therapy, and intrapulmonary percussive ventilation. An additional bronchoscopy was performed despite the initiation of these therapies due to recurrent right lung atelectasis. Treatment with inhaled tPA was reinitiated following worsening atelectasis, and azithromycin and bosentan were added to the drug therapy regimen. Despite the addition of these therapies, atelectasis persisted and subsequently surgical Fontan fenestration with drainage into the left atrium was performed. Although the patient's clinical status improved following this surgical intervention, episodes of atelectasis did recur, in turn warranting the continuation of airway clearance therapy. This therapeutic intervention was continued at home following hospital discharge, and included the use of IPV, albuterol, N-acetylcysteine, dornase-alfa, hypertonic saline, azithromycin, bosentan, and sildenafil. Clinical stability was being maintained on this regimen at six months follow-up.

Patient 3 showed significant clinical improvement following treatment with aerosolized tPA, and no adverse events were associated with its use [6]. Bronchiolar cast impaction was markedly decreased on repeat fiberoptic bronchoscopy, and improvements in oxygenation and pulmonary compliance were also noted. Subsequent chest radiographs following treatment with inhaled tPA showed gradual improvement in lung aeration. The patient was discharged on a drug regimen of aspirin, furosemide, albuterol, and aldactone, and at the time of publication of the report the patient was doing well on these therapies.

Patient 4 demonstrated gradual clinical improvement following treatment with tPA [5]. Areas of atelectasis were cleared and expectorated bronchiolar casts were notably thinner. Formations of new fibrin casts and subsequent symptoms of respiratory distress did not recur until the frequency of tPA administration was reduced from every four hours to every six hours after two weeks of treatment on the former regimen. Symptoms resolved once the dosing frequency of every four hours was reinstated. Additional attempts at decreasing the dosing frequency of tPA resulted in the increased formation of fibrin casts. Eventually, the frequency of dosing was successfully reduced to every six hours with no noted increases in cast formation. Several months later, another attempt was made to reduce the dosing frequency to every eight hours.
However, this attempt was unsuccessful. Patient 4 was still receiving inhaled tPA every six hours with no reported side effects at the time of publication.

Administration of tPA through aersolization and direct instillation of the medication onto casts in the airway has also been documented in a PB patient (patient 5, Table 1) with underlying asthma and no history of CHD [7]. The patient initially presented with respiratory distress and cough, and chest radiographs showed opacification in the left hemithorax. He was placed on a treatment regimen of hypertonic saline, dornase-alfa, bronchodilators, systemic corticosteroids, and ceftriaxone. Following expectoration of a "mucous plug" the patient was discharged with albuterol on an as-needed basis. The patient presented again four months later with respiratory distress, at which point flexible bronchoscopy was performed. A cast was discovered in the airway that was subsequently extracted, and bronchoalveolar lavage was negative for viral, fungal, and

mycobacterial pathogens. Eventually histological examination of the extracted cast was performed, which revealed that the cast was composed of fibrin, mucus, and eosinophils. Azithromycin was subsequently added to the treatment regimen.

There was no improvement in the patient's clinical status over the following two weeks despite the use of a wide range of aggressive airway clearance therapies [7]. Following the review of multiple case reports outlining the use of tPA, the decision was made to initiate treatment with this medication. Prior to administering aerosolized tPA into the airways, direct comparisons of dornase-alfa and tPA on casts were conducted by directly instilling 2.5 mg of each drug onto separate casts present in the bronchioles. The cast treated with tPA began to dissolve immediately, subsequently allowing the patient to expectorate multiple casts still present in the airways. The cast fragment treated with dornase-alfa remained unchanged. The patient's forced vital capacity (FVC) improved by nearly 50% following treatment with tPA, and chest radiographs showed a significant reduction in opacification. No adverse events were reported as a result of this treatment intervention. The patient was discharged on a treatment regimen of oral prednisone every other day, azithromycin, inhaled beclomethasone, and levalbuterol.

A decline in the patient's FVC at a follow-up pulmonary visit four months following discharge warranted an examination of the airways through flexible bronchoscopy, subsequently leading to the discovery of another bronchiolar cast. TPA (3.5 mg) was directly instilled onto the cast during the flexible bronchoscopy, and treatment with 5 mg of aerosolized tPA every eight hours was initiated. The patient's FVC improved to 81% after one week on this treatment regimen. Direct instillation of an additional 2 mg of tPA was performed to dissolve cast fragments still present in the airways. Improved aeration of the lungs was noted following this procedure. The patient was discharged on the same treatment regimen utilized previously, although the dosage of prednisone that he was receiving was increased from 0.25 to 0.5 mg/kg. At the time of publication of this case report the patient was reported to be doing well with an FVC of 93%, although the patient does still expectorate casts on occasion.

Most recently, a case of PB in a 13-year-old Fontan patient was reported [8]. The patient had a history of respiratory illness for which he was treated with nebulized albuterol and azithromycin. Following his presentation of respiratory distress, the patient underwent bronchoscopy, which revealed the presence of obstructive PB casts. Despite removal of the casts, the patient's symptoms worsened and he was treated with nebulized dornase alfa in addition to nebulized albuterol and antibiotic therapy with azithromycin and ceftriaxone. A repeat chest radiograph suggested re-accumulation of an obstruction in the left bronchus and he underwent another bronchoscopy for removal of the newly formed casts. Pathology showed the casts to be a fibrinous inflammatory exudate and nebulized tPA was initiated. The tPA (2 mg/mL) was initially dosed based on the regimen used by Costello et al. [9], starting with a dose of 12mg followed 1 hour later by a 10mg dose. Two hours later, a 5mg dose was given and continued every 2 hours for a total of 17 doses. Following dose 17, because of symptomatic improvement and oxygen saturation levels consistently above 94%, the dosing interval was increased to 3 h and therapy was continued for 21 additional doses. Nebulized t-PA therapy was discontinued on hospital day 6 and a bronchoscopy at that time showed no casts in the airways. The patient did have subsequent cast formation which required re-initiation of inhaled tPA therapy but he was eventually discharged from the hospital without tPA therapy. No additional information was given regarding recurrence

of PB in this patient.

Patient 11, a 27-month old with HLHS, was initially treated with tPA instilled directly into the airways with favorable results [21]. This was followed by treatment with a regimen of inhaled tPA (5mg) four times daily that did not lessen the frequency of cast expectoration but resulted in significantly fewer episodes of airway obstruction. This suggests that the use of inhaled tPA reduced cast size. Details for the clinical courses of three other patients (12-14) were not described in the published report [21].

Patient 15 was treated with a 5mg Q6h regimen of nebulized tPA and responded favorably as evidenced by a decline in PB cast size and frequency of production. Finally, patient 16, who presented with PB 9 years after Fontan palliation, had limited response to inhaled tPA but no details about the dosing regimen were provided in the case report. No adverse drug reactions related to tPA administration were reported in either case [23].

2.5 Study Rationale

Presently there are no FDA-approved pharmacotherapies for the treatment of PB. Nevertheless, inhaled tPA is routinely and effectively used to treat this serious pediatric illness. The rationale for this study is the need for safety and efficacy data for this therapy so that it can be confidently employed for the treatment of all children with PB.

2.6 Safety

Major bleeding events are the primary safety concern with tPA use [25]. Toxicology studies have been conducted to examine adverse events for both pulmonary and systemic administration of this drug.

2.6.1 Pulmonary Toxicology

Pre-clinical toxicology studies were performed in male and female B6C3F1 mice examining both short and long-term dosing schemes [20, 26]. A species-specific pulmonary formulation of tPA (pf-mtPA, Molecular Innovations, Novi, MI) was utilized for these studies. A range of doses (0.3-1.61 mg/kg/d) and dosing frequencies (up to 6 times/day) were tested.

In the short-term accelerated dosing study, mice received either single or accelerated dosing regimen of intratracheal (IT) nebulized pf-mtPA (3 mg/kg) of [20]. Accelerated dosing regimens consisted of single doses of pf-mtPA administered every two hours over the course of a four-, eight-, or twelve-hour time period. The mice entered a two-week recovery period following the completion of each respective dosing regimen during which animals were monitored for signs of hemorrhage.

All animals survived both the dosing and recovery periods of the study, and there were no notable changes in animal behavior that were indicative of hemorrhage, neurologic or respiratory defects [20]. Microscopic assessment of vital organs and gross necropsy showed no evidence of hemorrhage at the conclusion of the study. Estimated hematocrit values were measured at baseline prior to the initiation treatment with pf-mtPA and again at the conclusion of the study. These values provided an additional measure for detecting prolonged or asymptomatic hemorrhage. A decrease in mean estimated hematocrit was detected in female mice receiving the

pf-mtPA over the longest treatment interval of twelve hours. However, a similar drop in mean hematocrit was observed in female mice receiving six doses of placebo as well. One male assigned to the twelve-hour dosing regimen experienced a significant decrease in mean hematocrit. Despite this observation, no clinical or pathologic evidence of hemorrhage was noted upon further examination. Subsequently, this decline in mean hematocrit remained unexplained. Overall, no significant changes in estimated mean hematocrit were observed in male mice receiving the twelve-hour dosing regimen.

Indicators of lung injury were also assessed through examinations of protein and lactate dehydrogenase (LDH) activity in bronchoalveolar lavage fluid (BALF) [20]. No significant differences in BALF cell counts, protein, or LDH activity were noted between pf-mtPA and placebo treatment groups. Mean BALF cell and protein concentrations were higher in male mice than in females. However, this trend has been observed in other animal studies that have examined inflammatory responses to pulmonary administration of lipopolysaccharides (LPS). Furthermore, BALF cell concentrations in males were similar between untreated, pf-mtPA, and placebo-treated mice. Macrophages comprised the vast majority of cells identified with the differential in both males and females across all treatment groups. LDH activity across all treatment groups and both sexes fell below detection levels. These results collectively indicate that treatment with the dosing regimens of pf-mtPA utilized in this study did not contribute to lung inflammation or injury. Collectively, these data show that there were no negative effects of the test article at the studied doses.

The prolonged treatment of pf-mtPA was carried out in mice over the course of a twenty-eight day period. Both male and female mice received twice-daily dosing of either low-dose (0.3 mg/kg/day) or high-dose (0.6 mg/kg/day) IT administered nebulized pf-mtPA [26]. The low-dose treatment group tolerated the dosing regimen well, and no adverse reactions to treatment were observed. However, fatal pulmonary hemorrhage did occur in both males and females in the high-dose treatment group.

Within the high-dose treatment group, males suffered pulmonary hemorrhage six days after the initiation of treatment (8% incidence, n=1), and this same adverse reaction occurred in females on day twelve of treatment (16% incidence, n=2) [26]. The higher incidence of fatal lung hemorrhage in females can be attributed to differences in mean body weights of each sex, subsequently resulting in the females receiving a higher dose on average. Despite the administration of higher mean doses of mtPA to females, accumulation of mtPA in bronchoalveolar lavage fluid (BALF) occurred in higher concentrations in males. Due to the differences observed between sexes with regard to tPA accumulation in the lungs and the onset of pulmonary hemorrhage, it is possible that males may be at an increased risk of tPA-induced hemorrhage. Additionally, two male mice (one high-dose saline and one high-dose pf-mtPA) displayed signs of pulmonary hemorrhage following histology at the conclusion of the twenty-eight day dosing regimen. Despite the occurrence of these fatal adverse events, it is important to note that low-dose treatment groups showed no evidence of pulmonary hemorrhage over the twenty-eight day study period.

Indicators of lung injury were examined by measuring BALF cell counts and albumin concentrations [26]. An increase in BALF albumin concentrations were observed in both male pf-mtPA treatments groups in comparison to saline controls. However, BALF albumin

concentrations in the low-dose treatment group was still lower than the levels observed in mice receiving pulmonary administration of inflammatory cytokines known to cause lung injury [26]. BALF cell concentrations were higher in males and females in the high-dose treatment group in comparison to high-dose saline controls as well, but these values still fell below those associated with lung injury. No differences in cell differentials were noted between pf-mtPA- and saline-treated mice, and macrophages comprised the majority of cells identified (≥75%). Based on the results yielded from this study, it is possible that high-dose pf-mtPA may contribute to an increased risk of pulmonary hemorrhage. Although the exact mechanism by which this occurs remains unknown, degradation of the extracellular matrix as a result of proteolysis by tPA through indirect or direct means constitutes one potential explanation for this event.

2.6.2 Systemic Toxicology (Intravenous Administration)

To date, there have not been any reports of bleeding associated with inhaled tPA. This mimics our clinical experience. The bleeding events that have been reported with tPA are those which have occurred with its IV administration. As mentioned previously, tPA aids in clot breakdown within the body through the fibrinolytic property that it possesses, in turn yielding fibrin degradation products (FDPs). FDPs possess potent anticoagulant and antiplatelet effects, further contributing to heightened risks for bleeding events [28]. Subsequently, multiple studies have been conducted to examine hematologic and clinical factors that may contribute to the occurrence of these events.

2.7.0 Dose Rationale

A dosing regimen of 5 mg tPA (1mg/mL) nebulized every 6 h (+/- 30 min) for approximately 72 h or up to a total of 12 doses will be employed in this study. This regimen is was derived from experimental studies and published pre-clinical data (Figure 1) as well as extensive clinical experience of the investigators from using this regimen of inhaled tPA to treat children with PB.

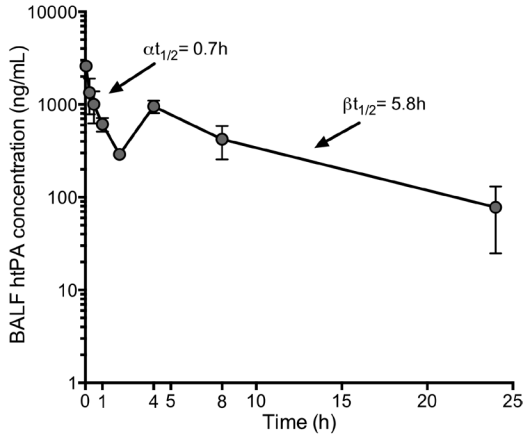

Figure 1: Time versus concentration curve of human tPA measured in the bronchoalveolar fluid (BALF) of mice following a single intratracheal (IT) dose (0.3mg/kg) of nebulized pulmonary formulation tPA (see text). Data are the mean (± SEM) of 3-4 mice at each time point.

Elaborate pharmacokinetic studies of lung delivered tPA have not been conducted. However, we recently completed a study using a healthy mouse model in which a single IT dose (0.3 mg/kg) of tPA (formulated for pulmonary delivery by the addition of a small amount of Tween 80) resulted in measurable concentrations of tPA in the BALF and an estimated terminal half-life of 5.8 h with an associated range of 2.6-8.0 h [19]. These data suggest that lung delivered tPA follows non-linear pharmacokinetics.

The Q6h dosing interval is shorter than the calculated value from these data because it takes into account the expected increase in clearance that will occur in the presence of airway fibrin. We expect that in the presence of fibrin there will be additional degradation of tPA due to the generation of plasmin. This fibrin-induced increase in tPA clearance in the lung compartment will likely reduce the elimination half-life of the drug.

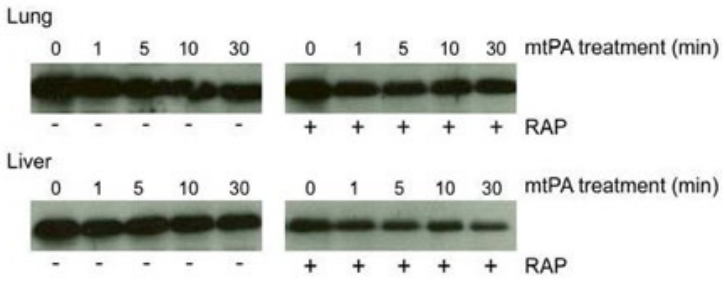

Figure 2: Uptake of mouse tPA (mtPA) is similar in whole lung and liver cell suspensions over 30 min. In addition, mtPA uptake in both cell types is reduced in the presence of receptor antagonist protein (RAP), an inhibitor of LRP1.

In this recently completed study, we also learned that the lungs have comparable amounts of low density lipoprotein receptor-related protein 1 (LRP1) to the liver (**Figure 2**) and that LRP1-mediated uptake of tPA occurs to a similar extent in isolated lung cells as it does in liver cells [19]. These data suggest that the lungs have a previously unrecognized metabolic capacity to execute LRP1-receptor mediated endocytosis of tPA. This is, in part, an LRP1-mediated event because the LRP1 inhibitor, receptor antagonist protein (RAP), reduced mtPA uptake in both cell types. The extent to which this mechanism participates in the lung clearance and metabolomics of tPA is not fully understood.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Objectives

- To assess the safety of inhaled nebulized tPA for the treatment of acute PB.

3.2 Endpoints

3.2.1 Primary Endpoint (safety)

- Development of new, active bleeding that is systemic and/or pulmonary
- New hematuria (defined as gross hematuria)

3.2.2 Secondary Endpoints (safety and efficacy)

- Changes in oxygen saturation (as determined by pulse oximetry)
- Changes in pulmonary function tests (PFTs) which will be done in accordance with American Thoracic Society guidelines [29]
- Frequency of production/expectoration and size of airway casts (weight and length)
- Changes in the chest x-ray (CXR)
- Requirement for urgent or emergent bronchoscopy and/or mechanical ventilation
- Measurement of fibrin and mucin content of airway casts
- Detection of fibrin degradation products (FDPs) in the systemic circulation
- Assessment of a patient-centered outcome measure (CFQ-R; see manual of operations (MoP))

3.2.3 Tertiary Endpoints

- Measurement of urine and blood metabolites (as measured by nuclear magnetic resonance (NMR) metabolomics) to identify potential biomarker candidates of drug response

4 STUDY DESIGN

This study will be a multi-center, phase II, open-label, single-arm study to assess the safety of inhaled tPA for the treatment of acute exacerbation of PB. A randomized, placebo-controlled trial is neither feasible nor ethical. Plastic bronchitis is exceedingly rare and based on the evidence that PB airway casts are most often primarily composed of fibrin, the majority of clinical centers, including ours, have adopted the practice of treating PB with inhaled tPA.

The study will be conducted in two phases. The first phase, screening (see **Figure 3**), can be conducted in advance of or at the time of an acute exacerbation of PB depending on whether the patient has underlying congenital heart disease. In parallel, a group of control subjects will be recruited for the purpose of collecting baseline blood and urine samples for metabolite assays, pulmonary function tests (PFT) and clinical labs for comparison to PB patients (see **Figure 4**).

In order for non-CHD patients to advance to the treatment phase, acquired PB airway casts must be primarily composed of fibrin. There is substantial and sufficient evidence that this is the case for patients with underlying CHD. However, PB patients who do not have underlying CHD, will be required to have pathologic confirmation of an airway cast to confirm that it is primarily fibrin. In parallel with the screening and treatment phases of the study, a cohort of healthy controls, a cohort of Fontan patients with protein losing enteropathy (PLE; a lymphatic mediated disease like PB) that occurs in Fontan patients) and a cohort of otherwise healthy Fontan patients (see text) will be enrolled for the purpose of acquiring baseline blood and urine samples that will serve as negative controls for metabolomics biomarker assays as well as for comparators for PFT and clinical laboratories. A blood and urine sample will also be acquired for this purpose from eligible patients with an initial acute exacerbation of PB.

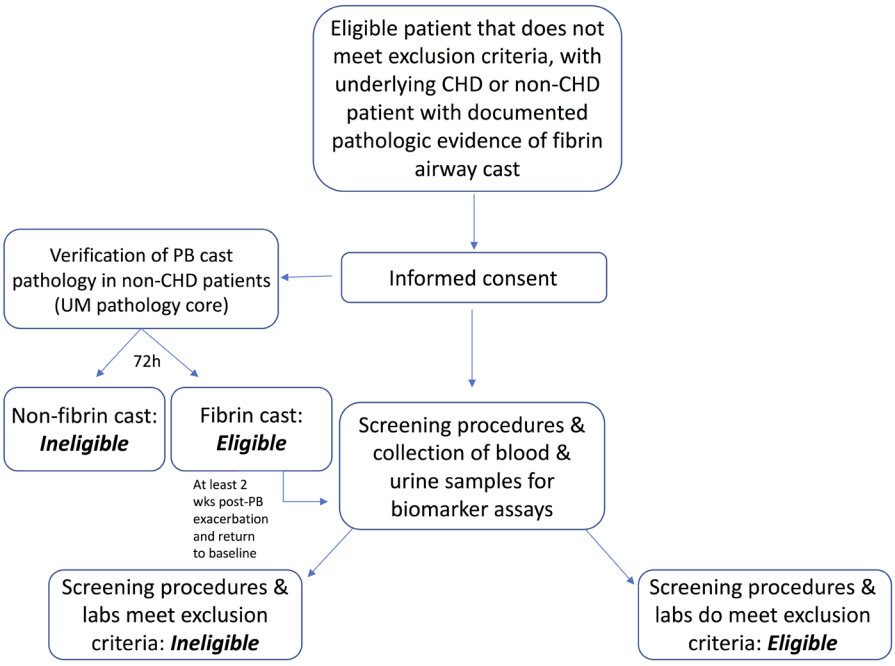

Figure 3. Scheme of events for PB patients entered into the screening phase of the study.

Following informed consent, patients with underlying CHD can proceed to screening procedures; expectorated or bronchoscopy-acquired PB casts from non-CHD patients will be sent to the study's pathology core laboratory (University of Michigan). A blood and urine sample will be collected and sent to the study's NMR Metabolomics Laboratory (University of Michigan; see MoP). Cast samples will be assessed for fibrin content within 72 h of receipt. If the cast is primarily composed of fibrin, the patient will be deemed eligible for the treatment phase of the study. This will be reported to the clinical site as either "treatment phase eligible" or "treatment phase ineligible" using REDCap, an electronic data capture (EDC) system that is secure, HIPAA compliant, and web-based. Alternatively, non-CHD patients known to have PB who have previous pathologic evidence of fibrin airway cast production can also be considered eligible for enrollment into the treatment phase of the study. Respective clinical sites will be responsible for providing pathological documentation of fibrin cast production to the study's pathology core lab.

In parallel, healthy subjects, Fontan patients with PLE and Fontan patients with no evidence of PB or other Fontan-associated complications will be recruited and enrolled for the purpose of the collection of a blood and urine sample (**Figure 4**) for biomarker assays (see MoP). We are including the latter group because, although Fontan physiology is not a study eligibility criterion, we expect that most of the enrolled patients will have Fontan physiology. These samples will serve as negative control (healthy and healthy Fontans) and positive (Fontan with PLE) data for the samples that will be collected from patients with PB. With the exception of the PLE patients, all control subjects will be enrolled at the University of Michigan.

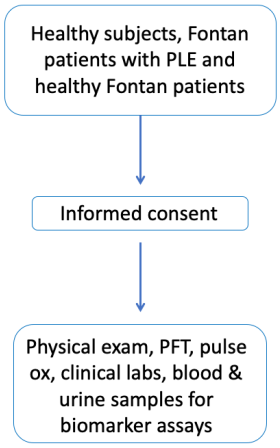

Figure 4. Scheme of events for healthy subjects, PLE Fontan patients and healthy Fontan patients

The treatment phase will consist of an approximately 72 h regimen of inhaled tPA 5mg every 6 h (+/- 30 min) during which measurements of safety and efficacy will be made. Treatment with inhaled tPA (5mg every 6 h) can continue at the discretion of the treating physician.

This study will also include the acquisition of blood and urine samples for the purpose of conducting biomarker assays. Specifically, we will assay these samples using an untargeted nuclear magnetic resonance (NMR metabolomics platform as previously described [30]). The purpose of this work is to identify potential candidate biomarkers that may be indicative of PB and PB response to inhaled tPA (see MoP).

5 SUBJECT SELECTION

5.1 Subject Recruitment

Patients with an acute exacerbation of PB or with a history of PB, defined as the expectoration of or a bronchoscopy retrieved fibrin PB cast at participating clinical centers in the study will be recruited for the screening eligibility. We plan to enroll up to 13 patients with acute exacerbation of PB will be enrolled into the treatment phase of the study. A separate set of healthy controls, and healthy Fontan patients will be recruited only from the University of Michigan and Fontan patients with PLE (recruited from all clinical sites) for the purpose of collecting PFT, clinical labs, and a urine and blood sample for biomarker assays.

5.1.1 Inclusion Criteria for Screening Phase

1. ≥ 5 years of age but ≤24 years of age and weigh at least 18.6 kg (41 lbs)
2. Patients with CHD that have a history of PB with previous airway cast production
3. Patients without CHD that present with an acute exacerbation of PB, defined as the expectoration of, or a bronchoscopy retrieved, fibrin PB cast that causes acute respiratory distress (e.g., severe coughing, difficulty breathing, dyspnea) or a history of PB with pathologic evidence of fibrin airway cast production. Either a cast sample (at least 2 cm [~0.8 inches]) or a pathology report that documents PB cast fibrin content must be submitted to the UM pathology core.
4. Must be able to use a mouthpiece nebulizer
5. Informed consent (with parental consent if age ≥14 years) or assent for age ≥10 and < 14 years old with parental informed consent

5.1.2 Exclusion Criteria for Screening and Treatment Phases

1. Known contraindication(s) to the use of tPA, including:
   - active internal bleeding;
   - history of cerebrovascular accident;
   - recent intracranial or intraspinal surgery or trauma;
   - intracranial neoplasm, intracranial arteriovenous malformation or intracranial aneurysm;
   - known bleeding diathesis;
   - and/or severe uncontrolled hypertension
2. Body weight ≥ 100th percentile or BMI > 30
3. Known cystic fibrosis
4. Currently receiving dornase-alfa and/or inhaled unfractionated or low molecular weight heparin and/or a direct acting oral anticoagulant (e.g., dabigatran, rivaroxaban)
   - Inhaled unfractionated or low molecular weight heparin must be discontinued at least 72h prior to the start of the treatment phase. Inhaled dornase alfa should be discontinued no later than the time of the start of enrollment in the treatment phase. If the patient is receiving inhaled tPA, this regimen must be discontinued and transitioned to the inpatient dosing regimen (5mg Q6h) of study drug (see MoP).
   - Direct acting oral anticoagulants must be discontinued one week prior to the start of enrollment in the treatment phase.

5. Protein losing enteropathy
6. Liver dysfunction (defined as ≥ 3X the normal levels of one or both liver transaminases, AST and ALT)
   - Transaminase levels acquired within the last 9 months can be used to assess liver function. If previously normal and there is no clinical indication that liver function has worsened, the patient can be enrolled. If there are no transaminase values within the last 9 months, they need to be acquired as part of screening.
7. Need for concomitant intravenous or sub-cutaneous anti-coagulation with resulting anti-Xa levels > 0.5 (low molecular weight heparins) or > 0.3 (unfractionated heparin)
8. International normalized ratio (INR) > 2.0 if not receiving warfarin
9. Patients being actively treated for thrombosis
10. Concomitant use of a thienopyridine class antiplatelet agent (e.g., clopidogrel)
11. A platelet count of < 100,000 platelets/μL
12. A hematocrit <30%
13. Gross hematuria on screening urinalysis
14. Pregnant or lactating women (negative pregnancy test required for girls/women of childbearing potential at the time of inhaled tPA administration). All women of child-bearing potential must be willing to practice appropriate contraception throughout the study.
15. Subjects who are known positive for, or are hospitalized with COVID-19 caused by the new coronavirus, SARS CoV-2, at the start of the treatment phase.
16. Suspected or active concurrent infectious illness.

5.1.3 Inclusion Criteria for Treatment Phase

1. Patients with CHD are eligible for the treatment phase if they have a history of previous airway cast production and/or present with symptoms of an acute exacerbation (e.g., difficulty breathing, dyspnea) of PB that requires hospitalization. An acute exacerbation of PB is defined as either respiratory symptoms suspicious for airway cast formation and/or the expectoration of, or a bronchoscopy retrieved, fibrin PB cast.
2. For non-CHD patients, there must be pathological evidence that the cast: 1) is primarily composed of fibrin based on fibrin-specific staining; 2) have associated inflammatory cells limited to lymphocytes and macrophages; and 3) do not have significant numbers of eosinophils, Charcot-Leyden crystals, acute inflammation, or inspissated mucus as determined by the study's pathology core laboratory during the screening phase of the study or patients with PB who have previous pathologic evidence of fibrin cast production.
3. Inclusion criteria for screening phase are met.

5.1.4 Inclusion Criteria for Healthy Controls

1. Healthy children ≥ 5 years of age but ≤18 years of age with no other underlying concomitant illness or chronic medication use (with the exception of vitamin supplements)
2. Weigh at least 18.6 kg (41 lbs)

5.1.5 Inclusion Criteria for Healthy non-PB Fontan Controls

1. Children ≥ 5 years of age but ≤18 years of age with uncomplicated Fontan physiology with no history of PB, other Fontan-associated complications (e.g., hepatopathy, PLE), or other concomitant illnesses (e.g., asthma).
2. Weigh at least 18.6 kg (41 lbs)

5.1.6 Inclusion Criteria for PLE Fontan Controls

1. Children ≥ 5 years of age but ≤18 years of age with Fontan physiology, no history of PB and a diagnosis of PLE defined as clinically symptomatic hypoproteinemia and/or enteral protein loss.
2. Weigh at least 18.6 kg (41 lbs)

5.1.7 Exclusion Criteria for Healthy, non-PB Fontan Controls and PLE Fontan Controls

1. Exceeds the 100th percentile for body weight or has a BMI greater than 30.
2. History of post-operative chylothorax following any palliation surgery (except for PLE controls).
3. Liver dysfunction (ALT &AST) (defined as ≥ 3X the normal levels of one or both liver transaminases, ALT & AST))
4. COVID-19 positive within the last 14 days prior to the scheduled visit and/or the presence of symptoms consistent with COVID-19 at the time of the visit
5. Suspected or active concurrent infectious illness

5.2 Women of Childbearing Potential

Women of childbearing potential will not be excluded from participating in the treatment phase of this study but pregnant or lactating women will. For study eligibility, girls/women will need to have a negative pregnancy test and agree to the use of appropriate contraceptives (either barrier or oral contraceptive).

6 STUDY TREATMENTS

6.1 Screening Phase for plastic bronchitis patients only

The initial phase of the study is the screening phase (Figure 3), which is designed to acquire the needed evidence for inhaled tPA treatment eligibility. Patients with CHD and known PB are eligible for screening because their casts are known to be primarily composed of fibrin and they are routinely treated with inhaled tPA for PB exacerbations. For non-CHD patients, documentation of fibrin cast production is required. This can be accomplished by submitting a cast sample (***at least 2 cm [~0.8 inches]***) to the pathology core laboratory at the University of Michigan (Figure 3). This will be done using PB cast collection kits that will be provided to each clinical site. Samples will be sent by express, next day air directly to the core lab. Upon receipt samples will be logged and prepared for cryopreservation. Sections from these samples will be evaluated by a pathologist with pulmonary expertise and accordingly stained as deemed appropriate. The pathologist will render a decision as to whether the sample is primarily composed of fibrin as defined under 5.1.3 and enter "treatment phase eligible" or "treatment phase ineligible" into REDCap (http://project-redcap.org/).

If the sample is primarily composed of fibrin, two-weeks following the PB exacerbation and when the patient's symptoms and treatment are back to baseline as determined by the patient's primary physician, a baseline urine and blood sample will be collected for the purpose of metabolite biomarker assays. These samples will be batched and sent to the NMR metabolomics core laboratory at the University of Michigan. These samples will be used for research purposes only.

A urine and blood sample will be collected at the initial presentation for the purpose of NMR

metabolomics assays. These samples will also be batched and shipped to metabolomics core laboratory at the University of Michigan. These samples will be used for research purposes only.

Alternatively, non-CHD patients that have a pathology report of a PB cast that documents fibrin content can be submitted to the UM pathology core. Non-CHD PB patients must have documentation that their airway casts are primarily composed of fibrin by pathological assessment to be eligible for the treatment phase of the study. This requirement and that CHD patients routinely produce fibrin airway casts highlights the unethical nature of including a placebo arm since we and others have demonstrated that fibrin airway casts are responsive to tPA treatment [1, 7, 8].

6.2 Treatment Phase for plastic bronchitis patients only

This is an open-label treatment study. All non-CHD patients who are determined to be eligible for inhaled tPA during the screening phase of the study will enter into the treatment phase at the time of the patient's next PB hospital admission. CHD patients known to have PB can either be screened in advance for eligibility or can be screened at the time of presentation of acute exacerbation for enrollment into the treatment phase of the study. Respective clinical sites are responsible for providing pathological document of fibrin cast production to the study's pathology core lab for non-CHD patients.

In the event that a CHD patient presents with an acute PB exacerbation that requires urgent treatment, screening (pre-treatment) clinical laboratories (blood and urine) should be acquired but treatment can commence before results are obtained, if, in the clinical judgement of the site PI, the risk for bleeding is low (e.g., no medical history of blood diathesis). However, study eligibility of the patient must be re-assessed once screening laboratory results are available. In the event that exclusion criteria are met, the patient must be withdrawn from the study.

6.3 Drug Supplies

6.3.1 Formulation, Preparation and Dispensing

Recombinant human tissue-type plasminogen activator (tPA, alteplase, Activase®, Genentech, South San Francisco, CA) will be utilized for this study. Each clinical site will be responsible for their own reconstitution of tPA for inhalation. The following information is available in the Activase® (Alteplase) package insert [31].

Alteplase (Activase®) is available as a sterile, lyophilized powder in 50 mg (29 million IU, NDC 50242-044-13) vials containing vacuum and in 100 mg (58 million IU, NDC 50242-085-27) vials without vacuum. The 100 mg Activase® vial contains one transfer device. The 50mg vial will be used for this study.

Reconstitution of tPA will be carried out using aseptic technique at each clinical site. Reconstitution should be carried out using a large bore needle (e.g., 18 gauge) and a syringe, directing the stream of Sterile Water for Injection, USP, into the lyophilized cake. DO NOT USE IF VACUUM IS NOT PRESENT. Slight foaming upon reconstitution is not unusual; standing undisturbed for several minutes is usually sufficient to allow dissipation of any large bubbles.

No other medication should be added to solutions containing Activase. Any unused solution should be discarded. Before administration, the product should be visually inspected for particulate matter

and discoloration whenever solution and container permit. If a precipitate is seen, the product should not be used. The product will be labeled in accordance with FDA requirements (please refer to the MoP).

6.3.2 Drug Storage and Drug Accountability

Store lyophilized Activase at controlled room temperature not to exceed 30°C (86°F), or under refrigeration (2°–8°C/36°–46°F). Protect the lyophilized material during extended storage from excessive exposure to light. Do not use beyond the expiration date stamped on the vial. Because Activase contains no antibacterial preservatives, it should be reconstituted immediately before use. The solution may be used for administration within 8 hours following reconstitution when stored between 2°–30°C (36°–86°F). Before administration, the product should be visually inspected for particulate matter and discoloration. Activase is stable for up to 8 hours. Exposure to light has no effect on the stability of these solutions. Excessive agitation during dilution should be avoided; mixing should be accomplished with gentle swirling and/or slow inversion. Do not use other infusion solutions, e.g., Sterile Water for Injection, USP, or preservative-containing solutions for further dilution.

Drug use and accountability will be performed in accordance with this protocol and FDA requirements. The investigational drug service (IDS) at each clinical site will be employed for drug accounting. In addition, each IDS will be responsible for developing and implementing procedures for the proper control and handling of the investigational drug, including procurement, storage, medication labeling and dispensing, drug inventory management, and other distribution and control functions.

6.3.3 Administration

At the time a patient is enrolled into the treatment phase of the study, the IDS will be notified and the needed tPA doses will be readied. The 5mg (5mL) dose will be dispensed into a jet nebulizer (Salter Labs, NebuTech HDN nebulizer, Ref 8960-7; https://www.salterlabs.com/8960-7-50) (see MoP). The dose will be administered by attaching the associated mouthpiece to the nebulizer head. Nebulization will commence by connecting the nebulizer's tubing to the hospital's internal compressed oxygen system via a flow meter (8 LPM) at which time the patient will be instructed to put the mouthpiece in his/her mouth and breathe slowly and deeply. This will continue until the nebulizer's reservoir is empty which typically takes approximately 7-10 min. Inhaled tPA administration will be standard across all of the study's participating clinical sites (see MoP). Efficacy measurements will be scheduled so they do not directly coincide with tPA administration.

6.4 Concomitant medications

Concomitant medication regimens will be prescribed at the discretion of the treating physician. However, the concomitant use of certain drugs that meet exclusion criteria should *not* be administered:

- Dornase-alfa (Pulmozyme)
- Intravenous or sub-cutaneous anti-coagulation with resulting anti-Xa levels ≥ 0.5 (enoxaparin) or ≥ 0.3 (unfractionated heparin)
- Thienopyridine class antiplatelet agent (e.g., clopidogrel)

- Inhaled unfractionated or low molecular weight heparin
- Direct acting oral anticoagulants

No medications should be co-administered with alteplase including bronchodilators and vibrating vests for airway clearance or any other form of airway clearance should not be used for 2 hours before or after the administration of alteplase.

7 STUDY PROCEDURES

7.1 Screening Phase

Patients will be screened for the treatment phase at each participating clinical center based on inclusion and exclusion criteria (**5.1.1** and **5.1.2**).

7.1.1 Screening Phase Visit 1: Enrollment for PB patients

Informed consent will be sought from study eligible patients. Consent will give the treating physician permission to submit the acquired cast sample or pathology report for pathological evaluation by the University of Michigan (coordinating clinical trial center) pathology core lab for non-CHD patients and a blood and urine sample to the University of Michigan's NMR Metabolomics Laboratory. Cast samples will be submitted via express, next day mail using a cast collection kit (see MoP). Within 72 h of receipt the core lab will report whether the patient is eligible or ineligible for the treatment phase of the study based on the determined fibrin content of the cast sample (see MoP). For CHD patients, informed consent will give the treating physician permission to perform screening activities to determine study eligibility (see schedule of activities table in section 7.3).

Non-CHD patients who are deemed eligible for the treatment phase following screening visit 1, are at least two weeks post an episode of PB exacerbation and have returned to baseline state and medical regimen, will be scheduled for the second screening visit. Respective clinical sites are responsible for providing documentation of fibrin cast production to the University of Michigan pathology core lab to meet eligibility requirements.

CHD patients with PB and non-CHD patients who have pathological documentation of fibrin cast production that meet screening phase criteria are eligible for the treatment phase at the time of an acute exacerbation of PB. They will only have 1 screening visit which is also the enrollment phase.

Healthy controls and healthy Fontan controls will be recruited and enrolled at the study's coordinating center (University of Michigan). PLE Fontan controls will be recruited at all the study's clinical sites. Their participation **(see inclusion and exclusion criteria, 5.1.4-5.1.7)** will consist of the procedures and tests outlined under screening visit 2.

7.1.2 Screening Phase Visit 1/2: Enrollment for PB subjects and healthy, PLE Fontan and non-PB Fontan controls

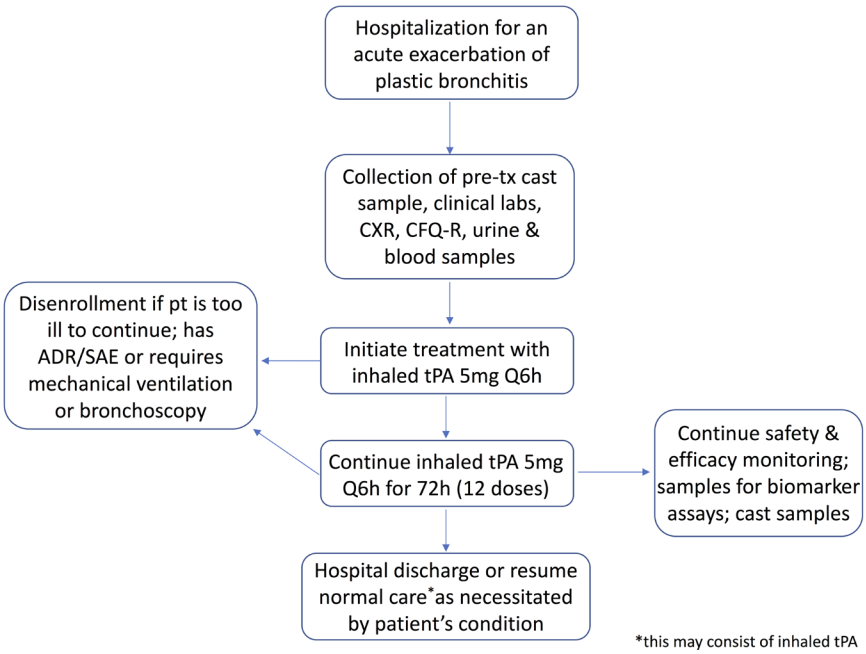

Figure 5. Scheme of events for PB patients entered into the treatment phase of the study

During this visit, the following procedures/tests will be performed:

1. Physical exam (including a pre-treatment abbreviated neurological exam as described in the MoP; PB patients only), vital signs, medical history (including medication regimen)
2. Pulmonary function tests
3. Pulse oximetry for measurement of baseline oxygenation
4. Urinalysis
5. Venipuncture for blood hematocrit (Hct), platelet count, fibrin degradation products (FDP), INR, PTT, IgA, M & G levels and fibrinogen and tPA concentrations (FDP, IgA, G & M levels & fibrinogen and tPA concentrations - not for clinical use; see the MoP for details about immunoglobulin levels and tPA concentrations) and;
6. the acquisition of a blood sample for the purpose of biomarker assays (not for clinical use).
7. Collect urine sample for the purpose of biomarker assays (not for clinical use).
8. Determine if the PB subject continues to meet the inclusion/exclusion criteria based on the screening criteria. If not, they will be excluded from the treatment phase of the trial. However, all biomarker specimens obtained at Screening Visit 2 will be retained.

7.2 Treatment Study Period: Treatment phase for Plastic Bronchitis Patients only.

The scheme for the treatment phase of the study is shown in **Figure 5**. Details of the procedures and tests involved in the treatment phase of the study can be found in the text. Blood and urine samples will be collected for the purpose of conducting metabolomics (biomarker) assays.

There is no time limitation between the screening and treatment phases of the study. As long as the study is active, patients who have completed the screening phase and are eligible for the treatment phase can be entered. In the event that a CHD patient presents with an acute PB exacerbation that requires urgent treatment, screening (pre-treatment) clinical laboratories (blood and urine) should be acquired but treatment can commence before results are obtained, if, in the clinical judgement of the site PI, the risk for bleeding is low (e.g., no medical history of blood diathesis). However, the eligibility of the patient must be re-assessed once screening laboratory results are available. In the event that exclusion criteria are met, the patient must be withdrawn from the study.

For enrolled patients who have met screening phase criteria, the treatment phase will consist of the following:

7.2.1 Treatment phase, pre-treatment/treatment (Day 1): Acute exacerbation of PB or Hospital Admission

Prior to treatment with study drug, the following assessments will be made:

1. Physical exam including a full neurological exam as described in the MoP
2. Vital signs
3. A chest x-ray (PA & lateral)
4. Pretreatment oxygen saturation (pulse oximetry)
5. Bed side pulmonary function tests (if feasible, should be obtained prior to tPA treatment and within 24h of tPA administration) if the patient admission happens over the weekend then the initial PFT should be done on Monday.
6. The collection of a blood sample for the purpose of biomarker assays (not for clinical use)
7. Collection of a urine sample for the purpose of biomarker assays (not for clinical use)
8. *A pre-treatment blood sample for Hct, platelet count, FDP, INR, PTT, and AST and ALT (if applicable); IgA, G & M levels and fibrinogen & tPA concentrations (FDP, IgA, G & M levels & fibrinogen & tPA concentrations – not for clinical use)
9. *A pre-treatment urinalysis and pregnancy test
10. Documentation of concomitant medication regimen
11. Initiation of therapy with inhaled tPA, 5mg Q6h (+/- 30min) for approximately 72 h or up to 12 doses during hospitalization
12. The collection and submission of all expectorated airway casts to the core pathology lab
13. Monitor and document any adverse effects
14. Patient-centered outcome measure (CFQ-R; see MoP)

Please see section 5.1.2 for exclusion criteria for the treatment phase of the study. *If screening phase, visit 1/2 laboratory tests were collected within 2 weeks of the treatment phase, pre-treatment, day 0, they do not need to be repeated with the exception of a pregnancy test in female patients of childbearing potential.

7.2.2 Treatment Phase, treatment (Days 2-3)

In addition to routine clinical monitoring, following the initiation of inhaled tPA treatment, **daily** monitoring will consist of:

1. Physical exam including an abbreviated neurological exam as described in the MoP

2. Vital signs
3. Oxygenation (pulse oximetry); see the MoP regarding these measurements
4. Pulmonary function tests (PFTs); see the MoP regarding PFTs
5. The collection and submission of all expectorated airway casts to the core pathology lab
6. Urinalysis
7. Measurement of blood Hct, platelet count, FDP, INR, PTT & fibrinogen & tPA concentrations (FDP, fibrinogen & tPA concentrations – not for clinical use)
8. Documentation of concomitant medication regimen
9. Assessment and categorization of potential adverse events
10. Acquisition of a blood sample for the purpose of biomarker assays (not for clinical use).
11. Collection of a urine sample for the purpose of biomarker assays (not for clinical use).
12. Dosing regimen of 5 mg tPA (1mg/mL) nebulized every 6 h (+/- 30min) for approximately 72 h or up to a total of 12 doses

7.2.3 Follow-up, hospital discharge (Day 3 or hospital discharge)

Within 24h of completion of the administration of the last dose of inhaled tPA, whether study drug or that administered as part of clinical care, or at hospital discharge, whichever occurs first, patients will have the assessments listed below. If hospital discharge is the same day as the day of the administration of the 12th (or last) dose of study drug, daily monitoring labs can be obtained after administration of the 12th (or last) dose along with all other hospital discharge scheduled activities:

1. Physical exam including a full neurological exam as described in the MoP
2. Vital signs
3. Documentation of concomitant medication regimen
4. Oxygenation (pulse oximetry)
5. Pulmonary function tests (done once at the time of the discontinuation of tPA)
6. Chest x-ray (PA & lateral) (done once at the time of the discontinuation of tPA)
7. Urinalysis
8. Blood Hct, platelet count, and FDP, INR, PTT, IgA, G & M levels & fibrinogen & tPA concentrations (FDP, fibrinogen & tPA concentrations on day 4 or up to 24 h after the discontinuation of inhaled tPA; IgA, G & M levels once between days 4-12; not for clinical use)
9. Acquisition of a blood sample for the purpose of biomarker assays (not for clinical use).
10. Collection of a urine sample for the purpose of biomarker assays (not for clinical use).
11. Assessment and categorization of potential adverse events
12. The collection and submission of all expectorated airway casts to the core pathology lab
13. Patient-centered outcome measure (CFQ-R; see MoP) (done within 24 h of the completion of study drug or just prior to hospital discharge, whichever comes first)

At hospital discharge, the medication regimen will be documented and patients will receive a "cast diary" to document the expectoration of any casts and hospitalizations for PB exacerbations that occur between hospital discharge and the study close out visit.

7.2.4 Closeout (Day 30 +/- 7 days)

The final study visit must be done during a time when the patient's PB is quiescent (i.e., there is

no active cast production). This should be performed as close to 30 days (+ 7 days) after the completion or termination of inhaled tPA as possible. This follow up visit will consist of the following tests/procedures:

1. Physical exam including an abbreviated neurological exam as described in the MoP
2. Vital signs
3. Venipuncture for blood Hct, platelet count, FDP, IgA, G & M levels & fibrinogen (FDP, IgA, G & M levels & fibrinogen– not for clinical use)
4. Pulmonary function tests, and;
5. Assessment and categorization of potential adverse events
6. Documentation of concomitant medications
7. Patient-centered outcome measure (CFQ-R; see MoP)
8. Review of the cast and hospitalization visit diary
9. Medical History

Alternatively, if an in-person 30d visit cannot be achieved because of unforeseen circumstances including limitations imposed by the COVID-19 pandemic, a telemedicine visit can be done, and a local/external clinical laboratory can be used for the acquisition of the needed clinical laboratory values. Under this scenario, a physical exam, vital signs, and pulmonary function tests will not be able to be acquired. The cast and hospitalization diaries and completed patient-centered outcome forms can be sent from the subject to the site by mail.

7.2.5 Assessment of Patient-Centered Outcomes (PCO)

We will assess PCO by utilizing a quality of life (QoL) tool that has been validated in cystic fibrosis [33]. The Cystic Fibrosis Questionnaire-revised (CFQ-R) has versions for an interview format for children (age 6-11 years), a self-report for children (age 12-13 years), a self-report for adolescents and adults aged 14 years and older and a caregiver version that will be used for all caregivers and children under the age of 6 [34] (see MoP). We will plan to obtain PCO data using the CFQ-R from patients and caregivers at multiple time points during the study.

7.3 SCHEDULE OF ACTIVITIES for enrolled study patients with plastic bronchitis

Protocol Activity	Screening Phase		Treatment Phase		Follow-up	Closeout
	Visit 1	Visit 1+2[1]	Pre-tx[2] (day 1)	Treatment (days 2-3)	Hospital Discharge (day 3 or hospital discharge)	Day 30[10] (+/- 7 days)
Informed Consent	X					
Medical History		X				X
Collection and submission of cast	X		X	X	X	
Physical exam and vital signs		X	X	X	X[3]	X
Abbreviated neurological exam		X		X		X
Full neurological exam			X		X[3]	
Chest X-ray			X		X[3]	
Laboratory:						
Hct and platelet count[4]		X	X	X	X[3]	X
INR & PTT[4]		X	X	X	X[3]	X
Transaminases (AST and ALT) [5]		X				
FDP[4]		X	X	X	X[3]	X
fibrinogen concentration[4]		X	X	X	X[3]	X
tPA concentration[6]		X	X	X	X[3]	
Urinalysis and pregnancy test (if applicable)[4]		X	X	X	X[3]	
Immunoglobulins (IgA, G and M)[4]		X	X		X[3]	X[7]
Pulmonary function tests		X	X	X	X[3]	X
Blood & urine for biomarker assays	X	X	X	X	X	
Pulse oximetry		X	X	X	X[3]	X
Treatment with study agent			X	X		
Adverse event assessment	X[8]	X[8]	X[8]	X	X	X
Concomitant Medications		X	X	X	X	X
Cast production & hospitalization diary				X	X[9]	X
Patient Centered Outcome Assessment			X		X[3]	X

[1]for non-CHD patients with plastic bronchitis, visit 1 and visit 2 (screening phase) should be at least two weeks apart (see study protocol); for CHD patients, visit 1 and 2 can be combined
[2]if screening visit 2 laboratories are within two weeks of the pre-treatment (day 0) visit, they do not need to be re-collected with the exception of a pregnancy in female patients
[3] Within 24h of completion of the administration of the last dose of inhaled tPA, whether study drug or that administered as part of clinical care, or at hospital discharge, whichever occurs first, patients will have all the required discharge assessments listed. If hospital discharge is the same day as the day of the administration of the 12th (or last) dose of study drug, daily monitoring labs can be obtained after administration of the 12th (or last) dose along with all other hospital discharge scheduled activities
[4]these assays will be performed by each center's clinical laboratory; a pregnancy test in female patients of childbearing potential will be performed once at the pre-treatment (tx) visit
[5]see exclusion criteria section 5.1.2 regarding assessment of liver function
[6]this assay will be performed by the UM DCC laboratory and is not for clinical use
[7]if applicable, patients with elevated anti-tPA antibodies will be followed until levels return to baseline; see MoP for details.
[8]Only adverse events considered possibly related to research procedures will be collected prior to the dosing of inhaled tPA
[9]at hospital discharge, patients will be given a diary to document episodes of cast expectoration and/or subsequent hospitalizations. This will be reviewed at the 30d visit. The estimated blood volume that will be collected per patient is included in the MoP
[10]If necessary, the 30d visit can be accomplished using telemedicine and a local laboratory for clinical laboratory (see protocol and MoP).

7.4 SCHEDULE OF ACTIVITIES for enrolled healthy, PLE Fontan, and non-PB Fontan controls

Protocol Activity	Controls
	Visit 1
Informed Consent	X
Medical History	X
Collection and submission of cast	--
Physical exam, vital signs	X
Chest X-ray	--
Laboratory:	
Hct and platelet count	X
INR & PTT	X
FDP	X
fibrinogen concentration	X
Urinalysis	X
Immunoglobulins (IgA, G and M)	X
Pulmonary function tests	X
Blood & urine for biomarker assays	X
Pulse oximetry	X
Treatment with study agent	--
Adverse event assessment	X[1]
Concomitant Medications (Fontan and PLE patients)	X

[1]Only adverse events considered to be possibly related to the research procedures will be collected for enrolled healthy and non-PB Fontan controls
The estimated blood volume that will be collected per patient is included in the MoP.

8 ASSESSMENTS

8.1 Primary Endpoint Safety Assessments

- Development of new, active bleeding that is systemic and/or pulmonary
- New hematuria (defined as gross hematuria)

8.2 Secondary and Tertiary Efficacy Assessments:

- Changes in oxygen saturation (as determined by pulse oximetry): we will make repeated measures of oxygenation beginning before and then after the initiation of tPA treatment.
- Changes in pulmonary function tests (PFT): we will assess PFT prior to and after tPA treatment; we expect that tPA treatment will improve pulmonary function
- Frequency of production/expectoration and size of airway casts (weight and length): we expect that tPA will reduce the frequency of airway cast production/expectoration
- Changes in the chest x-ray (CXR): we will assess the CXR using the Brasfield score. We expect scores to improve with tPA treatment.
- Requirement for urgent or emergent bronchoscopy and/or mechanical ventilation: we expect that few, if any, patients will require urgent or emergent bronchoscopy and/or mechanical ventilation
- Measurement of fibrin and mucin content of airway casts: we expect that pathologic assessment will show that tPA treatment reduces fibrin content compared with pre-treatment casts
- Detection of fibrin degradation products (FDPs) in systemic circulation: we will determine whether FDP are detected in the blood secondary to fibrinolysis of airway casts
- We will assess the impact of inhaled tPA treatment on PCO
- Measurement of urine and blood metabolites for the identification of potential biomarker candidates of drug response

8.2.1 General Medical History/Physical Exam

A medical history will be performed at Visit 1/2 (screening phase) and the Closeout visit. Physical examinations including an abbreviated neurological exam (see MoP) and vital sign measurements will be done at Visit 1/2 and then daily during the treatment phase until hospital discharge and again at day 30 (see chart of study activities **7.3**).

8.2.1 Concomitant Medication Use

Concomitant medications will be assessed during the screening phase and during tPA treatment and again at the study close-out (day 30).

8.2.3 Adverse Events

Assessment for potential adverse events will be made beginning from the time of signed informed consent through hospital discharge and again on day 30. Only adverse events that are categorized as "possibly related" to testing procedures will be captured until dosing with inhaled tPA begins.

Only adverse events that are categorized as "possibly related" to testing procedures will be

captured for enrolled healthy and non-PB Fontan controls.

8.2.4 Patient Centered Outcomes

Patient centered outcomes will be assessed at the time of acute PB exacerbation (pre-treatment, day 0), after tPA treatment (day 4 or hospital discharge) and at the 30 day closeout visit as described under section **7.2.5**. See the MoP.

9 ADVERSE EVENT REPORTING

9.1 Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) all events of death, and any study specific issue of concern.

9.1.1 Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with plastic bronchitis that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).

If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.

Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

9.1.2 Serious Adverse Events

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

9.2 Methods and Timing for Assessing and Recording Safety Variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, are collected and reported to the University of Michigan Data and Clinical Coordinating Center (UM DCCC), and appropriate IRB(s). The UM DCCC will report to the FDA and Genentech, Inc. in accordance with CFR 312.32 (IND Safety Reports) and will prepare and submit reports to the DSMB.

9.2.1 Adverse Event Reporting Period

Assessment for potential adverse events will be made beginning from the time of signed informed consent through hospital discharge and again on day 30. Only adverse events that are categorized as "possibly related" to testing/screening procedures will be captured until dosing with inhaled tPA begins.

The study period during which all AEs and SAEs must be reported begins from the initiation of dosing until 30 days after dosing, or until a subject's participation in the trial is considered complete or when a subject is discontinued/withdrawn, whichever is latest. After this period, investigators should only report SAEs that are attributed to prior study treatment.

Only adverse events that are categorized as "possibly related" to screening and testing procedures will be captured for enrolled healthy and non-PB Fontan controls.

9.2.2 Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the Activase (tPA; see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

9.2.3 Yes

There is a plausible temporal relationship between the onset of the AE and administration of the Activase (tPA), and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the Activase (tPA); and/or the AE abates or resolves upon discontinuation of the Activase (tPA) or dose reduction and, if applicable, reappears upon re-challenge.

9.2.4 No

Evidence exists that the AE has an etiology other than the Activase (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to Activase (tPA) administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

9.3 Procedures for Eliciting, Recording, and Reporting Adverse Events

9.3.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

9.3.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

9.3.2.1 Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is okay to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

9.3.2.2 Deaths

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

9.3.2.3 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be reassessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

9.3.2.4 Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

9.3.2.5 Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 30 days after the last dose of study drug, a report should be completed and expeditiously submitted to the UM DCC which will submit the report to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the Activase (tPA) should be reported as an SAE.

9.3.2.6 Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior Activase (tPA) exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

9.3.2.7 Case Transmission Verification of Single Case Reports

The Sponsor-investigator agrees to conduct the Case Transmission verification to ensure that all single case reports have been adequately received by Genentech via the Sponsor emailing Genentech a Quarterly line-listing documenting single case reports sent by the Sponsor to Genentech in the preceding time period.

The periodic line-listing will be exchanged within seven (7) calendar days of the end of the agreed time period. Confirmation of receipt should be received within the time period mutually agreed upon.

If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution. The Sponsor shall receive reconciliation guidance documents within the 'Activation Package'.

Following Case Transmission Verification, single case reports which have not been received by Genentech shall be forwarded by the Sponsor to Genentech within five (5) calendar days from request by Genentech.

At the end of the study, a final cumulative Case Transmission Verification report will be sent to Genentech.

9.3.2.8 AEs of Special Interest (AESIs)

AESIs are a subset of Events to Monitor (EtMs) of scientific and medical concern specific to the product, for which ongoing monitoring and rapid communication by the site investigators to the Sponsor is required. Such an event might require further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the Sponsor to other parties (e.g., Regulatory Authorities) may also be warranted.

Adverse events of special interest for this study include the following:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law:
    - Treatment-emergent ALT or AST > 3 × ULN in combination with total bilirubin > 2 × ULN
    - Treatment-emergent ALT or AST > 3 × ULN in combination with clinical jaundice
- Data related to a suspected transmission of an infectious agent by the study drug (STIAMP), as defined below:

  Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

9.3.2.9 Exchange of single case reports

The Sponsor-investigator will be responsible for collecting all protocol-defined Adverse Events (AEs)/Serious Adverse Events (SAEs), AEs of Special Interest (AESIs), Special Situation Reports (including pregnancy reports) and Product Complaints (with or without an AE) originating from the Study for the Product.

The Sponsor must report all the above mentioned single case reports adequately to Genentech within the timelines described below. The completed MedWatch or CIOMS I form or Genentech approved reporting forms should be faxed/emailed immediately upon completion to Genentech at the following contacts:

All protocol-defined AEs, SAEs, AESIs, Special Situation Reports (including pregnancy reports) and Product Complaints *with* an AE should be sent to:

Fax: 650-238-6067
Email: usds_aereporting-d@gene.com

All Product Complaints *without* an AE should be sent to:

Phone: (800) 334-0290 (M-F: 5 am to 5 pm PST)

Transmission of these reports (initial and follow-up) will be either electronically or by fax and within the timelines specified below:

Type of Report	Timeline
Serious Adverse Events (related and not related to the product)	30 calendar days from awareness date
Special Situation Reports (with or without AE and pregnancy)	
Product Complaints (With or without AE)	
AESI	

- **Special Situation Reports**

  Pregnancy reports

  While such reports are not serious AEs or Adverse Drug Reactions (ADRs) per se, as defined herein, any reports of pregnancy (including pregnancy occurring in the partner of a male study subject), where the fetus may have been exposed to the Product, shall be transmitted to Genentech within thirty (30) calendar days of the awareness date. Pregnancies will be followed up until the outcome of the pregnancy is known, whenever possible, based upon due diligence taken to obtain the follow-up information.

  Pregnancies in Female Partners of Male Patients

  Male patients will be instructed through the Informed Consent Form to immediately inform the investigator if their partner becomes pregnant during the study or within 30 days after the last dose of study drug. A Clinical Trial Pregnancy Reporting Form should be completed and submitted to Genentech within thirty (30) calendar days of the awareness date.

- **Other Special Situation Reports**

  The following other Special Situations Reports should be collected even in the absence of an Adverse Event and transmitted to Genentech within thirty (30) calendar days:
  - Data related to the Product usage during breastfeeding
  - Data related to overdose, abuse, misuse or medication error (including potentially exposed or intercepted medication errors)
  - In addition, reasonable attempts should be made to obtain and submit the age or age group of the patient, in order to be able to identify potential safety signals specific to a particular population

- **Product Complaints**

  A Product Complaint is defined as any written or oral information received from a complainant that alleges deficiencies related to identity, quality, safety, strength, purity, reliability, durability, effectiveness, or performance of a product after it has been released and distributed to the commercial market or clinical trial.

9.3.3 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic (Section A) and suspect medication information (Section C & D), the report should include the following information within the Event Description (Section B.5) of the MedWatch 3500A form:

- Protocol number and title description

- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics (section B.6)
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

9.3.3.1 Follow-up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e., D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at https://www.fda.gov/media/69876/download

9.3.3.2 Reporting to Regulatory Authorities, Ethics Committees, and Investigators

The Sponsor of the study will be responsible for the expedited reporting of safety reports originating from the Study to the Regulatory Authorities (FDA) where it has filed a clinical trial approval, in compliance with local regulations.

The Sponsor will be responsible for the expedited reporting of safety reports originating from the Study to the Ethics Committees and Institutional Review Boards (IRB), where applicable.

The Sponsor will be responsible for the distribution of safety information to its own investigators, where relevant, in accordance with local regulations.

Follow-up with investigational site personnel will be conducted by UM DCCC to obtain any additional information needed to complete the reporting of the event.

9.3.4 Additional Reporting Requirements for IND Holders

For Investigator-Sponsored IND Studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

The UM DCCC will coordinate with the Michigan Institute for Clinical and Health Research (MICHR) IND/IDE Investigator Assistance Program (MIAP) office for the reporting of any and all

IND safety reports to the FDA as per the requirements outlined in 21 CFR 312.32.

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

9.3.4.1 Seven Calendar Day Telephone or Fax Report

The sponsor-investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the sponsor-investigator to be possibly related to the use of Activase (tPA). An unexpected adverse event is one that is not already described in the Activase (tPA) Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and Genentech within 7 calendar days of first learning of the event.

9.3.4.2 Fifteen Calendar Day Written Report

The sponsor-investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of Activase (tPA). An unexpected adverse event is one that is not already described in the Activase (tPA) investigator brochure.

Written IND Safety reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the sponsor-investigator with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.
Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, Genentech, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 form, but alternative formats are acceptable (e.g., summary letter).

9.3.4.3 FDA fax number for IND Safety Reports:

Fax: 1 (800) FDA 0178

9.3.4.4 All written IND Safety Reports submitted to the FDA by the sponsor-investigator must also be faxed to Genentech Drug Safety (see MoP)

Fax: (650) 225-4682 or (650) 225-4630
Email: usds_aereporting-d@gene.com

9.3.4.5 And reported to the University of Michigan and Local Site IRB as per institutional policy:

University of Michigan Medical School Institutional Review Board (IRBMED)
2800 Plymouth Road
Building 520, Room 3214
Ann Arbor, MI 48109-2800
Telephone: 734-763-4768 (For International Studies: US Country Code: 001)
Fax: 734-763-1234
e-mail: irbmed@umich.edu

A summary of all non-expedited safety reports will be submitted in the FDA IND annual report.

AE reporting will follow institutional (IRB) and FDA regulatory guidelines.

9.3.4.6 For questions related to safety reporting, please contact Genentech Drug Safety

Copies of such reports should be emailed to Genentech at: Genentech Drug Safety CTV mail box: ctvist_drugsafety@gene.com

9.3.5 IND Annual Reports

9.3.5.1 Copies to Genentech:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-463
Aggregate reports: The Sponsor-investigator will forward a copy of the Final Study Report to Genentech upon completion of the Study.

Other Reports

The sponsor will forward a copy of the Final Study Report to Genentech upon completion of the Study.

9.4 Study Close-Out

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be sent to the following Clinical Operations mailbox: lytics-gsur@gene.com and to Genentech Drug Safety CTV oversight mail box at: ctvist_drugsafety@gene.com.

10 DATA ANALYSIS/STATISTICAL METHODS

10.1 Sample Size Determinations

Plastic bronchitis patients: Standard power calculations cannot be adequately completed for this study because of the lack of knowledge regarding the expected primary safety endpoint of clinically-relevant bleeding. The bleeding incidence of systemically administered tPA, extrapolated from adult studies of MI, is 5% [28]. This incidence is attributable to loss of fibrinolytic homeostasis that is induced by direct administration of tPA into the circulation. Given that presently, there is no evidence that administration of inhaled tPA results in measurable amounts of tPA in the circulation, we expect the incidence of systemic bleeding to be much less. Taking a liberal view, we expect an incidence of systemic bleeding that is approximately 1/3 of that which occurs with systemic administration of tPA. Using this estimate, enrollment of 24 subjects with PB with 0% bleeding will result in a 95% confidence interval that spans 0%-14.3%. Enrollment of 20 subjects with PB with 0% bleeding will result in a 95% confidence interval that spans 0%-16.8%. Enrollment of 24

subjects with PB with 1 child (4%) experiencing bleeding with 95% confidence, the population bleeding incidence is expected to be in the interval 0.1%-21.1%. Enrollment of 20 subjects with PB with 1 child (5%) has bleeding, with 95% confidence, the population bleeding incidence is expected to be in the interval 0.13%-24.9%. Based on a safety assessment following the enrollment and treatment of 6 PB subjects, no tPA-treated subject has reached a study safety end point (systemic bleeding). Given this, the planned study enrollment was adjusted. Specifically, considering the 0% observed rate of systemic bleeding in the 6 PB subjects who have completed the study as of November 2020, we assume the same bleeding incidence rate for the additional enrolled PB subjects. As such, enrollment of a total of 11 to 13 subjects with PB with no participant experiencing systemic bleeding, with 95% confidence, the population bleeding incidence is expected to be within 0% - 28.5% to 0% - 24.7% intervals. Therefore, the previously tolerated upper 95% confidence limit of 25% with a sample size of 13 would be maintained and modestly increase this limit with enrollment of 11 PB subjects.

Study sites in addition to our own institution will be recruited through the pediatric research consortium as well as other major CHD centers with whom our center has had previous multi-center collaborations. In addition, our institution is a primary member of the Pediatric Heart Network, a clinical research consortium dedicated to enhancing clinical treatment of CHD. The Pediatric Heart Network has previously demonstrated a significant commitment to research in the single ventricle CHD population, and the institutions that make up this consortium may have interest in participating in the trial. Due to the rarity of PB, we expect each center would likely contribution 3-4 patients, making 6-8 participating centers necessary. We expect our institution's research ties makes recruiting this number of institutions and patients feasible.

Healthy (non-Fontan), PLE Fontan controls and non-PB Fontan controls: Will be utilized for the purpose of benchmarking clinical laboratories, including immunoglobulin levels, PFT and the tPA response biomarker discovery portion of this study(see 7.3 and 7.4). Based on our previous work in the field of metabolomics (the technique that will be applied for biomarker discovery in this study), we expect that there will be large effect sizes (i.e., Cohen's D-statistic) ranging from 1.0 to 2.8 between PB patients and healthy, PLE Fontan and non-PB Fontan controls. Since we will control subjects to our PB cohort, we will account for two covariates that are known to influence the metabolome [30, 35, 36]. However, we do acknowledge that since so little is known about the metabolome in children, we have little prospective knowledge of variance. Nevertheless, we expect by enrolling 12 healthy controls, 12 PLE Fontan, 12 non-PB Fontan patients, and ~12 PB subjects, we expect to be able to detect trends in metabolic differences induced by PB and inhaled tPA. Healthy and non-PB Fontan control subjects will be recruited and enrolled at the study's coordinating site, the University of Michigan; PLE Fontan patients will be recruited at all clinical sites.

10.2 Safety Analysis

This is an open-label safety study that includes a group of healthy subjects that will serve as controls for biomarker assay development. For the safety analysis, the incidence of new, active bleeding events will be assessed.

10.2.1 Analysis Populations

All subjects enrolled into the study will be included in the analysis. Information pertaining to patients who continue inhaled tPA beyond 72 hours (+/- 30 min) will also be included in the analysis. Patients that complete the screening phase but are not dosed with inhaled tPA will be

replaced.

10.2.2 Analysis of Primary Endpoint (safety)

- Development of new, active bleeding that is systemic and/or pulmonary
- New hematuria (defined as gross hematuria)

Due to the nature of the study, the majority of analysis will be descriptive. For this primary endpoint, the number of clinically relevant episodes of both active bleeding and gross hematuria will be compiled. The proportion of patients meeting the safety endpoint will be reported with a 95% confidence interval.

10.2.3 Analysis of Secondary Endpoints (efficacy)

- Oxygen saturation (as determined by pulse oximetry)

Oxygen saturation values will be determined as described previously in the protocol. Descriptive statistics will be used to summarize the pre- and post tPA oxygen saturations.

- Pulmonary function test (PFT)

The change in pulmonary function from pre- to post- tPA treatment will be assessed for each patient.

- Frequency of production/expectoration and size of airway casts

Descriptive statistics on the frequency and production and size of airway casts will be used. If a subset of subjects in whom frequency of cast production is previously documented, the difference in pre- and post tPA cast production will be compared.

- Chest x-ray (CXR)

tPA treatment-induced changes in the CXR will be assessed.

- Requirement for urgent or emergent bronchoscopy and/or mechanical ventilation.

Descriptive statistics will be used to report this incidence of these complications.

- Fibrin and mucin content of airway casts

After laboratory calculation of the cast composition (previously described), descriptive statistics will be used to summarize the cast fibrin and mucin content for casts collected before and after tPA treatment.

- Detection of fibrin degradation products (FDP) and changes in systemic fibrinogen levels

The presence of FDPs will be viewed as a binary (yes/no) variable. The proportion of subjects in whom FDPs are found will be reported with an accompanying 95% confidence interval.

- Assessment of PCO

We will assess PCO using an established and validated QOL tool that has been used in CF.

10.2.4 Tertiary Endpoints

- Measurement of urine and blood metabolites for the identification of potential biomarker candidates of drug response

In this case-control portion of the study, the measurement of each metabolite will be compiled and reported for each group using appropriate statistics. Following this, appropriate parametric and non-parametric 2-group comparison will be used to compare biomarker metabolites between each group. These data will also be subjected to bioinformatics analysis which may lead insight into potential off-target drug response [37, 38].

10.3 Safety Analysis

The number of clinically relevant episodes of both active bleeding and hematuria will be compiled and reported with a 95% confidence interval.

11 MONITORING

11.1 Data Safety and Monitoring Board (DSMB)

An institutionally-initiated Data Safety Monitoring Board will be established for this trial. Briefly, members will include those with expertise in pediatric cardiology/pulmonology, plastic bronchitis and a biostatistician. The DSMB will monitor the progress of the trial with a scheduled meeting or conference call. The first DSMB meeting will occur after three patients have completed the treatment phase of the study or 6 months following study initiation, as long as at least one patient has entered the treatment phase, whichever comes first. In addition to reviewing Serious Adverse Events (SAEs), the first DSMB meeting will focus on over all safety of the trial and study agent and will make a determination as to whether or not the study should proceed. DSMB meetings will be scheduled quarterly, but they will only occur if at least 3 patients have been enrolled since the prior meeting. This is because of the study's small sample size and that the cadence of enrollment is expected to be slow. Unexpected and related or possibly related SAEs will be reported to the DSMB chair and the committee will convene at his discretion.

A DSMB will be named prior to initiation of the study.

11.2 Monitoring Plan and Information

To assure adequate protection of the rights of human subjects, per 21 CFR §312.50, 312.53, this study will be monitored by the University of Michigan Institute for Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. An initiation visit will take place, followed by routine monitoring visits. Additional visits can be scheduled at the request of the Project Manager. Monitoring visits may be in the form of a site visit or a review of the documents submitted to the Multi Site Coordinator. During a monitoring visit to a site, access to relevant hospital and clinical records must be given by the site investigator to the MICHR representative conducting the monitoring visit to verify consistency of data collected on the CRFs with the original source data. It is expected that the relevant investigational staff be available to facilitate the conduct of the visit, that source documents are available at the time of the visit, and that a suitable environment will be provided for review of study-related documents. Any issues identified during these visits will be communicated to the site and are expected to be resolved by the site in a timely manner.

The established monitoring plan will ensure the quality and integrity of the data through pre-investigation visits and periodic site visits to verify adherence to the protocol, completeness and

accuracy of study data and samples collected, proper storage, dispensing and inventory of study medication, and compliance with regulations.

12 DATA HANDLING AND RECORD KEEPING

12.1 CRFs / Electronic Data Record

Data will be protected by several different measures throughout the life of the study. Paper documents and records will be stored in a secured location with restricted access to authorized personnel only. Electronic study documents and data will be kept in a password protected environment, whereby access rights will be terminated at the request of the PI when study members leave the project.

A secure, regulatory-compliant, web-based electronic data capture (EDC) system designed specifically for investigators and their research teams will be used for data collection and management. The EDC is built on a flexible and extensible data model that can accommodate input of diverse clinical or laboratory data. The system possesses the ability to maintain an audit trail of the entire study, enabling traceability of entries and modifications to research-related data. Role-based access to the application, the databases and archives, and the underlying systems infrastructure comply with industry best practices and meet HIPAA security and privacy requirements, governed by HIPAA's "minimum necessary" principle.

12.2 Record Retention

Per 21 CRF §312.62, study records will be retained for 2 years after the investigation is discontinued and the FDA has been notified.

13 ETHICS

13.1 IRB/FDA

Prior to study commencement, an Investigator Initiated Investigational New Drug (IND) will be submitted to the Food and Drug Administration (FDA), for review and approval. The study will also be reviewed and approved by the Institutional Review Board (IRBMED, University of Michigan, Ann Arbor, MI) and the IRBs of all participating clinical sites.

13.1.1 Institutional Review Board (IRB)

Before implementing this study, the protocol, the proposed informed consent form and other information to be provided to subjects, must be reviewed by a properly constituted Institutional Review Board (IRB). Any amendments to the protocol must be reviewed and approved by IRBMED and submitted to the FDA as required under 21 CFR 312.30.

13.2 Ethical Conduct of the Study

The trial will be conducted in accordance with the principles of the Declaration of Helsinki.

13.3 Subject Information and Consent and Assent

A study team member will explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject will be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician. Study subjects that are age 14 years or older will be given the informed consent while subjects younger than 14 years but at least 10 years of age, will be given an assent form, and for children younger than 10 years of age, parental consent only will be obtained (or in accordance with each institution's IRB guidelines). For assent, the study will be briefly described as well things that could go well (potential benefits) and things that could go wrong (potential risks). In both situations, parental written informed consent will be required.

This informed consent will be given by means of a standard written statement, written in non-technical language in English only. The subject will be asked to read and consider the statement before signing and dating it, and should be given a copy of the document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form and assent form are considered to be part of the protocol, and will be submitted for IRB approval.

14 STUDY DISCONTINUATION CRITERIA

14.1 Stopping Rules for Safety reasons

The Data Safety Monitoring Board (DSMB) will review all Serious Adverse Events (SAEs) and make recommendations regarding the continuation or discontinuation of the study, as appropriate.

If an individual subject experiences moderate pulmonary bleeding (as defined below) and/or a moderate, non-pulmonary hemorrhage, over a 24h period treatment, study treatment should be stopped.

If an individual subject experiences either a severe pulmonary or non-pulmonary bleeding event, as defined below, study treatment should be stopped.

If three of the first 10 subjects experience an episode of moderate pulmonary bleeding over a 24h period and/or four or more episodes of moderate non-pulmonary bleeding or one or more severe bleeding events (pulmonary or non-pulmonary) that is determined to be related to treatment with study drug, then the study will be immediately stopped.

Any incidence of fatal bleeding (pulmonary or non-pulmonary) that is determined to be related to treatment with study drug will cause the study to be immediately stopped.

Pulmonary Hemorrhage: A disorder characterized by bleeding from the bronchial wall and/or lung parenchyma

Grading System for Pulmonary Hemorrhage*

Grade	Defined by:
1-Mild (scant)	hemoptysis of ≤ 5ml of bright red blood for which intervention not indicated
2-Moderate	Up to three episodes in a 24h period of hemoptysis of > 5ml ≤ 100 ml of bright red blood or a single episode of >100 < 240 ml for which medical intervention is necessary and a radiologic assessment (e.g., chest x-ray) should be considered
3-Severe	hemoptysis of ≥240 ml and/or suspected pulmonary bleeding for which transfusion, radiologic, endoscopic, or operative intervention is indicated (e.g., hemostasis of bleeding site), or there is life-threatening respiratory or hemodynamic compromise for which intubation or urgent intervention is indicated

*Adapted from CTCAE v.4.0 and reference [39]

Other (Non-Pulmonary) Hemorrhage

Grading System for Other (Non-Pulmonary) Hemorrhage

Grade	Defined by:
1-Mild	non-actionable bleeding that does not require treatment by a healthcare professional
2-Moderate	any overt, actionable sign of bleeding (e.g., gross hematuria) that is more than clinically expected but does not meet the severe criteria below; meets at least one of the following: 1) requires nonsurgical, medical intervention by a healthcare professional; 2) prolongs hospitalization or an increased level of care; 3) prompts evaluation (e.g., bronchoscopy, imaging)
3-Severe	Overt bleeding with a bleeding related drop of 20% or more in pre-treatment hematocrit that cannot be attributed to volume overload or an overt bleeding event that requires transfusion, surgical intervention or the use of intravenous vasoactive agents. Intracranial hemorrhage or intraocular bleeding the compromises vision.

Anaphylaxis Reaction: including laryngeal edema, rash, orolingual angioedema, and urticarial
If a subject experiences a mild or moderate anaphylaxis reaction, as defined below, study drug will be discontinued.

If three of the first 10 subjects experience severe anaphylaxis events as defined below, then the study will be immediately stopped.

Grading system for generalized hypersensitivity reactions*

Grade	Defined by:
1-Mild (skin and subcutaneous tissues only)+	Generalized erythema, urticaria, periorbital edema, or angioedema
2-Moderate (features suggesting respiratory, cardiovascular, or gastrointestinal involvement)	Dyspnea, stridor, wheeze, nausea, vomiting, dizziness (presyncope), diaphoresis, chest or throat tightness, or abdominal pain
3-Severe (hypoxia, hypotension, or neurologic compromise)	Cyanosis or SpO2 < 92% at any stage, hypotension (SBP < 90 mm Hg in adults), confusion, collapse, LOC, or incontinence

+adapted from: Brown, SG.

Acute Change in Mental Status and/or Signs and Symptoms of Intracranial Hemorrhage
If an individual subject develops an acute change in mental status such as confusion or disorientation, loss of alertness or abrupt change in motor function, that, in the judgment of the treating physician, warrants further neurological evaluation, study drug should be discontinued pending this evaluation. If an individual subject develops signs and symptoms consistent with stroke (e.g., slurred speech, facial drooping), or in the event that neurological evaluation is suggestive of stroke, study drug should be discontinued.

Any incident of confirmed intracranial hemorrhage will cause the study to be stopped.

Following discontinuation of the study, all study patients will be followed in accordance with the protocol requirements.

14.2 Discontinuation of a Subject

If a patient reaches a study safety end point using the criteria described above, study drug will be discontinued and care will be continued based on the preference of the attending physician. In the event a patient experiences an AE, drops out of the study or is discontinued due to protocol violations, all attempts will be made to exit the patient in accordance with the protocol requirements.

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